Hippocampal shape variations at term equivalent age in very preterm infants compared with term controls: perinatal predictors and functional significance at age 7

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Hippocampal shape in preterm infants

Abstract:

The hippocampus undergoes rapid growth and development in the perinatal months. Infants born very preterm (VPT) are vulnerable to hippocampal alterations, and can provide a model of disturbed early hippocampal development. Hippocampal shape alterations have previously been associated with memory impairment, but have never been investigated in infants. The aims of this study were to determine hippocampal shape differences between 184 VPT infants (<30 weeks' gestation or <1250 g at birth) and 32 full-term infants, effects of perinatal factors, and associations between infant hippocampal shape and volume, and 7 year verbal and visual memory (California Verbal Learning Test- Children's Version and Dot Locations). Infants underwent 1.5T magnetic resonance imaging at term equivalent age. Hippocampi were segmented, and spherical harmonics-point distribution model shape analysis was undertaken. VPT infants' hippocampi were less infolded than full-term infants, being less curved toward the midline and less arched superior-inferiorly. Straighter hippocampi were associated with white matter injury and postnatal corticosteroid exposure. There were no significant associations between infant hippocampal shape and 7 year memory measures. However, larger infant hippocampal volumes were associated with better verbal memory scores. Altered hippocampal shape in VPT infants at term equivalent age may reflect delayed or disrupted development. This study provides further insight into early hippocampal development and the nature of hippocampal abnormalities in prematurity.

Key words: preterm infant, magnetic resonance imaging, hippocampus, morphology, memory

Abbreviations:

BPD	bronchopulmonary dysplasia
CVLT-C	California Verbal Learning Test, Children's Version
FT	full-term
GA	gestational age
IUGR	intrauterine growth restriction
IVH	intraventricular hemorrhage
MANCOVA	multivariate analysis of covariance
MRI	magnetic resonance imaging
PCS	postnatal corticosteroids
PD	proton density
SD	standard deviation
SPHARM-PDM	spherical harmonics-point distribution model
VPT	very preterm
WM	white matter
WMI	white matter injury

Introduction:

The hippocampus is part of the limbic system and is crucial to memory and learning. It is folded into an 'S' shape along the floor of the lateral ventricle. Embryologically, the hippocampus belongs to cerebral archicortex (phylogenetically primitive allocortex) which matures more slowly than cerebral neocortex (Kier et al., 1997a). This disproportional maturation results in medial displacement and internal inversion of the hippocampus into the temporal horn region. Infolding begins at about 15 to 16 weeks' gestational age (GA) when the dentate gyrus and cornu ammonis start to infold. By 18 to 20 weeks, the dentate gyrus and cornu ammonis have folded into the temporal lobe, the hippocampus and subiculum approximate each other across the hippocampal sulcus, and the hippocampal sulcus becomes reoriented from vertical to horizontal (Kier et al., 1997a; Okada et al., 2003). There is recent evidence to suggest that hippocampal inversion may not be complete until up to 25 weeks of gestation (Bajic et al., 2010). The most rapid growth and development of the hippocampus occurs in the perinatal months, continuing up to 2 years after birth, and then resembling adult morphology by 5 years (Insausti et al., 2010; Kretschmann et al., 1986). The developmental trajectory differs between males and females due to the hormonal responsiveness of the hippocampus (Giedd et al., 1996). Furthermore, the posterior region grows faster than the anterior (Gogtay et al., 2006; Insausti et al., 2010), and maturation of memory and learning functions is thought to parallel hippocampal growth and development (Seress, 2001). However, to date there are few studies that have examined early morphological development of the human hippocampus.

The investigation of the very preterm (VPT, <32 weeks' GA) infant hippocampus can assist our understanding of early hippocampal development, particularly in relation to pathology. Using prematurity as a model, we can examine the effects of stress, brain injury or perinatal treatments during development on hippocampal shape. There are numerous factors that may influence the morphology of the hippocampus in very preterm infants but the impact of such factors has not yet been established. For example, the hippocampus is selectively vulnerable to hypoxic-ischemic injury (Schmidt-Kastner and Freund, 1991) which is a common complication in preterm infants (Volpe, 2009). Dexamethasone, a corticosteroid used to prevent or treat bronchopulmonary dysplasia (BPD) in very preterm infants, is known to have a specific neurotoxic effect upon the hippocampus (Sapolsky et al., 1990), and is implicated in hippocampal injury (Murphy et al., 2001). The hippocampus is a target for glucocorticoid stress hormones, which may explain its

vulnerability to corticosteroids such as dexamethasone (Perlman, 2001). Furthermore, it is recognized that preterm infants display elevated stress symptoms (Peng et al., 2009), and this may also cause the hippocampus to be more susceptible to damage in VPT infants. Supporting this claim we have previously shown that this VPT infant cohort had smaller hippocampi than full term (FT) infants at term-equivalent age, particularly those infants with white matter injury (WMI) or postnatal corticosteroid (PCS) exposure (Thompson et al., 2008). Other groups have also shown that children and adolescents born preterm have reduced hippocampal volumes (Lodygensky et al., 2005; Nosarti et al., 2002; Peterson et al., 2000).

