# Auditory processing deficits in unilaterally and bilaterally injured hypoxic–ischemic rats

Melissa M. McClure,[1] Steven W. Threlkeld,[1] Glenn D. Rosen[2] and R. Holly Fitch[1,CA]

[1]Department of Psychology, Behavioral Neuroscience Division, University of Connecticut, Storrs, Connecticut; [2]Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Massachusetts, USA

[CA]Corresponding Author: Roslyn.h.fitch@uconn.edu

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Hypoxic–ischemic represents a common cause of damage to the prenatal brain and can co-occur with prematurity. Prematurity is associated with emergent language impairments, and it has been suggested that rapid auditory processing deficits play a causal role in language difficulties. We previously demonstrated rapid auditory processing deficits in juvenile rats receiving neonatal unilateral hypoxic–ischemic injury, but these deficits appeared to resolve by adulthood. The current study compared unilaterally and bilaterally injured hypoxic–ischemic rats on auditory tasks, to assess whether rapid auditory processing recovery in adulthood is related to this aspect of injury. Current results indicate that while neonatal unilateral and bilateral hypoxic–ischemic injury both lead to rapid auditory processing deficits in the juvenile period, only rats with bilateral hypoxic–ischemic injury exhibit deficits that persist into adulthood. *NeuroReport* 16:1309–1312 © 2005 Lippincott Williams & Wilkins.

**Key words**: Language impairment; Prematurity; Rapid auditory processing

## INTRODUCTION

Hypoxia–ischemia (HI) represents a common cause of damage to the perinatal brain, and is associated with premature birth. A significant percentage of premature or very low birth weight (<1500 g) infants demonstrate cognitive/behavioral impairments later in development, including disorders of language [1–4]. Auditory processing deficits have been suggested to play a causal role in later language impairments [5], and thresholds for rapidly changing auditory cues in infants with a family history of language impairments are significantly higher than for controls [6]. Rapid auditory processing (RAP) deficits have also been identified in premature children, with a correlation between auditory processing and later language scores [7]. Importantly, similar RAP deficits have been reported in a rodent model of HI injury ([8], see below).

Further association between degree of injury and later cognitive performance has been demonstrated in premature infants. For example, magnetic resonance imaging reveals a relationship between periventricular leukomalacia (a common neuropathological feature associated with prematurity) and poor performance on reading and spelling tasks [9]. A relationship between abnormalities of the ventricles, corpus callosum, and other areas of white matter and later reading problems has also been shown [10]. Patterns of brain activation also differ between those with perinatal HI injury versus normals. Adults with corpus callosum damage associated with premature birth have been shown to have different activation patterns in response to auditory and visual tasks [11].

Insight into perinatal injury in the human has been provided through the use of an animal model of HI injury, which entails the occlusion of the common carotid artery followed by a period of hypoxia [12]. This early injury model in the rat, employed on postnatal day 1 up to day 5 (P1–P5), produces injury comparable to that seen in preterm infants. Specifically, damage is seen in white matter regions, including in and around the internal capsule [13], corpus callosum [8], and to periventricular oligodendrocyte progenitors surrounding the lateral ventricles [14]. This HI procedure, performed during the early neonatal period in rats, further leads to RAP deficits similar to those seen in premature children. These behavioral deficits were seen during the juvenile period using a unilateral-HI rodent model [8], although these deficits appeared to resolve by adulthood. This led us to question whether adult 'recovery' is a result of cortical compensatory mechanisms resulting from the unilateral nature of injury. The current study sought to address this question by utilizing a unilateral-HI and a bilateral-HI procedure. The bilateral procedure was piloted on P1 but the mortality rate approached 100%. Therefore, P4 was chosen for the bilateral procedure to increase survivability, and because it has been shown that this age is still within the range comparable to premature birth in humans.

## MATERIALS AND METHODS

*Animals:* Thirty-six male Wistar rats, born at the University of Connecticut, underwent an HI or sham procedure on P1 or P4, were weaned on P21, and housed in a 12-h light/dark cycle with food and water available *ad libitum*. Auditory testing took place during juvenile (P25–P34) and adult periods (P55–P65).

