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Auditory processing deficits in rats with neonatal hypoxic-ischemic injury

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Abstract

Hypoxia-ischemia (HI) refers to reduced blood oxygenation and/or a diminished amount of blood perfusing the brain, and is associated with premature birth/very low birth weight (VLBW). HI represents a common cause of injury to the perinatal brain. Indeed, a significant number of premature/VLBW infants go on to demonstrate cognitive/behavioral deficits, with particularly high incidence of disruptions in language development. Auditory processing deficits, in turn, have been suggested to play a causal role in the development of language impairments. Specifically, the inability to identify fast elements in speech is purported to exert cascading detrimental effects on phonological discrimination, processing, and identification. Based on this convergent evidence, the current studies address auditory processing evaluation in a rodent model of HI injury induced on postnatal days 1, 7, or 10 (which in turn is well accepted as modeling HI-related injury to the perinatal human). Induced injuries were followed by a battery of auditory testing, and a spatial maze assessment, performed both during juvenile and adult periods. Results indicate that rats suffering from these early HI insults performed significantly worse than shams on tasks requiring rapid auditory processing, and on a test of spatial learning (Morris water maze (MWM)), although these effects were not seen on simpler versions of auditory tasks or on a water escape assessment (thus ruling out hearing/motor impairments). Correlations were found between performance on rapid auditory and spatial behavioral tasks and neuroanatomical measures for HI animals such as: the volume of the hippocampus, cerebral cortex, ventricles, and/or the area of the corpus callosum. Cumulative findings suggest that perinatal HI injury in the rat may lead to neurodevelopmental damage associated, in turn, with auditory processing and/or learning and memory impairments. As such, the current model may have critical implications for the study of neurophysiological underpinnings of cognitive deficits in premature/VLBW infants.

Keywords: Hypoxia-ischemia; Very low birth weight (VLBW); Morris water maze; Auditory; Prematurity; Language deficit

1. Introduction

Hypoxia/ischemia (HI), or a reduction in blood oxygenation and flow, represents a common cause of damage to the perinatal brain, and can occur via different etiologies (Vannucci, 2000). In premature infants, damage consistent with HI is associated with the rupture of blood vessels (often in the vascular bed of the subependymal matrix), which can produce tissue compression, disruption of cerebrospinal fluid flow, and dilation of the ventricles (Volpe, 2001). In term infants, HI injury is often associated with asphyxia, placental dysfunction, and prolonged labor and/or resuscitation. HI resulting from premature birth is also associated with lower than average birth weights. A significant percentage (approximately 10%) of very low birth weight infants (VLBW, <1500 g) go on to exhibit gross motor deficits such as cerebral palsy, and an even greater percentage (25–50%) go on to demonstrate cognitive and other behavioral deficits (Volpe, 2001). Some examples of these deficits include: hearing impairment and/or speech problems (Kenworthy et al., 1987); delayed language development (Vohr et al., 1988; Casiro et al., 1990); low IQ (Ross et al., 1985); and deficits in phonological short-term memory (Briscoe et al., 1998). Auditory processing deficits have also been reported in premature babies, and such deficits have been suggested to play a causal role in the development of language related impairments in this

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population (Downie et al., 2002). Term infants suffering from asphyxia/HI at birth also show motor and speech impairments (Largo et al., 1986), decreased motor and language skills, a lower IQ later in development, and an increase in the incidence of handicaps (Robertson and Finer, 1985).

From a medical perspective, injury at different stages of human perinatal development results in unique neuropathological sequelae, depending on the timing and severity of injury. For example, premature birth is associated with periventricular-intraventricular hemorrhage (PVH-IVH), which can result from bleeding in fragile, thin-walled endothelial-lined vessels in the subependymal germinal matrix (Hambleton and Wigglesworth, 1976; Ghazi-Birry et al., 1997). PVH-IVH can result in death of cells in the germinal matrix and its glial precursor cells, destruction of periventricular white matter, disruption of CSF, and hydrocephalus (Volpe, 1994). Another common pathology of premature birth is periventricular leukomalacia (PVL), defined as a lesion to white matter surrounding the lateral ventricles (i.e. decreased myelination) (Volpe, 2001). Contributing factors to PVL include developmental immaturity of the blood supply and cerebrovascular regulation to the white matter coupled with the vulnerability of early differentiating oligodendrocytes to the deprivation of glucose and oxygen, and free radical attack (Perlman, 1998; Levy et al., 1997; Volpe, 2001). Term infants subjected to HI episodes later show damage to gray matter structures, such as the thalamus, putamen, cortex, basal ganglia, the dentate gyrus, and the brain stem (Barkovich and Sargent, 1995; Johnston et al., 2001; Pulera et al., 1998). These age-related shifts in regional vulnerability may reflect early sensitivity to NMDA receptor overstimulation, based on the immaturity of glutamate receptors that open more readily and stay open longer early in development (Johnston, 1995; Jensen, 2002). Selective vulnerability of tissues with age may also reflect a regional shift in metabolic demand (i.e. vulnerability) across development (Tuor, 1991; Levison et al., 2001).

These neuropathological injuries associated with HI in infants have been modeled in animals. The Rice–Vannucci model (Rice et al., 1981), for example, entails unilateral common carotid artery ligation of neonatal rats, followed by exposure to a period of hypoxia (typically 6–8% O_2). This induction protocol leads to damage prototypical of premature infants when performed on P1, and damage prototypical of term HI injury when performed on P7. Such differences are consistent with indications that developmental events ongoing in the P1 rat brain parallel those of the human preterm infant at approximately 24–28 weeks (Volpe, 2001), while events ongoing in the P7 rat brain approximate neurodevelopmental events for a 34-week-old human fetus (i.e. the cerebral cortex has layered, the germinal matrix is involuting, and the myelination of the periventricular white matter has yet to occur; Vannucci et al. (1997)). Accordingly, researchers have shown that P1 HI injury in rats leads to observation of some damage to the cortex and caudate, and a significant loss of white matter in the ipsilateral hemisphere (Sheldon et al., 1996), death of periventricular oligodendrocyte progenitors (Back et al., 2002; Ness et al., 2001), as well as damage in regions of the subplate, and the intermediate and subventricular zones (consistent with PVL seen in premature infants) (McQuillen, 2003). Researchers further observe that HI injury on P7 tends to produce damage to the corpus callosum and cerebral cortex (Follett et al., 2000), hippocampus, striatum, globus pallidus, and amygdala (Towfighi et al., 1991), and the thalamus and the brainstem (Northington et al., 2001).

