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Research report

Rapid auditory processing and learning deficits in rats with P1 versus P7 neonatal hypoxic-ischemic injury

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Abstract

Hypoxia-ischemia (HI) is associated with premature birth, and injury during term birth. Many infants experiencing HI later show disruptions of language, with research suggesting that rapid auditory processing (RAP) deficits (i.e., impairment in the ability to discriminate rapidly changing acoustic signals), play a causal role in language problems. We recently bridged these lines of research by showing RAP deficits in rats with unilateral-HI injury induced on postnatal days 1, 7, or 10 (P1, P7, or P10 [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]). While robust RAP deficits were found in HI animals, it was suggested that our within-age sample size did not provide sufficient power to detect age-at-injury differences within the pooled HI group.

The current study sought to examine differences in neuropathology and behavior following unilateral-HI injury on P1 versus P7 in rats. Ages chosen for HI induction reflect differential stages of neurodevelopmental maturity, and subsequent regional differences in vulnerability to reduced blood flow/oxygen (modeling age-related differences in premature/term HI injury). Results showed that during the juvenile period, both P1 and P7 HI groups exhibited significant RAP deficits, but deficits in the P1 HI group resolved with repeated testing (compared to shams), while P7 HI animals showed lasting deficits in RAP and spatial learning/memory through adulthood. The current findings are in accord with evidence that HI injury during different stages of developmental maturity (age-at-injury) leads to differential neuropathologies, and provide the novel observation that in rats, P1 versus P7 induced pathologies are associated with different patterns of auditory processing and learning/memory deficits across the lifespan.

Keywords: Language impairment; Prematurity; Low birth weight

1. Introduction

Hypoxia-ischemic (HI) brain injury collectively refers to reduced blood flow and blood oxygenation to the brain, and can result from premature birth, or injuries that occur during term birth (such as asphyxial insults, prolonged labor and/or resuscitation, and placental and/or umbilical cord disruption). The outcome of reduced blood flow/depleted oxygen transport to the brain includes a fall in glucose levels, impairments in normal energy metabolism, and a dependence on anaerobic glycolysis as the sole means of energy production. Ultimately, ATP levels fall, leading to a cascade of detrimental mechanisms including membrane depolarization, the accumulation of excitotoxic amino acids, increases in intracellular Ca^{2+}, generation of free radicals, inflammation, activation of caspases and cytokines, and the activation of phospholipases, proteases and nucleases (see [39] for more details). These cumulative events lead to necrotic or apoptotic cell death in affected regions of the brain, with regional injury contingent on the timing of injury coupled with the developmental stage of the brain at that timepoint.

HI injury during the premature period leads predominantly to white matter damage, often presenting itself as necrosis of white matter surrounding the lateral ventricles (an injury termed periventricular leukomalacia in human infants). This lesion takes the form of a loss of white matter surrounding the lateral ventricles [39], a result of factors which include immature stage of development of blood supply, poor cerebrovascular regulation, and vulnerability of future myelin producing cells (oligoden-

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drocyte progenitors) to free radicals in this area [3,21,27,39]. Abnormalities in the corpus callosum are also seen following premature birth [29,34]. HI insults occurring later in development (closer to term birth) result in a different pattern of neuropathology, manifesting as injury to gray matter structures such as the thalamus and basal ganglia [32,33]. The latter pattern of injury results from vulnerability of gray matter to excitotoxicity in this timeframe (reflecting overexpression and/or immaturity of neuronal glutamate receptors [16–18]).

With regard to the use of a rat model of brain injury and development, the brain of a rat during the early postnatal period is approximately equivalent (in terms of landmark neurodevelopmental events) to a premature human, while a P7 rat brain is approximately equivalent to a near-term human (32–34 weeks gestation [38]). Accordingly, brain injury induced in the neonatal HI rodent model (see [30] for procedural details) has consistently been shown to parallel anatomical features and neuropathology of human premature or term human HI injury (depending on day of injury). As neuropathology in human infants varies depending on the age of injury, differential patterns of neuropathology are also seen for the rat HI model depending on age of injury. Injury induced between postnatal days 1–5 in the rat leads to white matter damage, including lesions in and surrounding the internal capsule [37], and death of periventricular oligodendrocyte progenitors surrounding the lateral ventricles [3]. Damage to the corpus callosum [24] as well as damage in regions of the subplate is also seen [25]. Conversely, damage to the cerebral cortex [10,23], hippocampus, striatum, globus pallidus [36], thalamus, and brainstem [28] has consistently been shown following P7 HI injury in rats.

Both premature and/or term HI-injured human infants frequently go on to demonstrate cognitive and behavioral deficits later in development, including language-related abilities [12,17,18,20,26,31,39] as well as deficits in spatial memory [7]. Premature subjects with diagnosed language abilities have also been reported to show difficulties with rapid auditory processing (RAP [9,15]), where RAP refers to the ability to discriminate between rapidly changing auditory stimuli (within tens of ms [4]). RAP has been shown to be critical to language acquisition, and has in turn been suggested to play a causal role in emergent language problems [35]. In support of this view, thresholds for rapidly changing auditory cues in infants with a family history of language impairments are significantly higher than for controls, and these indices of auditory processing predict later language performance in both impaired and control children [4]. Similar correlations between early RAP indices and later language outcome have been reported for premature children [9,15].

