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Research Report

Developmental timeframes for induction of microgyria and rapid auditory processing deficits in the rat

Steven W. Threlkeld[a], Melissa M. McClure[a], Glenn D. Rosen[b,c], R. Holly Fitch[a,*]

[a]Department of Psychology; Behavioral Neuroscience Division, University of Connecticut, 806 Babbidge Road, Storrs, CT 06269-4154, USA
[b]Department of Neurology, Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215, USA
[c]Harvard Medical School, Boston, MA 02115, USA

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ABSTRACT

Induction of a focal freeze lesion to the skullcap of a 1-day-old rat pup leads to the formation of microgyria similar to those identified postmortem in human dyslexics. Rats with microgyria exhibit rapid auditory processing deficits similar to those seen in language-impaired (LI) children, and infants at risk for LI and these effects are particularly marked in juvenile as compared to adult subjects. In the current study, a startle response paradigm was used to investigate gap detection in juvenile and adult rats that received bilateral freezing lesions or sham surgery on postnatal day (P) 1, 3 or 5. Microgyria were confirmed in P1 and 3 lesion rats, but not in the P5 lesion group. We found a significant reduction in brain weight and neocortical volume in P1 and 3 lesioned brains relative to shams. Juvenile (P27–39) behavioral data indicated significant rapid auditory processing deficits in all three lesion groups as compared to sham subjects, while adult (P60+) data revealed a persistent disparity only between P1-lesioned rats and shams. Combined results suggest that generalized pathology affecting neocortical development is responsible for the presence of rapid auditory processing deficits, rather than factors specific to the formation of microgyria per se. Finally, results show that the window for the induction of rapid auditory processing deficits through disruption of neurodevelopment appears to extend beyond the endpoint for cortical neuronal migration, although, the persistent deficits exhibited by P1 lesion subjects suggest a secondary neurodevelopmental window at the time of cortical neuromigration representing a peak period of vulnerability.

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1. Introduction

Rapid auditory processing (RAP) deficits are defined as difficulties in the ability to detect rapid changes in auditory stimuli (Tallal, 2004; Tallal and Piercy, 1973; Tallal, 1976). Many infants and children with RAP deficits go on to exhibit language and reading difficulties upon entering the education system, and early indices of auditory processing are highly predictive of long term language outcomes, both in normal and impaired children (Benasich and Tallal, 2002; Benasich et al., 2006; Tallal et al., 1993). Concurrent anatomical analysis of postmortem dyslexic brains has revealed the presence of cortical malformations such as molecular layer ectopias and microgyria, primarily in the left hemispheric perisylvian

* *Corresponding author*. Fax: +1 860 486 2706.
E-mail address: roslyn.h.fitch@uconn.edu (R.H. Fitch).

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region (Galaburda, 1993; Galaburda and Kemper, 1979; Galaburda et al., 1985). Evidence linking auditory processing with language ability confirms that adult dyslexic individuals also have a high rate of RAP deficits compared to controls (Farmer and Klein, 1995).

Animal models bridging these lines of research have revealed RAP deficits in rats and mice with induced and spontaneous cortical malformations, including microgyria and molecular layer ectopias (Peiffer et al., 2001, 2002a, 2004a,b; Fitch et al., 1994; Clark et al., 2000). Historically, these studies have focused on the experimental induction or intrinsic presence of developmental cortical malformations as a key factor underlying emergent RAP deficits (Herman et al., 1997; Fitch et al., 1994). Since evidence shows that microgyria and other cortical dysplasias can be traced to early injury, or some other genetic or epigenetic disruption of neuronal migration (Gleeson, 2001; Volpe, 2001; Rosen et al., 1992b, 2000; Barth, 1987; Dvorak and Feit, 1977; Dvorak et al., 1978), cortical malformations themselves may be directly related to deficits in RAP. Alternatively, these malformations could be acting as indicators of a more generalized disruption of brain development that in turn impacts auditory processing (Clark et al., 2000; Fitch et al., 1994; Rosen et al., 2000). Consistent with the latter view, rats with postnatal day (P) 1 induced microgyric malformations reveal ipsilateral and contralateral changes in cortical connectivity as a result of the damage (Giannetti et al., 1999, 2000; Di Rocco et al., 2001, 2002; Rosen et al., 2000, 2001), which suggests that a global reorganization of neuronal circuitry is induced by early developmental trauma, such as focal freeze lesions. The connectivity changes associated with focal ischemic damage resulting in malformations are thought to reflect disruption of organization that occurs during cortical plate formation (Rosen and Galaburda, 2000; Rosen et al., 1992a, 2000), with research suggesting that the cortical plate is fully formed around P4 in the rat (Paxinos, 2004; Rosen and Galaburda, 2000; Dvorak and Feit, 1977). However, neuronal migration is not the only process taking place during this developmental period. In this same timeframe, axonal projections are well on their way to target locations, and dendritic arborization is beginning for cortical neurons that have reached their final resting place (Catalano et al., 1991). Moreover, axonal targeting and synapse development extends beyond the cessation point for the formation of typical six layer cortical lamina in rats (Lopez-Bendito and Molnar, 2003; Agmon et al., 1993). In order to understand the complex relationship between the presence of cortical malformations and RAP deficits in rats, and to assess the role that developmental malformations play in the emergence of RAP deficits, studies including cortical injury induced at ages that do and do not lead to microgyria must be employed.