In the current study, we sought to capitalize on this established rat model of HI (which has been shown to produce anatomical correlates of human preterm/term injury), and extend the model to the assessment of long-term behavioral/cognitive outcomes. Given evidence of relationships between early auditory processing and later language development in humans (Downie et al., 2002; Tallal, 1976), we assessed rats subjected to early HI injuries on a variety of auditory processing tasks, inclusive of both ‘‘easy’’ as well as more demanding conditions requiring discrimination of very rapidly changing acoustic cues. Rats with these types of early injury have not (to our knowledge) been evaluated in a complex auditory processing paradigm to date. In addition, based on prior evidence of maze learning deficits for P7 HI rats on a simple T-maze (Ford et al., 1989) as well as evidence of learning and memory deficits in children who were premature and/or VLBW (Briscoe et al., 1998), we examined performance on an established measure of spatial learning in rodents (Morris water maze (MWM)). Evidence suggestive of deficits on the MWM as well as other measures of learning have been reported for related animal models of preterm injury. For example, learning and memory deficits were found in rodents exposed to early postnatal hypoxia and tested on the radial arm maze (Decker et al., 2003; Grojean et al., 2003), treated with muscimol (a $GABA_A$ receptor agonist) and tested on the MWM (Nunez et al., 2003), and exposed to prenatal (E17) HI injuries and tested on the Y-maze (Cai et al., 1999). HI injury on P7 has shown consistent effects on learning and memory. Specifically, MWM has shown results indicating a spatial deficit in HI animals, with an increase in mean escape daily latencies and a decrease in time spent in target quadrant (Lebedev et al., 2003; Kumral et al., 2004). Long-term reference memory is also impaired in these animals based on performance on plus maze and water maze tasks (Ikeda et al., 2001). Further, HI animals are impaired in T-maze acquisition, and water maze tasks (Balduini et al., 2000).

In the human literature, a relationship has been found between cognitive scores and degree of brain abnormalities in subjects with perinatal HI. For example, volume of sensorimotor and temporal cortical brain regions correlate significantly with assessments of cognition in premature children (Peterson, 2002). Similarly, a relationship has been shown between cognitive scores and abnormalities of the

corpus callosum in preterm subjects at 8 years of age (Roth et al., 1993). Differential patterns of brain activation in preterm subjects with damaged callosa have also been found when compared to term controls during an auditory task (Santhouse et al., 2002). Term infants who undergo HI injury also show a correlation between scores assessing neurologic status (neurological signs, motor function, and behavior) and severe basal ganglia and white matter lesions (Haataja et al., 2001). In rodents with HI injury, deficits in MWM performance correlate significantly with hippocampal volume (Wagner et al., 2002), and degree of cerebral atrophy (Ten et al., 2003). In the current study, we therefore performed correlations between neuroanatomical and behavioral measures (after converting within-group scores to *z*-scores).

2. Experimental procedures

2.1. Subjects

Subjects were Wistar rats, born to time-mated dams (Charles River Laboratories, Wilmington, MA) at the University of Connecticut. Pups were subjected to a hypoxic-ischemic or sham procedure (see below) on postnatal days 1, 7, or 10 (P1, P7 or P10). Subjects were weaned on P21, and housed in a 12 h light/dark cycle with food and water available ad libitum. The first group of auditory tests (performed during the juvenile time period) began on P24 and lasted until P41. In adulthood, subjects were tested on another battery of auditory tests lasting from P69 until P84. A learning and memory task (Morris water maze) was also given between P70 and P75. Subjects were sacrificed between P95 and P97 via transcardial perfusion and the brains were sent to GDR for histological analysis.

2.2. Induction of HI

All subjects were culled into litters of 10 (8 males and 2 females) on P1. Pups were randomly assigned to litters receiving either P1 surgery, P7 surgery, or P10 surgery, balancing sham surgery and HI procedure within litter. On the appropriate surgery day, HI pups were anesthetized with isoflurane (2.5%) and a midline incision was made longitudinally in the neck. The right common carotid artery was located, separated from surrounding tissue, cauterized, and the incision was sutured. Pups were marked with footpad injections, placed under a warming lamp, allowed to recover from anesthesia and returned to their dams for a period of 2 h. Animals were then placed in a container in which they were exposed to 8% humidified oxygen (balanced with nitrogen) for a period of 90 min. Sham animals received the same surgical procedure, but with no artery cauterization and no hypoxia (they were placed in chamber open to room air for an equivalent period of time). All HI groups received comparable treatment but at different ages. All pups were returned (HI and shams together) to their mothers after the procedure, and later underwent behavioral testing.

2.3. Behavioral testing: startle reduction

The startle reduction paradigm capitalizes upon the acoustic startle reflex (ASR), which is a large amplitude motor response to a startle eliciting stimulus (SES). When preceded by a benign pre-stimulus, the ASR to the SES is attenuated (also called pre-pulse inhibition). In the studies presented here, the SES was always a 105 dB, 50 ms white noise burst. The simplest version of this task employed a 75 dB, 7 ms, 2300 Hz tone pre-stimulus. Comparison between the ASR amplitude when no pre-stimulus was present (an uncued trial) and when the pre-stimulus preceded the SES (a cued trial) yields an objective measure of sensory detection (Marsh et al., 1975). The inter-trial interval between each SES was variable (range, 16–24 s.) but averaged 20 s.