Importantly, parallel RAP deficits have been demonstrated in a rodent model of HI injury [23]. Spatial and reference memory deficits have also been identified in a rodent model of HI as assessed by a T-maze [11], and the Morris water maze (MWM) [1,2]. Specifically, RAP deficits and spatial learning/memory deficits were reported during the juvenile period in rats undergoing unilateral HI on P1, P7, or P10. This report, however, failed to show differences in behavioral deficits among these three different age-at-injury induction groups (possibly attributable to the small *n* in each of the HI groups, which were pooled (see [23])). Although statistical power was sufficient to detect a robust HI main effect, a more detailed analysis of relative deficits within each age-at-injury was not possible.

The current study sought to address the question of whether differential injuries resulting from P1 versus P7 HI (which in turn reflect different stages of developmental maturity) in the rat may also lead to differential behavioral patterns – specifically in RAP and learning/memory deficits – across the lifespan. We addressed this question by inducing HI injury on P1 versus P7 in rats, using a larger *n* for each group, and a longer period of hypoxia than had been previously employed (120 min versus 90 min). We compared performance on various tests of auditory processing utilizing a startle reduction paradigm in both juvenile and adult periods, and also assessed performance for a subset of all groups on the Morris water maze (MWM) in adulthood. Postmortem anatomical assessments for all groups are also included.

2. Materials and methods

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 (HI) or sham procedure (see below) on postnatal days 1 or 7 (P1 or P7). 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 P23 and lasted until P54. In adulthood, subjects were tested on another battery of auditory tests lasting from P78–95. The MWM was also performed on a subset of animals ($n = 24$; 8 P1 HI, 8 P7 HI, and 8 sham) from P96–P100. Subjects were sacrificed on P100 via transcardial perfusion and the brains were subject to histological analysis.

2.2. Induction of HI

All subjects were culled into litters of 10 (eight males and two females) on P1 (two female pups remained to avoid all-male litters and standardize litter size). Male pups were randomly assigned to litters receiving either P1 or P7 surgery, balancing sham surgery and HI procedure within litter. On the appropriate surgery day, HI pups were anesthetized with isoflurane (2.5%), 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 then 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. HI subjects were then placed in a container in which they were exposed to 8% humidified oxygen (balanced with nitrogen) for a period of 120 min. Sham animals were also anesthetized with isoflurane, a midline incision was made longitudinally in the neck, the incision was sutured, and they were later placed in a chamber open to room air for a period of time equivalent to the hypoxic portion of the procedure (120 min). Both sham and experimental pups were returned 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 (or ASR), which is a large amplitude motor response to an acoustic startle-eliciting stimulus (SES). The ASR can be measured (in mV) by placing an animal on a calibrated load-cell platform and presenting the SES. When the SES is preceded by a benign prestimulus, the ASR is attenuated (also referred to as 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 (normal single tone) employed a 75 dB, 7 ms, 2300 Hz tone prestimulus. The comparison between ASR amplitude when no prestimulus was presented (an uncued trial) and when the prestimulus

preceded the SES (a cued trial) yields an objective measure of sensory detection [22]. Attenuation response (ATT) scores served as the dependent variable, representing relative measures of detection for each animal, and were derived by dividing the sum of the cued trials by the sum of the uncued trials and multiplying by 100, within a task for each subject. ATT scores are thus presented as percentages and scores close to 100% indicate poor detection of a cue (i.e., the ability to attenuate to the prestimulus), since responses on cued and uncued trials are equivalent. The inter-trial interval between each SES was variable (range, 16–24 s) but averaged 20 s. Different tasks (comprising alterations in stimulus properties) were used to look at different aspects of auditory processing. First, a normal single tone procedure (the "easiest" task) was used to assess baseline startle and hearing (i.e., to determine if any subject was deaf). Next, long duration (0–100 ms) and short duration (0–10 ms) silent gap procedures were used to identify deficits in processing brief acoustic signal changes. Finally, a more complex task – an FM sweep procedure – was used to identify any deficits in discriminating rapidly changing frequencies (i.e., as seen in human speech).

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 Macintosh computer. 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 200 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. Scores were subjected to further analysis by deriving ATT scores as a function of relative performance on cued and uncued trials for each condition, for each subject, as described above (cued/uncued × 100). Auditory stimuli were generated on a Pentium III Dell PC with custom programmed software and a Tucker Davis Technologies (RP2) real time processor, amplified by a Marantz integrated amplifier PM700, and delivered via Cambridge speakers.