Functional consequences may be expected with hippocampal alterations. Reduced hippocampal size in this VPT infant cohort has been related to impaired cognition at 2 years of age (Thompson et al., 2008) as well as impaired working memory, in particular spatial working memory (Beauchamp et al., 2008). In children and adolescents born preterm, smaller or abnormal hippocampi have been associated with impaired cognition and memory functioning (Abernethy et al., 2004; Gimenez et al., 2004; Isaacs et al., 2004; Isaacs et al., 2000; Lodygensky et al., 2005; Vargha-Khadem et al., 1997). Given the role of the hippocampus in memory and learning, (Bohbot et al., 2000) one may speculate that deficits in this domain, which are often observed in preterm children, may be associated with altered hippocampal development (Rose et al., 2005). To date, the relationship between hippocampal *shape* and memory and learning in the preterm population has not been investigated.

While volumetric analyses can assess global changes of the hippocampus, they are less able to explain structural changes such as bending or flattening, or to localize focal changes (Gerig et al., 2001). Shape analyses can reveal such changes, and therefore can provide new and sensitive information (Gerardin et al., 2009), which may yield biologically meaningful information about the development of the hippocampus (Ho and Magnotta, 2010). A common approach to morphological analysis of the hippocampus is to use the spherical harmonics-point distribution model (SPHARM-PDM), which describes shape deviations in a specific population using multiple points across the 3 dimensional surface (Shi et al., 2007; Styner et al., 2003).

While hippocampal shape changes associated with prematurity have never been reported, studies involving other neurological diseases suggest that hippocampal shape changes may have developmental origins and functional consequences. For example, early developmental disorders and congenital brain

malformations such as holoprosencephaly, lissencephaly, Fukuyama muscular dystrophy, agenesis of the corpus callosum, polymicrogyria, heterotopia, tuberous sclerosis and schizencephaly have been associated with an abnormal shape and orientation (vertical) of the hippocampus (Barkovich, 2002; Montenegro et al., 2006; Sato et al., 2001) and hypothesized to be related to a premature or incomplete infolding (Kier et al., 1997b; Lehericy et al., 1995b).

The aims of this study were to determine: 1) differences in hippocampal shape between VPT and FT infants at term equivalent age; 2) effects of specific perinatal factors on hippocampal shape within VPT infants; and 3) associations between hippocampal volume, shape and 7-year memory function. The hypotheses were that: 1) VPT infant hippocampi would have altered shape compared with FT neonates, with changes relating mostly to altered hippocampal infolding; 2) alterations to VPT hippocampal shape would be related to PCS therapy for chronic lung disease, and to WMI (a proxy for hypoxic-ischemic injury); and 3) infant hippocampal volume and shape would relate to memory performance at 7 years of age.

Methods:

Participants and scanning

227 VPT infants (<30 weeks' gestation or <1250 g at birth) and 46 FT neonates were recruited between July 2001 and December 2003 as part of a prospective cohort study examining the effects of very preterm birth on brain development, as previously described (Beauchamp et al., 2008; Thompson et al., 2008). Infants underwent magnetic resonance imaging (MRI) on a 1.5 T General Electric scanner at term equivalent age (38 - 42 weeks) without sedation. T2 and proton density (PD) weighted dual echo fast recovery fast spin echo sequences with interleaved acquisition [1.7 mm coronal slices; TR 4000 ms; TE 60 / 160 ms; flip angle 90°; FOV 180 × 135 mm^2; matrix 256 × 224 (zero-padded fast Fourier transform reconstruction to a 512 x 512 matrix)] were acquired. 184 stable VPT (90% < 30weeks' gestation) and 32 healthy FT infant scans (79% of those recruited) were able to be analysed for this study. The remaining infants either failed to be scanned within the term equivalent age range (6%), or scans were of insufficient quality to be analysed due to movement and imaging artifacts (15%) (Thompson et al., 2008).

Hippocampal segmentation

T2 and PD image volumes were combined by image addition to enhance contrast-to-noise ratio, as previously described (Thompson et al., 2008). Hippocampi were manually delineated on the coronal view by a single operator (DKT). The posterior boundary was defined, and then in-plane boundaries were traced sequentially on each slice until the anterior boundary was reached. A previously defined and validated approach was used to define hippocampal boundaries (Watson et al., 1992), and a detailed description is provided in Thompson et al., 2008. The hippocampus of each hemisphere was manually segmented twice (once in native orientation and once flipped in the right/left direction) using '3D slicer' software version 2.6 (http://www.slicer.org/), and the overlap of the two delineations was used as the final hippocampal mask, as previously described (Thompson et al., 2008). The intraclass correlation coefficients were 0.97 and 0.96 for the right and left hippocampi, respectively. Dice's overlap coefficients for 15 subjects (4 FT and 11 VPT) were 0.91 (range 0.85, 0.94) for the right and 0.91 (range 0.88, 0.94) for the left hippocampi.

Hippocampal shape analysis

The SPHARM-PDM shape analysis pipeline was applied to the binary hippocampal masks, to obtain a description of global and local shape (Gerig et al., 2001; Styner et al., 2006). Routine pre-processing involved resampling the hippocampal masks to isotropic resolution, filling any cavities via a binary closing operation, and minimal smoothing using level set based anti-alias smoothing to ensure spherical topology.

