Impaired hippocampal development and outcomes in very preterm infants with perinatal brain injury

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ABSTRACT

Preterm infants are at high risk for brain injury during the perinatal period. Intraventricular hemorrhage and periventricular leukomalacia, the two most common patterns of brain injury in prematurely-born children, are associated with poor neurodevelopmental outcomes. The hippocampus is known to be critical for learning and memory; however, it remains unknown how these forms of brain injury affect hippocampal growth and how the resulting alterations in hippocampal development relate to childhood outcomes. To investigate these relationships, hippocampal segmentations were performed on term equivalent MRI scans from 55 full-term infants, 85 very preterm infants (born ≤ 32 weeks gestation) with no to mild brain injury and 73 very preterm infants with brain injury (*e.g.*, grade III/IV intraventricular hemorrhage, post-hemorrhagic hydrocephalus, cystic periventricular leukomalacia). Infants then underwent standardized neurodevelopmental testing using the Bayley Scales of Infant and Toddler Development, 3rd edition at age 2 years, corrected for prematurity. To delineate the effects of brain injury on early hippocampal development, hippocampal volumes were compared across groups and associations between neonatal volumes and neurodevelopmental outcomes at age 2 years were explored. Very preterm infants with brain injury had smaller hippocampal volumes at term equivalent age compared to term and very preterm infants with no to mild injury, with the smallest hippocampi among those with grade III/IV intraventricular hemorrhage and post-hemorrhagic hydrocephalus. Further, larger ventricle size was associated with smaller hippocampal size. Smaller hippocampal volumes were related to worse motor performance at age 2 years across all groups. In addition, smaller hippocampal volumes in infants with brain injury were correlated with impaired cognitive scores at age 2 years, a relationship specific to this group. Consistent with our preclinical findings, these findings demonstrate that perinatal brain injury is associated with hippocampal size in preterm infants, with smaller volumes related to domain-specific neurodevelopmental impairments in this high-risk clinical population.

1. Introduction

Despite advances in neonatal and perinatal care, prematurely-born infants remain at high risk for brain injury (Ment et al., 2009) and neurodevelopmental impairment (Baron and Rey-Casserly, 2010). Indeed, outcomes for infants with forms of brain injury common in this population, including intraventricular hemorrhage (IVH), post-hemorrhagic hydrocephalus (PHH) and cystic periventricular leukomalacia (cPVL), are among the worst in newborn medicine, with rates of cognitive and motor deficits as high as 85% in some clinical populations (Rifai and Tawil, 2015). Recent advances in neuroimaging have enabled improved characterization of the deleterious effects of premature birth and brain injury on brain growth and development, demonstrating regional and brain-wide effects. While these previous

studies have demonstrated links between preterm brain injury and adverse neurodevelopmental outcomes, the mechanisms underlying these deficits remain unclear (Adams-Chapman et al., 2008; Anderson et al., 2017; Ment et al., 2005).

Across multiple neuroimaging investigations, reductions in both global and region-specific brain volumes have been demonstrated in preterm children in comparison to full-term (FT) peers. Due to its integral role in learning and memory and associations with neurodevelopmental disorders, many studies have focused on the hippocampus, a component of the limbic system which rapidly develops from mid-gestation through the first years of life (Cheong et al., 2016; Nosarti et al., 2002; Omizzolo et al., 2013; Thompson et al., 2008). Across these investigations, preterm children demonstrate impaired hippocampal growth (Beauchamp et al., 2008; Thompson et al., 2008) and folding (Thompson et al., 2013), changes attributed to clinical exposures including hypoxic-ischemic injury, glucocorticoid use and stress. Further, these changes may be clinically significant, with smaller neonatal hippocampal volumes associated with worse cognitive performance during childhood (Beauchamp et al., 2008; Thompson et al., 2008). While subjects with brain injury have been included in some prior studies, the effect of injury on hippocampal development was not an area of primary focus for these investigations. Further, while white matter injury was associated with altered hippocampal development, most investigations included only limited numbers of subjects with brain injury of varying types and severities (Beauchamp et al., 2008; Thompson et al., 2008).

The pathogenesis of preterm brain injury is complex, encompassing both direct injury and widespread indirect pathology (Volpe, 2009). IVH is the most common form of preterm brain injury, typically occurring in the first three days of life in up to 31% of very preterm infants (VPT; born ≤ 32 weeks gestation) (Christian et al., 2016; Gale et al., 2017; Radic et al., 2015; Stoll et al., 2010, 2015). The combination of intraventricular blood and periventricular hemorrhagic infarction results in suppression of cell proliferation, white matter injury and release of inflammatory cytokines and free radicals, disrupting neural cell migration and progenitor cell formation (Del Bigio, 2011; Strahle et al., 2012; Whitelaw, 2001). High-grade IVH (i.e., Papile grade III/IV) leads to hydrocephalus requiring neurosurgical intervention in up to 28% of affected neonates (i.e., post-hemorrhagic hydrocephalus; PHH) (Christian et al., 2016). PHH results from inflammation, blood breakdown product toxicity, ventricular wall disruption and cilia dysfunction, leading to impaired cerebrospinal fluid (CSF) absorption and flow through the ventricular system (Garton et al., 2016a; McAllister et al., 2017; Strahle et al., 2012). IVH-PHH has been strongly associated with poor outcomes, leading to disability across motor, cognitive, language and social domains in > 50% of infants (Adams-Chapman et al., 2008; Ment et al., 2005). In comparison, cPVL, the most severe form of white matter injury occurring in < 5% of VPT infants, results from focal, macroscopic necrosis which evolves to cystic change over weeks (Gale et al., 2017; Volpe et al., 2011). Although its pathogenic lesions are predominantly in white matter, cPVL also results in disrupted gray matter development and decreased gray matter volumes (Pierson et al., 2007; Tzarouchi et al., 2011). Despite its decreasing prevalence, the deleterious neurodevelopmental effects of cPVL are well-established and remain severe, with links to motor, cognitive, speech/language, hearing and vision impairment (Anderson et al., 2017; Hamrick et al., 2004).

While the pathogenesis of these forms of brain injury and their sequelae are well-characterized, their regional impact on developmentally important structures, including the hippocampus, remains unknown. Critically, the ventricles have a large surface area adjacent to numerous subcortical structures, including the hippocampus. Thus, ventricular/periventricular injury and inflammation, such as that occurring in association with IVH, PHH and cPVL, place the hippocampus and other periventricular structures at unique risk for injury due to their anatomic proximity (Cherian et al., 2003). In addition, our

preclinical models demonstrate increased neuronal death and smaller hippocampal volumes after IVH with PHH (Garton et al., 2016b; Lekic et al., 2012). Further, in rodent models, intracranial hemorrhage in combination with IVH, a scenario mimicking neonatal grade IV IVH, results in greater neuronal death in the hippocampus and worse cognitive outcomes compared to IVH alone (Chen et al., 2015). This constellation of preclinical findings suggests the hippocampus may be uniquely susceptible to clinically significant effects of IVH, PHH and cPVL (Cai et al., 2001; Debillon et al., 2000; Field et al., 1993; Hagberg et al., 2002; Marumo et al., 2001; Uehara et al., 1999). However, few studies examining the effects of preterm brain injury on morphological development of the human hippocampus have been performed.