2.3.2. Single tone procedure

The single tone test session consisted of 104 trials (cued or uncued), presented in a pseudo-random order on a single day (with 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. Silent gap procedure

Each silent gap session consisted of 300 trials. Silent gaps of variable duration (serving as cues) were embedded in continuous 78 dB broad-band white noise, with the end of a gap, followed 50 ms later by the SES. During the first two sessions (days) the silent gaps ranged from 0 (uncued) to 100 ms in length (0, 2, 5, 10, 20, 30, 40, 50, 75, and 100 ms), and from 0 to 10 ms (0, 2, 3, 4, 5, 6, 7, 8, 9, and 10) for the next four sessions (days). Duration of gaps were presented in a pseudo-random order so that no more than three similar trials occurred in a row.

2.3.4. FM sweep procedure

A single FM sweep session consisted of 104 trials and the procedure involved the repeated presentation of a background 75 dB, downward FM sweep (2300–1900 Hz) with an upward FM sweep as the cue (1900–2300 Hz). Sweeps were separated by a between stimulus interstimulus interval (ISI), which was always 200 ms greater than the sweep duration. The duration of sweeps lasted 225, 125, 75 or 50 ms and this variable remained constant within a test session (day). Subjects were tested for one session (day) on each stimulus duration.

2.4. Behavioral testing: water escape and Morris water maze

As a control assessment for potential differences in swimming ability and/or motivation between groups, subjects were tested in 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 70 °F (see [23]). 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 the single trial.

The Morris water maze (MWM) is a task used to test spatial learning and memory [5,8]. The day after water escape testing, MWM testing began. Testing took place in a 48 in. diameter tub with a hidden submerged 8 in. diameter platform (2 cm below the surface of the water). The tub had no intra-maze cues, while the room had a number of extra-maze cues. Each testing day consisted of four trials per animal, with each trial representing release from a different (quasi-random) 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 escape platform was consistently placed in the SW quadrant (4 days). Animals were allowed to remain on the platform for 10 s following completion of a trial, and if the platform was not reached during the 45 s trial time, animals were guided to the platform and allowed to remain for 10 s. The distance to reach the platform on each trial, and the release location, were recorded for each trial using a custom-programmed data recording station. All procedures conformed to approved University of Connecticut IACUC protocols.

2.5. Histology

At P100 animals were weighed, anesthetized, and transcardially perfused with fixative (10% buffered formalin phosphate). Brains were removed, placed in formalin and shipped to GDR at Beth Israel Deaconess Medical Center for anatomical analysis. Brains were weighed, embedded in celloidin, and serially sectioned in the coronal plane at 30 μm. A series of every fifth section was stained with cresyl violet for Nissl substance and an adjacent series stained for myelin using the Loyez method (see Fig. 1). Volumes of the following bilateral brain regions were examined using a Fisher Micromaster II digital microscope and measured by overlaying a grid using ImageJ and computed using Cavalieri's estimator of volume [13]: cerebral cortex, hippocampus, corpus callosum, hippocampal commissure, and anterior commissure. All measurements were performed blind to treatment group.

2.6. Statistics

For auditory testing procedures, ATT scores – the dependent variable – were compared between groups. For all analyses between HI and sham groups, one-tailed tests of significance (alpha set at $p < .05$) were used, based on prior findings of significant deficits in rapid auditory processing and spatial learning/memory for neonatal HI subjects as compared to shams (i.e., hypothesis driven based on a priori effects [23,24]). Two-tailed tests were used for within-treatment comparisons (e.g., P1 versus P7 HI) and for all volumetric analyses of brain regions. Sham animals (P1 and P7) were pooled for all analyses, based on a lack of significant differences for all repeated measures ANOVAs performed between the two sham age-at-injury groups.

3. Results

3.1. Histology

HI surgeries were performed on 35 male rats (16 P1 and 18 P7) and 17 had sham surgery, which resulted in volumetric analysis of 15 P1 HI, 18 P7 HI, and 16 sham brains (two brains were unusable). P1 HI animals showed significant differences in the volume of the hippocampal commissure [$t = 3.41$, $p < .01$] as compared to shams, with the volume being smaller in the P1 HI group (see Fig. 2). P7 HI and sham animals differed significantly for the volume of the corpus callosum, right cortex, right hippocampus, and hippocampal commissure [$t = 10.5$, 13.64, 14.77, and 10.23, $p < .01$], with all areas being smaller in the HI group (see Figs. 2 and 3). P7 HI animals also showed a decrease in the volume of the right anterior commissure compared to shams [$t = 2.05$, $p < .05$] (see Fig. 3).