2.3.1. Apparatus

During startle testing, each subject was placed on an individual load-cell platform (MED Associates, Georgia, VT). The output from the platform was amplified (linear amp PHM-250-60 MED Associates) and acquired by a Biopac MP100WS Acquisition system connected to a Power Macintosh 7200. The amplitude of each subject's ASR was recorded (in mV) following the onset of the SES, by extracting the maximum peak value from the 150 ms signal epoch following the presentation of the SES. These values were coded for cued and uncued trials, and represented the subject's absolute response amplitude for each trial (the raw dependent variable). Scores were subjected to further analysis by deriving attenuated response measures as a function of relative performance on cued and uncued trials at each condition, for each subject (see Section 3). Auditory stimuli were generated on a Power Macintosh 6100 with custom programmed software, and sound files were played using SoundHack 0.881NF and delivered via powered Yamaha YHT-M100 speakers.

2.3.2. Single tone procedure

A single tone test session consisted of 104 trials (cued or uncued) and presented in a pseudo-random order on a single day (no more than three of the same type of trial in a row). 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 followed 50 ms later by the SES.

2.3.3. Oddball procedure

An oddball test session consisted of 104 trials, and a total of four sessions (one per day over 4 days) were given to each subject. The procedure involved the repeated presentation of a standard stimulus, consisting of a 75 dB high/low two-tone sequence (2300–1100 Hz, each lasting 7 ms) separated by a

within-stimulus inter-stimulus interval (ISI) of variable duration (225, 75, 40, or 10 ms; one interval used per session). Each sequence was separated by a between sequence ISI, which was always 200 ms greater than the within-stimulus ISI 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 105 dB, 50 ms SES. On cued trials, an ''oddball'' stimulus (the reversal of the standard two-tone sequence, i.e. low/high instead of high/low), was followed by 50 ms of silence and the SES.

2.3.4. FM sweep procedure

An FM sweep session consisted of 104 trials, and sessions were repeated over multiple days for the same sweep duration due to greater difficulty of the task. The procedure involved the repeated presentation of a background 75 dB, downward FM sweep (2300–1900 Hz) and an upward FM sweep as the cue (1900–2300 Hz). Sweeps were separated by a between stimulus ISI, which was always 200 ms greater than the sweep duration. The duration of sweeps lasted 75 or 40 ms, and this variable remained constant within a test session.

2.4. Behavioral testing: water escape and Morris water maze

As a motor control, subjects first completed a water escape task involving the use of a visible platform (4 in. in diameter) placed in an oval galvanized tub (40.5 in. × 21.5 in.) filled with water at 72 °F. Subjects were placed in one end of the tub and timed until they swam and climbed onto the platform on the other side of the tub. Latency to escape was recorded for each subject. The following day, MWM testing (5 days) began, and took place in a 48 in. diameter tub with a hidden submerged 4 in. diameter platform, which was located in a constant location (SE quadrant). The tub had no intra-maze cues, while the room had a number of extra-maze cues (computer, experimenters, a chair and table, a room divider, etc.). Each testing day consisted of four trials per animal, with each trial representing release from a different (randomly selected) compass point (N, E, S, W). On trial 1 of day 1, the animal was first placed on the platform for 10 s, removed from the maze and then released from the appropriate starting location. The latency and distance to reach the platform on each trial, as well as the release location, were recorded for each trial using a custom-programmed data recording station.

2.5. Brain analysis

Following behavioral testing (P95–97), animals were weighed, anesthetized and transcardially perfused with fixative (10% buffered formalin phosphate). Heads were removed, placed in formalin and shipped to GDR at Beth Israel Deaconess Medical Center for anatomical analysis. Brains were removed, weighed, embedded in celloidin, and serially sectioned in the coronal plane at 30 μm. Every fifth section was mounted on glass slides, stained with cresyl violet, and coverslipped with Permount. The entire rostral to caudal extent of each brain was examined blind with respect to condition under a compound microscope (Zeiss Axiophot) at 25×, and damage and distortions of the cortex and were hippocampus noted. Volumes of the following bilateral brain regions were viewed with a Fisher Micromaster II digital microscope and measured by overlaying a grid using ImageJ and computed using Cavalieri's estimator of volume (Gundersen and Jensen, 1987): the hippocampus, cortex, anterior commissure, and the lateral ventricles. The midsaggital height of the corpus callosum was also measured for all coronal sections and area was estimated using Cavalieri's estimator of area (Gundersen and Jensen, 1987). All procedures conformed to approved University of Connecticut IACUC protocols.

3. Results

3.1. Histology

HI surgeries were performed on 29 male rats (11 P1, 11 P7, and 7 P10) and sham surgery on 11 male rats (5 P1, 2 P7, and 4 P10). Nineteen (5 P1, 10 P7, and 4 P10) of the HI animals had visual confirmation of lesions ipsilateral to the ischemic procedure, and these animals were included in all analyses, along with all 11 sham animals (who had no visible lesions). Further, distinct patterns of pathology were seen, which varied with the age of the induction of HI. In general, P1 HI injury produced enlarged ventricles with minimal cell loss, whereas P7 and P10 HI injury resulted in moderate gray matter damage (pockets of cortical dysplasia) to severe gray matter damage (porencephalic cyst formation and/or near complete cortical cell loss in the right hemisphere; see Fig. 1).

