

Developmental learning impairments in a rodent model of nodular heterotopia

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Received: 2 March 2009 / Accepted: 30 June 2009 / Published online: 16 July 2009
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Abstract Developmental malformations of neocortex—including microgyria, ectopias, and periventricular nodular heterotopia (PNH)—have been associated with language learning impairments in humans. Studies also show that developmental language impairments are frequently associated with deficits in processing rapid acoustic stimuli, and rodent models have linked cortical developmental disruption (microgyria, ectopia) with rapid auditory processing deficits. We sought to extend this neurodevelopmental model to evaluate the effects of embryonic (E) day 15

exposure to the anti-mitotic teratogen methylazoxymethanol acetate (MAM) on auditory processing and maze learning in rats. Extensive cortical anomalies were confirmed in MAM-treated rats *post mortem*. These included evidence of laminar disruption, PNH, and hippocampal dysplasia. Juvenile auditory testing (P21–42) revealed comparable silent gap detection performance for MAM-treated and control subjects, indicating normal hearing and basic auditory temporal processing in MAM subjects. Juvenile testing on a more complex two-tone oddball task, however, revealed a significant impairment in MAM-treated as compared to control subjects. *Post hoc* analysis also revealed a significant effect of PNH severity for MAM subjects, with more severe disruption associated with greater processing impairments. In adulthood (P60–100), only MAM subjects with the most severe PNH condition showed deficits in oddball two-tone processing as compared to controls. However, when presented with a more complex and novel FM sweep detection task, all MAM subjects showed significant processing deficits as compared to controls. Moreover, *post hoc* analysis revealed a significant effect of PNH severity on FM sweep processing. Water Maze testing results also showed a significant impairment for spatial but not non-spatial learning in MAM rats as compared to controls. Results lend further support to the notions that: (1) generalized cortical developmental disruption (stemming from injury, genetic or teratogenic insults) leads to auditory processing deficits, which in turn have been suggested to play a causal role in language impairment; (2) severity of cortical disruption is related to the severity of processing impairments; (3) juvenile auditory processing deficits appear to ameliorate with maturation, but can still be elicited in adulthood using increasingly complex acoustic stimuli; and (4) malformations induced with MAM are also associated with general-

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ized spatial learning deficits. These cumulative findings contribute to our understanding of the behavioral consequences of cortical developmental pathology, which may in turn elucidate mechanisms contributing to developmental language learning impairment in humans.

Keywords Auditory processing · Neuronal migration · Startle response · Methylazoxymethanol induced heterotopia · Hippocampal dysplasia · Spatial learning · Non-spatial learning

Introduction

Language related learning disabilities, including specific language impairment and reading impairment, have been associated with malformations of neocortex in humans [1–6]. Specifically, molecular layer ectopia and microgyria have been seen in the brains of human dyslexics *post mortem*, and microgyria have also been identified in the neocortex of children with specific language impairment [using MRI; 3–5, 7]. Further, mild forms of heterotopia—which appear as abnormal clusters of cells along the ventricular zone, white matter, or deep cortical layers—have also been associated with specific reading impairments [again using MRI; 1, 2]. These malformations are likely a result of interacting genetic, epigenetic and environmental risk factors that ultimately lead to the disruption of processes within a critical window of cortical development, spanning from the beginning of ventricular zone mitosis to the end of neuronal migration [8–12]. In rodent models, cortical developmental disruption during this critical window is associated with deficits in processing rapid and/or complex acoustic information, and similar acoustic processing deficits have also been reported in humans with reading and language impairments [8, 13–21].

Rodent models of cortical developmental disruption have thus provided behavioral and anatomical insight into possible mechanisms mediating language related learning disabilities. Recently, genetic association studies have identified several genes linked to a higher incidence of developmental dyslexia (e.g., DCDC2, KIAA0319, ROBO1 and DYX1C1; [22–25]). Anatomical studies in the rat indicate a direct role for these genes in mediating neuronal migration [26–28]. Importantly, embryonic day (E)14 RNA interference (RNAi) for homologs to several of these genes has been shown to lead to deep cortical, white matter, and/or periventricular heterotopia in rats [26, 29]. We have also shown that *in utero* RNAi of *Dyx1c1* in rats leads to subsequent deficits in processing complex acoustic stimuli. Finally, in addition to cortical anomalies, a subset of these animals exhibited hippocampal heterotopia and this

subset also demonstrated spatial learning deficits [9]. These convergent studies continue to support a direct relationship between abnormal neocortical formation and subsequent auditory processing and learning disabilities, which may in turn relate to the etiology of developmental language disorders in humans.

In addition to genetic influences, injury models of the developing rat brain, such as hypoxia ischemia and focal freezing lesions resulting in the formation of microgyria, have also been shown to lead to auditory processing and learning deficits [8, 14, 15, 30–32]. Such deficits are again similar to those observed in humans with language learning impairment [18–20, 33]. Given the heterogeneous profile of human learning impairments, rodent models should continue to be utilized to gain etiological insight into developmental pathology of the neocortex.

As one example, recent studies evaluating the effects of embryonic methylazoxymethanol acetate (MAM) teratogenic exposure in rats and ferrets have identified a wide range of resulting morphological anomalies within cortex and adjacent regions—such as the dorsal hippocampus, white matter, and the ventricular zones [12, 34–36]. MAM is an anti-mitotic agent that can be administered to a pregnant dam via intraperitoneal injection, and passes through the placenta to damage the DNA in dividing cells of the fetal central nervous system [37]. Depending on the dose and timing of MAM administration, the severity and region of neural disruption can be modulated. For example, embryonic day (E) 15 exposure to MAM has been shown to produce disruption of cortical lamination, periventricular nodular heterotopia (PNH), and hippocampal dysplasia, as well as other anomalies—all of which have been associated with human learning impairments [1, 2, 37]. Thus MAM models have attracted increasing interest from researchers studying the effects of abnormal cell division, migration, and differentiation—as well as the behavioral consequences of such cortical developmental disruption.

The current study sought to extend the rodent model of cortical developmental disruption, auditory processing and learning impairment, by evaluating E15 MAM exposed rats and controls for long-term auditory processing and learning abilities. In these studies we used a modified acoustic startle paradigm to assess acoustic processing at various levels of complexity/difficulty (silent gap in white noise, two-tone oddball, and FM sweep detection), as well as two water maze paradigms (spatial and non-spatial). Subjects were tested across juvenile (P21–42) and adult (P60–100) ages on successively more complex acoustic tasks, in order to assess potential maturational shifts in auditory processing. Maze testing was assessed in adulthood only. Influence of the severity of PNH on auditory processing was evaluated when statistically warranted.