In the present study, comparable postnatal focal bilateral freezing lesions were made to the cortices of male rat pups at three ages: P1, P3 and P5. This age range incorporates the window for the completion of neuronal migration to the cortical plate, and evidence suggests that damage at these ages result in different types of malformations (Dvorak and Feit, 1977). We subsequently assessed the effects of these injuries on auditory processing, in an attempt to uncover the behavioral consequence of developmental disruption at these different time points.

2. Results

2.1. *Statistical grouping*

In all initial analyses, Age at Treatment (P1, P3, P5) and Treatment (Sham/Lesion) were considered fixed factors. When a main effect or interaction was observed, simple effects analyses were conducted to determine which groups contributed to the effect. In all analyses, sham animals were found to show no differences in performance or histological measure as a function of Age at Treatment (P1, versus P3, versus P5). Thus, for all simple effects analyses, shams were pooled into a single group and compared to P1, P3 or P5 lesion conditions separately (multi-variate ANOVAs required the nested design including Age at Treatment for shams). Finally, all tests were two tailed unless otherwise indicated (one-tailed tests were used where prior findings predicted an effect in one direction only).

2.2. *Histology*

There was no evidence of cortical damage in any of the sham subjects ($n = 16$). Postmortem analysis revealed the presence of bilateral microgyria only in the P1 ($n = 13$) and P3 ($n = 17$) lesion groups. The P5 ($n = 17$) lesion group, which received a comparable freezing lesion treatment relative to the P1 and P3 groups, did not show evidence of microgyria. However, the P5 lesion group did show some necrosis (disrupted cortical lamination) in areas of cortex directly under the probe application points (see Fig. 1), with an appearance typical of ischemic damage. Lesions were seen mostly in sensorimotor cortex (SM-I), with some extension into frontal, temporal, or occipital cortices. The majority of double lesions in P1 and P3 conditions appeared as one continuous severe microgyric lesion. However, the P5 group showed a pattern of disruption that was centered on the specific areas of probe application. This pattern appeared as four relatively small distinct pockets of necrosis resulting from the early insult.

Univariate ANOVAs were computed for all anatomical measures using Age at Treatment (3 levels) and Treatment (2 levels) as fixed factors. Results showed a significant main effect of Treatment for bilateral neocortical volume [$F(2,57) = 21.05$, $P < 0.01$], with lesion subjects showing significantly smaller cortices as compared to shams. Further, a main effect of Age at Treatment [$F(2,57) = 13.28$, $P < 0.01$] revealed significantly smaller neocortical volumes in subjects who received an insult on P1 compared to P3, and on P3 as compared to P5, with P5 brains having the largest neocortical volume out of the lesion age groups. An Age at Treatment × Treatment interaction was also found for neocortical volume [$F(2,57) = 5.01$, $P < 0.05$], reflecting the fact that the main effect of Age at Treatment on neocortical volume was pulled by a reduction in P1 and P3 lesion subjects and not P1 or P3 shams. Specifically, simple effects analyses revealed significant differences between P1 and P3 [$F(1,28) = 8.42$, $P < 0.01$], P1 and P5 [$F(1,28) = 103.22$, $P < 0.001$], and P3 and P5 [$F(1,32) = 32.55$, $P < 0.001$] neocortical volumes for lesion subjects. Simple effects analysis showed no difference between shams at different ages, nor between P5 lesion and

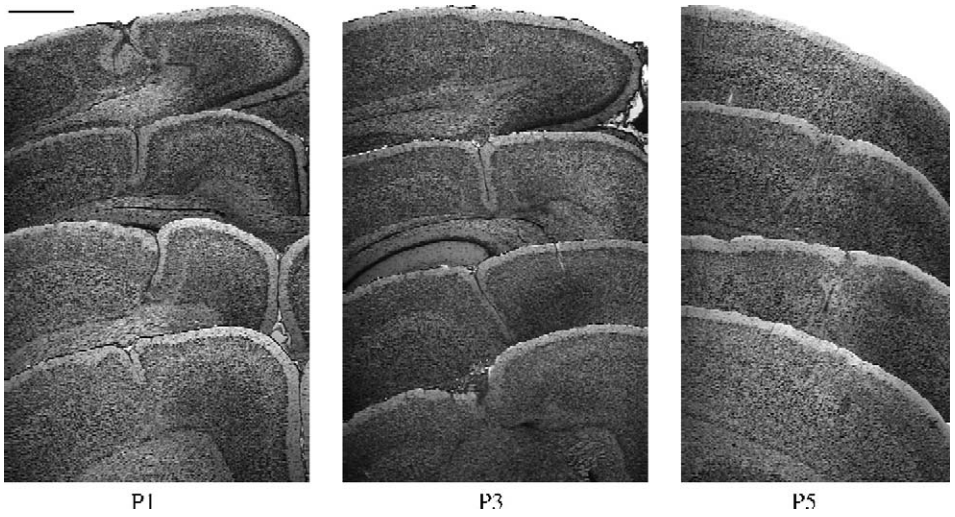

Fig. 1 – Serial photomicrographs (4×) of coronal slides showing extent of damage from anterior to posterior in P1, 3 and 5 lesion brains (note the distinct microsulcus in the P1 and 3 slides, and the absence of a microsulcus but clear necrotic scaring in the P5 case, scale bar, 500 μm). Normal cortex is shown in all cases adjacent to the malformation.

sham volumes, indicating that main effect differences between lesion and sham were a product of grossly reduced neocortical volumes in the P1 and P3 lesion conditions (see Fig. 2).