The hippocampal boundaries were converted to triangular surface meshes using the marching cubes algorithm (Lorensen and Cline, 1987). These triangular meshes were deformed to spheres using an algorithm that seeks to balance preservation of area while reducing angular distortion (Brechbuehler et al., 1995). These spherical parametrizations induce one-to-one mappings between spherical angular parameters of the vertices to their original cartesian coordinates. SPHARM represents the original surfaces using the coefficients of linear combinations of spherical harmonic basis functions defined on the spherical angle pairs of the spherical parametrizations. The coefficients of the spherical harmonic basis functions for the x, y, z coordinates of the original surfaces were estimated independently using ordinary linear least squares. The maximal spherical harmonic order was defined as 12.

Smoothed versions of the original surfaces were reconstructed by integrating spherical harmonic basis functions weighted by their estimated coefficients on uniform icosahedral polyhedra with a subdivision level

of 20. By reconstructing each surface with the same subdivision level, each reconstructed surface contained the same number of vertices. Initially, a reconstruction using the 0th and 1st order coefficients produced an ellipsoidal surface which was used for rotational alignment. Since the vertices of the reconstructed and aligned surfaces are in correspondence, they represent a PDM of the hippocampus across the cohort. A procrustes alignment was performed to remove any sources of residual linear alignment error (Styner et al., 2006). The VPT and FT hippocampi were separated into their own PDMs. For each vertex, the signed distance from the mean surface for each PDM was evaluated for statistically significant differences, indicating areas of local expansion or contraction.

Perinatal data

Perinatal data were collected by chart review. Data were recorded on: gender; GA; PCS (dexamethasone administered at a median total dose of 1.1 mg/kg) for the treatment of BPD; intrauterine growth restriction (IUGR), defined as birth weight z-score <-2 standard deviations (SD) from the mean, calculated relative to the British Growth Reference data (Cole et al., 1998); infection (necrotizing enterocolitis or sepsis); indomethacin treatment for a patent ductus arteriosus; WMI; and intraventricular hemorrhage (IVH). White matter (WM) maturation was qualitatively assessed by a neurologist using a modified version of a previously described grading system (Inder et al., 2003), assessing presence and extent of cystic lesions, focal signal abnormality, myelination delay in the posterior limb of the internal capsule and corona radiata, callosal thinning on the mid-sagittal slice, lateral ventricular diameter, and biparietal diameter. WMI was graded as follows: Grade I, normal WM; Grade II, mild WM abnormality; Grade III, moderate WM abnormality; Grade IV, severe WM abnormality. IVH was determined with cranial ultrasound scans performed on all infants serially throughout the neonatal intensive care course. The highest grade of IVH was recorded.

Memory

Visual and verbal memory were assessed at 7 years corrected age. Verbal memory was assessed according to performance on the long delay free recall task of the California Verbal Learning Test, Children's Version (CVLT-C) (Delis, 1994). The CVLT-C required children to recall a list of 15 words, which was presented by

the examiner over 5 trials. Approximately 20 minutes after the administration of the 5 trials the child was asked to recall the list of words. Verbal memory data was available for 152 preterm and 29 term children. Visual memory and learning was assessed based on performance on the long delay free recall of the Dot Locations subtest from the Children's Memory Scale (Cohen, 1997). Children were required to recall the placement of dots on a grid, across 3 trials, and approximately 20 minutes later the long delay free recall trial is administered. Visual memory data was obtained for 151 VPT and 29 FT children. Raw scores were used for both the visual and verbal memory tests.

Statistical analyses

Differences between the VPT and FT groups on neonatal and demographic variables were analysed with independent samples T-tests for continuous variables or χ^2 analyses for categorical variables.

Shape comparisons were made for the VPT vs. FT group for both the right and left hippocampi. In order to test for group differences in the spatial location of each vertex of the hippocampal surface, univariate Hotelling T^2 statistical testing was carried out on the corresponding boundary points across all subjects. Hippocampal volumes (for right and left hippocampi separately) were included in the shape analyses as a scaling factor, to correct for the effect of hippocampal size differences. Multivariate analysis of covariance (MANCOVA) was used to adjust VPT vs. FT local shape comparisons for WMI (grade I or II vs. III or IV), GA (< 26 weeks vs. ≥ 26 weeks), and PCS (Paniagua et al., 2009).

Within VPT infants, hippocampal shape was compared between male vs. female, GA < 26 weeks vs. GA ≥ 26 weeks, PCS exposure vs. no PCS exposure, IUGR vs. no IUGR, infection vs. no infection, indomethacin vs. no indomethacin, moderate / severe WMI (grade III or IV) vs. no or mild WMI (grade I or II), and IVH grade I or II vs. IVH grade III or IV. MANCOVA was used to adjust local shape changes due to different perinatal factors for WMI, GA, and PCS (included in the model as dichotomous variables, as described above).