We leverage advanced neuroimaging acquisition and analytic techniques to investigate the effects of preterm brain injury, including IVH, PHH and cPVL, on neonatal hippocampal development and neurodevelopmental outcomes at age 2 years in VPT children. For these investigations, we hypothesized that: 1) infants with grade III/IV IVH, PHH and cPVL would have smaller hippocampal volumes than VPT infants with no to mild brain injury and FT infants, with infants with PHH demonstrating the smallest hippocampi; 2) increased ventricular size due to brain injury would relate to smaller hippocampal volumes; and 3) smaller hippocampal size would relate to worse neurodevelopmental outcomes, with group- and domain-specific effects.

2. Materials and methods

2.1. Participants

In this longitudinal, observational study, VPT infants (born ≤ 32 weeks gestation) were recruited from the St. Louis Children's Hospital Neonatal Intensive Care Unit from 2007 to 2015. A subset of the VPT infants was specifically enrolled due to concern for brain injury (e.g., grade III/IV IVH, PHH, cPVL) identified on cranial ultrasound within the first month of life. Healthy, FT infants (born ≥ 36 weeks gestation) were recruited from the Newborn Nursery at Barnes-Jewish Hospital to serve as a comparison cohort. All FT infants had no history of in utero illicit substance exposure and no evidence of acidosis (pH < 7.20) on umbilical cord gas assessments. FT infants were sociodemographically matched to the VPT cohort. In both groups, infants were excluded if found to have chromosomal abnormality or suspected/proven congenital infection. The study was approved by the Human Research Protection Office of the study site. Parental informed consent was obtained for each subject prior to participation.

Detailed clinical information was systematically recorded for all infants. These data were used to develop markers of risk reflecting the severity of clinical illness for VPT infants based upon an established medical risk index (Woodward et al., 2012). Variables included: small for gestational age/intrauterine growth restriction (IUGR; weight ≤ 2 SD below mean), oxygen therapy at 36 weeks, maternal antenatal steroids (no = 1, yes = 0), received postnatal dexamethasone (no = 0, yes = 1), necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), retinopathy of prematurity (ROP), culture-positive sepsis, change in weight-for-height/length standard deviation score (SDS; ≥ 3 SD from birth to term equivalent) and length of total parenteral nutrition (above/below upper quartile). For each variable, data were dichotomized into those that met (1) and did not meet (0) clinical risk criteria. The dichotomous variables were summed across measures to create a Clinical Medical Risk Index (scale 0–10) (Lean et al., 2018).

2.2. Magnetic resonance imaging (MRI) scanning

VPT infants underwent MRI at term equivalent (36–40 weeks postmenstrual age). FT infants underwent scans during the first four days of life. All infants were imaged without sedation during natural sleep or while resting quietly (Mathur et al., 2007). The timing of scan acquisition for all VPT subjects was determined by clinical status and medical
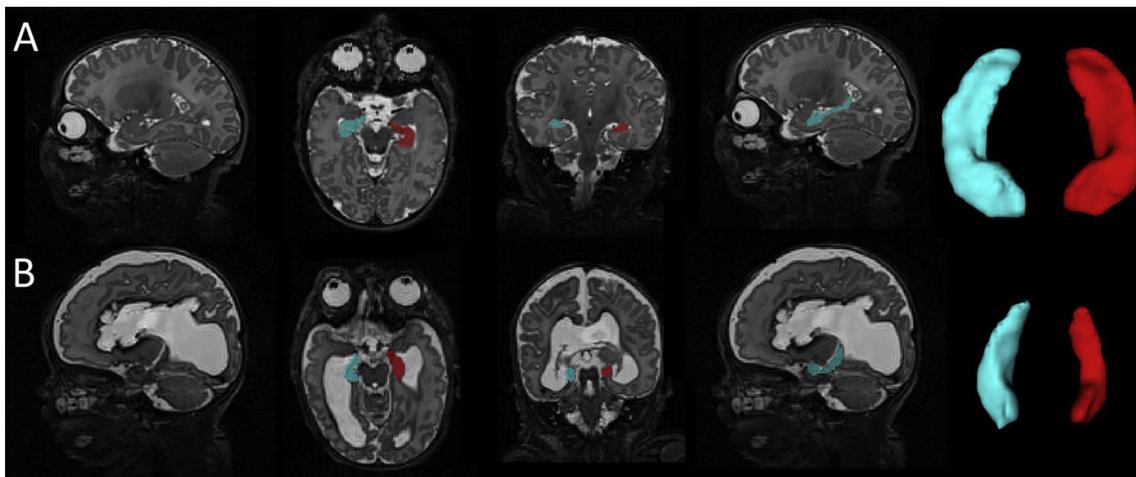
Fig. 1. Hippocampal segmentations in full-term infant and very preterm infant with brain injury. T2-weighted MRI images demonstrating axial, coronal and sagittal views and 3D volumetric reconstructions of segmented hippocampi bilaterally for a representative individual A) full-term infant and B) very preterm infant with post-hemorrhagic hydrocephalus. Note the gross difference in hippocampal volumes between the control and injured infants. Blue = right and red = left hippocampus.

course. MRI data were collected using a Siemens Trio 3 T scanner using an infant-specific head coil. T2-weighted data were acquired with the following sequence parameters: TR 8600 ms; TE 161 ms; voxel size $1 \times 1 \times 1$ mm^3. High-quality (*i.e.,* low motion) T2 data were available for all infants.

2.3. Volumetric measures

T2-weighted MRI data initially underwent automated segmentation using Morphologically Adaptive Neonatal Tissue Segmentation (MANTiS) (Beare et al., 2016), followed by manual editing of hippocampal results using ITK-SNAP software by a single highly-experienced rater (DA) (Fig. 1). For this procedure, outlines were inspected and adjusted in the coronal view of the T2-weighted image from posterior to anterior sections. The segmentations were subsequently modified in the axial and sagittal views. The left and right hemispheres were independently outlined. Anatomical boundaries generally followed the approaches of Watson and Thompson, with reference to an anatomic atlas (Duvernoy, 2005; Thompson et al., 2011; Watson et al., 1992). Intracranial volume (ICV) masks were also created in MANTiS with manual modifications. Hippocampal volumes were then divided by the ICV to generate standardized corrected hippocampal (cHC) volumes, which are displayed as ratios without units of measurement (Gilmore et al., 2010; L. G. Matthews et al., 2018; Paniagua et al., 2017; Thompson et al., 2008; Zacharia et al., 2006).

From the T2-weighted axial images, ventricular size was estimated using the Fronto-Occipital Horn Ratio (FOHR) (O'Hayon et al., 1998), a standard measure employed in the pediatric neurosurgical literature and validated *versus* ventricular volumes in infantile hydrocephalus (Ragan et al., 2015). Total maximal frontal and occipital horn widths of the lateral ventricles were measured manually by the same rater and used to calculate FOHR for each subject. To account for asymmetric hemorrhage and ventricular size, unilateral left- and right-sided FOHRs were also calculated, using the interhemispheric fissure as the central landmark.

2.4. Brain injury categorization

Three clinical raters (JS, RT, CS) reviewed T2-weighted images for structural abnormalities and presence/absence of focal, extensive or cystic white matter lesions. Multiple cranial ultrasounds obtained as part of routine clinical care for each subject were also reviewed for the presence of injury. All VPT subjects with moderate-severe white matter injury or IVH were further sub-categorized according to the presence of:

1) Papile grade III/IV IVH (Papile et al., 1978); 2) Papile grade III/IV IVH with PHH requiring neurosurgical intervention prior to term equivalent (TE) MRI (*e.g.,* reservoir, ventriculoperitoneal shunt placement, endoscopic third ventriculostomy with choroid plexus cauterization [ETV-CPC]); or 3) cPVL based upon the maximum injury grade reported (Fig. 2).