A univariate ANOVA computed for bilateral lesion volume was performed, excluding sham subjects (since no lesion was present). Results showed a significant main effect for Age at Treatment [$F(2,44) = 222.35$, $P < 0.001$], with simple effects analyses revealing significant differences between P5 and P3 [$F(1,32) = 611.32$, $P < 0.001$], and P5 and P1 [$F(1,28) = 214.57$, $P < 0.001$] groups. In both comparisons, insults at younger ages were associated with larger areas of dysplasic cortex.

Finally, results showed a significant main effect of Treatment for brain weight [$F(1,57) = 23.27$, $P < 0.001$], with lesion subjects having lighter brains. Analysis also revealed a main effect of Age at Treatment [$F(2,57) = 13.81$, $P < 0.001$], which

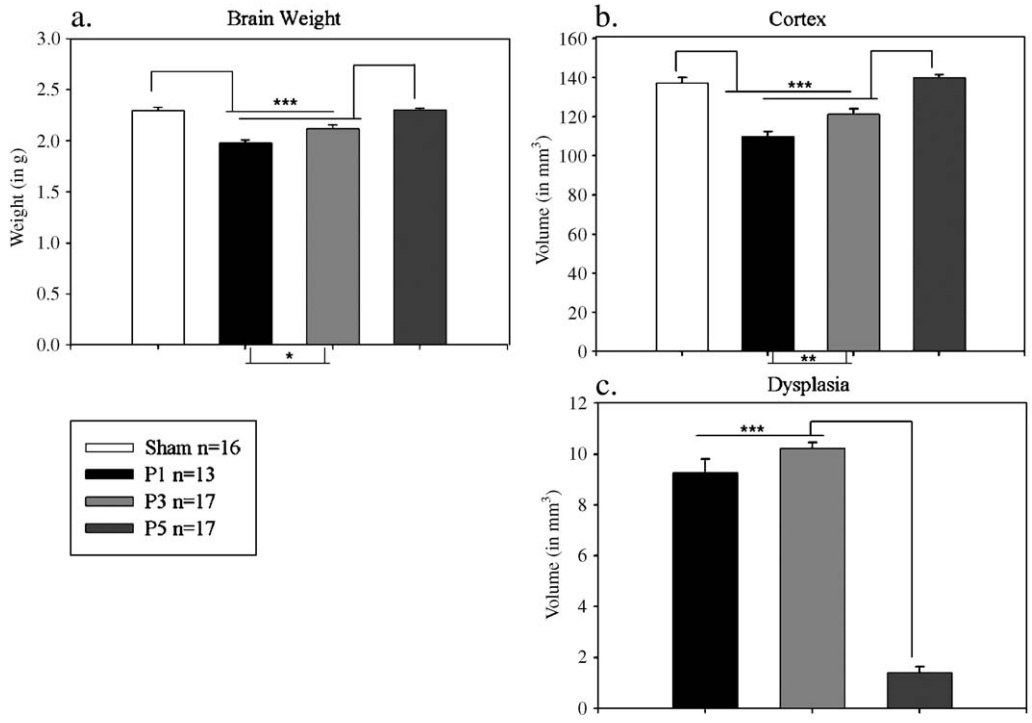

Fig. 2 – Histograms depicting differences in histology as a result of the age (P1, 3, 5) at which cortical injury occurred: (a) brain weight, (b) cortical volume, and (c) malformation volume for all applicable groups (with standard error bars). Shams were pooled for histological comparison, since no differences were seen between any sham age groups. Stars indicate a significant difference (*$P < 0.001$, **$P < 0.01$, *$P < 0.05$) for each comparison.**

reflected significantly lighter brain weight in P1 and P3 lesion subjects as compared to the P5 and sham groups. Simple effects analysis again indicated significant differences between the P1 and P5 [$F(1,28) = 95.74$, $P < 0.001$], P3 and P5 [$F(1,32) = 17.91$, $P < 0.001$] and P1 and P3 [$F(1,28) = 7.23$, $P < 0.05$] lesion groups, with no difference between sham groups or between P5 lesion and sham brain weight. Overall, the P1 lesion group showed the lightest brain weight.

2.3. *Juvenile: normal single tone*

Significant differences were found between mean cued and uncued startle response (ASR) scores for all groups, as shown by paired samples *t*-tests ($P < 0.05$), indicating significant discrimination of the single tone by all groups. Further, results from a univariate ANOVA with Treatment (2 levels) and Age At Treatment (3 levels) showed neither main effects nor an interaction for attenuated response scores (ATT; calculated by dividing the mean cued response amplitude by the mean uncued response amplitude and multiplying by 100). Thus, results showed no differential performance on a basic auditory discrimination task, indicating that the lesion treatment did not impact baseline hearing or the acoustic startle response.