The relationship between VPT infant hippocampal *volume* and 7-year memory performance was determined by linear regression, including adjustment for age at assessment and perinatal variables, as necessary. Hippocampal volume was corrected for intracranial volume, as described in Thompson et al., 2008. Intracranial volume was calculated by creating a brain versus non-brain mask on the T1 image,

including all brain tissue and cerebrospinal fluid (Kikinis et al., 1992). MANCOVA was used to associate local *shape* difference from the mean hippocampal surface with 7-year memory performance in the whole cohort. Pearson's correlations were performed between the memory measure (included in the model as a continuous standard score) and the magnitude of the projections of the difference vectors from the mean. An interaction between group (PT vs. FT) and the memory measure was tested to determine if memory outcomes were associated with the shape difference.

All group-wise comparisons were adjusted for multiple comparisons by permutation testing, using the family-wise error rate. All MANCOVA p values were adjusted for multiple comparisons using Bonferroni correction.

Results:

Very preterm versus full term infants

The perinatal and demographic characteristics of the cohort are shown in Table 1. There were no significant differences between VPT and FT infants for either the GA at the time of MRI scan (t = -1.8, p = 0.07) or the male-to-female ratio between groups (t = 0.59, p = 0.6). However, corrected age at 7 year assessment was older in FT compared with VPT children (t=2.1, p=0.03). Visual memory performance at 7 years was impaired in VPT compared with FT children [mean (SD): FT 5.59 (0.78); VPT 4.89 (1.33); p= 0.007], as was verbal memory [mean (SD): FT 8.52 (2.80); VPT 7.22 (3.26); p= 0.046].

There were various areas of shape difference within the hippocampal head and tail and the lateral edges of the body for both the right and left hippocampi of VPT compared with FT infants. As evident from the mean group overlay maps (Fig. 1A), the VPT infants had straighter and less arched hippocampi, with outward (lateral) and upward (superior) displacement of the head and tail. The areas of significant shape change appeared to correspond with regions of expansion along the lateral edges, coupled with contraction of the medial edge of VPT compared with FT hippocampi (Fig. 1A). Once corrected for WMI, PCS and GA with MANCOVA, many of the same regions of shape difference remained, especially for the left hippocampi (Fig. 1B).

Perinatal factors

Within the VPT infants, there were several diffuse regions of significant shape change associated with WMI, within the tail of both the right and left hippocampi. The mean overlay and displacement maps suggested that these areas of significant difference correspond mainly to hippocampi of VPT infants with WMI III/IV being outwardly (laterally) displaced, making the body and tail appear straighter, or less inwardly curved than those with WMI I/II (Fig. 2A). The significant regions of shape difference associated with WMI remained after adjusting for PCS and GA, and indeed the associations appeared stronger (Fig. 2B).

There were various regions across the whole right hippocampi that were significantly displaced in VPT infants exposed to PCS relative to those not exposed, particularly along the lateral edge. Again medial regions were contracted, while lateral regions expanded, leading to a less curved right hippocampus in VPT infants who received PCS vs. no PCS (Fig. 3B). Once adjusting for the contribution of WMI and GA, there were more regions of significant shape difference uniquely associated with PCS, including for the left hippocampus (Fig. 3B).

VPT infants born more immature, at a GA of <26 weeks, had only two small but significant regions of shape difference on the head and body of the left hippocampus (Fig. 4A) compared with those born more mature. These regions corresponded to areas of expansion in those born <26 weeks' GA compared with those born later. The mean overlay maps suggest that these alterations may also be a reflection of the less curved nature of the left hippocampus in infants born at <26 weeks' GA (Fig. 4A). However, none of the regions on the left hippocampus remained significant after adjusting for WMI and PCS, but a small region of significance was found on the right inferior hippocampus (Fig. 4B).

There were no significant regions of shape difference between VPT infants who were female vs. male, who had IUGR or not, who were exposed to infection or not, who were treated with indomethacin or not, or who had or had not experienced grade III or IV IVH, for either the left or right hippocampi (data not shown).

Memory & Learning

There was a significant association between larger left and right infant hippocampal volume and better 7 year verbal memory scores (Fig. 5). This association remained significant after adjusting for age at

assessment, gender and WMI. There was also a positive association between left (but not right) hippocampal volume and visual memory performance; however this did not remain significant after adjusting for age at assessment, gender and WMI (Table 2).

There were no significant correlations between the magnitude of the difference vectors projected from the mean hippocampal surface at term and 7 year memory performance (verbal or visual). Neither was there a significant interaction between memory performance and the VPT vs. FT shape comparison.

Discussion:

Summary of results

The shape of both the right and left hippocampus was different in VPT infants compared with FT infants being straighter (lateral-medially) and less arched (inferior-superiorly). Within VPT infants, hippocampal shape was associated with WMI, PCS, and to a lesser extent immaturity at birth (GA < 26 weeks). To our surprise, hippocampal *shape* changes at term were not related to visual or verbal memory performance at age 7 years. However, larger hippocampal *volumes* at term were associated with better memory performance, particularly for verbal memory.

Very preterm versus full term infants

VPT infant hippocampi were straighter, or less 'inverted' than FT hippocampi. Since hippocampi were scaled for volume, there was no effect of hippocampal size on the VPT vs. FT infant shape comparison. The adjusted model showed that hippocampal shape alterations in VPT infants could not be entirely explained by perinatal complications such as WMI, PCS, or immaturity at birth.