### 2.5. Neurodevelopmental and behavioral testing

Subjects returned for standardized developmental testing at age 2 years, corrected for prematurity. A blinded psychometrician assessed subjects using the Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III), generating composite scores in three domains: motor, language and cognitive performance (Bayley, 2006).

Parents also completed the Infant and Toddler Social Emotional Assessment (ITSEA) questionnaire. The ITSEA generated t-scores in the domains of Externalizing Behavior, Internalizing Behavior, Dysregulation and Competence (Carter et al., 2003). The Competence domain was most relevant to this study, as low Competence is related to symptoms of autism or attention deficit hyperactivity disorder, both of which have been correlated with hippocampal size and have increased incidence among children born VPT and/or with perinatal brain injury (Hoogman et al., 2017; Movsas et al., 2013; Rogers et al., 2012; Schendel and Bhasin, 2008; Stjernqvist and Svenningsen, 1999; Sussman et al., 2015).

Parents provided standardized sociodemographic information, which was used to define sociodemographic risk using a composite index modeled after Hack and Whitaker (Hack et al., 1992; Whitaker et al., 1996). The following five characteristics were coded as 1 (present) or 0 (absent) and summed to yield an index: 1) not a high school graduate, 2) African-American, 3) public insurance, 4) gave birth at age 18 or younger and 5) single parent household. When data were missing for one or two components, the mean of the remaining components was substituted for the missing one(s) in calculating the sum. Sociodemographic risk was not calculated for individuals missing three or more items (Lean et al., 2018).

2.6. Statistical analysis

Analyses were performed using SPSS version 24 (IBM Corporation, New York). Left and right cHC volumes and neurodevelopmental outcomes (Bayley-III composite and ITSEA competence scores) were compared in the context of demographic and clinical factors. Independently significant variables were determined using stepwise
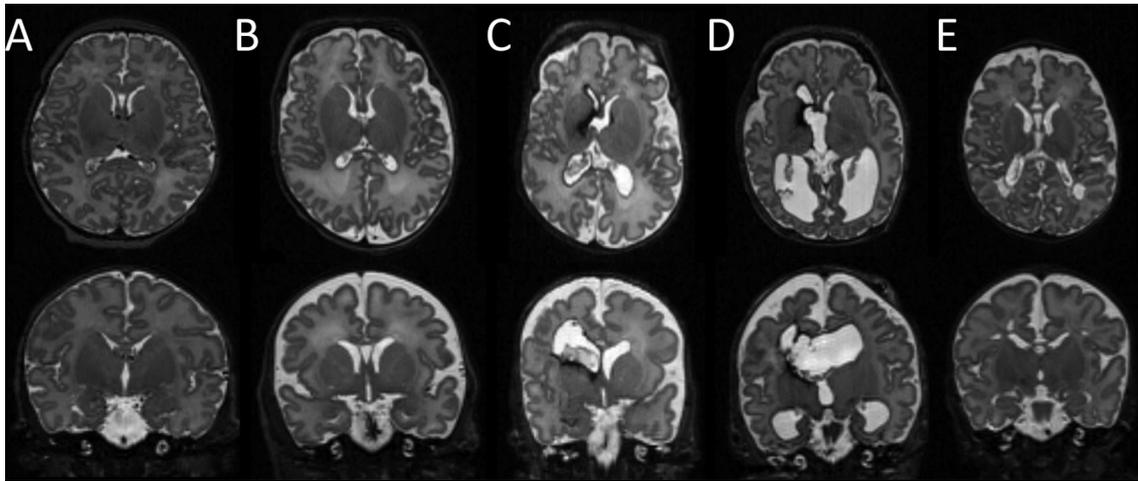
Fig. 2. Injury categories determined by MRI results. Axial T2-weighted MRI scans demonstrating representative individual infants in each brain injury category. A) Full-term infant; B) very preterm infant without BI, note dolichocephalic shape and decreased folding but otherwise normal anatomy; C) very preterm infant with grade III/IV intraventricular hemorrhage (IVH), note presence of intra- and periventricular hemorrhage; D) very preterm infant with IVH and post-hemorrhagic hydrocephalus (PHH) requiring neurosurgical intervention, note dilated lateral ventricles; and E) very preterm infant with cystic periventricular leukomalacia (cPVL), note cysts surrounding the lateral ventricles. All MRI scans performed at term equivalent postmenstrual age.

linear regression and run individually for left and right cHC volumes as dependent variables with group (full-term, VPT, brain injury [BI]), medical risk score, sex, race, gestational age at birth and postmenstrual age at scan entered into models in these analyses. Gestational age at birth and birthweight were found to be collinear; therefore birthweight was excluded from all models. Surviving variables were entered into linear mixed models with clustering between siblings entered as a random effect in the model.

Stepwise linear regression was repeated using Bayley-III motor, language and cognition and ITSEA competence scores as the dependent variables and group, left and right cHC volumes, sex, medical risk score, sociodemographic risk score, gestational age at birth and postmenstrual age at scan as independent variables. Race and sociodemographic risk scores were found to be collinear; therefore, race was excluded from all models. Surviving variables were again corrected for sibling effects using linear mixed models.

The outlined injury sub-categories were assessed individually in secondary analyses of VPT infants. Further, clinical variables shown to have significant effects on hippocampal development in prior studies, including components of the medical risk composite score, were investigated in secondary analyses. These included exposure to antenatal and postnatal steroids, indomethacin, necrotizing enterocolitis (NEC), confirmed sepsis and chorioamnionitis (Hatfield et al., 2011; Thompson et al., 2008).

3. Results

3.1. Clinical characteristics

A total of 213 infants were recruited and satisfied entry criteria. The FT group included 55 infants (29 male) with a mean gestational age of 39.2 weeks. The VPT group included 85 infants with no to mild BI (37 male) with a mean gestational age of 26.7 weeks. The BI group included 73 infants with BI (46 male) with a mean gestational age of 25.4 weeks. Clinical characteristics across each group are included in Table 1.

3.2. Hippocampal volumes

3.2.1. Brain injury

Stepwise linear regression demonstrated differences in corrected hippocampal volumes between males and females and between FT, VPT and BI infants (Table 2). In these analyses, males had smaller left and

right cHC volumes than females across all groups (left $p = .005$, right $p = .004$). VPT and BI infants had smaller cHC volumes than FT infants (VPT left 7% reduction, $p = .039$; right 7% reduction, $p = .002$; BI left 19% reduction, $p < .001$; right 21% reduction, $p < .001$; Fig. 3). Further, BI infants had smaller cHC volumes than VPT infants (left 12% reduction, $p < .001$; right 15% reduction, $p < .001$). Linear mixed models were performed to control for sibling confounders and evaluate for interactions between sex and infant group. No interactions were identified.

To evaluate for potential unanticipated effects from ICV correction, uncorrected HC volumes were also analyzed using identical procedures. These analyses demonstrated comparable findings across all results (Supplementary Table 1).

3.2.2. Ventricular size

Unilateral FOHR was used to investigate the effects of unilateral or asymmetric injury on cHC size. Across all three infant groups, larger ventricular sizes were associated with smaller cHC volumes (left $r = -0.70$, right $r = -0.67$), with significant associations for VPT and BI infants (Fig. 4). Among BI infants, these negative relationships were strongest for infants with IVH alone and PHH (Supplementary Table 2).