Methods

Subjects and treatment

Purchased-time-mated Wistar dams (Charles River, Wilmington, MA) received a single intraperitoneal injection of 25 mg/kg MAM (Midwest Research Institute, USA) diluted to 1 mL in .9% NaCl, on E15. Subjects were injected under light isoflurane anesthesia. Control dams received a single saline injection (also under light isoflurane). At birth, subjects were culled into litters of 10 (eight males and two females), to control for litter size and sex ratio effects. At P21, subjects were right or left ear marked and housed into like-treated pairs. At P60, animals were single housed prior to adult behavioral testing. All subjects were maintained on a 12:12 light/dark cycle with food and water available *ad libitum*. After weaning, a total 59 male rats (MAM $n=36$, control $n=23$) were utilized for behavioral testing. 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.

Behavioral testing: startle reduction

Acoustic testing involved a startle response paradigm that has been discussed extensively elsewhere (also called pre-pulse inhibition (PPI; see [15, 16, 38])). Briefly, 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. In the current experiments, the SES was a 105 dB, 50 ms white noise burst. The most basic version of this task used a 75 dB, 7 ms, 2300 Hz tone pre-stimulus. Relative comparison between the ASR amplitude in the presence (cued trial) and absence (uncued trial) of a pre-stimulus represents an objective measure of sensory detection [38]. The duration of each trial (i.e., between each SES) varied between 16–24 s, to eliminate subjects' ability to predict SES onset.

Startle reduction: apparatus

For testing, each subject was placed on a load cell platform (Med Associates, Georgia, VT, USA), which measured the subject's ballistic motor response to the SES in mV. Each pair of platforms had one speaker centered and mounted 50 cm above it (Cambridge Sound Works MC105, sound levels calibrated by sound-level meter [38]). Auditory

stimuli were delivered through the speakers using a Pentium 4 Dell PC with custom programmed software and a Tucker Davis Technologies (RP2) real time processor, played through a Niles SI-1260 amplifier (Niles Audio Corporation, Miami, FL) connected to all nine speakers.

Output signals from individual load cell platforms were acquired and passed through a linear load cell amplifier (PHM-250-60) into a Biopac MP100WS acquisition system (Biopac Systems, Santa Barbara, CA) connected to two Macintosh computers. The computers recorded each subject's movement on each trial, in the form of mV signals. The maximum peak value defining the absolute response score (ARS) for each trial was extracted by algorithm, from the 200 ms following the onset of the SES. This ASR represents one dependent variable for analysis of acoustic tasks. In addition, mean attenuated response scores (ATT) were calculated using the formula: $([\text{mean cued response} / \text{mean uncued response}] \times 100)$. In this formula, the ARS (as measured by load-cell displacement for each subject's startle response) for cued and uncued trials are expressed as a ratio, and then multiplied by 100. These ATT scores represent an average percentage, with one ATT score per stimulus condition per session per subject. ATT scores were analyzed as a second dependent variable for all acoustic tasks.

Startle reduction: normal single tone procedure

The normal single tone (NST) session was comprised of 104 trials (cued or uncued), presented in a pseudo-random order. 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). All subjects received NST as the initial auditory test on P23, and again on the first day of testing in adulthood (P61). Based on evidence of Treatment effects on juvenile NST scores, each subject's juvenile NST value was used as a covariate for all subsequent juvenile acoustic data analyses to control for any group differences in hearing, baseline startle, or baseline pre-pulse inhibition (PPI). Note that since no treatment effects for NST were seen in adulthood, this value was *not* used as a covariate in the analysis of adult auditory data.

Startle reduction: silent gap procedure

A silent gap procedure (similar to single tone) was utilized to assess simple auditory temporal processing (a commonly used tool for this purpose; [8, 19]). Each session included 300 trials, each consisting of the presentation of a variable duration 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 [39, 40]. Importantly, discrimination of stimuli (white noise vs. silence) is required to detect change, leading to PPI. Thus, if stimuli are discriminated (variable duration silent gap from white noise), and the stimulus change is detected, subjects will exhibit inhibition of the startle response.

Startle reduction: oddball procedure

An oddball session was comprised of 104 trials, with a total of two sessions (i.e., one per day over 2 days) administered in the juvenile and adult periods. This procedure involved the repeated presentation of a background 75 dB, high-low tone sequence (2300–1100 Hz, respectively) separated by a within-stimulus inter-stimulus interval (ISI) of variable duration (275 or 225 ms; one interval used per session). Each sequence was separated by a between sequence ISI, which was always 200 ms greater than the inter-stimulus interval to maintain perceptual contiguity of the tone-pair. On uncued trials, the last tone sequence was followed by 50 ms of silence, then by the 105 dB/50 ms SES. On cued trials, a reversal of the tone sequence occurred (low-high, 1100–2300 Hz) followed by 50 ms of silence, and then the SES. Again, if stimuli are discriminated (high-low tone pair from low-high), and the stimulus change is detected subjects will show inhibition of the startle response to the SES.

Startle reduction: FM sweep procedure

An FM sweep session was comprised of 104 trials, with a total of two sessions (one per day across 2 days) administered in adulthood only. This procedure involved the repeated presentation of a background 75 dB, downward FM sweep (2300–1900 Hz) separated by a within-stimulus inter-stimulus interval (ISI) of variable duration (225 or 175 ms; one interval used per session). Each sequence was separated by a between sequence ISI, which was always 200 ms greater than the sweep duration. On uncued trials, the last FM sweep was followed by 50 ms of silence, followed by the 105 dB/50 ms SES. On cued trials, an upward FM sweep (the reversal of the standard sweep, 1900–2300 Hz), was followed by 50 ms of silence and then the SES.

Behavioral testing: water escape, maze learning

At the completion of acoustic testing a subset of subjects (control $n=14$, MAM $n=36$) were tested on: water escape (1 day); Morris water maze (MWM, spatial learning, 5 days); and a non-spatial water maze task (non-spatial learning, 5 days). Due to testing constraints on the number

of animals that could be tested per day, coupled with an expectation of pathological and behavioral variability in the MAM group, the control group was reduced in number by random selection for this testing phase. Importantly, preliminary analysis of auditory detection scores for the subset of controls used for maze testing was found to be comparable to those of the full control group.

As a motor, visual and motivation control, all subjects were first tested on a water escape task. The water escape task involved the use of a visible platform (8 in. in diameter) placed at one end of an oval tub (40.5 in. \times 21.5) filled with water (8 in) maintained at room temperature (22°C). Subjects were released in the opposite end of the tub from the platform, and the time taken to reach the platform (latency) was recorded.