Univariate ANOVA's were computed for all anatomical measures with Age (3 levels) and Treatment (2 levels) as fixed factors (see Fig. 2). Results indicated a significant main effect for Treatment for corpus callosum area [$F(1, 24) = 13.1$, $p < .01$], with HI animals having significantly smaller callosal measures. An Age × Treatment interaction was also found for the corpus callosum [$F(2, 24) = 3.85$, $p < .05$], and within the HI groups simple effects analyses revealed significant differences between the P1 and P7 HI groups ($p < .01$) with the P7 HI animals having smaller callosal measures. The right hippocampal volume showed a significant main effect for Treatment [$F(1, 24) = 12.69$, $p < .01$], with the hippocampus being smaller in the HI group, and a significant main effect for Age [$F(2, 24) = 4.22$, $p < .05$]. Simple effects analysis again revealed a significant difference between the P1 and P7 HI groups ($p < .01$), with the P7 animals having smaller hippocampal volumes. A

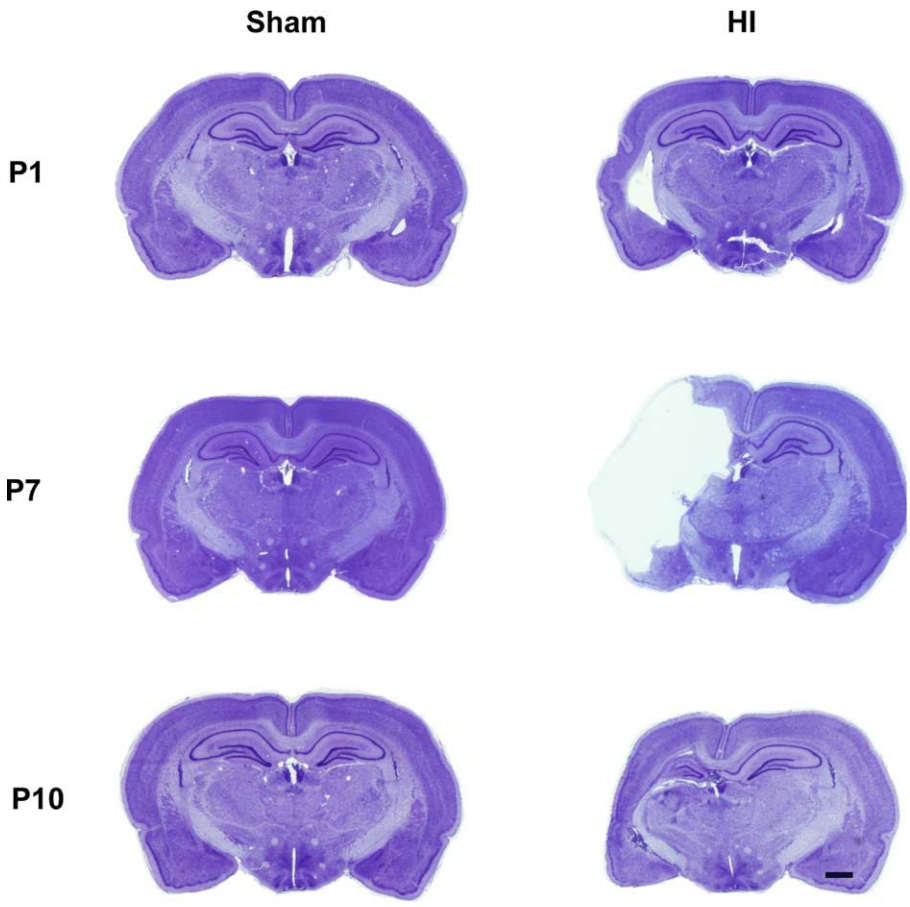

Fig. 1. Sections from P1, P7, and P10 sham and HI rats showing different patterns of pathology. P1 HI injury produced enlarged ventricles with minimal cell loss, whereas P7 and P10 HI injury resulted in moderate to severe gray and white matter damage. Scale bar: 1 mm.

univariate ANOVA for right cortical volume revealed a main effect for Treatment [$F(1, 24) = 10.00$, $p < .01$], a main effect for Age [$F(2, 24) = 5.09$, $p < .05$], and an Age × Treatment interaction [$F(2, 24) = 4.81$, $p < .05$]. Simple effects analyses showed that the group with the smallest cortical volume (P7) differed significantly from both the P1 and P10 HI groups ($p < .01$). A univariate ANOVA for the right ventricular volume revealed a main effect for Treatment [$F(1, 18) = 7.409$, $p < .05$] and results from a Tukey HSD showed that HI animals had significantly larger ventricular volumes. The left ventricular volume showed a main effect for Treatment [$F(1, 23) = 4.47$, $p < .05$] (with HI animals having larger ventricles) as well as a main effect for Age [$F(2, 23) = 4.48$, $p < 05$]. Results from a Tukey HSD revealed a significant difference between P1 and P10 HI animals ($p < .05$), with P1 HI animals having larger ventricular volumes. Although the P7 HI group showed the most damage to the most number of areas, the P1 HI group had more damage to white matter structures, including a smaller left and right anterior commissure as compared to the P10 group [$t = 4.16, 3.17, p < .05$]. The P10 group was found to have more damage to grey matter structures (such as the right hippocampus) as compared to the P1 HI group [$t = 2.39$, $p < .05$].

3.2. Auditory discrimination

3.2.1. Single tone, juvenile testing (P24)

Significant differences were found between cued and uncued absolute response amplitude scores for all groups as shown by paired samples t-tests ($p < .05$), indicating significant discrimination of the single tone. Moreover, results from a univariate ANOVA with Treatment (2 levels) and Age (3 levels) as fixed factors showed neither a main effect of Treatment [$F(1, 24) = 0.186$, $p > .1$] nor an interaction between Treatment and Age [$F(2, 24) = 0.667$, $p > .1$] for attenuation response scores (calculated by taking the cued response amplitude/the uncued response amplitude and multiplying by 100). Based on a lack of significant differences in responses to the single tone, we conclude that HI treatments did not impact baseline hearing or the general startle response. Thus, all groups performed equivalently on the detection of a 7 ms, 2300 Hz tone.

