Neonate Hippocampal Volumes: Prematurity, Perinatal Predictors, and 2-Year Outcome

Deanne K. Thompson, BSc(Hons),[1–3] Stephen J. Wood, PhD,[4] Lex W. Doyle, MD,[3,5] Simon K. Warfield, PhD,[6] Gregory A. Lodygensky, MD,[2,7] Peter J. Anderson, PhD,[3,5,8] Gary F. Egan, PhD, MBA,[1] and Terrie E. Inder, MD[2,3]

Objective: To compare preterm (PT) and full-term (FT) infant hippocampal volumes and to investigate the relations among PT hippocampal volume, perinatal risk factors, and neurodevelopmental outcome.

Methods: A total of 184 PT and 32 full-term infants underwent magnetic resonance imaging at term equivalent age with manual segmentation of the hippocampi on coronal slices. Perinatal data were collected and 2-year neurodevelopment was evaluated with the Mental Development Index and Psychomotor Development Index on the Bayley Scales of Infant Development.

Results: PT and FT infant hippocampi did not significantly differ after controlling for head size, and percentage reductions in PT hippocampi (3.4%) were less than for cortical (7%) and deep nuclear gray matter (13%), and total brain tissue volume (4.7%). PT hippocampal volumes were significantly lower in infants with moderate-to-severe white matter injury ($p < 0.001$), exposure to postnatal steroids (right, $p = 0.001$; left, $p = 0.008$), and indomethacin treatment (right, $p = 0.01$; left, $p = 0.03$). PT infant hippocampal volumes correlated with the Mental ($p < 0.001$) and Psychomotor Development Indices (right, $p = 0.001$; left, $p = 0.002$) after correcting for head size and sex, but remained significant only for the Mental Development Index and left hippocampi ($p = 0.04$) after additionally adjusting for white matter injury and steroids.

Interpretation: Hippocampal volumes were reduced in PT infants exposed to several perinatal events but were preserved in PT infants without these exposures. Smaller PT hippocampal volumes were indirectly associated with delayed development at 2 years.

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Very preterm (PT; <28 weeks' gestational age) or extremely low-birth-weight (BW) infants (<1,000gm BW) face a high risk for adverse neurodevelopmental outcomes including cerebral palsy, and cognitive and behavioral deficits.[1,2] One cognitive domain that is commonly found to be impaired in PT children is memory,[3–7] and consistent with this finding, a substantial proportion of this population experiences educational difficulties.[1,8,9] Given that the hippocampus is intimately involved in learning and memory,[10] injury or impaired hippocampal development within PT infants may be a critical contributor to their neurodevelopmental burden.

Magnetic resonance imaging (MRI) studies in older PT cohorts, from 8 years to adolescence, have demonstrated that hippocampal volumes are smaller than term born infants.[11–15] The mechanisms for reductions in hippocampal volumes are unclear, although injury to the hippocampi may occur through hypoxic-ischemic or metabolic insults.[16,17] Other perinatal complications, including drug therapies, may also play a role. Postnatal dexamethasone is known to have a specific neurotoxic effect on the hippocampus[18] because it is a target for glucocorticoid stress hormones.[19] Reductions in hippocampal volumes appear to have functional implications because they have been associated with impaired memory and learning in older PT children.[15,20–23]

From the [1]Howard Florey Institute, Centre for Neuroscience, University of Melbourne, Melbourne, Victoria, Australia; [2]Department of Pediatrics, St. Louis Children's Hospital, Washington University in St. Louis, St. Louis, MO; [3]Murdoch Childrens Research Institute, Royal Children's Hospital, Parkville; [4]Department of Psychiatry, Melbourne Neuropsychiatry Centre; [5]Department of Obstetrics and Gynecology, Royal Women's Hospital, University of Melbourne, Carlton, Victoria, Australia; [6]Department of Radiology, Children's Hospital, Harvard Medical School, Boston, MA; [7]Department of Pediatrics, Children's Hospital of Geneva, Geneva, Switzerland; and [8]Department of Psychology, University of Melbourne, Melbourne, Victoria, Australia.

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Address correspondence to Ms Thompson, Howard Florey Institute, Level 2, Alan Gilbert Building, 161 Barry Street, Carlton South, VIC 3053, Australia. E-mail: deanne.thompson@florey.edu.au

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Hippocampal volumes have not been extensively studied at term-equivalent age (TEA). In contrast, significant global and region-specific gray matter volume reductions have been noted in PT infants.[24] Cortical gray matter was the most reduced in the sensorimotor and orbitofrontal regions, whereas the parietooccipital and subgenual regions showed reduced deep nuclear gray matter compared with full-term (FT) infants. Therefore, this study extends these more general volumetric findings to examine a more specific gray matter region of interest, the hippocampus.

There were three aims of this study: (1) to utilize three-dimensional MRI to quantify and compare hippocampal volumes between FT and PT infants at TEA, (2) to determine the association between PT hippocampal volumes and perinatal risk factors, and (3) to investigate the association of PT hippocampal volumes with neurodevelopmental outcomes at 2 years' corrected age. We hypothesized that PT birth may adversely affect hippocampal development by TEA.

Subjects and Methods

Subjects

A prospective, observational cohort study of neonatal MRI was conducted between July 2001 and December 2003 at the Royal Women's Hospital in Melbourne, Australia. All 348 PT infants with either BW less than 1,250gm or gestational age at birth (GA) less than 30 weeks surviving to TEA were eligible for recruitment, and 233 (67%) were recruited. Infants with congenital anomalies were excluded (3%). Inability to obtain parental consent (22%) was the most common reason for failure to recruit, often attributed to early hospital transfer or long distance from the hospital. There were no significant differences in infants recruited or not recruited according to sex, GA, intraventricular hemorrhage, cystic periventricular leukomalacia, or bronchopulmonary dysplasia (oxygen requirement beyond 36 weeks' postmenstrual age). Fifty-one FT infants delivered at more than 37 weeks' gestation were also recruited nonrandomly from the postnatal wards of the Royal Women's Hospital and via response to advertisements in the recruiting hospitals. These infants had an unremarkable antenatal course and labor, and had no major neonatal complications. No FT children had congenital or chromosomal anomalies, and all children were retained regardless of presence or absence of developmental concerns at follow-up. Informed parental consent was obtained, and the study was approved by the Research and Ethics Committee at the Royal Women's Hospital.