3.2.3. Clinical variables

As both VPT and BI infants displayed smaller cHC volumes, we evaluated the association of clinical characteristics, including medical risk composite score, sex, race, gestational age at birth and postmenstrual age at scan with cHC volumes across groups. For these analyses, birth weight was excluded based on collinearity with gestational age at birth ($r = 0.79$, $p < .001$). In these results, left cHC volumes were related to group, postmenstrual age at scan and gestational age at birth ($\beta = -0.46$, $p < .001$; $\beta = -0.25$, $p = .001$; $\beta = -0.20$, $p = .007$, respectively). Right cHC volumes were related to group, postmenstrual age at scan, gestational age at birth and sex ($\beta = -0.48$, $p < .001$; $\beta = -0.22$, $p = .002$; $\beta = -0.22$, $p = .002$; $\beta = 0.14$, $p = .03$, respectively). There were no significant interactions. As in prior analyses, males had smaller left and right cHC volumes than females across both groups (Table 3). All infants with brain injury had smaller cHC volumes (left and right) than infants with no to mild brain injury. Specifically, infants with PHH had smaller cHC volumes (left and right) than infants with IVH alone and those with cPVL (Table 3, Fig. 3). In this cohort, postmenstrual age at scan and medical risk composite score were moderately correlated ($r = 0.51$, $p < .001$), with

Table 1
Clinical characteristics.

	Total $N = 213$	Full-Term n = 55	Very Preterm $n = 85$	Brain Injury $n = 73$
GA (weeks), mean (SD)	–	39.2 (1.2)**	26.7 (1.8)**	25.4 (1.9)**
PMA at time of scan (weeks), mean (SD)	–	39.4 (1.2) ‡	37.8 (1.5) ‡†	39.2 (2.4) †
Birthweight (grams), mean (SD)	–	3314 (452)‡**	951 (258)‡	874 (222)*
Male, n (%)	112 (53)	29 (53)	37 (44)	46 (63)
African American, n (%)	110 (52)	33 (60)	38 (45)	53 (54)
Siblings, n (%)	33 (15)	1 (3)	27 (32)	5 (7)
Sociodemographic risk score, mean (SD)	–	1.85 (1.5)	1.37 (1.2)†	2.0 (1.4) †
IVH, n (%)	–	–	–	33 (45)
PHH, n (%)	–	–	–	27 (37)
cPVL, n (%)	–	–	–	13 (18)
Clinical medical risk index, mean (SD)	–	–	1.9 (1.8)†	3.1 (1.9)†
Intrauterine Growth Restriction (IUGR), n (%)	–	–	5 (6)†	2 (3)†
Oxygen at 36 weeks, n (%)	–	–	45 (53)†	60 (82)†
No antenatal steroids, n (%)	–	–	8 (9)†	26 (36)†
Postnatal dexamethasone, n (%)	–	–	11 (13)	9 (12)
Necrotizing enterocolitis, n (%)	–	–	5 (6)†	22 (30)†
Patent ductus arteriosus, n (%)	–	–	46 (54)†	56 (77)†
Retinopathy of prematurity, n (%)	–	–	11 (13)†	22 (30)†
Sepsis, n (%)	–	–	21 (25)†	37 (51)†
Change in weight-for-height/length, n (%)	–	–	2 (2)	2 (3)
Prolonged TPN, n (%)	–	–	13 (15)†	26 (36)†
Chorioamnionitis, n (%)	–	–	32 (38)	27 (37)

Variables expressed using means were compared using ANOVA or t-tests. Variables expressed as frequencies compared using Chi-square.
Italicized variables were factored into clinical medical risk index.

** $p \leq .01$ when comparing between the three groups.
‡ $p \leq .01$ between FT and VPT.
* $p \leq .01$ between FT and BI.
† $p \leq .01$ between VPT and BI.

higher medical risk composite scores among patients with greater postmenstrual age at scan. This was driven by the fact that sicker patients (*i.e.*, greater medical risk composite) were often scanned later in their NICU course (*i.e.*, at later postmenstrual age); this may also explain the negative relationships between postmenstrual age at scan and cHC volumes, as smaller left and right cHC volumes were related to later postmenstrual age at scan (left and right $r = -0.35$, $p < .001$).

Relationships between cHC volumes and exposure to antenatal and

Table 2
Brain and neurodevelopmental measures.

|	FT $n = 55$ [95% CI]	VPT $n = 85$ [95% CI]	BI n = 73 [95% CI]	df	β	P
Left cHC volume	0.0027 [0.0026–0.0028]	0.0025 [0.0025–0.0026]	0.0022 [0.0021–0.0023]	211	−0.49	< 0.001**
Right cHC volume	0.0028 [0.0027–0.0029]	0.0026 [0.0025–0.0027]	0.0022 [0.0021–0.0023]	211	−0.54	< 0.001**
Motor score	103.5 [97.6–109.5]	85.2 [82.3–88.0]	68.4 [64.6–72.2]	134	−0.51	< 0.001**
Language score	102.0 [94.9–109.1]	89.3 [86.3–92.3]	77.5 [73.1–82.0]	132	−0.50	< 0.001**
Cognition score	93.9 [88.9–98.8]	86.0 [83.5–88.5]	75.0 [71.4–78.6]	137	−0.39	< 0.001**
ITSEA competence	49.5 [44.2–54.8]	43.1 [39.8–46.4]	36.5 [32.0–41.0]	132	−0.30	< 0.001**
Left FOHR	0.36 [0.36–0.37]	0.40 [0.39–0.40]	0.46 [0.44–0.48]	210	F 52.8	< 0.001**
Right FOHR	0.36 [0.36–0.37]	0.40 [0.39–0.40]	0.47 [0.45–0.49]	210	61.1	< 0.001**
	Males $n = 112$ [95% CI]	Females $n = 101$ [95% CI]		df	β	P
Left cHC volume	0.0024 [0.0023–0.0024]	0.0025 [0.0025–0.0026]		211	0.17	0.004*
Right cHC volume	0.0024 [0.0024–0.0025]	0.0026 [0.0025–0.0027]		211	0.18	0.002*

cHC = corrected hippocampal volume (ratio).
FOHR = fronto-occipital horn ratio.
Motor, Language and Cognition scores were generated using the Bayley Scales of Infant and Toddler Development, 3rd edition.
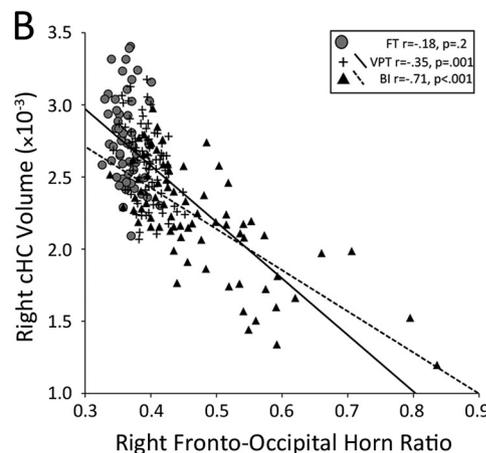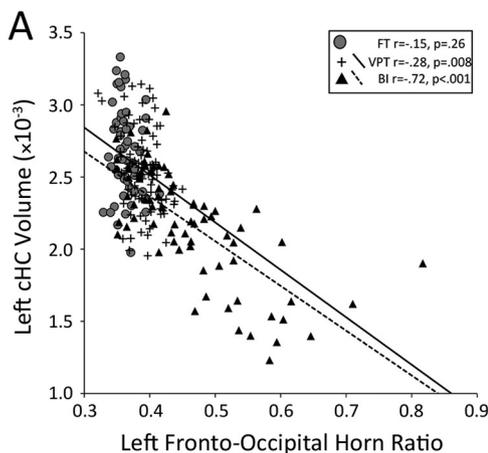All values corrected; those denoted with β-coefficients were derived from stepwise linear regression models including sex, race and medical risk composite score, while all values denoted with F statistics were derived from ANOVA.