3.2.2. Oddball procedure, juvenile testing (P25–28)

Absolute and attenuated response scores were collected over 4 days for the oddball test, using four ISIs (225, 75, 40, and 10 ms, one per session/day). Initial analyses showed significant differences between absolute response

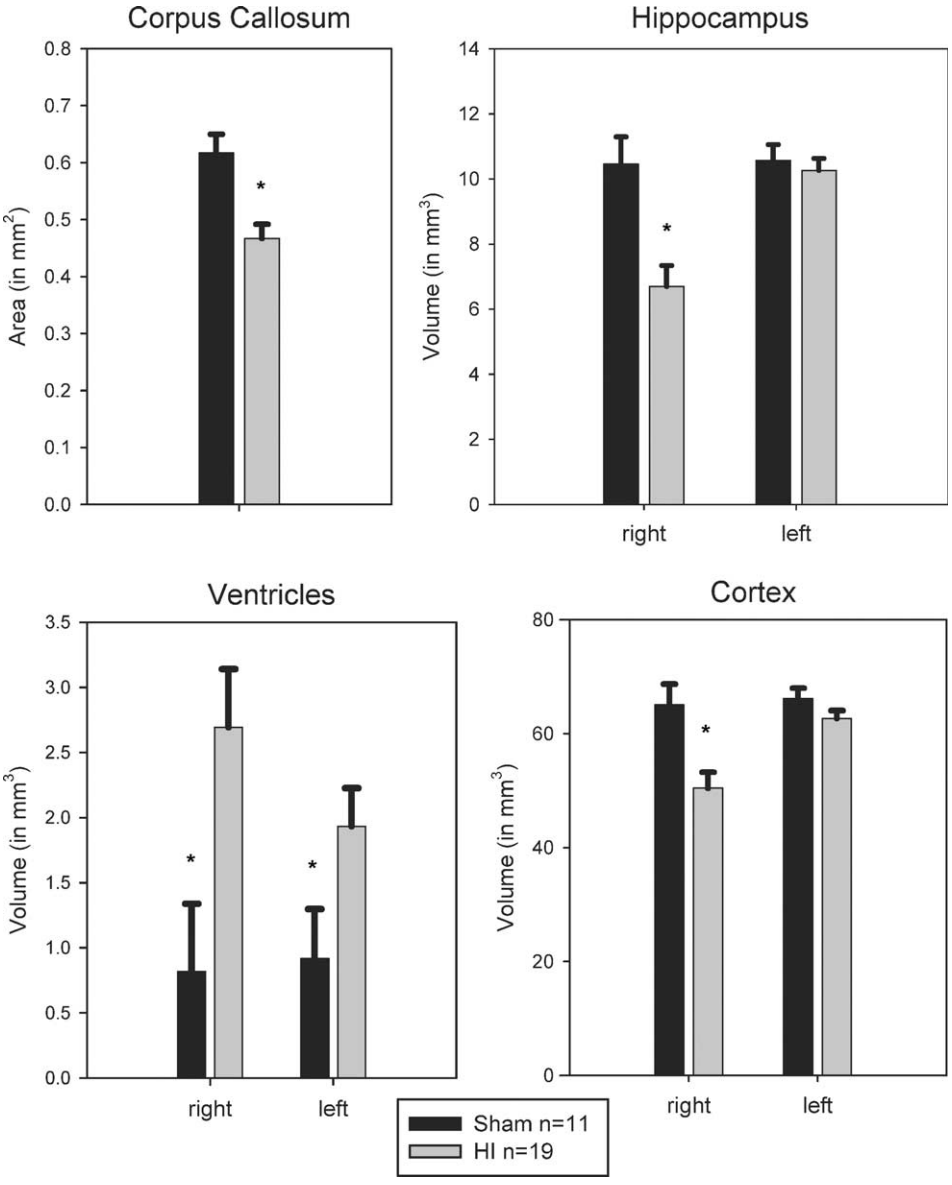

Fig. 2. Mean volumes of both right and left hippocampus, cortex, and ventricles and area of the corpus callosum in HI and sham animals. *Note*: $^{*}p < .05$.

amplitude scores, as shown by paired-samples t-tests, for HI and sham groups at all ISI durations. These findings show significant detection of the cue at all four ISIs by all groups.

A 4 (ISI) × 2 (Treatment) × 3 (Age) repeated measures ANOVA was conducted on attenuated response scores, and revealed a significant ISI × Treatment interaction [$F(3, 22) = 3.97$, $p < .05$]. A significant main effect for ISI was also found [$F(3, 22) = 4.52$, $p < .05$]. Probing the source of the interaction using univariate ANOVA's with Age (3) and Treatment (2) as fixed factors revealed a significant main effect for Treatment [$F(1, 24) = 5.7$, $p < .05$] at the shortest oddball duration [(10 ms), $t = 2.75$, $p < .05$], with all HI animals performing worse than shams (see Fig. 3).

3.2.3. FM sweep procedure, juvenile testing (P30–36, 38–41)

The 75 ms sweep was presented over 6 days in the juvenile period, followed by presentation of a shorter stimulus duration FM sweep (40 ms) for 3 days. Significant differences were found using paired-samples *t*-tests between absolute response amplitude scores for the 75 ms FM sweep for shams on all days, and for HI animals on days 1, 4, 5 and 6, indicating significant detection of the cue. The 40 ms FM sweep did not show significant differences between cued and uncued response amplitudes for either group on any testing day, suggesting no detection of this cue by any group.

A 6 (Day) × 2 (Treatment) × 3 (Age) repeated measures ANOVA then was conducted on attenuation response scores for the 75 ms FM sweep duration and a significant main

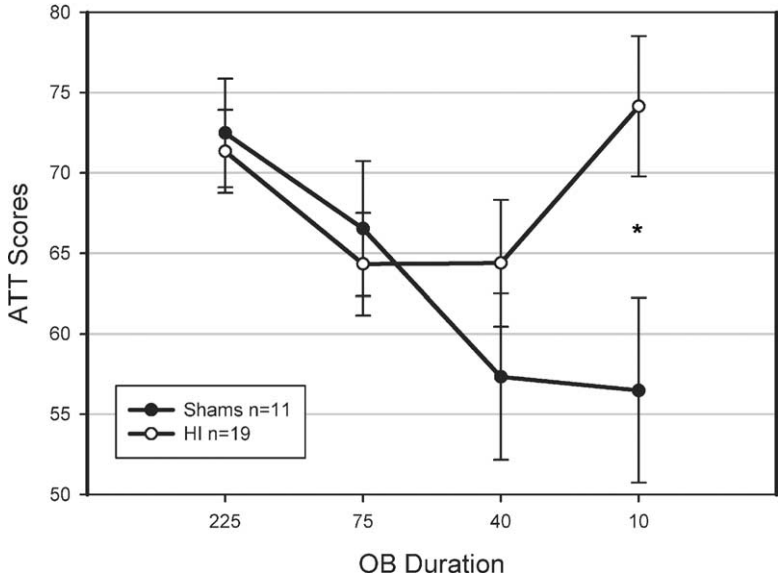

Fig. 3. Attenuation response scores for juvenile HI and sham animals on the oddball task over 4 ISI durations (in ms). *Note*: *$p < .05$.