2.4. *Juvenile: long silent gap results*

Juvenile (P27–39) long silent gap ATT scores were analyzed using a Treatment (2 levels) × Age at Treatment (3 levels) × Day (4 levels) × Gap (9 levels) repeated measures ANCOVA (using normal single tone scores (NST) as a covariate for the 0–100 ms SG procedure). Results showed no significant effect of Age of Treatment [$F(1,57) < 1$, ns] for the P1, P3 or P5 shams over four test sessions, nor among P1, P3 or P5 lesion groups. There were also no main effects or interactions with Treatment.

2.5. *Juvenile: short silent gap results*

Juvenile short silent gap ATT scores were analyzed using a Treatment (2 levels) × Age at Treatment (3 levels) × Day (4 levels) × Gap (9 levels) repeated measures ANCOVA (again using NST as a covariate). Results revealed a significant main effect of Treatment [$F(1,57) = 6.07$, $P < 0.05$], with shams showing overall better gap detection than P1, 3 or 5 lesion animals across the four short gap test sessions (see Fig. 3). Results showed no main effect of Age at Treatment [$F(2,57) < 1$, ns], nor an Age at Treatment × Treatment interaction [$F(2,57) < 1$, ns], indicating similar performance for all lesion subjects across ages. Repeated measures ANOVAs were computed between control and lesion groups at individual gaps, to assess differences in performance for each gap duration (no differences were found between the 3 sham groups, which were pooled for these analyses). Simple effects analyses at each gap duration revealed significant differences between lesion and sham groups on the 3, 4, 5, 6, 7, 8, and 10 ms gaps ($P < 0.05$), with shams consistently performing better. Further, the sham group detected gaps down to 3 ms in duration, while lesion groups were unable to detect gaps below 5 ms in duration (as indicated by paired samples *t*-test for mean cued and uncued startle responses (ASR) at each gap duration).

2.6. *Adult: short silent gap results*

Results for adult testing (P60–64) revealed improved performance for all groups on the short-duration (0–10 ms) silent gap procedure as compared to data from the juvenile period, consistent with previous findings (Peiffer et al., 2004c). A repeated measures ANCOVA on short silent gap ATT scores was performed using Treatment (2 levels) × Age at Treatment (3 levels) × Gap (9 levels) (NST used as a covariate). Results showed no main effect of Treatment [$F(1,56) < 1$, ns] nor interaction for this single session of short silent gap testing in the adult period. This lack of effects reflects the fact that P3 and P5 lesion rats closed the performance disparity relative to shams that had been seen in the juvenile period. However, a Treatment (2 levels) × Gap (9 levels) repeated measures ANOVA (using NST as a covariate) revealed that the performance of pooled sham versus P1 lesion animals still approached a significant difference [$F(1,26) = 2.11$, $P = 0.056$,

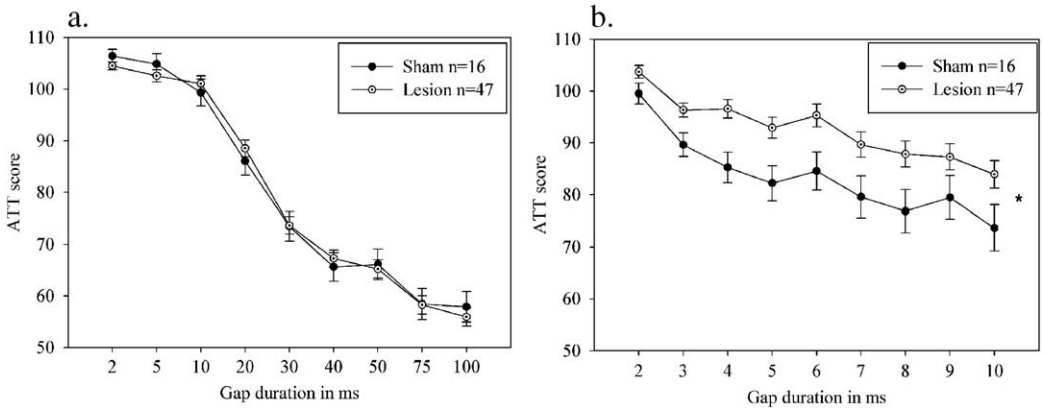

Fig. 3 – Juvenile Long (0–100 ms; a) and Short (0–10 ms; b) silent gap attenuated response scores for all lesion ages, compared to shams. Mean attenuated response scores for each group across all gap durations were collapsed over 4 days of testing. Shams showed significantly better gap detection compared to all lesion groups on the short-duration gap detection task. The star indicates a significant difference between shams and the three lesion groups ($P < 0.05$).