A total of 184 stable PT and 32 healthy FT infant scans (76% of those recruited) underwent volumetric MRI analysis of the hippocampus, with the remainder of scans either not of sufficient quality because of movement and imaging artifacts, or failing to be scanned within the TEA range (38–42 weeks' postmenstrual age).

Perinatal data were obtained by chart review. Apart from variables already listed, data were also recorded on antenatal steroids, multiple births, postnatal steroids (PNS; dexamethasone administered at a median total dose of 1.1mg/kg), the need for inotropic support, indomethacin treatment for a patent ductus arteriosus, episodes of sepsis, and necrotizing enterocolitis. BW *Z*-scores were computed relative to the British Growth Reference data,[25] and intrauterine growth restriction was defined as a BW *Z*-score less than −2 standard deviations (SDs). Cranial ultrasound scans were obtained serially throughout the neonatal intensive care course in all infants within the first 48 hours and at ages 4 to 7 days and 4 to 6 weeks as a minimum. If an abnormality was detected, weekly cranial ultrasound assessments were undertaken. All ultrasound scans were reported independently of clinical details and magnetic resonance scans. The highest grade of intraventricular hemorrhage was recorded, and the presence of cystic periventricular leukomalacia was noted.

Magnetic Resonance Imaging

Scanning took place within a 1.5-Tesla General Electric Signa MRI scanner (Milwaukee, WI). All infants were scanned at TEA (38–42 weeks' postmenstrual age). Infants were fed and swaddled, placed in a vacuum fixation bean bag, outfitted with earphones, and scanned while sleeping to minimize motion artifacts. No sedation was administered.

Whole-brain images were acquired, applying two different imaging modalities within the same session: three-dimensional T1 spoiled gradient recalled (1.2mm coronal slices; flip angle, 45°; TR, 35 milliseconds; TE, 9 milliseconds; field of view, 21 × 15cm^2; matrix 256 × 192) and T2 dual-echo fast spin-echo sequences with interleaved acquisition (2mm coronal; TR, 4,000 milliseconds; TE, 60/160 milliseconds; field of view, 22 × 16cm^2; matrix 256 × 192, interpolated 512 × 512).

Qualitative Image Analysis

Images were analyzed qualitatively for white matter injury (WMI) grading by two blinded independent readers including a qualified neonatal neurologist (T.E.I.) and a trained neonatologist. White matter (WM) was graded with a score of 1 to 4 according to WM signal abnormality, WM volume, presence of cystic abnormality, quality of the corpus callosum, and myelin maturation (WMI grade 1 = normal; grade 2 = mild noncystic abnormality; grade 3 = moderate-to-severe noncystic abnormality; grade 4 = severe cystic abnormality).[26] Intrarater and interrater group assignment for WMI scores were more than 90%.

Quantitative Image Analysis

TOTAL BRAIN SEGMENTATION. All postacquisition MRI analyses were undertaken on Sun Microsystems workstations (Palo Alto, CA). The total brain volume (TBV) was measured according to the following steps. First, a brain versus nonbrain mask depicting the intracranial cavity (ICV; including all cerebrospinal fluid within the ventricles and subarachnoid space) was created on the T1-weighted image.[27] Second, the T1-weighted image underwent image registration to dually acquired T2-weighted and proton density–weighted images using linear transformation algorithms, to undergo subsequent tissue classification.[28] Brain tissue was separated into myelinated and unmyelinated WM, cortical and deep nuclear gray matter, and cerebrospinal fluid according to previously described criteria.[24] The total gray matter

volume included all the cortical and deep nuclear gray matter. TBV included all the gray matter and WM within the skull, including the cerebellum, but excluded all cerebrospinal fluid.

HIPPOCAMPAL SEGMENTATION. The operator (D.K.T.) was blinded to all perinatal data including to which group the images belonged: FT or PT. The hippocampus was manually outlined in the coronal view on the combined raw T2- and proton density–weighted image volumes to increase contrast for optimal visualization of hippocampal boundaries. As these images were acquired dually within the same imaging session, registration was not required for this volume addition. Each infant imaging underwent hippocampal segmentation twice, once in original orientation and then again after being flipped in the left/right direction, to take into account possible hemispheric bias in the operator. The hippocampal volume used in subsequent analyses was the average of these two segmentations (original and flipped). The hippocampi on the right of the screen were always traced first. 3D slicer 2.5 software (http://slicer.org/) was used, and reference was made to anatomical atlases.[29,30] In general, anatomic boundaries followed Watson and coworkers'[31] approach. Once the posterior boundary at the hippocampal tail was defined (the slice where the fornix was seen in its entirety), the in-plane boundaries were traced sequentially on each slice until the anterior boundary at the head of the hippocampus was reached. This boundary was defined as the most anterior slice where cerebrospinal fluid was still seen temporally to the hippocampus. Tracing of each slice of the hippocampi proceeded from the medial edge to the inferior, then the temporal aspect, and finally to the superior edge of the hippocampus. Hippocampal volumes were delineated a second time (average of both original and flipped hippocampal segmentations) for 15 randomly chosen images. Intraclass correlation coefficients were then calculated for intraobserver reliability by the single operator (D.K.T.). The intraclass correlation coefficient was 0.97 for the right and 0.96 for the left hippocampus.