effect was found for Treatment [$F(1, 24) = 4.38$ $p < .05$], with HI animals performing worse than shams on all days (see Fig. 4). Univariate analyses for each day of the 75 ms FM sweep with Age (three levels) and Treatment (two levels) as fixed factors revealed that HI animals performed significantly worse than shams on day 5 [$F(1, 24) = 6.56$, $p < .05$], with a trend to perform worse on all other days.

3.2.4. FM sweep procedure, adult testing (P80–84)

The 75 ms sweep was presented to approximately half of the animals in adulthood over 2 days. These animals were chosen randomly from each group at the beginning of testing and confirmed for lesions following perfusion (sham $n = 6$; HI $n = 11$, 3 P1, 4 P7, and 4 P10; see Fig. 5). The 75 ms FM sweep procedure was followed by 2 days presentation of the 40 ms sweep. Significant differences were found between absolute response amplitude scores for the HI and shams for the 75 ms FM sweep and on the 40 ms FM sweep ($p < .05$).

A repeated measures ANOVA, 2 (Day) × 2 (Treatment) × 3 (Age), was carried out on attenuation scores for the 2 days of the 75 ms FM sweep duration given in adulthood. This analysis revealed a significant main effect for Day [$F(1, 11) = 15.50$, $p < .01$], but neither a main effect for Treatment, nor a Day × Treatment interaction. A repeated measures ANOVA, 2 (Day) × 2 (Treatment) × 3 (Age), was also carried out on attenuation scores for the 2 days of 40 ms FM sweep duration in adulthood. No significant differences

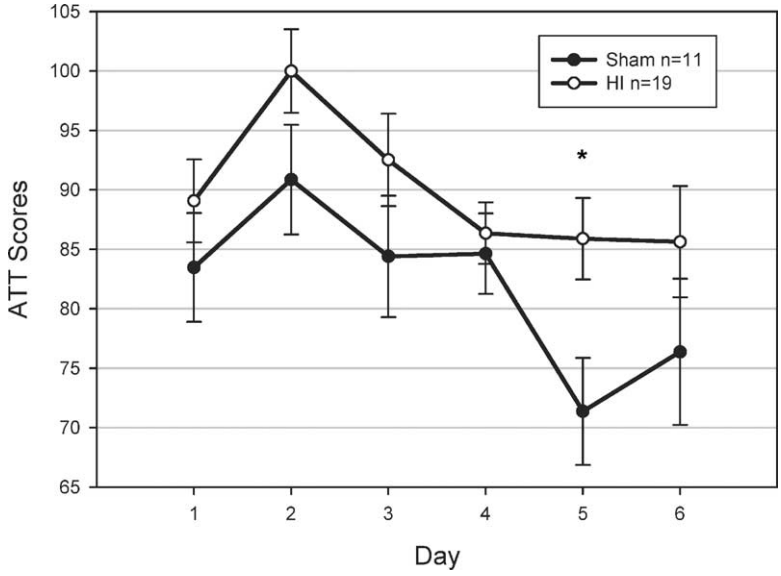

Fig. 4. Attenuation response scores for the 75 ms FM sweep procedure over 6 days in the juvenile period for HI and sham animals. *Note*: *$p < .05$.

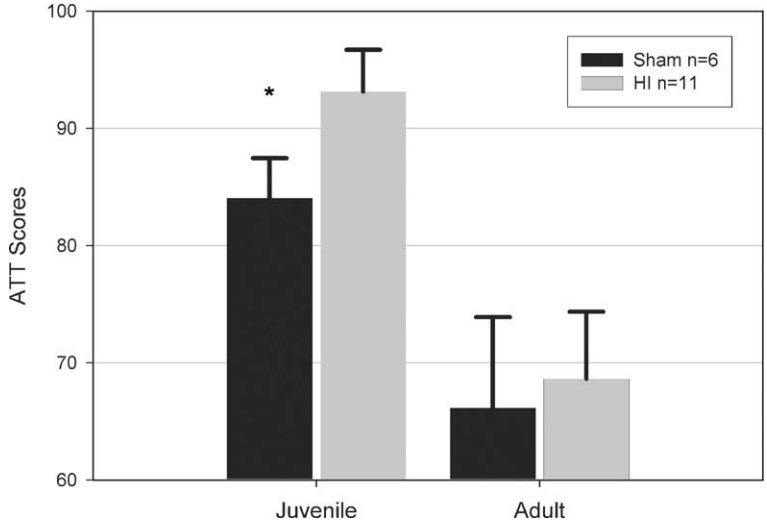

Fig. 5. Average attenuation response scores for the 75 ms FM sweep procedure over 2 days compared between a subset of the same animals in the juvenile and adult period for HI and sham animals. *Note*: $*p < .05$.

were found overall, although HI animals performed worse on this task.

3.2.5. Auditory results, excluded animals

Analyses were also run on the subset of animals that were dropped due to the failure to confirm lesions. We infer that these subjects may have been exposed to hypoxia only if cauterization of the carotid artery was not absolute. Overall, these animals showed similar deficits to confirmed HI subjects on the shortest oddball duration [$F(1, 19) = 3.05$, $p < .1$] as well on the 75 ms FM task, with a significant main effect for Treatment using a repeated measures ANOVA [$F(1,15) = 6.10$, $p < .05$]. This group included 6 P1, 1 P7, and 3 P10 animals, with the P1 group performing the worst. Therefore, it is unclear as the whether the P1 animals who were initially excluded had neuroanatomical damage that was harder to detect, or whether hypoxia alone may contribute to a deficit in auditory performance. Future studies may address this issue.