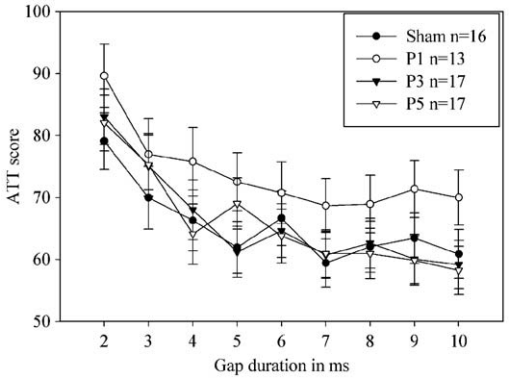

Fig. 4 – The graph illustrates adult gap detection performance. Adult short (0–10 ms) silent gap attenuation response scores for three ages of lesion (P1, 3, 5) versus shams. Adult P1 lesion subjects continue to show poor short gap detection performance relative to shams.

one tailed], with P1 lesion subjects still performing worse than shams on adult gap detection (see Fig. 4).

3. Discussion

3.1. *Overview*

Prior anatomical analyses of rat brains with early freezing lesion have suggested that disruption of neocortical neuronal migration represents a key component to the development of microgyric malformations (Rosen and Galaburda, 2000; Rosen et al., 1992a; Dvorak and Feit, 1977; Dvorak et al., 1978). This line of thought is supported in the current study, specifically by the presence of distinct microgyric formations in rats that received bilateral P1 and P3 freezing lesions during the postnatal period of neocortical neuronal migration, but not P5 rats that received a comparable lesion when neuronal migration to the cortical plate was completed.

Behavioral results from the present study reveal that juvenile rats with bilateral freezing lesions induced on postnatal days 1, 3 or 5 did not differ from each other or shams on a long-duration gap detection task (0–100 ms). However, during short-duration gap detection test sessions (0–10 ms), lesioned rats had significantly higher attenuation response scores as compared to shams (indicating worse discrimination). These results indicate poor rapid auditory processing for all of the juvenile lesion groups (P1, 3, 5) relative to shams. Results from adulthood reveal general improvement in gap detection across all conditions, but with P1 lesioned rats maintaining a trend of poor gap detection as compared to shams and, to a lesser degree, other lesion groups.

Brain weight and volumetric analysis of the three lesion conditions revealed a pattern of histological variation that may shed light on the behavioral findings. Results for brain weight and cortical volume showed significant differences between P1, 3 and 5 injured rats, with P1 animals having the lightest weight and smallest cortical volume followed by the P3 and P5 conditions respectively. Importantly, P3 and P5 lesion rats showed amelioration of rapid gap detection deficits in adulthood, whereas the P1 deficits persisted. It has been suggested that P1 represents a specific period of cell death vulnerability in the developing cortex of rodents. For example, Nakaya et al. (2005) showed that P1 mice exposed to radiation had a significantly greater percentage of cortical neuronal apoptosis compared to P7, P14 and P30 mice that underwent the same treatment. The present study lends further support to the hypothesis that specific time points of susceptibility to cell death may characterize early postnatal neurodevelopment and that these vulnerable periods may influence the relative impact of injuries on long term behavioral impairments.

3.2. *Brain changes and processing impairments*

The implications of the current findings to the study of early brain injury and behavioral outcome – with particular relevance to neurodevelopmental disruptions that may be linked to language-related disabilities characterized by rapid auditory processing (RAP) deficits – are significant. For example, the current findings support the view that it is not the presence of a specific type of malformation per se that is critical to the expression of processing deficits but rather, that early disruption of brain development – even that which fails to produce a microgyric sulcus – is nevertheless associated with later behavioral deficits.

The current report indicates that trauma to the neocortex during a more general period of development (i.e., extending outside the window for neuronal migration) may lead to RAP deficits. Although the cause of RAP deficits is still unknown, a change in the auditory relay architecture (e.g., MGN to auditory cortical connections) of the brain, or alterations to cellular properties of key auditory processing loci (e.g., MGN, auditory cortex), resultant from early pathology, are possible candidates (Peiffer et al., 2002b; Di Rocco et al., 2001, 2002; Rosen et al., 1999; Herman et al., 1997).

Research has shown changes in cortico-cortical and thalamo-cortical connectivity as a result of P1 freezing injury (Di Rocco et al., 2001, 2002; Giannetti et al., 1999, 2000; Rosen et al., 1999, 2000, 2001) that produces microgyria in the rat (Dvorak et al., 1978; Rosen et al., 1992a). Further, anatomical changes associated with the formation of microgyria, such as the cascade of cell death around the area of injury, are believed to contribute to altered patterns of connectivity (Rosen et al., 2000; Giannetti et al., 2000). However, it is possible that freezing damage similar to that experienced by postnatal day 5 lesion animals in the current study, which did not lead to microgyria, could still produce similar connectivity changes (or other top–down mediated cascades of structural or functional perturbation) ultimately affecting auditory processing. Support for this line of thought can be drawn from developmental studies of thalamocortical connectivity, which show that projection pathways and synapses are actively forming up to and beyond P5 in the rat (Lopez-Bendito and Molnar, 2003; Agmon et al., 1993).