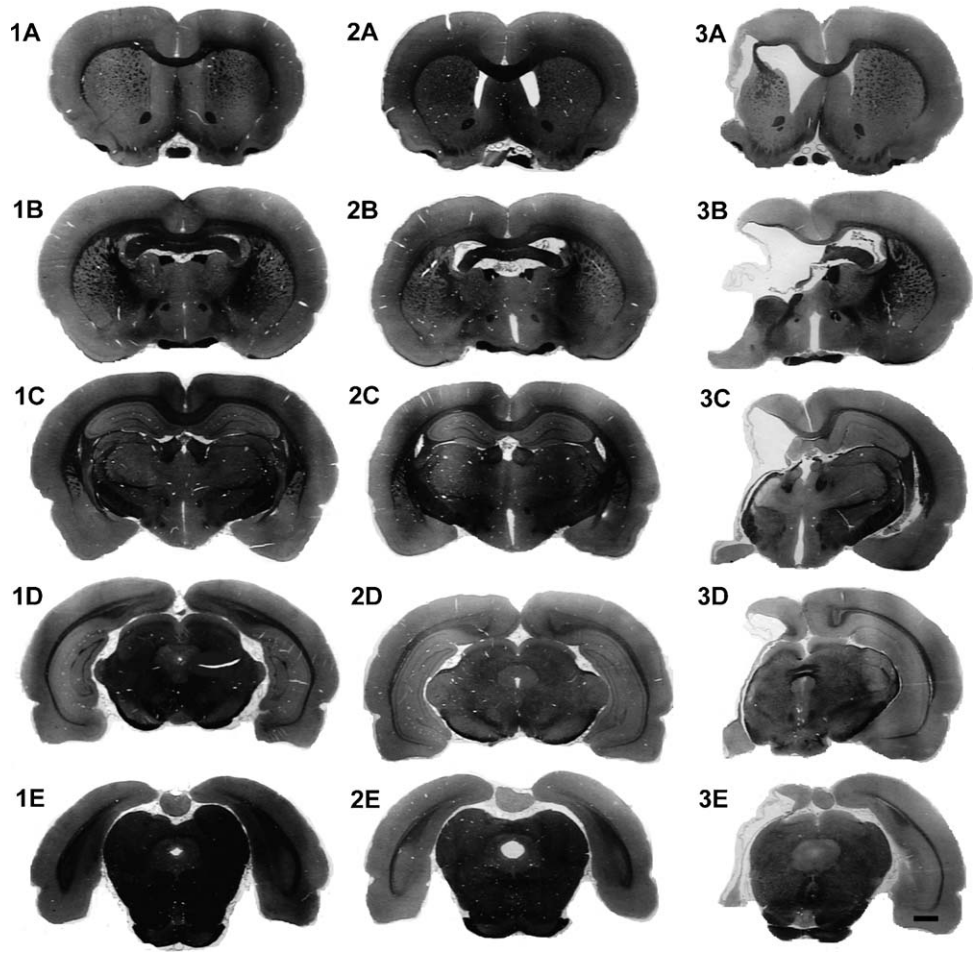

Fig. 1. Myelin stained coronal sections of a typical sham (1A–E), P1 HI (2A–E), and P7 HI (3A–E) animal. Scale bar = 1 mm.

3.1.1. Juvenile testing: normal single tone

Results from one-way ANOVAs showed no differences between the two age-at-injury within the treatment group or the sham group [$F(1,33) = .014$, $p > .1$, and $F(1,15) = .001$, $p > .1$, respectively]. A one-way ANOVA between pooled HI animals and pooled shams, however, revealed a significant difference on normal single tone scores [$F(1,50) = 7.64$, $p < .01$], with shams performing slightly better. Thus, all subsequent analyses (comparing HI and sham groups) were run using each subject's normal single tone ATT score as a covariate for other auditory processing indices.

3.1.2. Juvenile testing: silent gap (0–100 ms, 2 days and 0–10 ms, 4 days)

The treatment group (P1 HI versus P7 HI) underwent a 2 (age-at-injury) × 2 (day) × 9 (gap) repeated measures ANOVA for the long (0–100 ms) silent gap, which revealed a significant gap × age-at-injury interaction [$F(8,26) = 4.66$, $p < .01$]. A

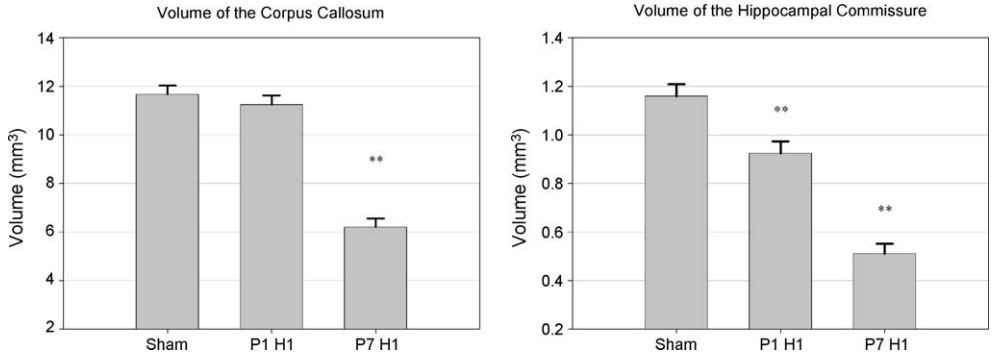

Fig. 2. Bar graphs comparing the mean volumes (±S.E.M.) of the corpus callosum and hippocampal commissure between sham, P1 HI, and P7 HI animals. P7 HI animals showed decreased volume in both the corpus callosum and the hippocampal commissure, while P1 HI animals only showed deceased volume in the hippocampal commissure. Note: $^{**}p < .01$.