3.3. Water escape and Morris water maze, adult testing (P69–75)

The same subset of adult subjects were run on water escape and MWM in adulthood, and the same HI animals with observed damage were included in subsequent analyses. A univariate ANOVA with 2 (Treatment) and 3 (Age) as fixed factors for escape latencies in the water escape task revealed no significant differences between groups ($p > .1$, ns). Further, no main effect for Treatment was seen on trial 1 on day 1 for escape latency or distance to escape ($p > .1$, ns). Thus, HI subjects did not appear to differ from shams in a basic swim-escape task.

For the MWM, a 5 (Day) × 2 (Treatment) × 3 (Age) repeated measures ANOVA was conducted on distance traveled. This analysis revealed a main effect for Treatment [$F(1, 11) = 7.09$, $p < .05$; see Fig. 6], an effect indicating that HI animals swam a greater distance than sham littermates to reach the platform. A similar main effect was seen for escape latency; HI animals took significantly longer to reach the platform than sham animals [$F(1, 11) = 8.94$, $p < .05$].

3.4. Correlation between anatomy and behavioral measures

Auditory scores on the oddball task (225, 75, 40, and 10 ms) and mean score over 6 days of FM75 in the juvenile period as well as anatomical measures were converted into z-scores for HI animals, and Pearson product–moment correlations were performed between anatomical and behavioral measures. Significant negative correlations were seen between scores on one or more of the short oddball tasks (75, 40, or 10 ms) and/or the 75 ms FM sweep task, and the following anatomical measures: corpus callosum, right hippocampus, left hippocampus, right cortex, and left ventricle (see Table 1). No significant correlations were found for the longest oddball duration (225 ms). These correlations indicate that smaller neuroanatomical measures predict worse performance, but on tasks incorporating rapidly changing auditory stimuli only.

Due to the small number of animals that were tested on the MWM, HI animals were pooled across ages and correlations were run for the mean distance over days 2–5 (converted into z-scores and correlated against z-scores of anatomical measures). Days 2–5 were used since no learning is typically evidenced on the first day of testing. Significant negative correlations were found between mean MWM distance and: corpus callosum area; right cortical volume; and near significant correlations with the right and left hippocampus. Also, a significant positive correlation was found between the mean MWM distance and right

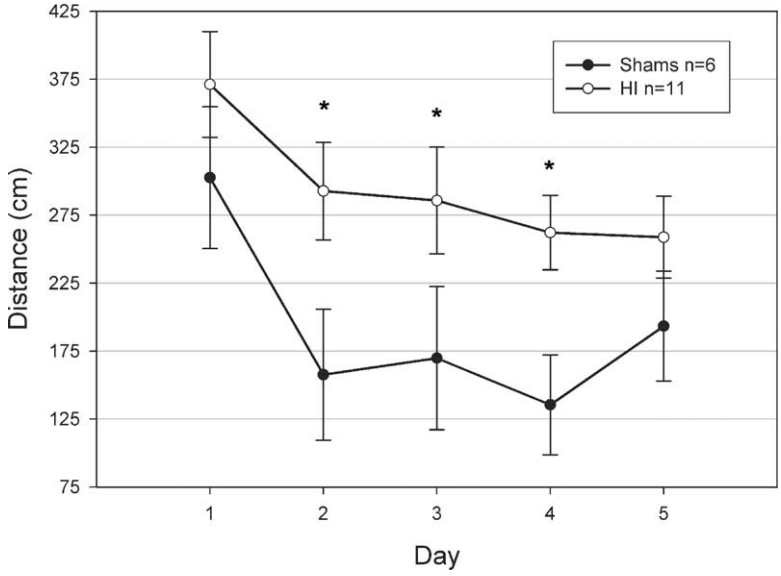

Fig. 6. Morris water maze distances over 5 days for HI and sham animals. *Note*: *$p < .1$.

ventricular volume (see Table 1). These findings indicate that higher MWM distance values (which indicate poorer learning), correlate with smaller white and gray matter structures and larger ventricles in HI subjects. No significant correlations were found between any behavioral and anatomical measure for sham animals.

4. Discussion

4.1.1. Behavioral results, auditory testing

Prior neuropathological analyses of long-term HI injury using the animal model described here show compelling parallels to perinatal HI injury seen in humans (including damage to cortex and hippocampus) (Johnston et al., 2001; Northington et al., 2001). With regard to behavioral findings, HI animal's baseline startle response (as shown by the single tone attenuated response scores) was unaffected by the treatment and therefore, the startle reduction paradigm was useful for more in depth assessment of subjects complex auditory processing ability. During juvenile auditory testing, a rapid auditory processing deficit was identified in HI animals compared to sham littermates for two separate auditory tasks (the short oddball and the FM sweep procedures). These deficits were seen on the oddball task at the shortest ISI duration (10 ms), and on the 75 ms FM sweep. However, at longer ISIs on the oddball task, groups performed similarly, indicating that the impairment was specific to rapid auditory processing, and not auditory processing in general.

In adulthood, a randomly selected subset of animals from each treatment group (approximately half of total *N*) was re-tested on the FM sweep procedure (75 and 40 ms). Surprisingly, no group differences were found on the 75 ms or the 40 ms FM sweep procedure for HI and shams (although a trend for worse performance for HI animals was seen). Comparison of the average of the first 2 days of the 75 ms FM sweep in the juvenile period to the average of 2 days of the 75 ms FM sweep given in adulthood (on the same subset of animals) revealed a significant improvement in attenuation scores for both sham and HI animals ($p < .05$).