Morris water maze (MWM)

After completing water escape (on the following day), subjects began Morris water maze (MWM) testing, which was administered over a period of five days. Testing was conducted in a round 48 in. diameter tub with an 8 in. diameter submerged (invisible) platform which was consistently placed in the southeast (SE) quadrant. Fixed, extramaze cues were abundant (computer, sink, door, table), while precaution was taken to eliminate intra-maze cues (tub and platform were painted black so the submerged platform blended into a consistent background; see [41]). On each of five testing days, subjects underwent four trials, with each trial starting from a different randomly selected compass point (N, S, E, W). On day one, trial one, each subject was placed on the platform for 10 s, removed from the platform, and then released from one of the starting locations. Each trial had a maximum time of 45 s. Subjects unable to reach the platform within this time window were guided to the target and allowed to remain for 5 s. The latency to reach the platform for each trial was recorded using a video tracking system (Smart Track, San Diego Instruments, San Diego CA).

Non-spatial water maze (NSWM)

The non-spatial water maze has been used to test reference learning, i.e., the ability to consistently locate a hidden platform using intramaze visual cues that are independent of extramaze space. Testing took place in the same 48-in. diameter tub as the spatial MWM, with the submerged 8-in. diameter platform located 1 in. below the water's surface, but also included an insert characterized by four black/white complex visual stimuli (which acted as intramaze cues uniquely identifying each quadrant of the outer maze wall). The intramaze patterns consisted of: black/white vertical stripes; black/white horizontal stripes; white dots

on a black background; and black dots on a white background (for more details, see [42]). The platform location was always paired with the vertical lines for each subject, such that escape required an association between the target intramaze stimulus (i.e., vertical lines) and the platform, irrespective of extramaze cues. While the platform remained in a constant within-maze position relative to the 4 quadrants, the maze itself was randomly rotated across trials with respect to the room. Subjects were released from the same compass point (N) on all trials, and latency to reach the platform was recorded for each trial (SmartTrack, San Diego, CA). All other testing parameters were similar to the spatial version of the MWM (number of trials, testing days, and length of time subject was left on platform).

Histology

At P100, subjects were weighed, anesthetized with ketamine/xylazine (100/15 mg/kg), and transcardially perfused with saline followed by 10% phosphate buffered formalin. Brains were extracted, placed in formalin, and shipped to GDR at Beth Israel Deaconess Medical Center for anatomical analysis. Brains were embedded in celloidin, and serially sectioned in the coronal plane at 30 μm . A series of every tenth section was stained with cresyl violet for Nissl substance. A screener identified the distribution and relative severity of the malformations without knowledge of treatment group or litter of origin. The most common anatomical anomalies consisted of disrupted cortical lamination, periventricular nodular heterotopia (PNH), and hippocampal dysplasia. PNH were ranked by severity (mild ($n=8$), moderate ($n=16$), and severe ($n=12$)), and this categorization was used for *post hoc* analyses of acoustic testing results. Hippocampal dysplasia was also ranked by severity (mild $n=6$, moderate $n=11$, severe $n=19$). However, hippocampal malformations were identified in all MAM treated subjects, and were typically more severe (with less variability) as compared to the overall PNH profile. In addition, volumes of the cerebral cortex, hippocampus, and corpus callosum were assessed using a Fisher Micromaster II digital microscope. Structural volumes were measured by overlaying serial images with a grid (ImageJ), and were computed using Cavalieri's estimator of volume [43, 44].

Statistics

For initial acoustic processing analyses, MAM subjects were analyzed as one group. When main effects of Treatment were observed, *post hoc* analyses were conducted using severity of PNH (three levels: mild, moderate, severe) as a between-subjects variable.

Results

Histology

Histological analyses were performed on the 36 MAM treated and 23 control brains. A blind screener documented the severity of neurological dysplasia and identified three categories of PNH; mild ($n=8$); moderate ($n=16$); and severe ($n=12$; see Fig. 1). All MAM treated subjects showed hippocampal dysplasia, as well as some degree of disrupted cortical lamination. There were no malformations in any of the control brains. MAM treated rats showed a significant difference in volume of the cerebral cortex [$F(1, 57)=181.5, p<0.001$] as compared to controls, with smaller cortical volume in the MAM group. MAM animals also differed significantly in the volume of corpus callosum [$F(1, 57)=225.2, p<0.001$] and hippocampus [$F(1,57)=91.1, p<0.001$] as compared to controls, again with both structures smaller in the MAM group (see Fig. 2). Interestingly, overall volume reductions in MAM subjects were not related to severity of PNH, or to severity of hippocampal heterotopia.

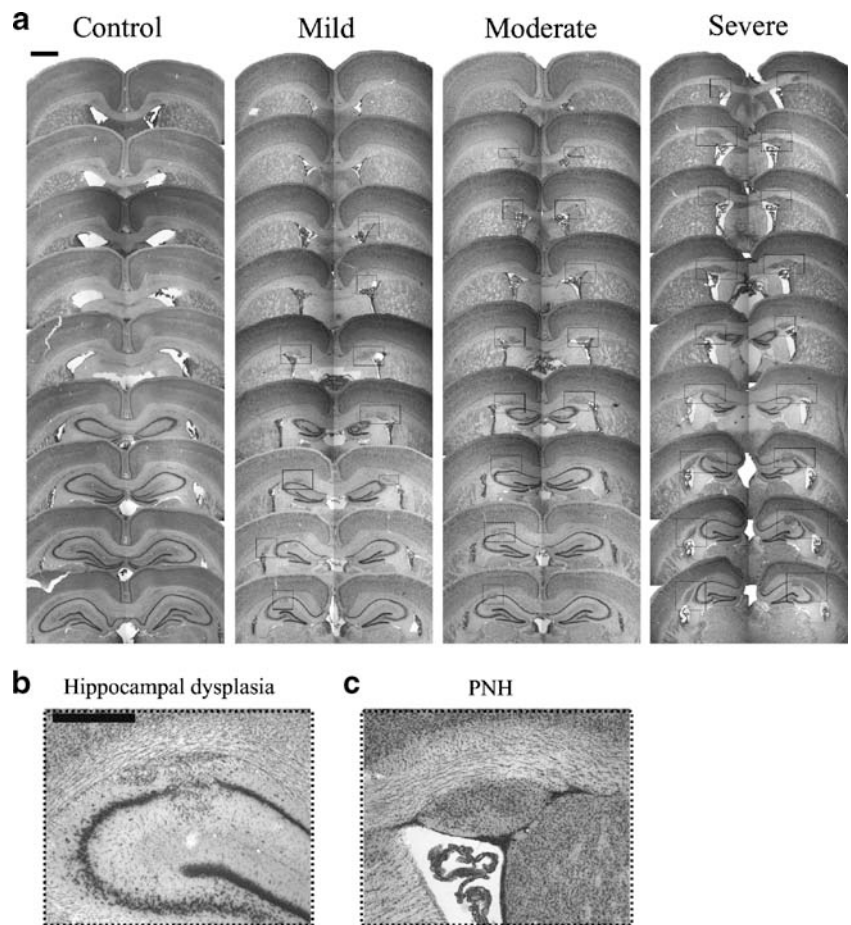
Juvenile testing: normal single tone (NST)