NEURODEVELOPMENTAL ASSESSMENT. At 2 years' corrected age, infants were assessed for developmental delay using the Bayley Scales of Infant Development (BSID-II).[32] Assessors were blinded to group membership (FT or PT) and to all other perinatal data. Cognitive development, including language, reasoning ability, memory, and learning, was assessed by the Mental Development Index (MDI). Motor development, including gross and fine motor skills, was evaluated by the Psychomotor Development Index (PDI). After all neurodevelopmental assessments, the parent/guardian who was present during the assessment was asked to comment regarding their child's performance and the validity of the assessment. In a small number of cases (n = 18), a second appointment was made for children who refused to participate at the first appointment, or when parents believed that the assessment was invalid. Consistent with procedures reported previously,[33,34] children who scored below the lower limit of published normative data were assigned a standardized score of 45. To distinguish these children from those who were too impaired to undertake the test, we assigned those unable to complete the test a standardized score of 40, or 4 standard deviations less than the mean.

Statistical Analyses

Data were analyzed using SPSS version 12.0.1 (SPSS, Chicago, IL). Initially, PT and term infants' left and right *raw* hippocampal volumes were compared with independent sample t tests, but it was considered imperative to correct for head size; therefore, a "corrected" hippocampal volume was created and used for all subsequent analyses according to a previously described formula[35]:

$$\mathrm{cHCV} = \mathrm{mHCV} - g(\mathrm{mICV} - \mathrm{aveICV}) \quad (1)$$

where cHCV = corrected hippocampal volume, mHCV = measured hippocampal volume, g = gradient of regression line between FT hippocampal volume & ICV, mICV = measured ICV, and aveICV = average/mean ICV of FT infants. Comparison of PT and FT infant hippocampal volumes was achieved by repeated-measures analysis of variance, where the repeated measures were the left and right hippocampal volumes, and the between-subject variable was group (PT or FT).

To test whether prematurity was having a unique effect on hippocampal volumes, we also compared the proportional effect of prematurity on cHCV to the effect of prematurity on the TBV and other gray matter volumes, which were also corrected for ICV according to Equation 1. PT and FT differences were compared for each of these volumes using independent sample t tests.

Within PT infants, the relations of cHCV with perinatal variables were explored initially by univariate analyses (t tests for dichotomous variables and simple linear regression for continuous variables), checking whether there were violations of the assumptions of normality or equality of variance between groups. Two continuous variables, days on parenteral nutrition and hours on positive pressure ventilation, violated the assumption of normality and were therefore dichotomized, choosing the median value as the cutoff. WMI was dichotomized into grades 1 and 2 and grades 3 and 4. Because 15 different variables were tested, Bonferroni adjustment for multiple comparisons was performed, and the α level of significance was set at $p < 0.003$ for the univariate analyses for each hemisphere of the hippocampus. Those variables statistically significant at $p < 0.05$ for either hippocampus were then analyzed by stepwise linear regression to determine the independently significant variables. A corrected total gray matter volume (cGMV) was calculated according to Equation 1 and used as a covariate to account for the fact that all gray matter is reduced in PT infants,[24] as we aimed to investigate the unique effect on the hippocampus.

MDI and PDI scores were related to PT cHCV (both right and left hemispheres) by linear regression, and then by stepwise linear regression to adjust for sex and other perinatal variables as necessary.

Results

Preterm Compared with Term Infants

A total of 184 PT infants and 32 term control infants' MRI scans were analyzed. There were no significant

differences between PT and FT infants for either the gestational age at the time of MRI ($p = 0.7$) or the male-to-female ratio between groups ($p = 0.6$). Table 1 shows the characteristics of the cohort.

Exploratory analysis demonstrated that FT and PT infant *uncorrected* hippocampi differed significantly in the right side [FT: 1.19 (0.12)cm^3; PT: 1.13 (0.16)cm^3; $p = 0.04$], but not the left side [FT: 1.16 (0.13)cm^3; PT: 1.12 (0.16)cm^3; $p = 0.12$]. After correcting for head size, there was no significant effect of prematurity on cHCV [FT: right, 1.19 (0.10)cm^3; left, 1.16 (0.12)cm^3; PT: right, 1.15 (0.13)cm^3; left, 1.13 (0.14)cm^3; $p = 0.09$)]. However, a hemispheric difference in the hippocampi was noted (Wilks' lambda = 0.86; $F_{1,214} = 35.96$; $p < 0.001$), but this difference between the right and left side did not differ between the FT and PT infants, as reflected by the lack of a significant group by hemisphere interaction ($p = 0.1$). In light of the noted hemispheric effect, left and right hippocampal volumes were subsequently analyzed separately.

Other gray matter volumes, as well as TBV, were significantly reduced in PT infants compared with term control subjects (Table 2). The percentage reduction in brain volumes related to prematurity was least for the cHCV, larger for corrected TBV and corrected cortical gray matter, and largest for corrected deep nuclear gray matter (see Table 2).

Preterm Perinatal Predictors of Hippocampal Volumes

On univariate analyses, there were no violations of the assumptions of normality or equality of variance between groups. In PT infants, there were significant reductions ($p < 0.003$) in cHCV with grade 3 and 4 WMI, and with PNS for both the right and left hippocampi, with a trend to significance in the right hippocampus only with indomethacin therapy (Table 3). Each side of the hippocampus was also tested against either of the two continuous variables with linear regression. There was no significant correlation between BW and hippocampal volume at TEA after Bonferroni adjustment for multiple comparisons (right: $r = 0.19$; $p = 0.01$; left: $r = 0.18$; $p = 0.02$). Likewise, immaturity (GA) had little impact on hippocampal volume (right: $r = 0.14$; $p = 0.06$; left: $r = 0.14$; $p = 0.06$). The remaining univariate analyses showed no significant relations with cHCV (see Table 3).