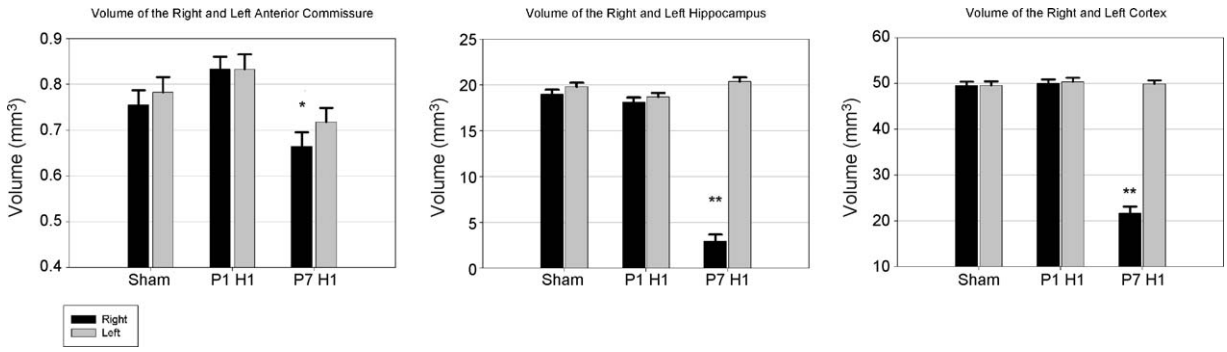

Fig. 3. Bar graphs comparing the mean volumes (±S.E.M.) of the anterior commissure, hippocampus, and cortex between sham, P1 HI, and P7 HI animals. P7 HI animals show reduced volume of the right anterior commissure, right hippocampus, and right cortex. Note: $^{*}p < .05$, $^{**}p < .01$.

2 (age-at-injury) × 4 (day) × 9 (gap) repeated measures ANOVA for the short (0–10 ms) silent gap also revealed a day × age-at-injury interaction [$F(3,31) = 5.39$, $p < .01$]. Therefore, these groups were not pooled, and P1 and P7 HI animals were separately compared to the pooled sham group.

A 2 (treatment) × 2 (day) × 9 (gap) repeated measures ANOVA for the long (0–100 ms) silent gap showed no significant differences for P1 HI subjects as compared to shams, and no difference between P7 HI subjects as compared to shams (see Fig. 4). Therefore, these groups did not differ in the detection of long duration silent gaps.

For the short (0–10 ms) duration silent gap, a repeated measures 2 (treatment) × 4 (day) × 9 (gap) was performed for P1 HI versus shams. This analysis revealed a day × treatment interaction [$F(3,28) = 3.34$, $p < .05$] (see Fig. 5). Probing the source of this interaction revealed a main effect for day 2 only (with HI performing worse than shams; see Fig. 6).

A repeated measures 2 (treatment) × 4 (day) × 9 (gap) ANOVA for P7 HI versus shams was also run for the short (0–10 ms) duration silent gap, and revealed a significant gap × treatment interaction [$F(8,26) = 2.04$, $p < .05$], as well as a significant main effect of treatment [$F(1,33) = 3.64$, $p < .05$], with HI subjects performing worse than shams on all days (see again Fig. 5).

3.1.3. Adult testing: silent gap (0–10 ms)

A repeated measures ANOVA comparing P1 and P7 HI subjects on a single day of short (0–10 ms) silent gap in adulthood revealed a significant gap × age-at-injury interaction [$F(8,26) = 2.4$, $p < .05$] and a marginal main effect for age-at-injury [$F(1,33) = 3.6$, $p < .1$], with P1 HI animals performing better than P7 HI at all gaps. No differences were seen between P1 and P7 shams. Thus, pooled shams were again compared to P1 and P7 HI groups separately.

Results from a repeated measures ANOVA between P1 HI and the sham group revealed no significant differences (see Fig. 7). A repeated measures ANOVA between the sham and P7 HI group, however, revealed a marginal main effect for treat-

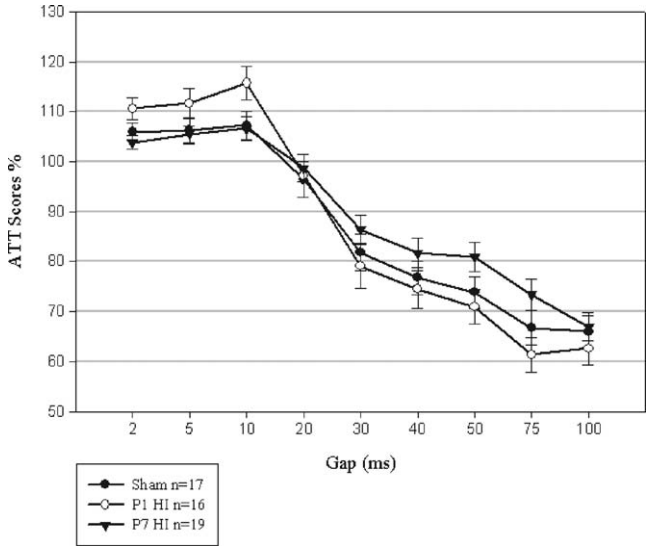

Fig. 4. Means (±S.E.M.) for attenuation response scores (ATT scores) on the 0–100 ms silent gap task over 2 days in the juvenile period for P1/P7 HI and sham animals. This task showed no differences between P1 HI, P7 HI, and sham animals, indicating no differences between groups on the detection of long duration silent gap.