Results from paired samples t-tests of absolute response scores (cued versus uncued) showed that both MAM [$t=6.99, p<0.001$] and control [$t=8.905, p<0.001$] rats were able to significantly detect the normal single tone cue (NST). However, a one-way ANOVA also showed a significant difference between MAM and control animals on the juvenile single tone detection task [$F(1, 57)=7.653, p<0.01$], with MAM subjects showing significantly worse detection as compared to controls (see Fig. 3a). *Post hoc* analysis using a Tukey HSD test revealed significant effects of PNH severity [$F(1,55)=3.49, p<0.05$], with more severe PNH leading to worse scores. Results from the *post hoc* analysis showed a significant difference between the severe PNH group and controls, with controls showing better detection ($p<0.05$). In contrast, the Tukey HSD test showed no difference between mild PNH or moderate PNH versus control animals on the single tone detection task—thus indicating similar performance ($p=\text{ns}$; see Fig. 3b). However, all subsequent juvenile auditory analyses (comparing MAM and control groups) were run using each subject's normal single tone ATT score as a covariate, to control for baseline juvenile group differences in auditory acuity and/or PPI.

Juvenile testing: silent gap (0–100 ms)

A 2 (Treatment) \times 9 (Gap) repeated measures ANCOVA for silent gap showed no significant difference between MAM

Fig. 1 Photomicrographs showing: **(a)** overlaid serial sections from control, mild, moderate and severe PNH conditions (boxes outline heterotopia; *scale bar* = 1 mm); **(b)** Hippocampal dysplasia taken from the moderate PNH series (*dotted lines* show cutouts from series); and **(c)** Enlarged PNH section taken from the severe PNH series (**b** & **c** *scale bar* = 500 μ m)



and control subjects, indicating that basic auditory temporal processing remained intact in MAM-treated subjects [$F(1, 56)=0.06, p=ns$]. Results from paired samples *t*-tests for absolute response scores showed that both MAM and control rats could significantly detect down to the 30 ms

silent gap [$t=3.31, p<0.05; t=2.5, p<0.05$, respectively; see Fig. 4].

Juvenile testing: oddball

Absolute and attenuated response scores were collected over 2 days for the oddball test, using two ISIs (275 and 225 ms, one ISI used per session/day). Initial analyses using paired-samples *t*-tests showed significant differences between absolute response amplitude scores for both MAM and control groups at both ISI durations ($p<0.01$), thus indicating significant detection of the oddball cues for both groups.

A 2 (ISI) \times 2 (Treatment) repeated measures ANCOVA was then conducted on attenuated response scores (ATTs). This analysis revealed a significant main effect of Treatment [$F(1,56)=9.14, p<0.01$], with MAM rats showing significantly worse oddball detection performance as compared to controls (see Fig. 5a). *Post hoc* analysis using a Tukey HSD test also revealed significant effects of PNH severity [$F(1,55)=8.11, p<.01$], with the severe PNH ($p<0.01$) and moderate PNH ($p<0.05$) groups as compared to controls. Controls showed significantly better detection than both PNH conditions. In contrast, no statistical

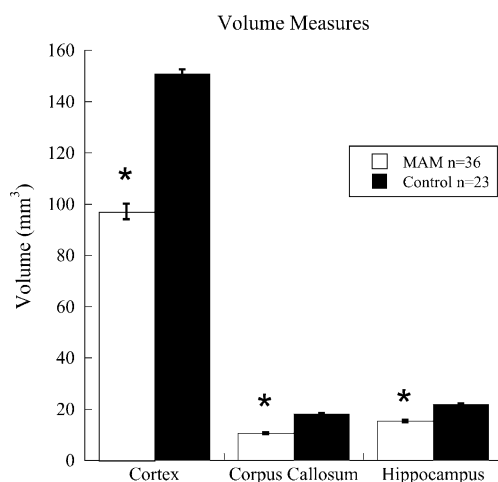


Fig. 2 Graph showing significant differences in hippocampal, corpus callosum, and cortical volumes for control and E15 MAM treated rats (* = $p<.01$)

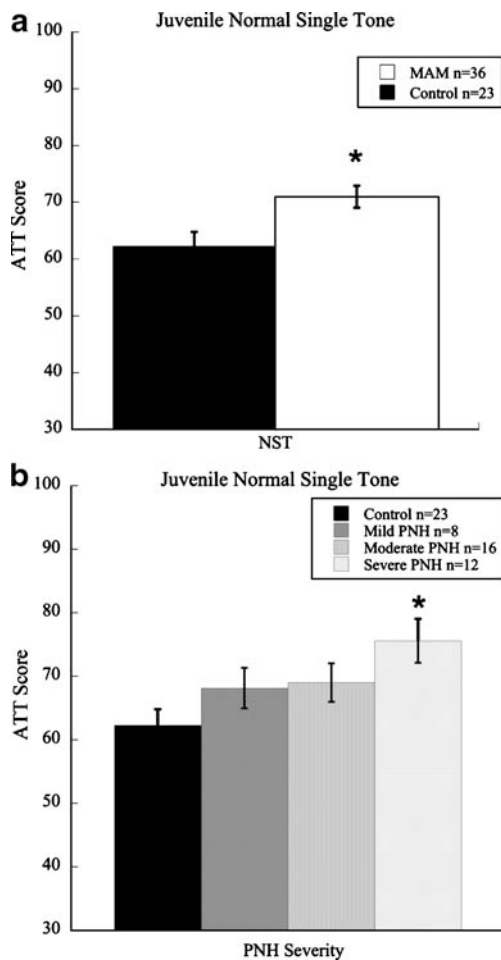


Fig. 3 Graphs showing: (a) performance on the single tone detection task for juvenile control and MAM treated rats; and (b) the performance of severity subgroups (mild, moderate, severe) for MAM treated rats and controls on the juvenile Single tone detection task (* $p < .05$)

difference was seen between control and mild PNH groups, indicating comparable oddball detection (see Fig. 5b).