***Induction of hypoxia–ischemia:*** Pups were culled into litters of 10 (eight males/two females) on P1 and were randomly assigned to litters receiving either unilateral-HI surgery/sham surgery (P1), or bilateral-HI surgery/sham surgery (P4). At surgery, HI pups were anesthetized with isoflurane (2.5%), a longitudinal midline incision was made in the neck, one (or both) of the common carotid arteries was cauterized, and the incision was sutured. After a period of 2 h with their dams (following footpad injections and recovery from anesthesia), unilateral-HI animals were placed in a chamber containing 8% humidified oxygen (balanced with nitrogen) for 90 min. Sham animals received a similar surgical procedure, but no artery cauterization and no hypoxia (placed in a chamber with room air for 90 min). Pups were returned to their mothers after the procedure. This procedure differed slightly for bilateral animals, who received bilateral cauterization, did not receive any hypoxia (owing to mortality concerns), and remained with their mothers after surgery, as did their same day (P4) sham littermates.

***Behavioral testing; startle reduction:*** The startle reduction paradigm capitalizes upon the acoustic startle reflex (ASR), a large amplitude motor response to a startle-eliciting stimulus (SES; a 105-dB, 50 ms white noise burst). When preceded by a benign prestimulus, the ASR to the SES is attenuated. Comparison between the ASR amplitude when no 'cue' is present (an uncued trial) and when a 'cue' stimulus (benign prestimulus such as an oddball reversal) precedes the SES (a cued trial) yields an objective measure of sensory detection [15].

***Apparatus:*** During startle testing (see [9] for apparatus details), the amplitude of each animal's ASR was recorded (in mV) by extracting the maximum peak value from the 150 ms signal epoch following the SES. These values were coded for cued and uncued trials, and represented the animal's absolute response amplitude for each trial. Attenuated response scores (ATT scores) were then derived by dividing the average response on cued responses by the uncued responses and multiplying by 100, which provides a measure of relative performance for each rat.

***Oddball and modified oddball procedures:*** The oddball procedure involved the repeated presentation of a standard stimulus, a 75-dB high/low two-tone sequence (2300–1100 Hz, each tone 7 ms), separated by an interval of variable duration (225, 75, 65, 40, or 20 ms). Each two-tone sequence was separated by an epoch 200 ms greater than the duration between tone pairs, to maintain perceptual contiguity of paired tones. On uncued trials, the last two-tone sequence was followed by 50 ms of silence, followed by the SES. On cued trials, an 'oddball' stimulus (reversal of standard two-tone sequence) was followed by 50 ms of silence and the SES. Similarly, the modified oddball (mob) procedure involved the repeated presentation of a 75-dB high/low two-tone sequence and these tones varied in duration both within and between tone pairs (40/140, 20/70, 10/60, and 3/60 ms).

***Histological analysis:*** Following behavioral testing (P80), animals were weighed, anesthetized, and transcardially perfused with fixative (10% buffered formalin phosphate); their heads were removed, placed in formalin, and shipped to G.D.R. for anatomical analysis. The brains were removed, weighed, embedded in celloidin, serially sectioned in the coronal plane at 30 μm, mounted on glass slides, stained with cresyl violet, and coverslipped with Permount. The entire rostral to caudal extent of each brain was examined blind to condition under a stereomicroscope, and damage and distortions were noted. All procedures conformed to approved University of Connecticut Institutional Animal Care and Use Committees protocols.

## RESULTS

***Histology:*** Animals for which we were able to confirm the presence of lesions were included in the subsequent analyses [P1 unilateral-HI $n$=18; P4 bilateral-HI $n$=5; and shams ($n$=15; P1 $n$=10, P4 $n$=5)]. Unilateral damage on P1 led to enlarged ventricles and minor cell loss in the cortex ipsilateral to the ischemic lesion. Damage from bilateral P4 HI injury resulted in cortical and hippocampal cell loss and/or ventricular enlargement in both hemispheres (see Fig. 1).