A large amount of hippocampal development occurs in the first postnatal year including synaptic development, dendritic and glial growth, myelination of WM associated with the hippocampus, and enhanced connectivity with the remainder of the neocortex (Insausti et al., 2010). It is, therefore likely that preterm birth would affect such developmental processes. The current study suggests that VPT infant hippocampal shape alterations are due to incomplete infolding of the hippocampus. Incomplete infolding has been previously associated with morphological abnormalities of the hippocampus (Kier et al., 1997a;

Lehericy et al., 1995a). Considering cortical-hippocampal connections are among the first connections to be established early in life (Hevner and Kinney, 1996), a potential cause for abnormal hippocampal infolding may be abnormalities of corticogenesis or WM damage, resulting in abnormal cortical-hippocampal connections (Bernasconi et al., 2005). Preterm infants are at risk of both cortical abnormalities and WM damage (Ramenghi et al., 2007), which may contribute to abnormal hippocampal shape in VPT infants. In fact, hippocampal deformations such as malrotation have previously been related to abnormalities of associated cortical regions as well as disrupted cortical-hippocampal connectivity (Qiu et al., 2010; Voets et al., 2011).

Although this is the first study to examine hippocampal shape in prematurity, early congenital brain anomalies can result in concurrent hippocampal abnormalities, and have been widely studied (Barkovich, 2002; Donmez et al., 2009). Sato et al.'s study found a high prevalence of hippocampal abnormalities (including incomplete inversion) in patients with polymicrogyria, heterotopias, tuberous sclerosis and schizencephaly (Sato et al., 2001). Epilepsy is a commonly studied developmental disease associated with 'hippocampal malrotation', including abnormal shape (pyramidal, vertically oriented, or globular), positioning (medially located) and vertical collateral sulcus of the hippocampus (Gamss et al., 2009; Lehericy et al., 1995a; Peltier et al., 2005). However, Bajic and colleagues proposed that 'incomplete hippocampal inversion' better describes this morphological disturbance in hippocampal development (Raininko and Bajic, 2010). They suggested that incomplete hippocampal inversion is a more common variant than once thought, and reflects a more general disturbance to cerebral development (Bajic et al., 2009). Although the current study suggests incomplete hippocampal infolding, it does not necessarily fit the full criteria for a diagnosis of 'hippocampal malrotation' which is rarely present in individuals without seizures (Gamss et al., 2009).

Perinatal factors

WMI was uniquely associated with less infolded hippocampi in VPT infants. Hypoxic-ischemic injury may damage both the cerebral WM and the hippocampus, and result in deafferentation of the hippocampus. Loss of cortical-hippocampal connections is a possible explanation for the mechanism by which WMI could affect hippocampal shape (Bernasconi et al., 2005). No studies have examined the effect

of WMI on hippocampal shape, however WMI has previously been associated with hippocampal *volume* reductions in VPT infants (Thompson et al., 2008) and adolescents (Nosarti et al., 2002). Although there is some plasticity demonstrated by the hippocampus after damage in adults (Braun et al., 2008), in cases of early perinatal brain injury, hippocampal damage appears irreversible (Insausti et al., 2010).

In addition to WMI, PCS exposure in VPT infants was independently associated with a more immature, less infolded hippocampal shape. Thus, PCS exposure may be detrimental to normal hippocampal development and infolding. PCS are administered to reduce the risk of BPD (Lin et al., 1999), but have been reported to be associated with adverse neurological effects and detrimental neurodevelopmental outcomes (Stark et al., 2001). Dexamethasone, the PCS used in this study, has been reported to have a specific neurotoxic effect upon the hippocampus (Halliday et al., 2009; Murphy et al., 2001; Sapolsky et al., 1990). Considering the hippocampus is a target for glucocorticoid stress hormones (Perlman, 2001), both PCS and the stress associated with VPT birth may act through the same mechanism to detrimentally affect hippocampal development. Stress elevates glucocorticoid levels to stimulate glutamate release which in turn inhibits production of granule cells, changing the structure of the dentate gyrus (Gould and Tanapat, 1999). However, there is exciting new research that shows that antioxidant treatment may diminish the unwanted effects of glucocorticoid excess, which may improve the safety of PCS therapy for BPD (Camm et al., 2011). PCS exposure has also been associated with reduced hippocampal *volumes* at term equivalent (Thompson et al., 2008).

Although there was some evidence for a lower GA affect on hippocampal shape, there were very few regions of significance for hippocampal shape change between VPT infants born < 26 weeks vs. ≥ 26 weeks GA, and these disappeared after adjusting for WMI and PCS. While this is the first study to examine the effect of GA on hippocampal shape, an association of hippocampal volume with GA or birth weight has been previously reported (Nosarti et al., 2002; Peterson et al., 2000).

Memory functioning

VPT vs. FT shape differences at term were not associated with memory performance at 7 years of age. This preliminary finding suggests that prematurity-related alterations of hippocampal shape and infolding do not adversely affect later memory functioning. It is likely that different developmental

trajectories of hippocampal subregions and their connections may parallel functional differences, which is particularly relevant to VPT infants who are vulnerable during critical stages of brain development (Gogtay et al., 2006). Gogtay et al. postulated that hippocampal shape changes in anterior regions such as the head would relate to fear, anxiety and associative memory functions considering their projections to the prefrontal cortex, amygdala and HPA axis (Gogtay et al., 2006). Whereas posterior regions of the hippocampus including the dentate gyrus and CA3 are thought to be involved in memory, as they receive visual-spatial input from primary sensory areas including the association, perirhinal and entorhinal areas (Gogtay et al., 2006).