Table 1. **Perinatal and Demographic Characteristics of the Total Cohort**

Characteristics	Term Infants (n = 32)	Preterm Infants (n = 184)
Mean gestational age at birth, wk (SD)	39.0 (1.2)	27.6 (1.9)
Mean gestational age at MRI, wk (SD)	40.5 (1.0)	40.1 (1.1)
Mean Birth weight, gm (SD)	3,289 (504)	964 (219)
Mean weight at MRI, gm (SD)	3,484 (490)	2,971 (531)
Male sex, n (%)	18 (56)	93 (51)
Multiple births, n (%)	1 (3)	79 (43)
Antenatal steroid administration, n (%)	0 (0)	164 (89)
Bronchopulmonary dysplasia,[a] n (%)	0 (0)	68 (35)
Inotropic support, n (%)	0 (0)	71 (39)
Postnatal steroid therapy,[b] n (%)	0 (0)	14 (8)
Intrauterine growth restriction,[c] n (%)	2 (6)	22 (12)
Necrotizing enterocolitis, n (%)	0 (0)	19 (10)
Proven sepsis, n (%)	1 (3)	80 (44)
Median positive pressure ventilation, hr (IQR)	0 (0, 0)	66.5 (3, 253)
Median parenteral nutrition, days (IQR)	0 (0, 0)	11 (5,16)
Indomethacin therapy, n (%)	0 (0)	63 (34)
White matter injury (grade III/IV), n (%)	0 (0)	31 (17)
Intraventricular hemorrhage, n (%)	0 (0)	24 (13)

[a]Required oxygen at 36 weeks gestational age.
[b]Postnatal dexamethasone, 0.15mg/kg per day, reducing over 10 days.
[c]*Z*-score > 2 standard deviations (SD) less than mean weight for gestational age.
MRI = magnetic resonance imaging; IQR = interquartile range.

Table 2. **Comparison of Preterm and Full-Term Infant Corrected Hippocampal Volumes (Left and Right Combined) with Those of Other Corrected Gray Matter Volumes Including Cortical Gray Matter and Deep Nuclear Gray Matter, as Well as Corrected Total Brain Volume**

Volume	Mean Corrected Volume, cm^3 (SD)		Mean Difference, cm^3	95% CI	*p*	% Difference
	PT	FT				
cHCV (L + R)	2.27 (0.26)	2.36 (0.21)	−0.08	−0.18, 0.01	0.09	3.4
cTBV	404.8 (21.6)	424.9 (9.8)	−20.0	−24.7, −15.3[a]	<0.001	4.7
cCGM	163.1 (23.7)	175.4 (23.5)	−12.3	−21.2, −3.3	0.007	7.0
cDNGM	13.7 (3.5)	15.7 (2.3)	−2.0	−3.0, −1.1[a]	<0.001	12.7

[a]Equal variances not assumed.
PT = preterm; FT = full term; SD = standard deviation; CI = confidence interval; cHCV = corrected hippocampal volumes; cTBV = corrected total brain volume; cCGM = corrected cortical gray matter; cDNGM = corrected deep nuclear gray matter.

On stepwise regression, WMI was the most statistically significant variable related to left and right cHCV. PNS exposure was also significantly related to cHCV in both sides, as was indomethacin therapy for a patent ductus arteriosus (PDA) (see Table 3). The covariate, cGMV, was statistically significantly related to cHCV, explaining 7.7% of variance on the right ($p = 0.002$) and 8.9% on the left side ($p = 0.001$).

Developmental Outcomes

The FT infants had a mean MDI of 102 (SD, 17) and PDI of 101 (SD, 9), suggesting their development was within the reference range and typical of the general population. PT infants had a mean MDI of 84 (SD, 20) and PDI of 87 (SD, 18). In PT infants, there was a positive correlation between cHCV at TEA and cognitive functioning at 2 years of age (Fig, A), where larger cHCV was associated with greater MDI scores (Table 4). There was also a positive correlation between hippocampal volume and PDI score (see Table 4; see Fig, B).

Female subjects scored significantly better on both the MDI (mean difference, 11.7; 95% confidence interval, 6.1–17.3; $p < 0.001$) and the PDI (mean difference, 7.2; 95% confidence interval, 2.0–12.4; $p = 0.007$). After adjusting for sex, the relation between cHCV on both sides remained statistically significantly related to both the MDI and the PDI (see Table 4).

When other perinatal variables in addition to sex were added to the stepwise regression, WMI grades 3 and 4 and PNS were significantly related to both MDI and PDI, and this reduced the size of the relations of neurodevelopmental scores with cHCV. The only significant relation that remained was that between the MDI and left cHCV (see Table 4).

Discussion

This study demonstrates that very PT infants do not have uniquely reduced hippocampal volumes by TEA compared with FT infants, once the reduced head size (ICV) and proportionately larger reduction in cerebral GM of PT infants are taken into account. However, several postnatal factors including the presence of WMI, exposure to PNS, and indomethacin were found to negatively affect cHCV. By 2 years of age, PT infants with reduced cHCV at TEA had reduced performance on the MDI (reflecting cognitive development) and the PDI (a measure of psychomotor development), independent of the effect of ICV and sex. However, these relations diminished (apart from that of MDI and left cHVC) after adjusting for WMI and PNS, suggesting that they are major pathways in the alterations in cHCV.

Comparison of Preterm and Full-Term Infants

There was no significant effect of prematurity on hippocampal volumes that could not be attributed to the overall smaller heads of PT infants. The hippocampus appeared to be the least affected of the gray matter volumes by PT birth and, therefore, could be perceived to be relatively spared. However, greater alterations in hippocampal volume may be apparent at later ages given that the major hippocampal "growth spurt" occurs between birth and 2 years of age.[36] Reductions in hippocampal volumes have been demonstrated in older PT children[11,12,20] and adolescents.[13–15] The fact that hippocampal deficits appear to be present in older PT populations but not at TEA has important implications for neuroprotective strategies in the neonatal period.

Previous studies have shown that the hippocampus is an asymmetrical structure,[37,38] and our results confirmed these findings. However, the effects of prematurity did not appear to be asymmetrical, as reflected by the lack of a group by hemisphere interaction. Others, including Isaacs and coauthors,[20] have also failed to detect a differential hemispheric effect in PT adolescent hippocampi.