3.3. *Implications for language learning impairment and dyslexia*

Data gained from studies of human LLI and dyslexic populations related to the current findings present a compelling case

for further examination of histological changes and aberrant sensory processing as a function of early injury in rodent models. Further, current debate regarding the causal factors implicated in the manifestation of LLI may be clarified by animal models of RAP impairment. For example, human infant studies have repeatedly shown an association between infants with a family history of language impairment and RAP impairment (Benasich et al., 2002, 2006; Leppanen et al., 2002). However, studies utilizing older subjects with LLI or dyslexia show a more variable pattern of RAP impairment (Ramus et al., 2003; Rosen and Manganari, 2001; McArthur and Bishop, 2001, 2004, 2005). Hautus et al. (2003) showed that dyslexic children between the ages of 6 and 9 had significant deficits in auditory temporal acuity compared to age matched controls as measured by gap detection, while rapid auditory temporal processing deficits were not seen when these same tests were given to older reading impaired children (ages 10–13 years) or adults (ages 23–25 years; Hautus et al., 2003). Our current findings indicate that subtle cortical perturbation occurring on P5 and more pervasive malformations (microgyria) produced from P1 or P3 freezing lesions result in comparable patterns of processing deficits when rodents are tested as juveniles. However, the improved performance of P5 and P3 injured rats compared to the P1 lesion condition in adulthood indicates that neuropathology, which influences RAP in the juvenile period, may become more heterogeneous when measured later in life. These findings have implications to current debates, such as for example whether RAP deficits are causal or co-morbid to language impairment. It is unclear whether RAP deficits ameliorate with time (although the damage to language development has occured), or are co-morbid and more likely to resolve with age as compared to higher-order language deficits (McArthur and Bishop, 2005; Tallal, 2004; Hautus et al., 2003). Further, it is possible that studies evaluating RAP in adult and even adolescent dyslexic and LLI humans may inadvertently introduce variability by not controlling for age, as well as pre- and postnatal developmental history (which could be influenced for example by exposure to teratogenic conditions or a family history of LLI). Future studies evaluating human populations with LLI and dyslexia, and rodent models of RAP impairment should seek to control for, and investigate variables such as age and task complexity as well as other factors that may play a role in the development of RAP impairments.

3.4. *Evidence for a critical period*

A related (though not strictly comparable) series of studies have been performed by Brian Kolb and colleagues over the past decade. In these experiments, focal aspiration lesions of frontal and motor cortex were made at various ages (ranging from embryonic day (E) 18 to adulthood) in the rat, and long-term behavioral consequences (using a variety of behavioral tasks) were assessed. These findings indicate significant, differential long-term consequences of aspiration lesions depending on the age of trauma in the rat (Kolb et al., 2004). For example, lesions on E18 or between P7 and 12 resulted in functional recovery, with "odd" regrowth of damaged cortex in the E18 animals, and dendrite and spine regrowth in the P7–12 age range. However, lesions acquired during the developmental window between P1 and 6 resulted in poor long-term functional compensation, as well as abnormal connectivity (Kolb et al., 2004). Based on these findings, Kolb et al. (2004) suggest that multiple periods critical to functional recovery after cortical injury may exist. The period of neurogenesis occurring around E18 may represent one window, while the second may extend from P7 to P10, when gliogenesis and synaptic formation are at their peak. Kolb et al. (1998) point out that the intermediate period between the two windows is typified by extensive differentiation, migration and arborization of cortical neurons. Thus, it has been suggested that interference with cortical development between P1 and P6 in the rat may have especially significant long-term consequences to the reorganization and recovery of function after injury (Kolb et al., 1998).

While the present findings support Kolb et al. (2004) idea of a critical window [P1–6], they also indicate that within that critical period, variations in behavioral profile of injured animals can occur. This is illustrated by a persistent RAP deficit in P1 microgyric rats as compared to P3 and P5 lesioned rats. The persistent RAP deficits of P1 rats may indicate that the most critical point of vulnerability falls around P1, within a broader period of susceptibility to long-term RAP impairment [P1–6]. Further support for this hypothesis is gained from studies showing a higher rate of cortical cell death susceptibility in the early postnatal period of development in rodents following exposure to various teratogens (Nakaya et al., 2005; Ikonomidou et al., 2000). Future studies exploring the physiological and cellular properties of injured cortex may shed light on the variability of deficit persistence following injury in the P1 to P6 range in rats.

3.5. *Implications for the study of brain malformations and RAP deficits*

The current findings suggest that the critical parameters relating to rapid auditory processing deficits stem from disruption of a general developmental process, rather than a specific type of malformation or anomaly. This assertion is consistent with evidence that both spontaneous neocortical ectopias and induced microgyria have been associated with rapid auditory processing deficits in both rats and mice (Peiffer et al., 2002a, 2004a; Clark et al., 2000; Fitch et al., 1994), and further, that the cortical location of these anomalies does not appear to modulate emergent rapid auditory processing (RAP) deficits (Peiffer et al., 2001, 2002a, Herman et al., 1997). This assertion is also consistent with evidence that other common, more pervasive, forms of early injury – for example, hypoxia–ischemia as seen in premature and very low birthweight infants – is also associated with later RAP deficits when induced in a neonatal rat model between P1 and P10 (McClure et al., in press).