Although the current study was unable to find *regional* hippocampal structure-function relationships in VPT infants, we were able to show that overall hippocampal volume at term-equivalent age is related to memory performance at 7 years of age. These results suggest that hippocampal volume may be more sensitive to detect structure-function relationships than shape. The current study showed that larger left and right hippocampal volumes in VPT infants at term-equivalent age predicted better verbal memory at 7 years of age. VPT infants have been shown to have specific deficits in verbal memory (Taylor et al., 2000), thus smaller hippocampi in the neonatal period may be a biomarker for such deficits. These findings are consistent with those of a previous study that showed a relationship between simultaneously-measured hippocampal volume and verbal memory in an adolescent preterm population (Gimenez et al., 2004). Traditionally, left hippocampal volumes have been associated with memory for verbal material (Zaidel, 1995), and right hippocampal volumes have been associated with non-verbal (i.e. visual) memory (Bohbot et al., 2000; Zaidel, 1995). However the current study found associations for both the right and left infant hippocampus with verbal memory in VPT children. Furthermore, larger left hippocampal volumes (but not right) in VPT infants predicted better visual long-term memory at 7 years, before adjusting for age at assessment, gender and WMI. There is existing evidence that memory function may be less lateralised that previously thought (Shinoura et al., 2011), especially in those with hippocampal injury (Ver Hoef et al., 2008), which may explain the findings of the current study. It is also possible that the abnormalities to normal hippocampal right-ward asymmetry shown to be present in VPT infants (Thompson et al., 2009) may lead to altered laterality of hippocampal functions. Alternatively, considering the brain becomes more localized and lateralized with maturity, the relationship between volume and memory may change with

subsequent growth of the hippocampus beyond term-equivalent age. It should be noted that since global brain injury may drive memory impairment, correction for intracranial volume may confound the results. Therefore, we repeated the regression analyses using raw uncorrected hippocampal volumes and were able to confirm the main findings, demonstrating that our findings are robust (data not shown). Furthermore, WMI may be driving both memory impairment and hippocampal pathology, which could possibly lead to a spurious correlation. For instance, a secondary consequence of WMI is widened lateral ventricles, which may reduce hippocampal volume. We attempted to separate the effect of WMI on hippocampal volume and memory associations by adjusting for WMI in our analyses. These findings suggest that WMI may have driven the significant (unadjusted) association between visual memory and left hippocampal volume, while verbal memory associations remained robust.

Apart from memory difficulties, early abnormalities of the hippocampus may predispose children to other disorders later in life. Abnormalities of the hippocampus have been associated with autism (Saitoh et al., 2001), language disorders (Agostini et al., 2010), mild cognitive impairment (Yassa et al., 2010), epilepsy (Bernasconi et al., 2005), and a range of neuropsychiatric disorders such as schizophrenia and bipolar disorder (Connor et al., 2004) and depression (Cole et al., 2010). Developmental disorders such as these have also been associated with preterm birth (Botting et al., 1997; Matsumoto et al., 2001), and damage to the hippocampus may be one of the links between prematurity and the higher incidences of such disorders.

Limitations and future directions

VPT infants have heterogeneous brains, including the hippocampi. Even so, the large numbers of subjects in this study provided enough power to detect characteristic differences between FT and VPT infant hippocampal shape. Considering a large amount of growth and development of the hippocampus occurs during the first postnatal year (Insausti et al., 2010) and continues into adolescence (Suzuki et al., 2005), it may not be possible to observe the full effects of prematurity on hippocampal shape until later. We hope to further elucidate hippocampal shape alterations in VPT 7 year-old children in our follow-up MRI study of this cohort.

Hippocampal shape analysis relies on good hippocampal boundary definition. A limitation to this study was the relatively large slice thickness of the T2 and PD images on which the hippocampi were traced.

Despite this, high reliability was achieved in the current study by tracing hippocampi according to a predetermined protocol, with a standardized neuroanatomical basis (Thompson et al., 2008). However, different studies often use different methods of boundary definition, different image acquisition parameters and differing magnetic field strengths (use of 1.5T or 3T scanners), thus making it difficult to compare findings across studies.

Shape analysis assumes perfect registration of all individual hippocampal surfaces into a common reference space for comparison. If the surfaces are poorly registered, the spatial distribution of statistically significant regions of shape change may be artifactual. It may be useful to confirm the current SPHARM shape analysis findings using medial models which explicitly model bending and do not operate based on boundary representations, making them less susceptible to bias as a result of registration errors (Heimann and Meinzer, 2009).

The future of shape analysis may be improved with the use of higher field MRI, enabling higher resolution and improved accuracy in defining hippocampal boundaries. A more detailed assessment of the subfields of the hippocampus will allow detection of more subtle hippocampal changes (Theysohn et al., 2009).