Table 3. **Corrected Hippocampal Volumes Related to Perinatal Variables in Preterm Infants**

Variable	Side	Mean Volume Corrected (SD), cm^3		Mean Difference (95% CI), cm^3	Adjusted Mean Difference[a] (95% CI), cm^3
WMI grade 3 or 4		Yes (n = 31)	No (n = 153)		
	R	1.03 (0.13)	1.16 (0.15)	−0.14 (−0.19, −0.09); $p < 0.001$	−0.12 (−0.16, −0.07); $p < 0.001$; additional variance 12.3%
	L	1.01 (0.12)	1.15 (0.13)	−0.17 (−0.22, −0.11); $p < 0.001$	−0.13 (−0.17, −0.08); $p < 0.001$; additional variance 12.8%
PNS		Yes (n = 14)	No (n = 170)		
	R	1.02 (0.10)	1.16 (0.13)	−0.14 (−0.21, −0.07); $p < 0.001$	−0.10 (−0.17, −0.04); $p = 0.001$; additional variance 5.8%
	L	1.02 (0.11)	1.14 (0.13)	−0.12 (−0.19, −0.05); $p < 0.001$	−0.09 (−0.16, −0.02); $p = 0.008$; additional variance 4.0%
Indomethacin		Yes (n = 63)	No (n = 121)		
	R	1.11 (0.13)	1.17 (0.13)	−0.06 (−0.10, −0.02); $p = 0.003$	−0.05 (−0.08, −0.01); $p = 0.01$; additional variance 2.7%
	L	1.09 (0.13)	1.15 (0.13)	−0.05 (−0.10, −0.01); $p = 0.01$	−0.04 (−0.08, −0.004); $p = 0.03$; additional variance 2.0%
Sepsis		Yes (n = 80)	No (n = 104)		
	R	1.12 (0.13)	1.17 (0.13)	−0.05 (−0.08, −0.007); $p = 0.02$	
	L	1.10 (0.13)	1.15 (0.14)	−0.04 (−0.08, −0.002); $p = 0.04$	
PPV > 66.5 hours		Yes (n = 92)	No (n = 92)		
	R	1.12 (0.13)	1.17 (0.13)	−0.04 (−0.08, −0.004); $p = 0.03$	
	L	1.11 (0.14)	1.15 (0.13)	−0.04 (−0.08, 0.004); $p = 0.08$	
BPD		Yes (n = 60)	No (n = 123)		
	R	1.12 (0.15)	1.16 (0.12)	−0.04 (−0.08, 0.004); $p = 0.08$	
	L	1.11 (0.15)	1.14 (0.13)	−0.03 (−0.07, 0.02); $p = 0.2$	
Inotropes		Yes (n = 71)	No (n = 113)		
	R	1.13 (0.13)	1.15 (0.13)	−0.03 (−0.06, 0.01); $p = 0.2$	
	L	1.11 (0.14)	1.14 (0.13)	−0.03 (−0.07, 0.01); $p = 0.2$	
PN >11 days		Yes (n = 85)	No (n = 99)		
	R	1.13 (0.13)	1.16 (0.13)	−0.02 (−0.06, 0.02); $p = 0.3$	
	L	1.12 (0.14)	1.14 (0.13)	−0.02 (−0.06, 0.02); $p = 0.4$	
ANS		Yes (n = 164)	No (n = 19)		
	R	1.15 (0.13)	1.13 (0.13)	0.02 (−0.05, 0.08); $p = 0.6$	
	L	1.13 (0.14)	1.10 (0.13)	0.03 (−0.04, 0.09); $p = 0.4$	
Sex		M (n = 93)	F (n = 91)		
	R	1.14 (0.14)	1.15 (0.12)	−0.02 (−0.06, 0.02); $p = 0.4$	
	L	1.12 (0.14)	1.13 (0.13)	−0.01 (−0.05, 0.03); $p = 0.6$	
IVH		Yes (n = 24)	No (n = 158)		
	R	1.13 (0.19)	1.15 (0.12)	−0.02 (−0.08, 0.04); $p = 0.5$	
	L	1.11 (0.19)	1.13 (0.13)	−0.02 (−0.08, 0.03); $p = 0.4$	
NEC		Yes (n = 19)	No (n = 165)		
	R	1.16 (0.11)	1.14 (0.13)	0.01 (−0.05, 0.08); $p = 0.7$	
	L	1.14 (0.11)	1.13 (0.14)	0.02 (−0.05, 0.08); $p = 0.6$	
IUGR		Yes (n = 22)	No (n = 162)		
	R	1.15 (0.11)	1.14 (0.13)	0.003 (−0.06, 0.06); $p = 0.9$	
	L	1.13 (0.11)	1.13 (0.14)	0.004 (−0.06, 0.07); $p = 0.9$	

[a]Adjusted for cGMV, and perinatal variables listed.
SD = standard deviation; CI = confidence interval; WMI = white matter injury; PNS = postnatal steroids PPV = positive pressure ventilation; BPD = bronchopulmonary dysplasia; PN = parenteral nutrition; ANS = antenatal steroids; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; IUGR = intrauterine growth restriction; L = left; R = right.