** $p < .001$ when comparing between three groups.
* $p < .005$ when comparing between two groups.
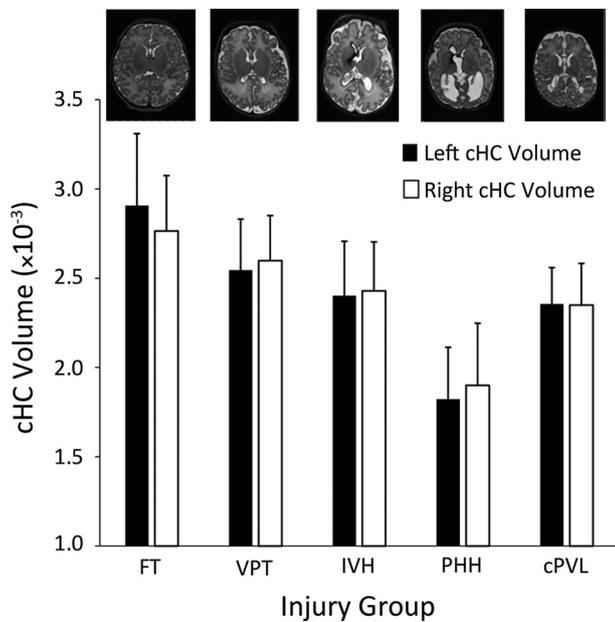
Fig. 3. Corrected hippocampal volumes across groups. Plots demonstrating corrected hippocampal volumes for each infant category, including full-term infants (FT), very preterm infants with no to mild brain injury (VPT), very preterm infants with grade III/IV intraventricular hemorrhage (IVH), very preterm infants with IVH and post-hemorrhagic hydrocephalus requiring neurosurgical intervention (PHH) and very preterm infants with cystic periventricular leukomalacia (cPVL). Overall, the PHH group had the smallest hippocampi. Black = left and white = right corrected hippocampal volumes (ratios). Error bars represent one standard deviation.

postnatal steroids, indomethacin, confirmed sepsis, chorioamnionitis and NEC were analyzed in a post-hoc fashion among VPT and BI infants. Exposure to antenatal steroids was associated with larger cHC volumes (left and right $p < .001$). In contrast, exposure to postnatal dexamethasone or hydrocortisone had no association with cHC volumes in either the VPT or BI groups. Infants with exposure to indomethacin ($n = 18$) also did not have significantly different cHC volumes. Further, there were no differences in cHC volumes among VPT or BI infants attributable to chorioamnionitis or sepsis, both in models correcting for associated variables and when using t-tests. Finally, while NEC was common in infants with brain injury, there were no differences in left or right hippocampal volumes in affected infants in the BI group (analyzed independently due to the low prevalence of NEC in the VPT group).

3.3. Neurodevelopmental outcomes at age 2 years

3.3.1. Motor

Stepwise linear regression demonstrated significant effects of infant group, medical risk scores and left and right cHC volumes with Bayley-III motor composite scores. Group, medical risk and left and right cHC volumes were subsequently included in a linear mixed model controlling for sibling effects. There were no significant interactions between these variables, indicating the relationship between larger left and right cHC volumes and higher motor scores was similar across all groups.

There were significant stepwise decreases in motor scores among FT, VPT and BI infants and significant differences in BI sub-groups (Supplementary Fig. 1A). Infants with BI had the lowest motor scores compared to FT and VPT infants, and those with cPVL scored lowest overall. As VPT and BI infants had lower motor scores, we used stepwise linear regression to evaluate the association of motor scores with more detailed clinical characteristics, including medical risk composite score, sex, social risk score, gestational age at birth, postmenstrual age at scan, group and cHC volumes. There were significant effects of group (as above; $\beta = -0.48$, $p < .001$) and medical risk composite scores (as above; $\beta = -0.23$, $p = .006$), with higher medical risk scores corresponding to lower motor scores ($r = -0.38$, $p < .001$).

3.3.2. Language

Stepwise linear regression revealed significant effects of infant group and social risk scores on language composite scores. There was no interaction between group and social risk. Secondary analyses demonstrated that language scores decreased across all three groups as social risk scores increased. The effect of cHC volumes was not significant, indicating there were no relationships between cHC volumes and language outcomes.

There were also significant stepwise decreases in language scores among FT, VPT and BI infants (Supplementary Fig. 1B). Infants with BI had the lowest language scores compared to FT and VPT infants. Among infants with BI, there were no significant differences in scores between injury sub-groups. As VPT and BI infants also had lower language scores, the same clinical characteristics were similarly evaluated using stepwise linear regression. In these analyses, there was again only a significant effect of group (as above; $\beta = -0.39$, $p < .001$).

3.3.3. Cognition

Stepwise linear regression revealed significant effects of infant group, social risk scores and left and right cHC volumes on cognitive scores. The resulting secondary analyses demonstrated that cognitive scores decreased across all three groups as social risk scores increased. In addition, there were significant interactions between infant group and left and right cHC volumes (F = 8.4, $p < .001$; F = 3.6, $p = .03$, respectively). Specifically, among the BI infants only, larger left and

Fig. 4. Corrected hippocampal volumes decrease as ventricular size increases. Scatter plots demonstrating A) left and B) right corrected hippocampal volumes (cHC; ratios) *versus* hemisphere-specific fronto-occipital horn ratios (FOHR) for infants grouped by injury category. Fit lines generated using Pearson's correlation. Circle = full-term infants (FT), cross = very preterm infants with no to mild brain injury (VPT) and triangle = very preterm infants with brain injury (BI).

Table 3
Brain and neurodevelopmental measures for brain injury groups.

|	IVH $n = 33$ [95% CI]	PHH $n = 27$ [95% CI]	cPVL $n = 13$ [95% CI]	df	β	p
Left cHC volume	0.0024 [0.0023–0.0025]	0.0018 [0.0017–0.0019]	0.0024 [0.0022–0.0025]	72	−0.24	0.036^
Right cHC volume	0.0024 [0.0023–0.0025]	0.0019 [0.0018–0.0020]	0.0023 [0.0022–0.0025]	72	−0.26	0.025^
Motor score	74.4 [69.6–79.2]	65.1 [59.0–71.2]	56.9 [45.4–68.3]	43	−0.50	< 0.001**
Language score	81.5 [74.5–88.5]	74.6 [68.7–80.5]	72.3 [56.1–88.4]	40	−0.22	0.06
Cognition score	80.4 [75.6–85.1]	69.7 [65.2–74.3]	69.4 [55.0–83.8]	44	−0.29	0.034^
ITSEA competence	41.9 [36.0–47.7]	37.9 [30.1–45.8]	21.7 [11.9–31.5]	42	−0.45	0.001**
Left FOHR	0.41 [0.39–0.43]	0.54 [0.50–0.57]	0.43 [0.39–0.47]	70	F 25.2	< 0.001**
Right FOHR	0.42 [0.40–0.44]	0.55 [0.51–0.59]	0.44 [0.40–0.47]	70	22.8	< 0.001**
	Male $n = 46$ [95% CI]	Female $n = 27$ [95% CI]		df	β	P
Left cHC volume	0.0021 [0.0020–0.0022]	0.0023 [0.0021–0.0025]		72	0.23	0.043^
Right cHC volume	0.0021 [0.0020–0.0023]	0.0023 [0.0022–0.0025]		72	0.24	0.034^