Conclusions

This study provides further insight into the development of the hippocampus and the impact of early environmental factors on hippocampal morphology. Furthermore, it provides insight into the nature of hippocampal abnormalities associated with prematurity, extending previous findings of hippocampal volume reductions in VPT infants, and the associated perinatal risk factors. VPT infants have altered hippocampal shape compared to FT infants, and alterations do not appear to be limited to a specific region of, but rather due to incomplete infolding of the hippocampus in the lateral-medial and inferior-superior aspects. This may reflect delayed or disrupted development. WMI and PCS exposure, in particular, appeared to contribute to altered hippocampal morphology in VPT infants. Hippocampal shape differences at term equivalent age did not appear to be predictive of long-term memory functioning at 7 years of age. It may be that the full effects of prematurity-related hippocampal shape alterations may not be apparent until later childhood or adulthood. Alternatively, smaller infant hippocampal *volume* independently predicted impaired verbal memory

performance at 7 years. Further investigation is warranted to understand the development of this important brain structure throughout life, and also to fully understand the role of the hippocampus in the high rates of cognitive impairments associated with prematurity.

Acknowledgements:

We gratefully acknowledge Marc Seal, Richard Beare and Jian Chen for helpful discussions on data analysis and interpretation. We also acknowledge the statistical help of Katherine Lee. Thanks to Leona Pascoe, Natalie Reidy and Shannon Scratch for performing the memory tests. We acknowledge the support of the Victorian Infant Brain Studies and Developmental Imaging teams at the Murdoch Childrens Research Institute and Royal Children's Hospital, University of Melbourne, Victoria. Thanks also to the families and children who participated in this study, as well as Michael Kean and the radiographers at the Royal Children's Hospital for acquiring the MRI scans.

Funding Support:

This study was also supported by the National Health and Medical Research Council (NHMRC) of Australia (Project Grant No. 237117 to T.E.I. and L.W.D.; No. 400317 to G.F.E.; Senior Research Fellowship No. 628371 to P.J.A.; Early Career Fellowship No. 1012236 to D.K.T.), the National Institute of Health, USA (Grant No. R01 RR021885, R01 GM074068, R01 EB008015, and P30 HD018655 to S.K.W.), the United Cerebral Palsy Foundation, USA, to T.E.I., the LeilaY. Mathers Charitable Foundation, USA, to G.F.E.), the Brown Foundation, USA, to G.F.E.), and the Victorian Government's Operational Infrastructure Support Program to P.J.A, C.A. and D.K.T.

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Displacement map with areas of positive expansion (red) or negative contraction (blue) of the PCS exposed hippocampal surface from the mean surface, overlaid on mean of all hippocampi. **(B)** Bonferroni corrected statistical map of hippocampal shape differences for very preterm infants with PCS vs. noPCS exposure, after correcting for white matter injury and gestational age at birth.

Fig. 4. Shape differences for very preterm infants born at less than 26 completed gestational weeks' (GA<26, n=34) vs. infants born at or after 26 week's gestational age (GA≥26, n=150), displayed for the right and left hippocampi. **(A)** Top: statistical p-value map of significant regions of shape difference after permutation testing overlaid on mean of all hippocampi. Middle: mean overlay of hippocampi of infants born at GA<26 weeks (blue) and GA≥26 weeks (red) hippocampi. Bottom: Displacement map with areas of positive expansion (red) or negative contraction (blue) of the GA<26 hippocampal surface from the mean surface, overlaid on mean of all hippocampi. **(B)** Bonferroni corrected statistical map of hippocampal shape differences for very preterm infants with GA<26 vs. GA≥26, after correcting for white matter injury, and postnatal corticosteroid exposure.

Fig. 5. Scatter plot of left and right very preterm infant hippocampal volume (corrected for intracranial volume) with 7 year verbal memory score, including fitted regression line and 95% confidence intervals.