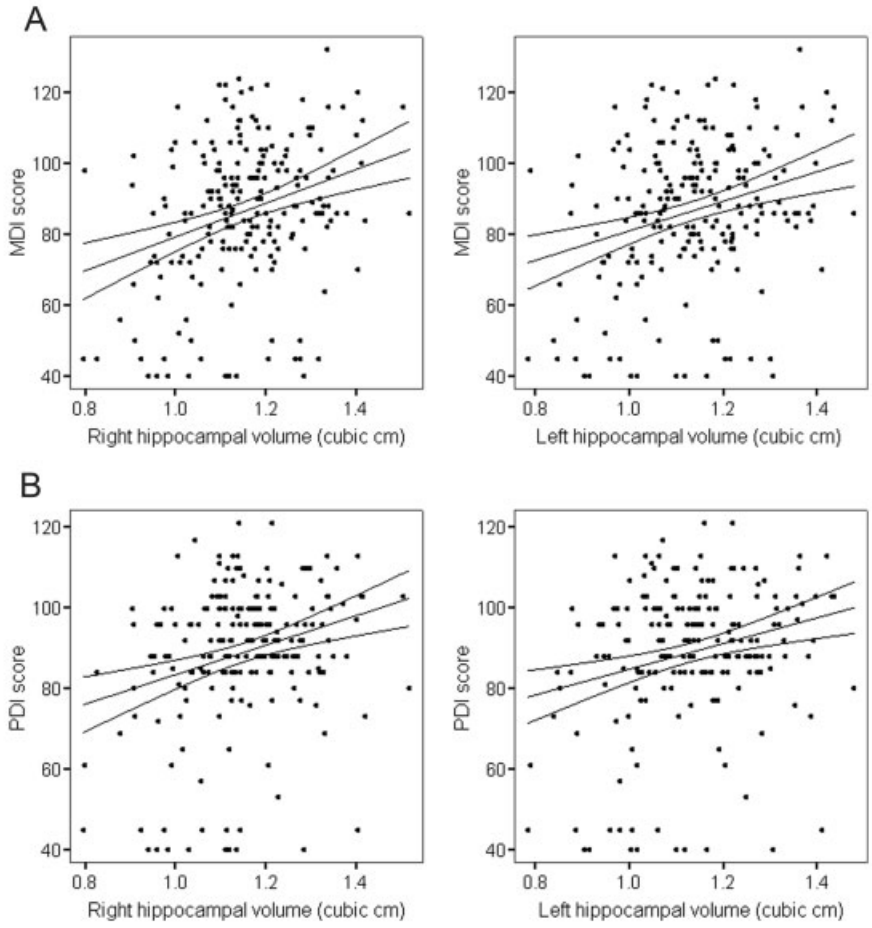

Fig. Scatter plot of right and left preterm corrected hippocampal volume with (A) Mental Development Index (MDI) score and (B) Psychomotor Development Index (PDI) score, with fitted regression line and 95% confidence intervals.

Preterm Perinatal Predictors

We investigated factors that may contribute to vulnerability of the hippocampus in PT infants by TEA. Our data demonstrate that the main perinatal factor associated with reductions in PT cHCV was cerebral WMI. This effect remained significant when covarying for cGMV and perinatal exposures. WMI has been previously demonstrated to adversely affect global cerebral development within this cohort of PT infants.[24,39] The association between cHCV reductions and WMI in PT infants may not necessarily reflect a direct causal pathway. Rather there are complex, interrelated causes. Hypoxic-ischemic injury may damage both the cerebral WM and the hippocampus, and both structures may be comarkers for this form of cerebral insult. Cerebral WMI may also result in a secondary insult to the developing hippocampus with a deafferentation of the hippocampus by the loss of axonal pathways. Serial imaging will be required to disentangle this relationship, as well as further analysis utilizing diffusion imaging techniques.

Postnatal dexamethasone is administered to reduce ventilator dependence in infants with immature lungs. PNS were independently associated with reduced cHCV in this cohort, suggesting a specific vulnerability of this structure to PNS. There may be a causative relation between PNS and detrimental hippocampal development. The doses of dexamethasone administered in this study were relatively low (median, 1.1mg/kg total dose), and the dexamethasone preparation did not contain sulfite preservative, which has been shown to contribute to the neurological impairments associated with dexamethasone.[40] Nonetheless, we noted a significant reduction in cHCV in PT infants who had received dexamethasone. Dexamethasone has previously been implicated in hippocampal injury.[41] In contrast with this, Lodygensky and colleagues[12] found no relation between hippocampal volume and the postnatal use of high-dose hydrocortisone in PT infants at age 8 years. This discrepancy in steroid findings may be because of either recovery of the hippocampus by mid-to-late childhood, or by hydrocortisone having a less detrimental effect on the hippocampus than dexamethasone.

Table 4. **Linear Relations of Mental Development Index and Psychomotor Development Index with Corrected Hippocampal Volumes, after Adjustment for Sex, and Then Adjusted for Other Perinatal Variables**

Outcome	Side	Unadjusted Coefficient[a] (95% CI)	Coefficient Adjusted for Sex (95% CI)	Coefficient Adjusted for Sex and Other Perinatal Variables[b] (95% CI)
MDI	R	4.40 (2.26–6.53); $p < 0.001$	4.17 (2.11–6.22); $p < 0.001$	2.14 (−0.09–4.37); $p = 0.06$
	L	4.20 (2.14–6.26); $p < 0.001$	4.07 (2.10–6.03); $p < 0.001$	2.19 (0.06–4.32); $p = 0.04$
PDI	R	3.37 (1.42–5.33); $p = 0.001$	3.23 (1.31–5.16); $p = 0.001$	1.67 (−0.46–3.79); $p = 0.12$
	L	3.10 (1.21–4.99); $p = 0.001$	3.02 (1.16–4.87); $p = 0.002$	1.54 (−0.49–3.57); $p = 0.14$

[a]Increase in Mental Development Index (MDI) or Psychomotor Development Index (PDI) per 0.1cm^3 increase in corrected hippocampal volume.
[b]White matter injury and postnatal steroids are the only additional perinatal variables that are statistically significant.
CI = confidence interval; L = left; R = right.

Indomethacin is a common therapy for persistence of the arterial duct. There is evidence that indomethacin may be deleterious for the PT brain,[42] and particularly the hippocampus, because it is a Cox2 gene inhibitor that causes downstream reduction of neuroprotective prostaglandin (PGE_2), and hence the neuroprotective EP2 receptor, which is abundant in the hippocampus.[43]

Differences in cerebral volumes within this cohort of PT infants have been previously reported to be related in part to immaturity, in particular, GA.[26,39] However, this study was unable to detect significant correlations between cHCV and GA at birth. BW was significantly associated with cHCV on univariate analysis; however, there was no significant correlation after controlling for cGMV and other perinatal predictors. These findings are consistent with those of other groups that have been unable to establish a relation between GA or BW and hippocampal volumes within children born PT.[11,13]

Similarly, this study was unable to confirm a significant sex difference in cHCV within PT infants. There has been no consistency in previous research regarding the role of sex in hippocampal development. Some groups have reported a sex discrepancy in hippocampal volumes,[12,44] whereas others have not.[31,45]

cHCV did not appear to be affected by any other perinatal variables, once significant contributors were accounted for in multivariate regression. Consistent with our findings, Peterson and colleagues[11] failed to find any correlation between PT hippocampal volumes and a range of perinatal variables at 8 years of age. These results infer that clinical risk factors such as intraventricular hemorrhage, severe respiratory problems (bronchopulmonary dysplasia), sepsis, or inotropic support for cardiac difficulties do not independently affect infant hippocampal development.