IVH = Papile grade III/IV intraventricular hemorrhage; PHH = post-hemorrhagic hydrocephalus requiring neurosurgical intervention; cPVL = cystic periventricular leukomalacia; cHC = corrected hippocampal volume (ratio); FOHR = fronto-occipital horn ratio.
Motor, Language and Cognition scores generated using Bayley Scales of Infant and Toddler Development, 3rd edition.
All values corrected; those denoted with β-coefficients were derived from stepwise linear regression models including sex, race and medical risk composite score, while all values denoted with F statistics were derived from ANOVA.
^ $p < .05$.
** $p \leq .001$.
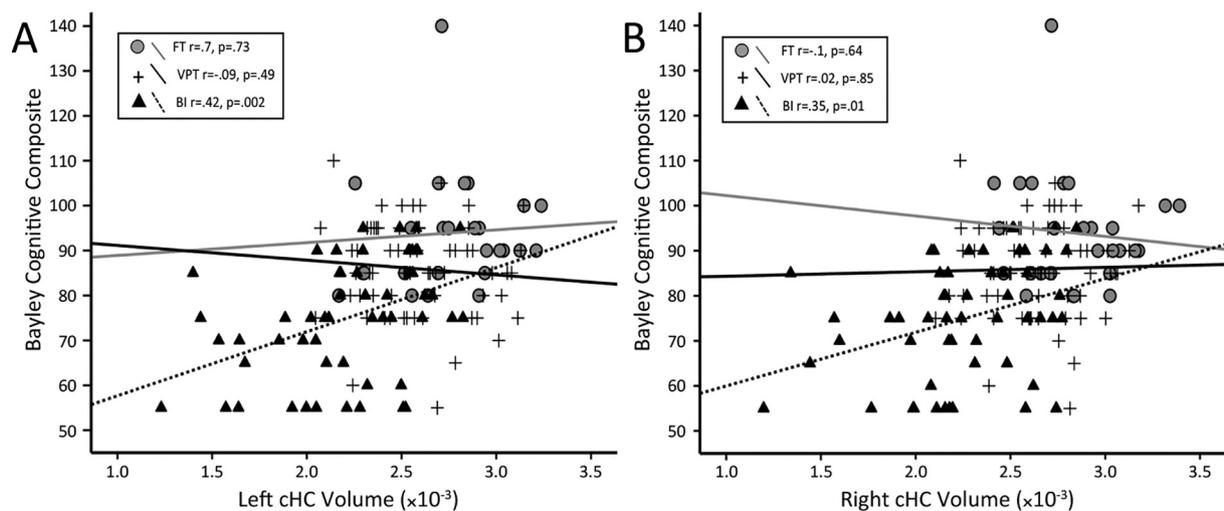
Fig. 5. Relationship between corrected hippocampal volumes and cognitive outcomes. Scatter plots demonstrating relationships between neonatal A) left and B) right corrected hippocampal volumes (cHC; ratios) and Bayley Scales of Infant and Toddler Development, 3rd edition cognitive composite scores at age 2 years for each group. Note that the very preterm infants with brain injury demonstrate strong positive relationships unique to this group. Circle = full-term infants (FT), cross = very preterm infants with no to mild brain injury (VPT) and triangle = very preterm infants with brain injury (BI).

right cHC volumes were associated with higher cognitive scores ($r = 0.42$, $p = .001$; $r = 0.35$, $p = .005$, respectively Fig. 5).

There were also significant decreases in cognitive scores among FT, VPT and BI infants without significant differences between BI subgroups (Supplementary Fig. 1C). Infants with BI had the lowest cognitive scores compared to FT and VPT infants, and those with cPVL and PHH scored lowest overall. For cognition scores, on stepwise linear regression including clinical characteristics, there were significant effects of group (as above; $β = −0.24$, $p = .019$), left and right corrected hippocampal volumes (as above; $β = 0.26$, $p = .008$; $β = 0.28$, $p = .005$, respectively) and gestational age at birth ($β = 0.19$, $p = .03$).

Earlier gestational age at birth corresponded to lower cognitive scores ($r = 0.28$, $p = .002$).

3.3.4. ITSEA competence

Stepwise linear regression demonstrated a significant effect of group and social risk scores on ITSEA competence scores. Secondary analyses demonstrated that ITSEA competence scores and social risk scores were negatively correlated ($r = −0.33$, $p < .001$). The effect of cHC volumes was not significant, indicating no relationship between cHC volumes and ITSEA competence scores.

Infants with BI had the lowest competence scores, with no

difference in scores between FT and VPT infants (Supplementary Fig. 1D). Among infants with BI, those with cPVL had the lowest competence scores, without significant differences between infants with IVH and PHH. When evaluating the effects of clinical characteristics on ITSEA Competence scores, there were significant effects of group (as above; β = −0.21, *p* = .03), social risk scores (as above; β = −0.28, *p* = .003) and sex (β = −0.22, *p* = .017). Females had lower competence scores than males. Additionally, there was a significant effect of the right cHC (β = 0.25, *p* = .008), with lower ITSEA competence scores related to smaller right cHC volumes (*r* = 0.21, *p* = .027).

4. Discussion

4.1. Summary of findings

Using advanced neuroimaging methods and gold standard manual hippocampal segmentations, we demonstrate that preterm brain injury, including IVH, PHH and cPVL, is associated with smaller hippocampal volumes. This work is consistent with our preclinical findings of hippocampal injury in IVH-PHH (Garton et al., 2016b). VPT infants with brain injury demonstrated smaller cHC volumes than VPT infants with no to mild brain injury and FT infants. Within the BI group, infants with PHH had the smallest hippocampal volumes. Further, in infants with brain injury, larger ventricle size was associated with smaller hippocampal volumes. Sex differences were noted across all infant groups, consistent with prior reports. While antenatal steroids related to larger hippocampal volumes, we found no relationship between postnatal steroid administration and hippocampal size. BI infants demonstrated the worst neurodevelopmental performance across all domains. Larger neonatal hippocampal volumes were associated with better motor outcomes at age 2 years across all infant groups. However, the relationship between larger hippocampal volumes and improved cognitive outcomes was unique to BI infants, a finding with important implications for improving our understanding of the pathophysiology underlying IVH-PHH and the relationships between these forms of brain injury and neurodevelopmental outcomes.

4.2. Relation to previous studies of hippocampal development in prematurely-born children

4.2.1. Hippocampal size

Recent studies in infants and older children have provided converging evidence of impaired hippocampal growth and development in children born preterm (Beauchamp et al., 2008; Nosarti and Froudist-Walsh, 2016; Omizzolo et al., 2013; Thompson et al., 2008, 2013). These investigations demonstrate both hippocampal volume and shape are altered in VPT infants in comparison to FT peers, differences attributed to exposures to stress, metabolic insults and hypoxic-ischemic injury. These patterns persist into childhood and adolescence, with VPT children continuing to have smaller hippocampi later in life (Nosarti et al., 2002), commonly in association with other volumetric reductions in the white and gray matter and cerebellum (de Kieviet et al., 2012). However, these differences did not remain significant across all studies when accounting for differences in brain size. This suggests the hippocampus is susceptible to the same deleterious factors impairing growth brain-wide, though whether it possesses unique vulnerability to these effects remains unclear. Our finding of smaller hippocampal volumes in the VPT and BI cohorts is consistent with these data, though we identified differences in both uncorrected values and those corrected for ICV. Further, the magnitude of the reductions in hippocampal volumes was greater in our cohort than the literature, which may relate to differences in medical risk.