***Juvenile oddball procedure:*** Results from a 2 (treatment; unilateral-HI vs. bilateral-HI) × 5 (oddball duration) repeated-measures ANOVA for ATT scores revealed no significant differences between groups; thus, the unilateral-HI and bilateral-HI groups were pooled and compared with shams. Results from a 2 (treatment; pooled HI vs. sham) × 5 (oddball duration) repeated-measures ANOVA on ATT scores revealed a significant difference between groups [$F(1,34)=3.6$, $p<0.05$ (one-tailed)] (see Fig. 2), with shams performing better at all durations and significantly so at the 40 ms duration [$F(1,34)=4.74$, $p<0.05$].

***Adult modified oddball procedure:*** Results from a 2 (treatment; bilateral-HI vs. sham) × 4 (mob duration) repeated measures ANOVA revealed a marginal effect for

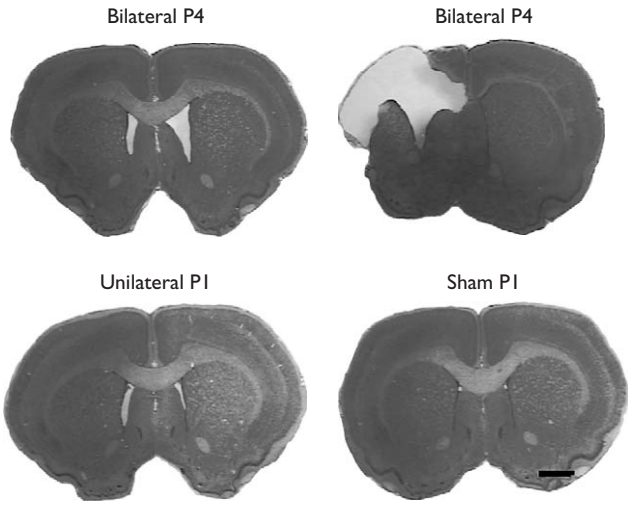


**Fig. 1.** Nissl-stained sections from two bilateral hypoxia–ischemia (HI), one unilateral-HI, and one sham animal. Note the ventricular enlargement in the unilateral-HI animal and the bilateral-HI animal (on the left) on the side(s) ipsilateral to the ischemic procedure. Also note cortical damage to the bilateral-HI animal on the right. Scale bar: 500 μm.

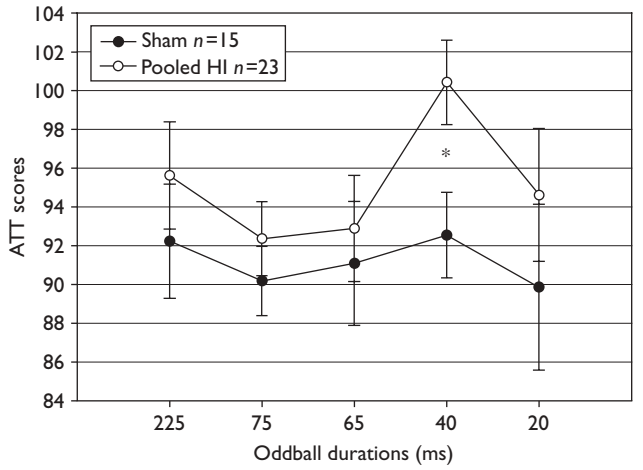


**Fig. 2.** Attenuation response scores (ATT scores) for pooled unilateral and bilateral hypoxic–ischemic (HI) versus sham animals for the variable duration oddball task in the juvenile period. The duration within each tone pair is on the *x*-axis. $*p < 0.05$.