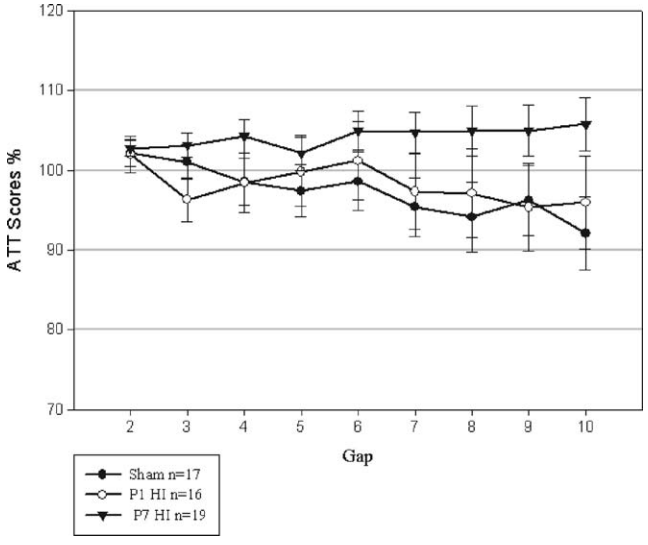

Fig. 5. Means (±S.E.M.) for attenuation response scores (ATT scores) on the 0–10 ms silent gap task over 4 days in the juvenile period for P1/P7 HI and sham animals. Over the 4 days of testing P1 HI animals achieved performance similar to shams, while P7 HI animals maintained poor performance relative to shams.

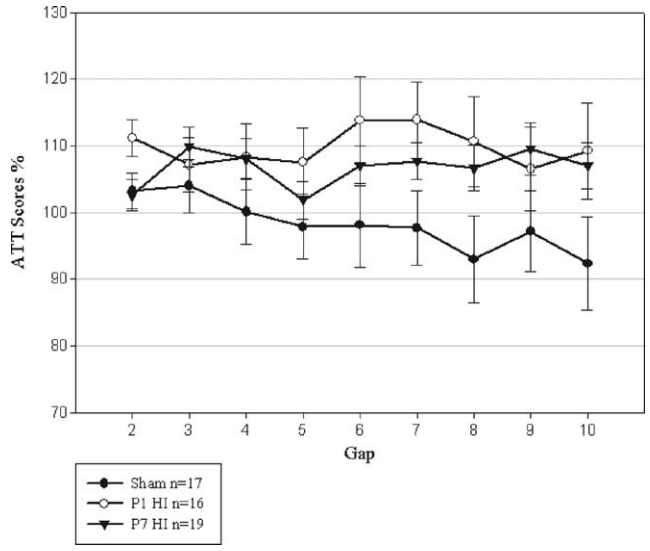

Fig. 6. Means (±S.E.M.) for attenuation response scores (ATT scores) on the second day of the 0–10 ms silent gap task in the juvenile period for P1/P7 HI and sham animals. Note the similar trend between the P1 and P7 HI groups, indicating poor performance on the detection of short duration silent gaps in comparison the sham animals.

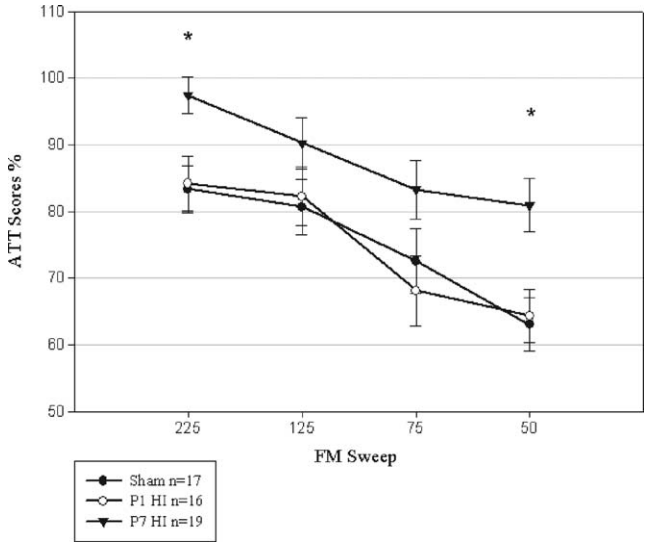

Fig. 8. Means (±S.E.M.) for attenuation response scores (ATT scores) on the variable duration FM sweep procedure in adulthood for P1/P7 HI and shams animals. P7 HI animals showed a main effect for treatment in comparison to sham animals, indicating impaired performance on this complex task. P7 HI animals $^{*}p < .01$, comparison between P7 HI and sham groups.

ment [$F(1,33) = 2.03$, $p < .1$], with shams performing better at all gaps (see Fig. 7).