4.2.2. Brain injury

Reductions in hippocampal volumes were most prominent in VPT infants with BI, with the magnitude of differences dependent upon injury type and severity. Prior studies in this population have included only limited numbers of infants with white matter injury assessed using qualitative injury scoring systems centered on white matter volumes, myelination and injury. These studies demonstrated white matter injury to be an important determinant of hippocampal size from infancy through adolescence (Nosarti et al., 2002; Thompson et al., 2008). However, the single study evaluating effects of high-grade IVH on hippocampal development included only seven affected children and failed to identify differences (Thompson et al., 2013). Our study includes large numbers of infants with high-grade IVH and PHH, an important clinical population with high rates of disability. Further, we investigated infants identified to have each of these common forms of injury. As hypothesized, injured infants were found to have smaller hippocampi than term and VPT infants with no to mild BI, with infants with PHH having the smallest hippocampi. These differences suggest subtype-specific injury relationships, consistent with our preclinical data (Garton et al., 2016b).

4.2.3. Other clinical variables

While brain injury was the focus of this investigation, we evaluated additional clinical and demographic predictors previously linked to hippocampal development and outcome. We found the right hippocampus to be larger than the left across groups, including in BI infants, similar to previous investigations (Pfluger et al., 1999; Thompson et al., 2009). Also consistent with prior reports, we found differences in hippocampal volumes based upon sex, with males having smaller hippocampi than females across all groups, including in injured infants. The exact mechanisms underlying these differences remain unknown, with prior reports implicating genetic and/or environmental variables (Peper et al., 2007; Stefanis et al., 1999). However, these findings highlight that these hemispheric and sex-related differences may begin *in utero* and persist even when growth trajectories are altered by premature birth and/or brain injury. Interestingly, this differs in comparison to other regional brain volumes where there have been no sex differences identified in preterm children (Thompson et al., 2007). Further, these differences may be clinically significant, with hippocampal size previously related to hyperactivity in girls but not boys (Rogers et al., 2012).

In our investigation, VPT infants with and without brain injury exposed to antenatal steroids had larger hippocampal volumes. However, it is worthwhile to note that more VPT infants with no to mild BI were exposed to antenatal steroids (91%) than VPT subjects with BI (64%). The literature is mixed with respect to antenatal steroids and the developing brain; while clinical studies showed improved outcomes and decreases in BI with a single dose of betamethasone (Baud et al., 1999; Roberts et al., 2017), preclinical studies demonstrated neuronal loss in the hippocampus (Bustamante et al., 2014; S. G. Matthews, 2001; Noorlander et al., 2014). The hippocampus is also known to be sensitive to corticosteroids during early development, and postnatal steroid administration may worsen the response to injury (Tombaugh et al., 1992). Prior studies demonstrated exposure to postnatal dexamethasone impaired hippocampal growth (Thompson et al., 2008), findings not apparent in our cohort. However, only modest numbers of subjects in our study were exposed to postnatal dexamethasone (27% of VPT infants with no to mild BI and 32% of VPT infants with moderate or severe BI). These discrepancies may also be secondary to differences in postnatal steroid selection (*i.e.,* hydrocortisone *versus* dexamethasone *versus* both) and administration practices and/or medical comorbidities in the uninjured VPT population between ours and prior studies (Kidokoro et al., 2014).

Finally, the effects of perinatal clinical risk factors common in preterm infants, including sepsis, NEC, chorioamnionitis and indomethacin exposure, have been extensively studied, with smaller hippocampi and larger ventricles among affected infants (Hatfield et al., 2011). While rates of some factors, such as NEC, were higher in the brain injury group, targeted *post hoc* investigations in our cohort

demonstrated no independent relationships between these clinical exposures and hippocampal volumes in the BI group.

4.2.4. Outcomes

The hippocampus is known to be critical to learning and memory (Bohbot et al., 2000; Cabeza and Nyberg, 2000; Nadel et al., 2000; Nelson et al., 2001). Longitudinal studies in VPT cohorts suggest neonatal hippocampal volumetric measurements may provide valuable information regarding cognitive and memory performance during early and middle childhood. For example, smaller hippocampal volumes have been associated with greater visual motor impairment in very low birthweight infants and worse cognition in small for gestational age infants (Martinussen et al., 2009). Further, prior studies demonstrated an association between developmental outcome measures and hippocampal size, where neonatal left hippocampal volumes predicted mental developmental index scores on the Bayley Scales of Infant Development, 2nd edition at age 2 years (Thompson et al., 2008). In our study, smaller hippocampal volumes were associated with worse motor outcomes across all infant groups, consistent with these prior studies which suggested this pattern may reflect global negative associations with perinatal events and/or treatments on both hippocampal development and early motor outcomes rather than a causative relationship (Thompson et al., 2008). However, we also found that smaller hippocampal size was associated with worse cognitive outcomes only in the BI group, suggesting a unique relationship may exist between brain structure and function in the setting of brain injury in this high-risk population.

The persistence and nature of these longitudinal relationships remains to be determined. In a longitudinal study of VPT children, larger neonatal hippocampal volumes were associated with better learning and memory performance at age 7 years, though similar results were not identified for hippocampal shape (Thompson et al., 2013). Further, across multiple studies of older children and adolescents born prematurely, smaller and/or altered hippocampi have been associated with impaired cognition and memory (Abernethy et al., 2004; Giménez et al., 2004; Isaacs et al., 2004, 2000; Lodygensky et al., 2005). For example, reductions in hippocampal volumes have been associated with worse verbal learning and recognition during adolescence (Giménez et al., 2004). Further, in neurologically normal children born preterm, those who displayed larger declines in intelligence quotient (IQ) from childhood to adolescence had smaller right and left hippocampi than those who exhibited smaller declines in IQ (Isaacs et al., 2004). Finally, early studies reported smaller hippocampal volumes were associated with cognitive deficits in preterm children (Isaacs et al., 2000; Peterson et al., 2000). However, similar relationships have not been identified in more recent studies investigating comparable measures obtained cross-sectionally during childhood or evaluating changes in hippocampal size and folding over time (Omizzolo et al., 2013). This suggests the need for additional targeted investigation to comprehensively define these relationships, including in children with brain injury.

4.3. Pathophysiology of brain injury effects on hippocampal development

This study demonstrated impaired hippocampal development and worse cognitive outcomes in VPT infants with common patterns of preterm brain injury, including grade III/IV IVH and PHH. The etiology of volumetric changes of the hippocampus and other brain structures in the uninjured preterm infant likely relate to disrupted development of pre-oligodendrocytes, subplate neurons and axons due to stresses on the neonate (Volpe, 2009). Our study is the first to delineate volumetric changes to the hippocampus after IVH and PHH, extending findings from our preclinical work (Garton et al., 2016b). The hippocampus is located adjacent to the temporal horn of the lateral ventricle; thus, it may be exposed to direct effects of IVH-induced ependymal injury in the temporal horn, presumably through an iron-mediated or inflammatory pathway based upon our rodent model data (Garton et al.,

2017). As the intraparenchymal component of hemorrhage results in greater injury to the hippocampus compared to IVH alone in preclinical models, there may also be other pathways involved (Chen et al., 2015). Relatedly, in preterm infants with germinal matrix hemorrhage alone, there is suppression of cell proliferation in the ganglion eminence which may be indicative of more widespread effects including a smaller hippocampus (Del Bigio, 2011). This constellation of findings suggests that following IVH and parenchymal perihemorrhagic infarction (i.e., grade IV IVH), there may be injury to the hippocampus and surrounding subcortical structures through several pathways related to iron, hemoglobin (Strahle et al., 2014) and inflammation (Gram et al., 2014); however, the exact mechanisms remain to be determined. Further, there may be other variables that play roles in modifying this relationship across clinical settings, including degree of prematurity, increased intraventricular/intracranial pressure and environmental and/or pharmacologic exposures (Morales et al., 2015; Urlesberger et al., 1991).