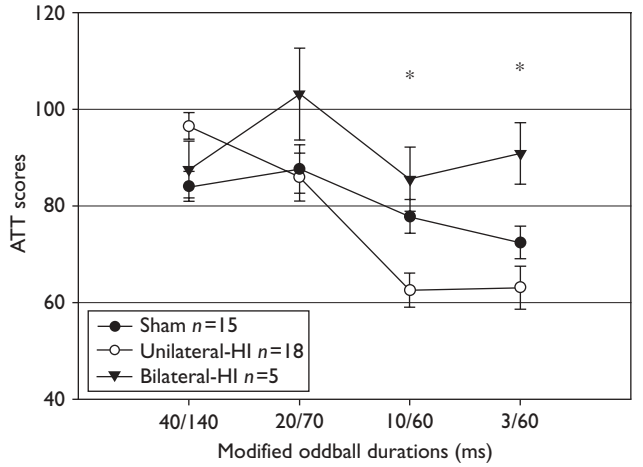


**Fig. 3.** Attenuation response scores (ATT scores) for unilateral and bilateral hypoxic–ischemic (HI) versus sham animals for the modified oddball task in adulthood. The duration within each tone pair/between tone pairs is on the *x*-axis. $*p < 0.01$, unilateral-HI versus bilateral-HI comparison.

Treatment [F(1,16)=2.42, $p<0.1$ (one-tailed)], with bilateral-HI rats performing worse than shams. A 2 (treatment; unilateral-HI vs. sham) $\times$ 4 (mob duration) repeated-measures ANOVA revealed a significant mob duration $\times$ treatment interaction [F(3,27)=6.79, $p<0.01$], indicating that unilateral-HI animals performed worse than shams at the 40/140 condition but better at the 10/60 and 3/60 conditions. Thus, no overall difference between unilateral-HI and sham performance was seen, nor unilateral-HI deficits at short durations.

Bilateral and unilateral-HI performance were then directly compared. A 2 (treatment; unilateral-HI vs. bilateral-HI) $\times$ 4 (mob duration) repeated-measures ANOVA revealed a significant interaction for treatment $\times$ mob duration [F(3,19)=5.09, $p<0.01$ (one-tailed)] and a significant main effect for treatment [F(1,21)=7.31, $p<0.01$ (one-tailed)], showing that bilaterally injured animals performed worse than unilaterally injured animals in adulthood. Results from an independent samples *t*-test showed significant differences at the shortest (10/60 and 3/60) mob tasks [$t$=2.29, $p<0.05$ and 3.88, $p<0.01$ (one-tailed)], with bilateral-HI animals performing worse than unilateral-HI animals (see Fig. 3).

## DISCUSSION

Anatomical/behavioral analysis of long-term HI injury in rats suggests compelling parallels to perinatal HI brain injury in humans. Previous findings from our lab have shown correlations between RAP performance and neuroanatomical damage (in ipsilateral cortex, hippocampus, and corpus callosum) in a rodent HI model [8]. Initially, we showed similar behavioral deficits in P1, P7, and P10 HI injured animals, although areas of major damage varied between these groups (with more damage to white matter areas at the earlier age, and more gray matter damage at later ages; [8]). Future studies may in fact reveal patterns of differences for age at unilateral-HI injury using a larger '*n*' and/or a longer duration of hypoxia.

In the current study, juvenile HI animals (both unilaterally and bilaterally injured) show deficits in RAP performance (with worse performance on rapid auditory discrimination procedures). This impairment parallels human literature in which premature infants with brain lesions show a deficit in auditory temporal processing, which may in turn contribute to difficulties in the discrimination of speech sounds [9]. Current results also show that unilateral-HI animals no longer display the same RAP deficit in adulthood as had been seen in the juvenile period. RAP deficits in bilaterally injured animals, on the other hand, appear to persist into adulthood.

The apparent recovery of RAP ability in unilateral-HI animals may be the result of cortical compensation, severity/location of damage, or some other factor. In a study involving preterm children, anatomical correlates of minor cognitive and motor disabilities were not found, although an increasing degree of damage to various brain regions did correlate with more severe cognitive and motor disabilities [16], supporting the idea that the greater the extent of damage the greater the incidence of disabilities. In rats, lesion size and degree of functional recovery demonstrate a significant inverse relationship [17]. Finally, it has been demonstrated in children with prenatal unilateral ventricular enlargement that over 90% of survivors show normal cognitive development, while subjects with bilateral ventricular enlargement have a significantly worse outcome [18].