Developmental Outcomes

PT cHCVs at TEA were related to cognitive development (MDI) at 2 years of age, and motor development (PDI), even after controlling for sex. These findings, particularly in relation to MDI, are consistent with previous studies that have reported significant associations between hippocampal volume with memory,[15,20] cognition,[12] and intelligence quotient[14] in PT school-aged children and adolescents. However, for the most part, the significant association of hippocampal volume with cognitive and motor development diminished after controlling for WMI and PNS. This suggests that hippocampal volume itself may not directly predict adverse outcomes, but rather that certain perinatal events and treatments such as WMI and/or PNS may adversely affect both hippocampal development and early cognitive and motor development. This explains the unexpected relation between impaired hippocampal development and delayed motor development. Furthermore, other cerebral structures are clearly important for early cognitive and motor development, such as the prefrontal cortex, sensorimotor, parietooccipital, and premotor regions. Although these regions have been shown to be related to general cognitive ability and memory functioning in PT children,[46] it is not yet known whether impaired development of these structures are linked with impaired hippocampal development. Given the concerns over the predictive validity of the Bayley Scales of Infant Development in relation to later cognitive functioning,[47] further follow-up of this cohort throughout childhood using more specific measures of memory and learning, as well as other cognitive domains, is in progress.

MRI provides good anatomical definition and can be utilized to define PT hippocampal integrity,[48] with proven reliability compared with postmortem histopathology.[49] Moreover, hippocampal volumes obtained

from MRI studies have correlated closely with histologically determined cell loss.[50,51] The hippocampi in this cohort were measured consistently according to a predetermined protocol, with a standardized neuroanatomical basis. Although neonatal volumes are small and the boundaries difficult to define because of poor image contrast in the unmyelinated infant brain, our method was optimized for superior visibility. Furthermore, our reliability measures were high and were comparable with those measured for adolescent hippocampi.[13] Considering each hippocampal measurement was the average of two separate segmentations (original and flipped orientation), this helped to improve reliability and reduce error. However, there remains considerable debate as to the most representative and reproducible method of tracing the hippocampi. An automatic method of reliably and objectively segmenting the hippocampus is required.

In conclusion, contrary to our hypothesis, very PT infants showed no reduction in hippocampal volumes at TEA compared with FT infants. However, there was no evidence of neuroprotection, as suggested by the adverse effects of certain perinatal exposures on PT hippocampi, and the negative associations of reduced cHCV to neurodevelopmental outcomes. WMI, PNS exposure, and indomethacin lead to smaller hippocampi. It may be that the full effect of prematurity and its associated adverse exposures may not be apparent until childhood or adulthood. The fact that hippocampal reductions were not apparent at TEA indicates important opportunities for intervention. Strategies to reduce PNS exposure and the negative effects of WMI may protect hippocampal growth. PT hippocampal deficits were indirectly associated with delayed cognitive and motor development at 2 years' corrected age. Further investigations with serial studies from birth through childhood are warranted to understand the development of this important brain structure throughout childhood in this vulnerable population, and to fully appreciate the role of the hippocampus in the high rates of cognitive impairments exhibited by PT children later in life.

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References

1. Anderson P, Doyle LW. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. JAMA 2003;289:3264–3272.
2. Hack M, Fanaroff AA. Outcomes of children of extremely low birthweight and gestational age in the 1990's. Early Hum Dev 1999;53:193–218.
3. Taylor HG, Minich NM, Klein N, Hack M. Longitudinal outcomes of very low birth weight: neuropsychological findings. J Int Neuropsychol Soc 2004;10:149–163.
4. Rose SA, Feldman JF. Memory and processing speed in preterm children at eleven years: a comparison with full-terms. Child Dev 1996;67:2005–2021.
5. Briscoe J, Gathercole SE, Marlow N. Everyday memory and cognitive ability in children born very prematurely. J Child Psychol Psychiatry 2001;42:749–754.
6. Vicari S, Caravale B, Carlesimo GA, et al. Spatial working memory deficits in children at ages 3-4 who were low birth weight, preterm infants. Neuropsychology 2004;18:673–678.
7. Taylor GH, Klein NM, Minich NM, Hack M. Verbal memory deficits in children with less than 750 g birth weight. Child Neuropsychol 2000;6:49–63.
8. Litt J, Taylor HG, Klein N, Hack M. Learning disabilities in children with very low birthweight: prevalence, neuropsychological correlates, and educational interventions. J Learn Disabil 2005;38:130–141.
9. Saigal S. Follow-up of very low birthweight babies to adolescence. Semin Neonatol 2000;5:107–118.
10. Bohbot VD, Allen JJ, Nadel L. Memory deficits characterized by patterns of lesions to the hippocampus and parahippocampal cortex. Ann NY Acad Sci 2000;911:355–368.
11. Peterson BS, Vohr B, Staib LH, et al. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. JAMA 2000;284:1939–1947.
12. Lodygensky GA, Rademaker K, Zimine S, et al. Structural and functional brain development after hydrocortisone treatment for neonatal chronic lung disease. Pediatrics 2005;116:1–7.
13. Nosarti C, Al-Asady MH, Frangou S, et al. Adolescents who were born very preterm have decreased brain volumes. Brain 2002;125:1616–1623.
14. Abernethy LJ, Palaniappan M, Cooke RW. Quantitative magnetic resonance imaging of the brain in survivors of very low birth weight. Arch Dis Childhood 2002;87:279–283.
15. Gimenez M, Junque C, Narberhaus A, et al. Hippocampal gray matter reduction associates with memory deficits in adolescents with history of prematurity. Neuroimage 2004;23:869–877.
16. Schmidt-Kastner R, Freund TF. Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 1991;40:599–636.
17. Kuchna I. Quantitative studies of human newborns' hippocampal pyramidal cells after perinatal hypoxia. Folia Neuropathologica 1994;32:9–16.
18. Sapolsky RM, Uno H, Rebert CS, Finch CE. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J Neurosci 1990;10:2897–2902.
19. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000;57:925–935.
20. Isaacs EB, Lucas A, Chong WK, et al. Hippocampal volume and everyday memory in children of very low birth weight. Pediatric Res 2000;47:713–720.
21. Isaacs EB, Vargha-Khadem F, Watkins KE, et al. Developmental amnesia and its relationship to degree of hippocampal atrophy. Proc Natl Acad Sci U S A 2003;100:13060–13063.