Adult testing: normal single tone (NST)

Again results showed differences in cued and uncued absolute response amplitude scores for MAM and control animals using paired samples t-tests ($p < 0.05$), thus indicating significant detection of the single tone (NST) by both groups. However, unlike juveniles, adult between group comparisons using one-way ANOVA showed *no* significant difference in ATT scores between MAM and control subjects [$F(1,58) = 2.4, p = ns$]. Thus differences in single tone detection observed in juvenile subjects were no longer present in adulthood (see Fig. 6a). As such, single tone (NST) scores were *not* used as a covariate for analysis of adult auditory processing data.

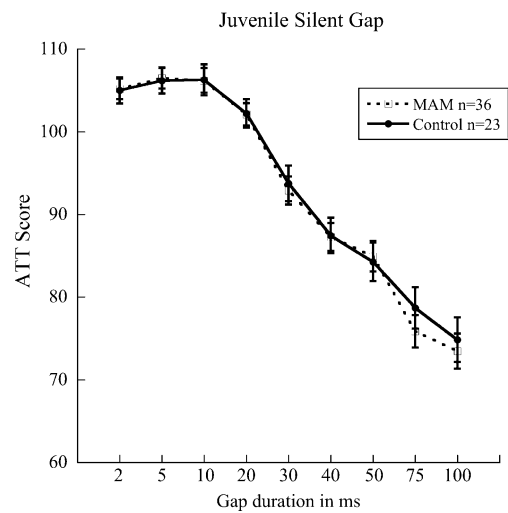


Fig. 4 Juvenile silent gap detection performance for control and MAM treated rats, showing no difference in gap detection between the groups (values reflect NST covariate)

Adult testing: oddball

Absolute and attenuated response scores were again collected over 2 days for the adult oddball test, using two ISIs (275 and 225 ms, one per session/day). Results showed significant differences between absolute response amplitude scores, as shown by paired-samples t-tests, for MAM and control groups at both ISI durations ($p < 0.01$). Again, a 2 (ISI) \times 2 (Treatment) repeated measures ANOVA was conducted on attenuated response (ATT) scores. This analysis showed no significant effect of Treatment for adult oddball testing [$F(1,57) = 1.76, p = ns$], indicating that oddball deficits observed in juvenile MAM-treated subjects did not persist into adulthood (see Fig. 6b). However, when each PNH group (mild, moderate, severe) was analyzed independently using a 2 (ISI) \times 2 (Treatment) repeated measures ANOVA, the severe PNH condition did show a significant effect of Treatment on adult oddball as compared to controls [$F(1,33) = 4.42, p = 0.043$]. This result indicates a persistent auditory processing impairment on the oddball task for severe PNH subjects, as compared to control and less severe PNH subjects (see Fig. 6c).

Adult testing: FM sweep

Significant differences were found using paired samples t-tests between absolute response amplitude scores for control subjects on both FM sweep ISI conditions (225 and 175 ms). However, MAM treated animals *did not* show differences between cued and uncued absolute response scores, thus indicating impaired processing at both ISIs. Accordingly, results from a 2 (Treatment) \times 2 (ISI) repeated measures ANOVA for attenuation response scores revealed a significant main effect of Treatment [$F(1,57) = 21.87,$

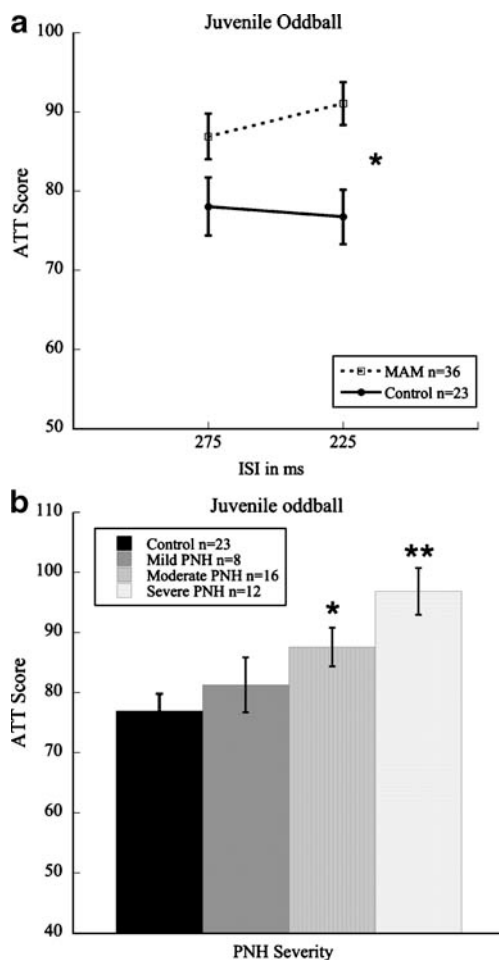


Fig. 5 Graphs showing: (a) significant difference between MAM treated and control subjects on the 275 and 225 ms ISI oddball task; and (b) combined juvenile oddball performance for the three PNH severity groups (mild, moderate and severe) and control rats, showing significant deficits in moderate and severe PNH conditions ($*p < .05$, $**p < .01$; values reflect NST covariate)

$p < 0.001$], with control rats showing significantly lower (better) attenuation scores compared to MAM treated rats (see Fig. 7a). *Post hoc* analysis using a Tukey HSD test revealed significant effects of PNH severity [$F(1,55)=9.45$, $p < 0.001$], with controls showing significantly better FM sweep detection compared to all three severity conditions (mild, moderate ($p < 0.05$), and severe ($p < 0.01$), respectively). Thus, unlike the oddball tasks, the novel FM sweep task elicited robust deficits from adult MAM-treated rats regardless of PNH severity (see Fig. 7b).

Water escape and maze learning (Morris water maze (MWM) & Non-spatial water maze (NSWM))

The same subset of subjects was run on water escape, MWM, and NSWM, at the conclusion of acoustic testing

(MAM $n=36$, Control $n=14$). A one-way ANOVA performed on escape latencies in the water escape task revealed no significant differences between groups, indicating that MAM subjects did not differ from controls in their motor (swim) behavior.