The current findings also support the notion that early disruption of brain development during a "critical window" may lead to perturbations in the formation of circuitry that is in turn crucial to the performance of rapid auditory discriminations. This idea is further consistent with neuroimaging evidence that reveals subtle and pervasive patterns of activational disruption, as well as evidence of white matter (connectional) anomalies, in language-disabled humans

(Deutsch et al., 2005; Silani et al., 2005; Temple et al., 2000, 2003), coupled with an absence of gross morphological anomalies in developmentally language-disabled human populations (Tallal et al., 1993, 1998; notwithstanding the cellular anomalies reported in postmortem analysis by Galaburda and Kemper, 1979, and Galaburda et al., 1985, 1994). Indeed, it has been suggested that the neuropathological anomalies reported by Galaburda et al. (1985, 1994) in human dyslexic brains may represent "flags" of underlying disruption to neural circuitry, rather than direct causal features in the expression of dyslexia in those same subjects (Fitch and Tallal, 2003; Clark et al., 2000; Fitch et al., 1994; Rosen et al., 2000). Such an assertion would be consistent with emergent literature revealing "dyslexia susceptibility genes," which are found with higher than expected incidence in human dyslexic populations. Specifically, these genes have consistently been found (in animal models) to modulate aspects of neuronal development and migration of neurons or axonal outgrowth (e.g., robo1, kiaa0319, dcdc2, dyx1c1; Cope et al., 2005; Hannula-Jouppi et al., 2005; Meng et al., 2005; Miller, 2005; Taipale et al., 2003).

In summary, it appears that disruptions in the formation of cortical neural circuitry during a critical period in development – as opposed to specific types or locations of injury – are related to subsequent deficits in rapid auditory processing (which have, in human populations, been consistently found to relate to later language deficits; see Benasich and Tallal, 2002; Benasich et al., 2006; Tallal, 2004; Tallal et al., 1998).

4. Conclusion

The current results show that the emergence of RAP deficits is not dependent on the formation of microgyria in rats, and the critical window for the acquisition of RAP deficits appears to extend outside the period of neuronal migration necessary for formation of microgyria. Our findings are consistent with work by Kolb et al. (2004) who suggest that the P1–6 period represents a period of critical vulnerability to cortical perturbation in the rat due to key neurodevelopmental processes ongoing at that time, although our results also suggest that P1 represents a period of peak vulnerability, based on persistent deficits in this subset of subjects. Future studies will address when the period of susceptibility to disruption of rapid auditory processing via focal neocortical damage has ended in the rat, as well as the relationship between auditory developmental maturation and RAP deficit persistence.

5. Experimental procedure

5.1. *Subjects*

Subjects included 63 male Wistar rats born to purchased dams (Charles River Laboratory, Wilmington, MA) at the University of Connecticut. Mating was staggered between dams so that births for the P5, P3 and P1 surgery fell on the same day. Subjects were right or left ear marked and housed into pairs at P21 using a 12:12 light/dark cycle with food and water ad libitum. Juvenile testing began at P23 for all subjects. All procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, including adequate measures to minimize pain and discomfort. The Institutional Animal Care and Use committee (IACUC) at the University of Connecticut approved all procedures.

5.2. *Induction of freezing lesion*

On the day of surgery, litters were culled to 10 pups (8 male, 2 female), with male pups randomly assigned to receive double-pair freezing lesion or sham surgery. Females were retained to equalize litter size and avoid all-male litters. Surgeries involved the placement of a 2 mm stainless steel probe cooled to –70 °C on the skull-cap. Focal lesions were induced as a double-pair (two to each hemisphere) as previously described (Peiffer et al., 2004a). This procedure was modeled after that developed by Dvorak and Feit (1977); Dvorak et al. (1978); and Humphreys et al. (1991). P1 rats received the freezing probe for 5 s, while the P3 animals were given 7 s and the P5 rats a 10-s lesion (to compensate for age-related increases in thickness of the skull). A subset of sham subjects was taken from each age group (P1, P3, P5) and received similar treatment with a room temperature probe. After surgery, pups were individually marked with inkpad injections, warmed under a lamp and placed back with the mother. Final *n*'s for each group were sham P1 (n = 5), sham P3 (n = 6), sham P5 (n = 5) (total sham = 16); P1 lesion (n = 13); P3 lesion (n = 17); P5 lesion (n = 17).

5.3. *Behavioral testing: startle reduction*

Juvenile auditory testing began on P23 and involved a startle response paradigm that has been discussed extensively elsewhere (Peiffer et al., 2002b, 2004a). The startle modification paradigm involves the presentation of an auditory cue prior to a startle-eliciting stimulus (SES). The SES elicits an acoustic startle reflex (ASR) and if the preceding auditory cue is detected, the intensity of the ASR is reduced accordingly.

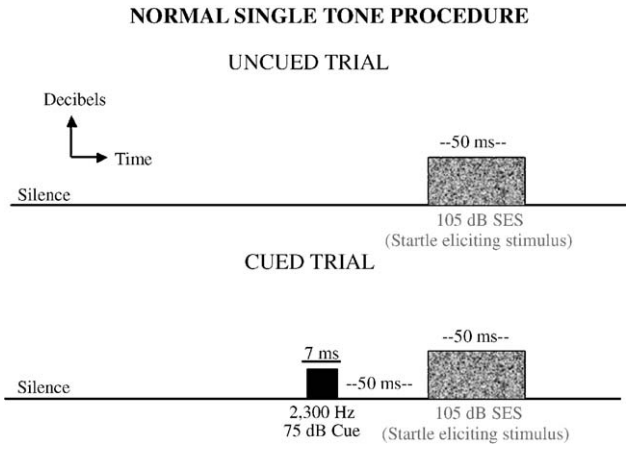

Fig. 5 – A schematic cartoon illustrating the normal single tone procedure.