22. Isaacs EB, Edmonds CJ, Chong WK, et al. Brain morphometry and IQ measurements in preterm children. Brain 2004;127: 2595–2607.
23. Abernethy LJ, Klafkowski G, Foulder-Hughes L, Cooke RWI. Magnetic resonance imaging and T2 relaxometry of cerebral white matter and hippocampus in children born preterm. Pediatric Res 2003;54:868–874.
24. Thompson DK, Warfield SK, Carlin JB, et al. Perinatal risk factors altering regional brain structure in the preterm infant. Brain 2007;130:667–677.
25. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med 1998;17:407–429.
26. Inder TE, Wells SJ, Mogridge NB, et al. Defining the nature of the cerebral abnormalities in the premature infant: a qualitative magnetic resonance imaging study. J Pediatr 2003;143: 171–179.
27. Kikinis R, Shenton ME, Gerig G, et al. Routine quantitative analysis of brain and cerebrospinal fluid spaces with MR imaging. J Magn Reson Imaging 1992;2:619–629.
28. Warfield SK, Kaus M, Jolesz FA, Kilinis R. Adaptive, Template moderated, spatially varying statistical classification. Med Image Anal 2000;4:43–55.
29. Mai JK, Assheuer J, Paxinos G. Atlas of the human brain. San Diego: Academic Press, 1997.
30. Duvernoy HM. The human hippocampus. An atlas of applied anatomy. Munchen, Germany: J.F. Bergmann Verlag, 1988: 166.
31. Watson C, Andermann F, Gloor P, et al. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic-resonance-imaging. Neurology 1992;42:1743–1750.
32. Bayley N. Bayley scales of infant development. 2nd ed. San Antonio, TX: The Psychological Corporation, 1993.
33. Doyle LW. Evaluation of neonatal intensive care for extremely low birth weight infants in Victoria over two decades: I. Effectiveness. Pediatrics 2004;113:505–509.
34. Woodward LJ, Anderson PJ, Austin NC, et al. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. N Engl J Med 2006;355:685–694.
35. Free SL, Bergin PS, Fish DR, et al. Methods for normalization of hippocampal volumes measured with MR. AJNR Am J Neuroradiol 1995;16:637–643.
36. Utsunomiya H, Takano K, Okazaki M, Mitsudome A. Development of the temporal lobe in infants and children: analysis by MR-based volumetry. Am J Neuroradiol 1999;20:717–723.
37. Giedd JN, Vaituzis AC, Hamburger SD, et al. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4-18 years. J Comp Neurol 1996;366:223–230.
38. Pfluger T, Weil S, Weis S, et al. Normative volumetric data of the developing hippocampus in children based on magnetic resonance imaging. Epilepsia 1999;40:414–423.
39. Inder TE, Warfield SK, Wang H, et al. Abnormal cerebral structure is present at term in premature infants. Pediatrics 2005;115:286–294.
40. Baud O, Laudenbach V, Evrard P, Gressens P. Neurotoxic effects of fluorinated glucocorticoid preparations on the developing mouse brain: role of preservatives. Pediatr Res 2001;50: 706–711.
41. Murphy BP, Inder TE, Huppi PS, et al. Impaired cerebral cortical gray matter growth after treatment with dexamethasone for neonatal chronic lung disease. Pediatrics 2001;107:217–221.
42. Harding DR, Humphries SE, Whitelaw A, et al. Cognitive outcome and cyclo-oxygenase-2 gene (-765 G/C) variation in the preterm infant. Arch Dis Child Fetal Neonatal Ed 2007;92: F108–F112.
43. McCullough L, Wu L, Haughey N, et al. Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. J Neurosci 2004;24:257–268.
44. Bhatia S, Bookheimer SY, Gaillard WD, Theodore WH. Measurement of whole temporal-lobe and hippocampus for Mr Volumetry—normative data. Neurology 1993;43:2006–2010.
45. Cook MJ, Fish DR, Shorvon SD, et al. Hippocampal volumetric and morphometric studies in frontal and temporal lobe epilepsy. Brain 1992;115(pt 4):1001–1015.
46. Woodward LJ, Edgin JO, Thompson D, Inder TE. Object working memory deficits predicted by early brain injury and development in the preterm infant. Brain 2005;128:2578–2587.
47. Hack M, Taylor HG, Drotar D, et al. Poor predictive validity of the Bayley Scales of Infant Development for cognitive function of extremely low birth weight children at school age. Pediatrics 2005;116:333–341.
48. Naidich TP, Daniels DL, Haughton VM, et al. The hippocampal formation and related structures of the limbic lobe: anatomic-magnetic resonance correlation. In: Gouaze A, Salamon G, eds. Brain anatomy and magnetic resonance imaging. Berlin: Springer-Verlag, 1988:32–64.
49. Felderhoff-Mueser U, Rutherford MA, Squier WV, et al. Relationship between MR imaging and histopathologic findings of the brain in extremely sick preterm infants. AJNR Am J Neuroradiol 1999;20:1349–1357.
50. Lee N, Tien RD, Lewis DV, et al. Fast spin-echo, magnetic resonance imaging-measured hippocampal volume: correlation with neuronal density in anterior temporal lobectomy patients. Epilepsia 1995;36:899–904.
51. Jack C Jr, Bentley M, Twomey C, Zinsmeister A. MR imaging-based volume measurements of the hippocampal formation and anterior temporal lobe: validation studies. Radiology 1990;176: 205–209.