For the MWM, a 2 (Treatment; MAM & Control) \times 5 (Day) repeated measures ANOVA was conducted on latency to reach the platform. This analysis revealed an overall effect of Treatment [$F(1,48)=4.05$, $p=0.05$], with MAM subjects showing longer latencies. However, *post hoc* analysis based on severity of hippocampal dysplasia (mild $n=6$, moderate $n=11$, severe $n=19$) did not relate to MWM latency scores [$F(3,46)=1.77$, $p=ns$], thus indicating that MAM treatment and associated generalized hippocampal malformations (as seen in all MAM subjects) resulted in an overall spatial learning impairment relative to controls (see Fig. 8).

For the NSWM, a 2 (Treatment, MAM & Control) \times 5 (Day) repeated measures ANOVA was conducted on latency to reach the platform. In contrast to the spatial condition, no Treatment effect was observed between MAM-treated and control rats [$F(1,48)=.59$, $p=ns$], suggesting intact non-spatial reference learning in the MAM group.

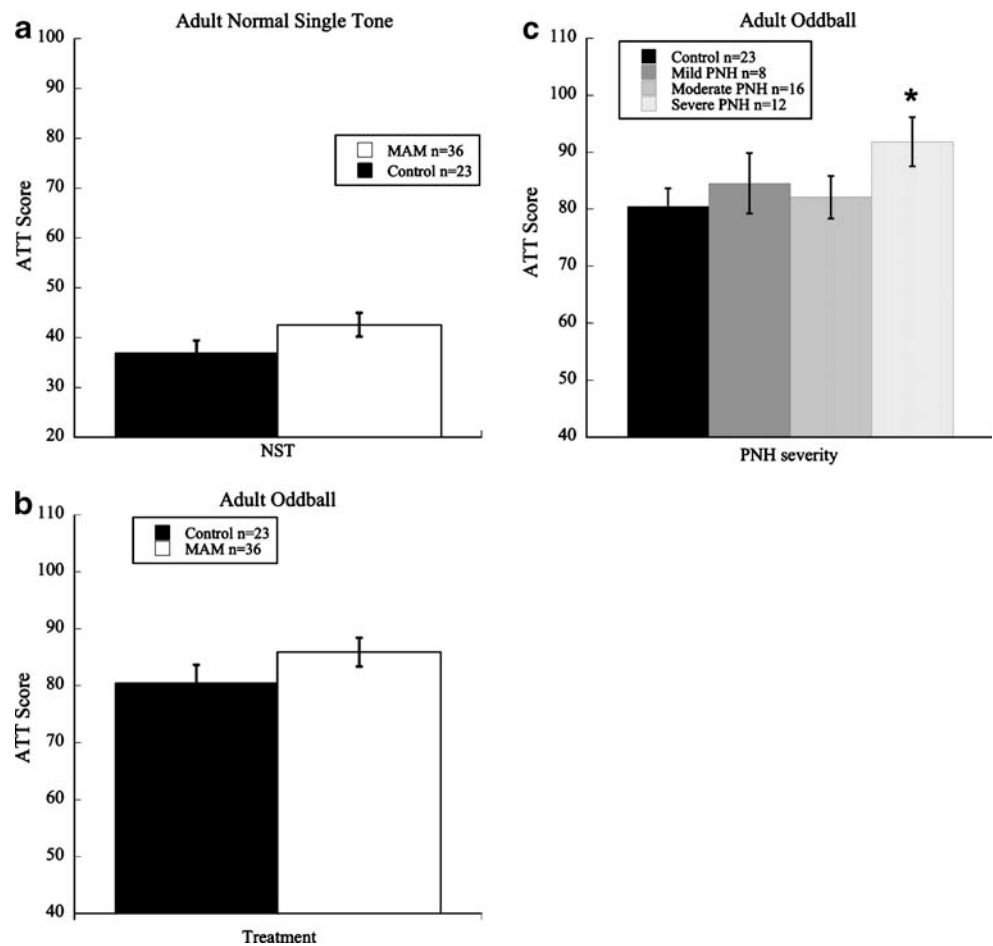
Discussion

Auditory processing

The present results demonstrate novel findings of auditory temporal processing deficits in rats with periventricular nodular heterotopia (PNH) stemming from the disruption of early cortical neurogenesis and migration. Specifically, our results showed that E15 MAM exposure led to deficits in processing spectral and temporally complex acoustic stimuli. Moreover, the severity of these behavioral deficits was directly related to the severity of PNH. We also showed spatial learning deficits in MAM-treated subjects, although these did not appear to correlate with PNH severity.

Despite juvenile MAM-treated subjects showing a baseline deficit in pre-pulse inhibition or NST (which was associated with severe PNH), deficits in temporally relevant processing were only seen on the more complex oddball and FM sweep detection tasks—even after covariance for baseline pre-pulse inhibition (PPI). Importantly, neither MAM-treated nor control subjects differed in their ability to detect silent gaps embedded in broadband white noise (a commonly used task to assess temporal processing in human language impaired populations and rodent models; [8, 19, 39]). This equivalent performance by groups indicates that basic acoustic processing and hearing were intact in the MAM-treated group. In addition, juvenile

Fig. 6 Graphs showing: (a) no significant difference between MAM and control conditions on adult normal single tone; and (b) no significant difference on adult oddball (combined 275 and 225 ms ISI), suggesting that overall auditory processing deficits observed in juvenile subjects ameliorate with maturation, at least in the mild and moderate PNH conditions; with (c) the severe PNH condition showing persistent deficits on the oddball task



oddball results revealed a significant effect of MAM treatment on detection of the 275 and 225 ms ISI two-tone sequence (as evidenced by detection of a change in tone order), with controls performing significantly better than MAM rats on this task. When effects were examined as a function of severity of PNH (mild, moderate and severe), results revealed significant impairments of oddball detection—specifically in rats with moderate and severe PNH as compared to controls. In contrast, juvenile MAM exposed rats with mild PNH did not show significant deficits in processing the oddball cue as compared to controls. These results parallel previous findings with regard to the behavioral effects of cerebral cortical microgyria, wherein the severity or extent of postnatal day one (P1) freeze-lesion-induced microgyria (double-bilateral versus single-bilateral pair) was related to degree of deficits seen for rapid acoustic processing [15].

In adulthood (P60+), subjects were again tested for basic pre-pulse inhibition (NST), as well as oddball tone-pair detection (i.e., detection of a tone-pair reversal). Unlike severe PNH juvenile subjects, adult MAM subjects did not

show any impairment in the detection of a single tone (NST). These findings may reflect a developmental delay of PPI circuitry in rats with severe cortical developmental disruption, one that may “catch up” with typically developing rats by adulthood. In fact, additional work will be needed to determine if the initial PPI deficit in juvenile MAM-treated rats is a product of abnormal or maturationally delayed startle circuitry. Nonetheless, given that juvenile MAM-treated rats were able to significantly detect silent gaps in white noise at a performance level comparable to controls, the interpretation of a general developmental delay in PPI (as opposed to any deficits in hearing or gross startle response) seems the most likely cause of altered NST scores in MAM-treated juveniles.