Animal models of PVL also demonstrated injury and microglial activation in the hippocampus following experimental PVL; however, the exact mechanism of these changes is not yet clear (Cai et al., 2001; Debillon et al., 2000; Field et al., 1993; Hagberg et al., 2002; Marumo et al., 2001; Uehara et al., 1999). In these models, periventricular white matter injury is induced through infectious as well as hypoxic-ischemic models of PVL, and the same pathophysiologic processes that result in injury to the white (and gray) matter around the ventricle likely play a role in injury to the adjacent hippocampus. Oligodendrocyte progenitor cells and activated microglia are implicated in the pathogenesis of PVL and have been targeted therapeutically in preclinical models with erythropoietin and minocycline, respectively (Fan et al., 2005; Mizuno et al., 2008).

4.4. Post-hemorrhagic hydrocephalus and ventricular size

CSF volume (both intra- and extra-axial) in preterm infants is approximately twice that of term infants (Thompson et al., 2007). In this population, ventriculomegaly in the setting of IVH is likely a distinct clinical entity from PHH requiring surgical treatment, often persisting into adolescence and adulthood (Nosarti et al., 2002). As infants with ventricular dilation secondary to hydrocephalus after IVH are known to be a unique high-risk population, we evaluated infants requiring clinical neurosurgical intervention (e.g., reservoir, ventriculoperitoneal shunt, ETV-CPC) as a separate group. Indeed, we found that among all infants, those with PHH had the smallest cHC volumes. This remained true even when comparing uncorrected hippocampal volumes, eliminating the possibility this finding was confounded by increases in ICV secondary to hydrocephalus. Ventricular size was analyzed separately for VPT infants with IVH and cPVL, and similarly found to be inversely related to hippocampal size. In these infants, larger ventricle size may relate to ependymal expansion or ex vacuo changes from injury around the ventricle wall. Thus, forms of injury resulting in larger ventricle size may similarly affect the adjacent hippocampus. While prior reports investigating ventricular size in IVH have shown smaller deep gray matter and cerebellar volumes at term equivalent age in preterm infants with larger ventricles, the hippocampus has not been examined (Brouwer et al., 2016). These findings across groups, injury types and structures suggest the relationship between smaller hippocampal volume and larger ventricle size in infants with PHH may be the result of a complex pathophysiologic process, including contributions from variables such as degree of prematurity, inflammation, increased intraventricular pressure, clinical and/or pharmacologic exposures in the NICU, and factors related to surgical treatment of PHH including anesthesia (Morales et al., 2015; Urlesberger et al., 1991; Wellons et al., 2013). Further, our data demonstrate these infants have the worst outcomes among all neonatal groups, with cognitive outcomes associated with hippocampal size in VPT infants with BI. These findings are consistent with previous reports detailing worse neurodevelopmental

outcomes in IVH-PHH dependent upon ventricular size (Srinivasakumar et al., 2013).

Based upon the anatomic proximity of the hippocampi to the lateral ventricles, prior literature suggests increases in intracranial pressure in the setting of PHH may also contribute to this effect, although the relationship is likely complex, as even those infants who do not require treatment for hydrocephalus are likely to have transiently elevated intracranial pressures (Morales et al., 2015; Urlesberger et al., 1991; Wellons et al., 2013). Although some have proposed definitions of ventricular size cut-offs based on normative values for ventriculomegaly (Davies et al., 2000; Levene, 1981), there is not a universally accepted cut-off nor one that is known to predict worse outcomes. However, as our understanding of the etiology of PHH is expanding, there are likely widespread processes that concurrently contribute to brain injury and hydrocephalus. For example, blood breakdown products within CSF circulate throughout the ventricular system in infants with PHH resulting in injury mediated through the toxic effects of iron or white blood cell-associated inflammation (Garton et al., 2016a). This suggests the pathway to improved neurodevelopmental outcomes in this population extends beyond decreasing ventricular size and maintaining lower intracranial pressures alone (Kulkarni et al., 2017).

4.5. Caveats, limitations and future directions

Our rigorous data quality criteria resulted in a sample size comparable to prior investigations, though with a much greater number of injured infants. Despite this relative large total sample size, the number of infants in each injury subcategory was small for some diagnoses, limiting statistical power for some group comparisons. This cohort demonstrated medical comorbidities common in this clinical population (Table 1). Across all groups there was an imperfect balance with respect to sex, and there was a greater proportion of male infants in the brain injury cohort than other groups. While relationships between these clinical variables and key results were carefully examined, their effects may be incompletely assessed using the medical risk composite score. In addition, while the hippocampi were identified using gold standard manual segmentations generated by a highly experienced rater using images acquired with MRI sequences optimized for tissue contrast, segmentation of small structures in infants with heterogeneous brain injury is technically challenging. We employed standardized neuroanatomical boundaries, rigorous data quality criteria and methods designed to reduce error in measured results. However, it remains possible our methods may not fully account for variations in anatomy across subjects. An automatic method for accurately segmenting the hippocampus objectively in this clinical population, including in infants with brain injury, would be beneficial (Thompson et al., 2011).

Finally, while we identified longitudinal relationships between neonatal hippocampal measures and early childhood outcomes, including associations specific to infants with brain injury, it is uncertain if these relationships will persist and/or evolve in later childhood in light of recent reports (Thompson et al., 2014). Assessments at later ages enable more detailed assessments across domains known to be associated with hippocampal function such as memory and learning. In addition, the majority of prior studies in this domain have been performed in children from Australian families from higher socioeconomic backgrounds (Beauchamp et al., 2008; Kidokoro et al., 2014; Omizzolo et al., 2013; Thompson et al., 2008), in contrast to our American socially-disadvantaged preterm sample.

Further longitudinal study in this and related cohorts of VPT children extending into later childhood remain necessary to comprehensively characterize the relationships identified in this investigation and characterize population-based differences which may contribute to findings. Additional detailed analysis of the effects of brain injury on hippocampal shape, as well as incorporation of advanced neuroimaging

modalities, including assessment of microstructural and functional connectivity of the hippocampus to other brain structures, will further expand upon our findings. Finally, our clinical observations regarding the association between brain injury and hippocampal size and cognitive outcomes requires validation in preclinical animal model investigations designed to define the pathophysiology underlying the relationship and the effects of other clinical variables of interest (*e.g.*, intracranial pressure, anesthesia, inflammation, neurosurgical intervention).

5. Conclusions

Characterization of early hippocampal development in VPT infants with brain injury, including IVH, PHH and cPVL, adds to the growing body of research exploring the role of the hippocampus in neurodevelopmental outcomes and its susceptibility to the effects of premature birth. Our results reveal VPT infants with brain injury demonstrate smaller hippocampal volumes in comparison to full-term and VPT infants with no to mild injury, consistent with our preclinical work in models of IVH-PHH. Furthermore, we demonstrated unique longitudinal associations in this group, with smaller hippocampal volumes related to worse cognitive outcomes during early childhood. Infants with PHH demonstrated both the smallest hippocampal volumes and worst neurodevelopmental outcomes. In infants with brain injury, larger ventricular size was associated with smaller hippocampal volumes, a relationship which may inform future studies. Further investigation of the longitudinal relationships between neonatal hippocampal measures and domain-specific outcomes during later childhood remains necessary to better define the role of the hippocampus in neurobehavioral development in this high-risk population.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2019.101787.

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