Table 1
Correlational matrix of *z*-scores of auditory measures [oddball 75, 40 and 10 ms and FM75 sweep over 6 days in the juvenile period (ZOB75, ZOB40, ZOB10, ZFM75), *z*-scores of the mean distance from days 2 to 5 of the MWM (ZMWM)], and *z*-scores of anatomical measures for HI animals

	Corpus callosum	Right hippocampus	Left hippocampus	Right cortex	Right ventricle	Left ventricle
ZOB75	−0.496**	−0.442*	−0.676***	−0.506*	0.095	−0.336
ZOB40	−0.455**	−0.462**	−0.211	−0.610**	0.427	−0.069
ZOB10	−0.333	−0.361	−0.480**	−0.257	0.165	−0.364
ZFM75	−0.590***	−0.544**	−0.544**	−0.729***	0.299	−0.574** (18)
ZMWM	−0.754*** (11)	−0.549* (11)	−0.569* (11)	−0.811** (11)	0.854*** (8)	−0.085 (11)

Eighteen animals were included for correlations with the left ventricle due to complete loss of cortex in one animal. $N = 19$ unless otherwise indicated (in parentheses).

* $p < .1$.
** $p < .05$.
*** $p < .01$.

This indicates that the 75 ms sweep task may have been too easy to elicit deficits in adult HI subjects. Accordingly, the same adult HI animals showed a deficit (albeit not significant) on the 40 ms FM sweep task. Apparently, by altering the complexity and/or duration of the task we were able to tap into the rapid auditory processing ability in rats, thus revealing a rapid auditory deficit in juvenile animals with neonatal HI injury. While a shorter duration task was suggestive of a similar deficit in adult animals, future research will need to employ more difficult tasks to elicit a difference between adult HI and sham animals.

4.1.2. Behavioral results, MWM

Spatial learning and memory were also assessed in adult sham/HI subjects through the use of a Morris water maze. Approximately, half of all subjects (selected randomly from each Treatment and Age group) were tested on the MWM (the same animals tested on FM sweeps in adulthood). Animals were first tested on a water escape task to assess swimming/motor ability. Both groups performed similarly, and were consequently tested on 5 days of the MWM procedure. HI animals performed significantly worse over the 5 days when compared to shams, as indicated by longer swimming distances and escape latencies, thus suggesting a learning/memory impairment in HI animals.

4.2. Correlations between behavior and anatomy

Correlations between juvenile auditory scores and post-mortem anatomical assessment revealed significant relationships between performance on harder tasks such as: the oddball at 75, 40, and 10 ms, and the FM75 ms sweep task; and neuropathological indices. Area measures of the corpus callosum revealed significant negative correlations with these auditory measures. Both the right and left hippocampus, the right cortex, and the left ventricle also showed significant correlations with some if not all of these tasks. It is interesting to note that the longest oddball task (225 ms) showed no correlations with any of these measures, indicating that damage to these brain areas affected only rapid auditory processing and not general auditory processing. The MWM (scores for distance and time averaged over days 2–5) showed similar negative correlations with callosal area, and the right cortical volume, and also showed a significant positive correlation with right ventricular volume. Interestingly, the volume of the hippocampus did not correlate significantly with MWM results; however, as a caveat, only four P7 HI animals (the group with the greatest damage to the hippocampus) were included in this analysis.

4.3. Relationship between HI in humans and animals

The current results indicate that neonatal HI injury leads to deficits in rapid auditory processing ability. This impairment parallels the human literature, in which premature infants with brain lesions show a deficit in auditory temporal processing (Downie et al., 2002). These auditory problems may further contribute, in humans, to an inability to discriminate speech sounds (Tallal, 1976). Interestingly, similar damage to specific brain areas such as the cortex, hippocampus, and corpus callosum significantly correlate with rapid auditory performance in HI animals, areas that do not show similar correlations in sham animals. Furthermore, HI animals (in adulthood) show an impairment in MWM performance, indicating a spatial learning/memory deficit that is assumed to have persisted through the juvenile period. These results are consistent with evidence of learning and memory deficits found in other animal models of premature/term injury (Cai et al., 1999; Decker et al., 2003; Grojean et al., 2003; Lebedev et al., 2003; Nunez et al., 2003). Working memory deficits have also been associated with language disability in humans (Bishop et al., 1999; Farmer and Klein, 1993). Overall, deficiencies in auditory processing and spatial memory found in the current study provide evidence that these deficits are associated with the neuropathology of HI. Given that similar anatomical damage and auditory/memory deficits have been seen in humans, this animal model of HI in rats may provide a useful instrument through which to research the effects of early brain injury in humans, as well as variables modulating the long-term neurobehavioral consequences of such injury.

Finally, juvenile auditory processing deficits appear to persist in adulthood but at a more subtle level, possibly as a result of developmental maturation and/or cortical compensation. In the current study, the use of a more temporally demanding auditory task was suggestive of adult auditory deficits in HI animals (reminiscent of deficits seen in the juvenile period). This developmental pattern is similar to that seen for another developmental injury model, the induction of microgyria by freeze injury to the neonatal cortical plate (Humphreys et al., 1991). Specifically, more complicated behavioral tasks (i.e. a modified version of the oddball task) are required to elicit a deficit in adult microgyric animals compared to tasks used in the juvenile period (Peiffer et al., 2004). Neonatal HI injury has also been shown in this study to impact spatial learning/memory in adulthood. It can be assumed that this deficit persists into adulthood from the juvenile period, however, direct evidence must be obtained to support this assumption. Future studies will include testing spatial learning and memory in the juvenile period for HI animals. In addition, the use of the same auditory tasks in both the juvenile period and in adulthood, and the development of more demanding tasks to administer in adulthood, should shed some light onto the maturational process associated with HI injury. Further, the lack of behavioral differences in this study between animals who underwent the HI procedure at different developmental timeponts (P1, P7, or P10) may be attributable to the relatively small *n*'s in the HI groups. By increasing the number of animals in these groups we hope to uncover more significant age-based differences on

behavioral tasks in future studies. Finally, a bilateral HI procedure is under development to address the issue of cortical compensation, and to relate this HI model more closely to cognitive consequences of HI in humans.

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