Interestingly, combined adult oddball results also indicated that juvenile oddball two-tone processing deficits ameliorate over time in the moderate PNH condition—either as a result of previous experience, or normal maturation. This is in contrast to the persistent oddball deficits seen in the severe PNH condition. The contrast between improved performance in mild PNH subjects, and

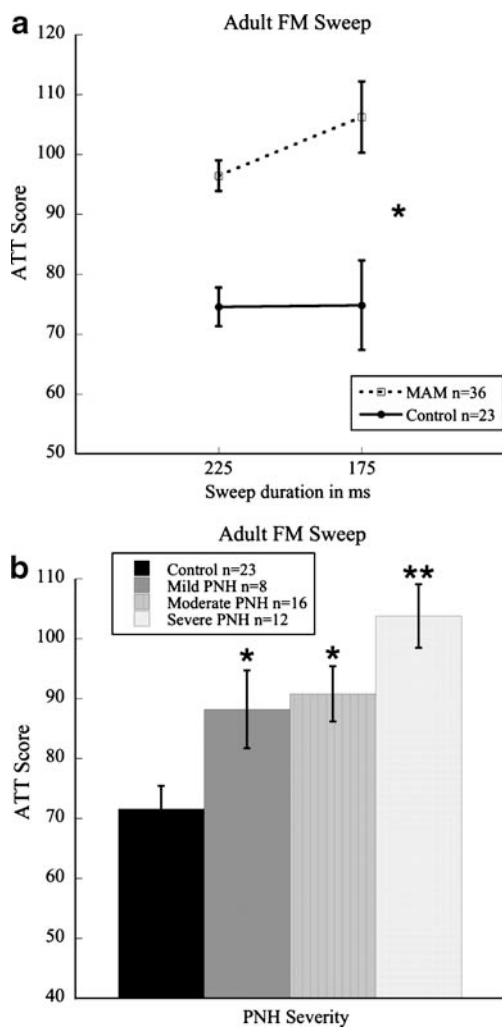


Fig. 7 Graphs showing: (a) significant difference between MAM treated and control adult subjects on the 225 and 175 ms FM sweep conditions; and (b) combined adult FM sweep performance for the three PNH severity groups (mild, moderate and severe) and control rats, showing significant deficits in all PNH conditions ($*p < .05$, $**p < .01$)

persistent oddball deficits in subjects with severe PNH, supports the notion of a deficit spectrum—one in which severity of processing impairments is directly related to the severity of neurodevelopmental disruption. With regard to the improved performance of mild PNH subjects in adulthood, we have previously reported that both maturation and experience can improve long-term acoustic processing. Specifically, Peiffer and colleagues [39] reported that juvenile rats with bilateral microgyria exhibited deficits in detecting short silent gaps (2–10 ms) in broadband white noise as compared to adults with no prior experience, suggesting that auditory temporal acuity improves with developmental maturation. We also reported that adult microgyric rats with prior acoustic experience show significantly better acoustic processing than adult rats with no prior experience [45]. These cumulative studies

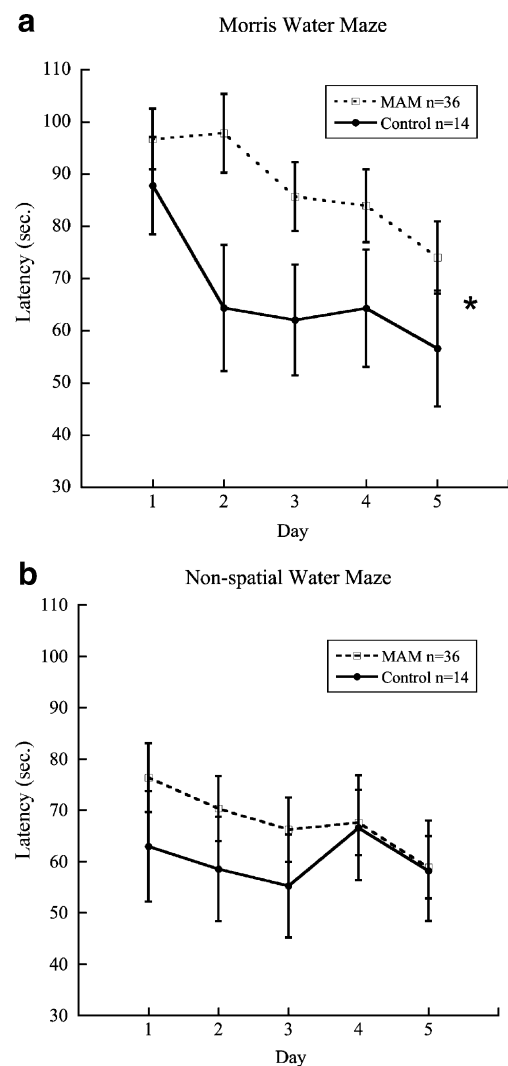


Fig. 8 Graphs showing: (a) MAM treated rats with a significant spatial learning deficit on the Morris water maze as compared to controls ($*p = .05$); and (b) no difference between the two Treatment groups on the non-spatial water maze

suggest that both maturation and experience may have contributed to the improvement of MAM treated rats with moderate PNH on the oddball task, across juvenile and adult testing periods.

However, adult subjects were also tested on a novel FM sweep task, and results for the FM sweep procedure (225 and 175 ms) revealed a significant effect of MAM treatment—with all MAM subjects performing significantly worse than controls. These results indicate that MAM rats *do* exhibit persistent deficits in rapid auditory temporal processing, but these deficits may require increasingly more complex stimuli to be elicited with increasing experience and/or age. Interestingly, comparison of PNH severity revealed significant impairments for all severity classes (mild, moderate, severe) versus control subjects on the adult FM task, even though there was only a trend for mild

PNH subjects to perform worse than controls on the juvenile oddball task (no statistical difference was observed). In sum, a novel and complex cue detection task (FM sweep, requiring discrimination of a sweep reversal) was able to elicit deficits, even in only mildly disrupted MAM subjects.