For testing, subjects were placed on a load cell platform (Med Associates, Georgia, VT, USA), which measured the subject's ballistic motor response to the SES in mV. Signals were acquired and passed through a linear load cell amplifier (PHM-250-60) into a Biopac MP100WS acquisition system (Biopac Systems, Santa Barbra, CA) connected to two Macintosh computers, which recorded the subject's movement and ASR as a mV signal. The maximum peak value defining the ASR for each trial was extracted from the 150 ms following the onset of the SES, and this ASR represents a dependent variable. Auditory stimuli were generated using a Pentium 4 Dell PC with custom programmed software and a Tucker Davis Technologies (RP2) real-time processor. Stimulus files were played through a Marantz integrated amplifier connected to nine Cambridge Sound Works speakers, with sound levels calibrated by sound-level meter (Peiffer et al., 2002b). Each pair of platforms had one speaker centered and mounted 30 cm above. Attenuated response scores (ATT) were calculated from the peak ASR using the formula (mean cued response/mean uncued response] × 100). In this formula, absolute response scores (as measured by load-cell displacement for each subject's startle response) for cued and uncued trials are expressed as a ratio, multiplied by 100 (thus ATT scores represent a percentage). ATT scores were analyzed as a second dependent variable for all tasks.

5.4. *Single tone*

In the current study, all subjects received 1 day of a normal single tone procedure (NST; Peiffer et al., 2002b). The single tone test session was comprised of 104 trials (cued or uncued), presented in a pseudo-random order on 1 day. Uncued trials consisted of a silent background followed by the 105 dB, 50 ms SES. On cued trials, a 75 dB, 7 ms, 2300-Hz tone was presented 50 ms prior to the SES. Trials were variable in duration (16–24 s, 20 s on average). Individual scores on the single tone task were used as a covariate to control for baseline startle response differences in the analysis of performance on higher order auditory discrimination (gap detection) procedures (see Fig. 5).

5.5. *Juvenile, long silent gap*

A long silent gap procedure (similar to single tone) was utilized to assess gap detection over 4 days. The long session (0–100 ms) included 300 trials, each consisting of the presentation of a variable duration long silent gap (0, 2, 5, 10, 20, 30, 40, 50, 75, or 100 ms) embedded in continuous 75 dB broadband white noise. The gap was presented 50 ms prior to a 105 dB burst of white noise. The uncued trials used a "gap" of 0 ms. The cue–burst interval for each task was maintained at 50 ms (Friedman et al., 2004; Peiffer et al., 2004c).

5.6. *Juvenile, short silent gap*

A short silent gap procedure (using gap durations of 0, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ms) was also presented for 4 days, in order to assess the shortest detectable gap as a function of treatment (Friedman et al., 2004). All parameters except for the gap durations were identical to those in the long silent gap condition.

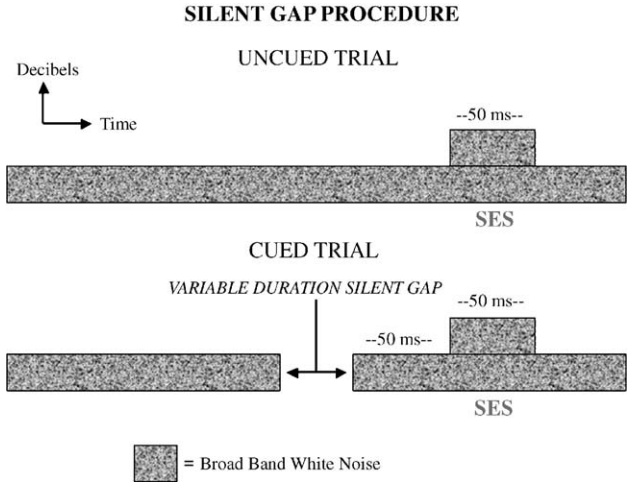

Fig. 6 – A schematic cartoon illustrating the gap detection procedure.

5.7. *Adult, short silent gap*

Adult short silent gap testing was identical to the juvenile procedure, except that adult subjects were presented with the task for 1 test day (see Fig. 6).

5.8. *Brain analysis*

At the end of behavioral testing, subjects were weighed, anesthetized with ketamine/xylazine (100/15 mg/kg), and transcardially perfused with saline followed by 10% phosphate-buffered formalin. Heads were removed, bottled in formalin and shipped to GDR at Beth Israel Deaconess Medical Center for histological preparation. The brains were removed, weighed, lesions visually assessed and location confirmed. Celloidin embedding was used on whole brain tissue and sections were made in the coronal plane at 30 μm. Every fifth section was mounted, stained with cresyl violet, and coverslipped before shipment back to the University of Connecticut for further analysis. Volumes of bilateral cortex and lesion were visualized using a Fisher Micromaster II digital microscope. Measurements were derived from a grid overlay using ImageJ, and computed using Cavalieri's estimator of volume (Gundersen and Jensen, 1987).

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