## CONCLUSION

Neonatal unilateral-HI injury in rats (P1) produces RAP deficits in the juvenile period, which appear to resolve by adulthood and neonatal bilateral-HI injury (P4) produces similar deficits that persist into adulthood. Given the similarity to patterns of anatomical damage, auditory deficits, and language difficulties seen in humans, the current animal model of HI may provide a useful instrument through which to research effects of early HI brain injury in humans.

## REFERENCES


1. Volpe JJ. *Neurology of the Newborn*. Philadelphia: Saunders; 2001.
2. Largo RH, Molinari L, Comenale Pinto L, Weber M, Duc G. Language development of term and preterm children during the first five years of life. *Dev Med Child Neurol* 1986; **28**:333–350.
3. Frisk V, Whyte H. The long-term consequences of periventricular brain damage on language and verbal memory. *Dev Neuropsychol* 1994; **10**: 313–333.
4. Kilbride HW, Thorstad K, Daily DK. Preschool outcome of less than 801-gram preterm infants compared with full-term siblings. *Pediatrics* 2004; **113**:742–747.
5. Tallal P. Rapid auditory processing in normal and disordered language development. *J Speech Hear Res* 1976; **19**:561–571.
6. Benasich AA, Tallal P. Infant discrimination of rapid auditory cues predicts later language impairment. *Behav Brain Res* 2002; **136**:31–49.
7. Downie AL, Jakobson LS, Frisk V, Ushycky I. Auditory temporal processing deficits in children with periventricular brain injury. *Brain Lang* 2002; **80**:208–225.
8. McClure MM, Peiffer AM, Rosen GD, Fitch RH. Auditory processing deficits in rats with neonatal hypoxic–ischemic injury. *Int J Dev Neurosci* 2005; **23**:351–362.
9. Downie AL, Jakobson LS, Frisk V, Ushycky I. Periventricular brain injury, visual motion processing and reading and spelling abilities in children who were extremely low birth weight. *J Int Neuropsycol Soc* 2003; **9**: 440–449.
10. Stewart AL, Rifkin L, Amess PN, Kirkbride V, Townsend JP, Miller DH *et al*. Brain structure and neurocognitive and behavioral function in adolescents who were born very preterm. *Lancet* 1999; **353**: 1653–1657.
11. Santhouse AM, Ffytche DH, Howard RJ, Williams SC, Stewart AL, Rooney M *et al*. The functional significance of perinatal corpus callosum damage: an fMRI study in young adults. *Brain* 2002; **125**:1782–1792.
12. Rice JE, III, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic–ischemic brain damage in the rat. *Ann Neurol* 1981; **9**: 131–141.
13. Uehara H, Yoshioka H, Kawase S, Nagai H, Ohmae T, Hasegawa K *et al*. A new model of white matter injury in neonatal rats with bilateral carotid artery occlusion. *Brain Res* 1999; **837**:213–220.
14. Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J *et al*. Selective vulnerability of late oligodendrocyte progenitors to hypoxia–ischemia. *J Neurosci* 2002; **22**:455–463.
15. Marsh RR, Hoffman HS, Stitt C, Schwartz GM. The role of small changes in the acoustic environment in modifying the startle reflex. *J Exp Psychol Anim Behav Process* 1975; **1**:235–244.
16. Krageloh-Mann I, Toft P, Lunding J, Andresen J, Pryds O, Lou HC. Brain lesions in preterms: origin, consequences and compensation. *Acta Paediatr* 1999; **88**:897–908.
17. Kolb B, Cioe J. Recovery from early cortical damage in rats, VIII. Earlier may be worse: behavioural dysfunction and abnormal cerebral morphogeneis following perinatal frontal cortical lesions in the rat. *Neropharmacology* 2000; **39**:756–764.
18. Senat MV, Bernard JP, Schwarzler P, Britten J, Ville Y. Prenatal diagnosis and follow-up of 14 cases of unilateral ventriculomagaly. *Ultrasound Obstet Gynecol* 1999; **14**:327–332.


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