3.1.4. Adult testing: FM sweeps

For the FM sweep procedure, a 4 (FM sweep duration; 225, 125, 75, and 50 ms) × 2 (age-at-injury) repeated measures ANOVA was run for the HI groups. Results showed a significant main effect for age-at-injury [$F(1,33) = 7.95$, $p < .01$], indicating differences between the P1 and P7 HI animals (with P7 HI performing worse). A 4 (FM sweep duration) × 2 (treatment) repeated measures ANOVA showed no differences between the P1 HI and pooled shams, but a significant main effect of treatment between the P7 HI group and pooled shams [$F(1,33) = 7.12$, $p < .01$] (see Fig. 8). Individual univariate ANOVAs for each FM sweep revealed significant differences between the sham and P7 HI group at the 225 and 50 ms durations [$F(1,33) = 10.43$ and 8.00, $p < .01$], with the P7 HI group performing worse on all FM durations. Interestingly, the P7 HI group also performed worse than the P1 HI group on all FM durations, and significantly so at the 225, 75 and 50 ms durations [$F(1,33) = 6.14$, 5.36, and 8.88, $p < .05$].

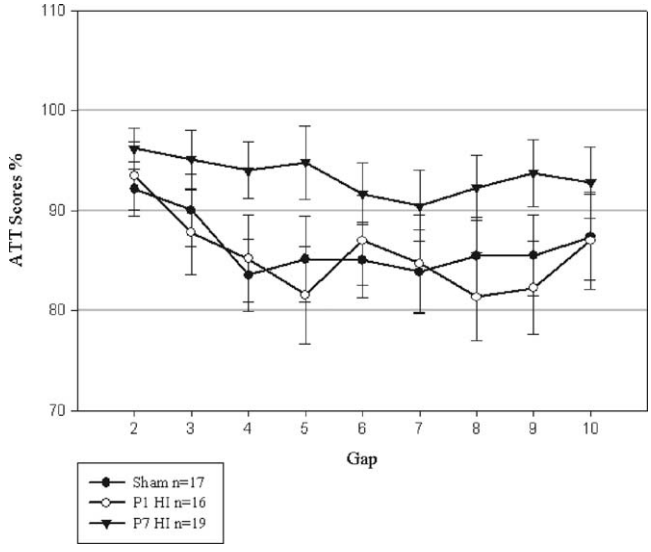

Fig. 7. Means (±S.E.M.) for attenuation response scores (ATT scores) on the 0–10 ms silent gap task over 2 days in adulthood for P1/P7 HI and sham animals. Note the P7 HI group performing worse than shams, showing a marginal treatment main effect ($p < .1$), indicating that deficits in detection of short duration silent gaps extended into adulthood.

3.1.5. Adult testing: water escape and Morris water maze (MWM)

A subset of animals (8 sham, 8 P1 HI, and 8 P7 HI) were randomly selected from each group and tested on a water escape task and the MWM. Results from the water escape revealed no differences between HI and sham animals, thus, HI subjects did not appear to differ from shams in swim-speed or motivation.

For the MWM, a 4 (testing day) × 2 (age-at-injury) repeated measures ANOVA was computed for the HI groups, using average distance swam (averaged across the four trials in each day). Analyses revealed a significant testing day × age-at-injury interaction for the P1 and P7 HI animals [$F(3,12) = 5.61$, $p < .05$; see Fig. 9]. Probing the source of this interaction between HI subjects with independent samples t-tests revealed significant differences on day 4 [$t = 2.98$, $p < .05$], with P7 animals swimming longer distances on all days compared to P1 HI subjects. A 4 (testing day) × 2 (treatment) repeated measures ANOVA between P1 HI and the sham group showed no differences between groups, while a comparable analysis showed a marginal main effect of treatment for P7 HI animals versus shams [$F(1,14) = 1.83$, $p < .1$], with P7 HI animals swimming significantly longer distances than shams on day 4 [$t = 4.86$,

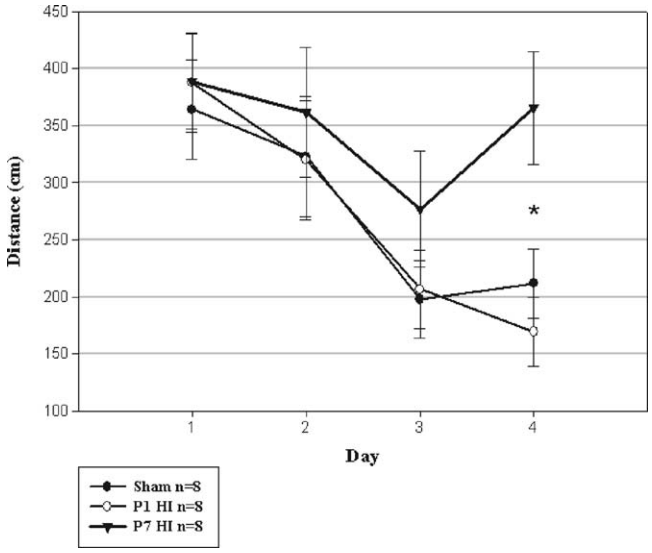

Fig. 9. Average distance swam to reach platform (±S.E.M.) over 4 trials/day for 4 days for P1/P7 HI and sham animals on the Morris water maze (MWM). The platform was placed in SW quadrant and extra-maze spatial cues remained consistent over days. $^{*}p < .05$, comparison between P7 HI and sham groups, and the P7 HI and P1 HI groups.