Maze learning

Spatial and non-spatial learning were also assessed in adult MAM and control subjects, through the use of two different water maze paradigms. In order to insure adequate testing time, control subjects were reduced in number by random selection of a subset, while all MAM-treated rats were retained for maze learning (MAM $n=36$, control $n=14$). Animals were initially tested on a water escape task to evaluate swimming/motor ability, and the two groups performed similarly. However, MAM treated rats were found to perform significantly worse than controls on a spatial maze task (MWM), as evidenced by longer latencies. Interestingly, however, no effects of PNH severity on this task were found. In contrast to MWM, no significant differences were observed between the groups on escape latency in the non-spatial condition (NSWM). The application of these findings to the role of cortical disruption in human language disability *per se* is unclear, but may well relate to the broader relationship between generalized developmental cortical disruptions, and generalized learning deficits (such as mental retardation (MR)). However, in the present model (Single maternal MAM dose, 25 mg/kg on E15) we do not show generalized cross-task impairment (as is seen in human mental retardation) given observations of intact silent gap detection and non-spatial learning. Future studies utilizing increased dose or injection regimes may provide additional insight into thresholds for neurodevelopmental disruption and global behavioral impairments representing more severe clinical sequelae.

Importantly, these convergent results parallel other findings that E15 MAM treated rats have spatial learning impairments relative to controls [46–49], although this result has not been consistent across studies of spatial learning in MAM-treated rats (possibly due to inadequate design power, or variability in the severity or location of migratory anomalies; [11]). In the current study, it is likely that spatial learning impairments resulted from the observed migratory anomalies in dorsal hippocampus of E15 MAM-treated rats, given that this region has repeatedly been implicated in spatial learning [50, 51]. Interestingly, in contrast to auditory processing data, we did not observe a relationship between severity of hippocampal dysplasia and spatial learning scores. Future studies may seek to identify a threshold for spatial learning impairment and dorsal hippocampal dysplasia through the use of varying doses of

E15 MAM exposure, coupled with a more in depth histological analysis. However, overall results suggest that E15 MAM treatment leads to spatial but not non-spatial learning impairment as compared to controls.

Implications for human learning impairment

Rodent models of cortical developmental disruption have repeatedly shown deficits in processing rapid and/or complex acoustic stimuli [8, 9, 15, 16]. Importantly, while not without controversy, deficits in processing rapid auditory stimuli have been extensively documented in human learning impaired populations [17, 19, 21, 52–54]. Moreover, auditory temporal processing has been identified as a significant predictor of language outcome in typical infants as well as those with a family history of language impairment [33, 55]. Further, EEG/ERP and fMRI data relating cortical physiology to rapid auditory processing thresholds reveal disruptions of cortical activity in individuals with language learning impairment, as well as infants with a family history of language deficits [20, 21, 55, 56]. Additional evidence that cortical developmental perturbation may lead to language learning impairments in humans is based on *post mortem* assessment of dyslexic brains, which revealed diffuse ectopic clusters of neurons and microgyric malformations in left perisylvian language regions and frontal cortex [4]. In addition, microgyria have been seen in the neocortex of children with specific language impairment [3, 5], and PNH have been related to reading impairments despite normal intelligence in human clinical patients [1, 2].

The current findings have significant implications for the study of human learning impairment based on the striking parallels between rodent models of cortical developmental pathology and predictors of human language learning disabilities. The present study provides evidence that generalized cortical developmental disruption leads to auditory temporal processing impairments (while leaving baseline temporal acuity intact, i.e., the detection of silent gaps in white noise). These disruptions are also associated with deficits in spatial learning, although the latter may more directly reflect more homogeneous MAM-induced disruptions in the hippocampus.

Previous work in our lab, along with clinical observations, continue to suggest that a variety of pathogens can produce a diverse phenotype of cortical anomalies, and these are in turn related to processing and learning impairments across human clinical populations and rodent models. Thus, it does not appear that the *mode* of pathological disruption—including genetic (E14 *in utero* RNAi of *Dyx1c1*), epigenetic (ectopia in autoimmune deficient BXSb/MpJ and NZB/BINJ mice), injury-induced (P1 freeze lesion induced microgyria), or

teratogen-induced (E15 MAM exposure)—exclusively determines the subsequent behavioral outcome in rodent models [8–10, 15, 16]. However, the onset *timing* of the disruption within a broad window—between cortical neurogenesis, and the completion of neuronal migration—appears to be of major importance in determining long-term behavioral sequelae [8–10, 15, 16]. Further, while heritability can account for more than half of the variance in reading impairments [57–59], the present study suggests that environmental factors can also have a significant effect on neural systems mediating language-related processes, and highlights the unlikely prospect of identifying a single cause (genetic or otherwise) of language learning impairments related to auditory temporal or other processing mechanisms.

In addition, we have observed that the presence of more severe PNH is related to more significant impairments in acoustic processing in MAM exposed rats. In humans, severe cortical developmental disruption is related to pervasive learning deficits and global cognitive impairments [60, 61]. However, several studies evaluating milder forms of PNH and microgyria in humans suggest that there is a significant relationship between the extent of cortical disruption, and the severity of language related learning deficits, even within relatively mild cases [1–3, 5, 6]. These findings lend further support for the use of rodent models to help identify factors that might influence the heterogeneous profile of human learning impairments.

Finally, we observed a general improvement in oddball detection from the juvenile period to adulthood in E15 MAM treated rats. However, in adulthood, deficits were still observed when MAM rats were presented with a novel and complex FM sweep task. The notion of maturational influence on both auditory processing, and the changing profile of language impairments from childhood to adulthood in individuals with language difficulties, have been well documented, and parallel observations of maturational improvement seen in the current study. For example, Hautus and associates [19] showed that six and seven year old reading impaired children had deficits in detecting silent gaps in broadband white noise (a measure of temporal processing), whereas reading impaired individuals age ten to adult did not show these same deficits. Yet, studies have found significant temporal processing deficits in adults with reading impairment using more complex tone order association tasks similar to the oddball and FM sweep procedures used in the current experiments [52, 54, 56].

In conclusion, we sought to extend the model for developmental learning impairment to evaluate embryonic teratogen exposure leading to neurodevelopmental malformations. Our findings reinforce the notion that early cortical developmental disruption leads to fundamental problems in processing temporally relevant acoustic information, with relative effects across a severity spectrum.

Conversely, generalized deficits in spatial learning associated with MAM exposure do not appear to be related to severity of PNH caused by MAM exposure, and may reflect a more homogenous disruption of the hippocampus as seen across affected (MAM-treated) subjects.

These and related findings continue to provide insight to human clinical data, by supporting a link between: (1) cortical developmental pathology, (2) rapid auditory processing deficits, (3) spatial learning deficits, and (4) developmental language/learning impairments.

Acknowledgements This research was supported by NIH Grant HD20806.

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