$p < .05$]. These findings indicate that P7 HI subjects swam significantly longer distances to reach a submerged platform than shams or P1 HI, and thus appear to have been significantly impaired on a spatial learning and memory task.

4. Discussion

Relatively little is known about the relationship between age-at-injury in human neonates and long-term cognitive/behavioral outcome. In addition to poor neuropathological measures of damage for infants, there is a paucity of longitudinal analyses for later neuropathological and functional impairment. Further, with the confound of incidence (i.e., the risk of HI injury increasing with each additional week of premature birth), it appears that increasing prematurity is associated with a worse outcome. Although differential overall "outcome risk" has been identified between premature and term HI injured infants, it is not clear whether a comparable HI injury during these time periods leads to comparable functional impairments.

Many infants who undergo HI early in development go on to exhibit language impairments and memory problems [7,12,17,18,20,26,31,39]. RAP deficits have been suggested to play a causal role in such language impairments [35], and a RAP deficit has been identified in premature children with language problems (with correlations seen between RAP indices and language scores [9,15]). RAP and memory deficits have also been identified in a rodent model of HI [1,2,11,23,24]. Given parallels in deficits and neuropathological sequelae between rodents and humans who undergo HI injury, the current study sought to compare these deficits (specifically modeling HI injury in rats at ages comparable to premature and near-term human infants) to see if differential behavioral consequences/gross brain malformations can be related to different maturational stages at the time of injury. The auditory tasks employed in this study were chosen to assess different aspects of auditory processing associated with different patterns of damage. While the neurophysiological underpinnings of complex and/or rapid auditory processes have both cortical and subcortical contributions (i.e., medial geniculate body of the thalamus [6]), it can be inferred that increasingly demanding tasks rely more heavily on cortical input. For example, rats with cortical spreading depression can still detect longer (but not short) silent gaps in a startle reduction paradigm [14]. Similarly, electrophysiological evidence of mismatch negativity can be seen for some stimulus contrasts at the level of MGN in guinea pig, while other contrasts are thought to rely on cortical processing [19]. As such, it represented an important goal to associate patterns of damage with observed deficits using tasks of varying acoustic demand.

The current findings present novel results which: (1) replicate evidence of differential neuropathology depending on age-at-injury, with P7 HI animals showing gross anatomical damage to the corpus callosum, hippocampal commissure, right cortex, right hippocampus, and right anterior commissure, while P1 HI animals only exhibit a reduction in the hippocampal commissure; and (2) further, show differential behavioral outcome measures depending on age-at-injury, with HI injuries sustained on P7 leading to more persistent rapid auditory processing and spatial learning and memory deficits as compared to similar injuries incurred on P1. Even in the juvenile period, P1 HI subjects were found to show recovery of performance over the 4 days of the 0–10 ms silent gap, while P7 HI did not. Deficits in the auditory procedures indicate the inability of animals to detect, and therefore attenuate their startle response, to specific auditory stimuli. The stimuli presented here include a silent gap procedure, which revealed deficits among HI subjects only for short duration stimuli (indicating deficits in RAP of short duration silent gaps, a discrimination comparable to processing short-stop gap syllables in speech). Deficits in P7 HI animals were also seen on the FM sweep procedure, a task utilized to identify deficits in discrimination/processing of rapidly changing frequencies (again similar to those seen in human speech). The MWM, a task used to assess spatial learning and memory in rats (specifically the ability for animals to learn, remember, and find a platform in relation to extra-maze cues [5]), revealed deficits for P7 HI animals over the 4 days of testing (as measured by increased swim distance to find the submerged platform). These findings indicate an impairment in the ability to learn/remember the placement of an escape platform in reference to spatial cues over repeated trials/days.

As a caveat, the relationship between behavioral deficits and age-at-injury may not be solely related to the maturational stage of the brain when injury took place, but could also be attributable to the differential degree of damage between HI injury on P1 and P7 (with P7 sustaining a much more severe injury). This possibility is interesting in itself, given that the same procedure induced on P1 produces less measurable apparent anatomical damage than on P7. This interpretation of our behavioral findings still supports the notion that premature infants may be more resilient than term infants to comparable HI episodes.

In summary, the findings presented here have profound clinical significance in suggesting in that, despite the markedly lower incidence of HI injuries among term as compared to severely premature infants, when these injuries are sustained at term they may exert more dramatic and persistent deleterious behavioral/cognitive consequences. Thus, the brain of a premature infant may be more resilient to the same duration/degree of HI episode compared to a term infant.

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