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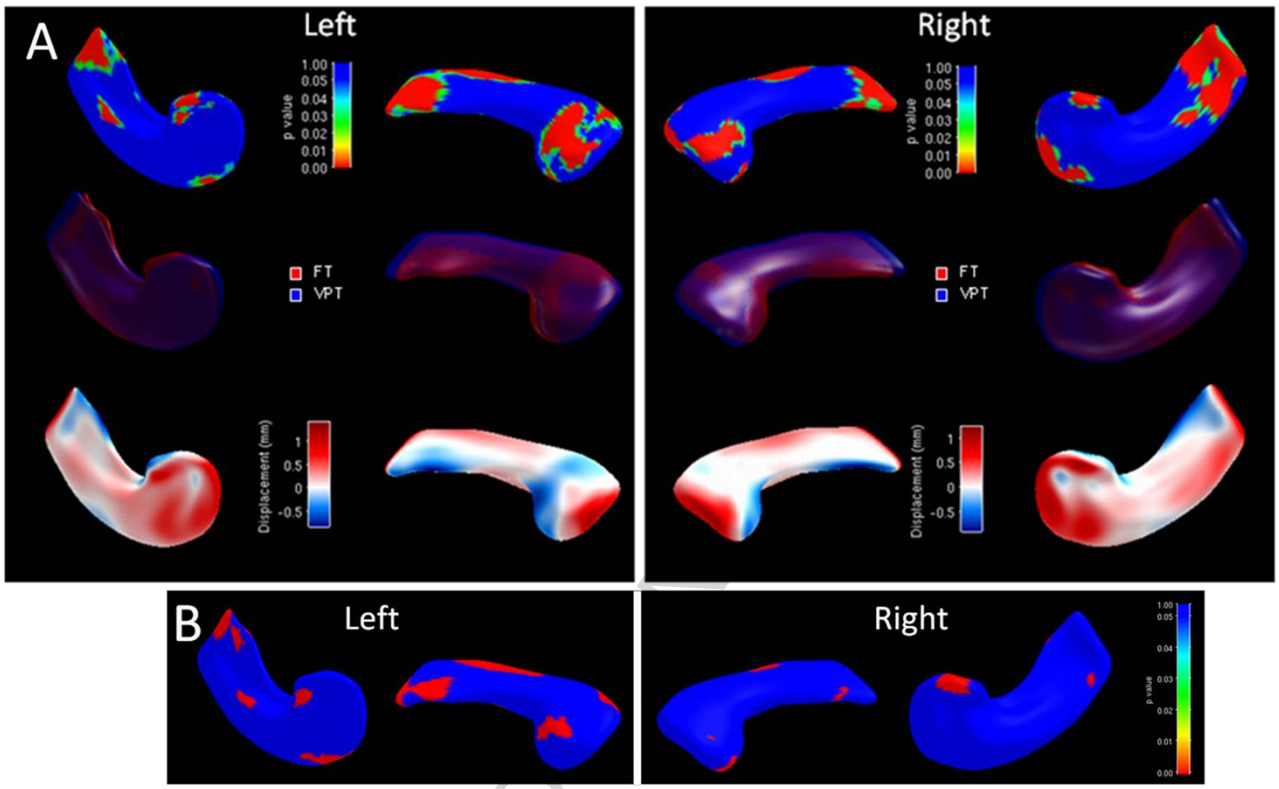

Figure 1

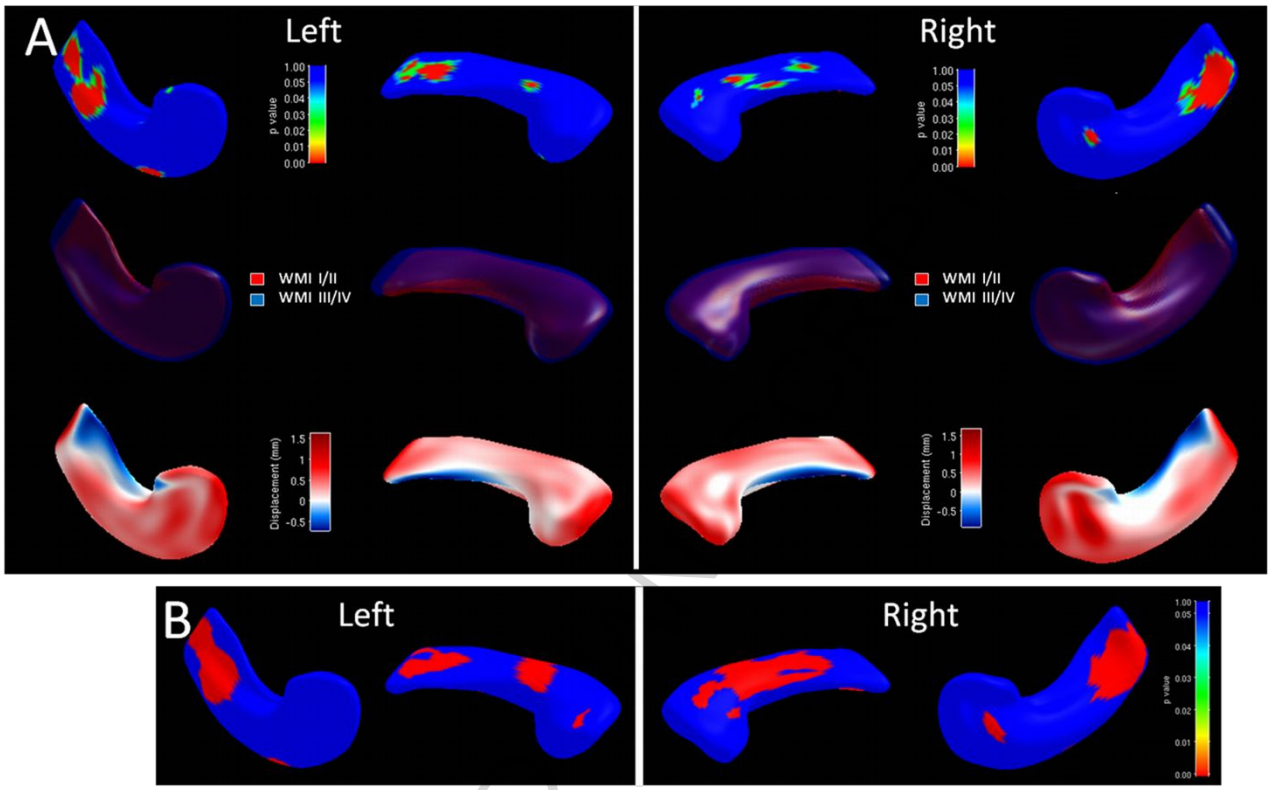

Figure 2

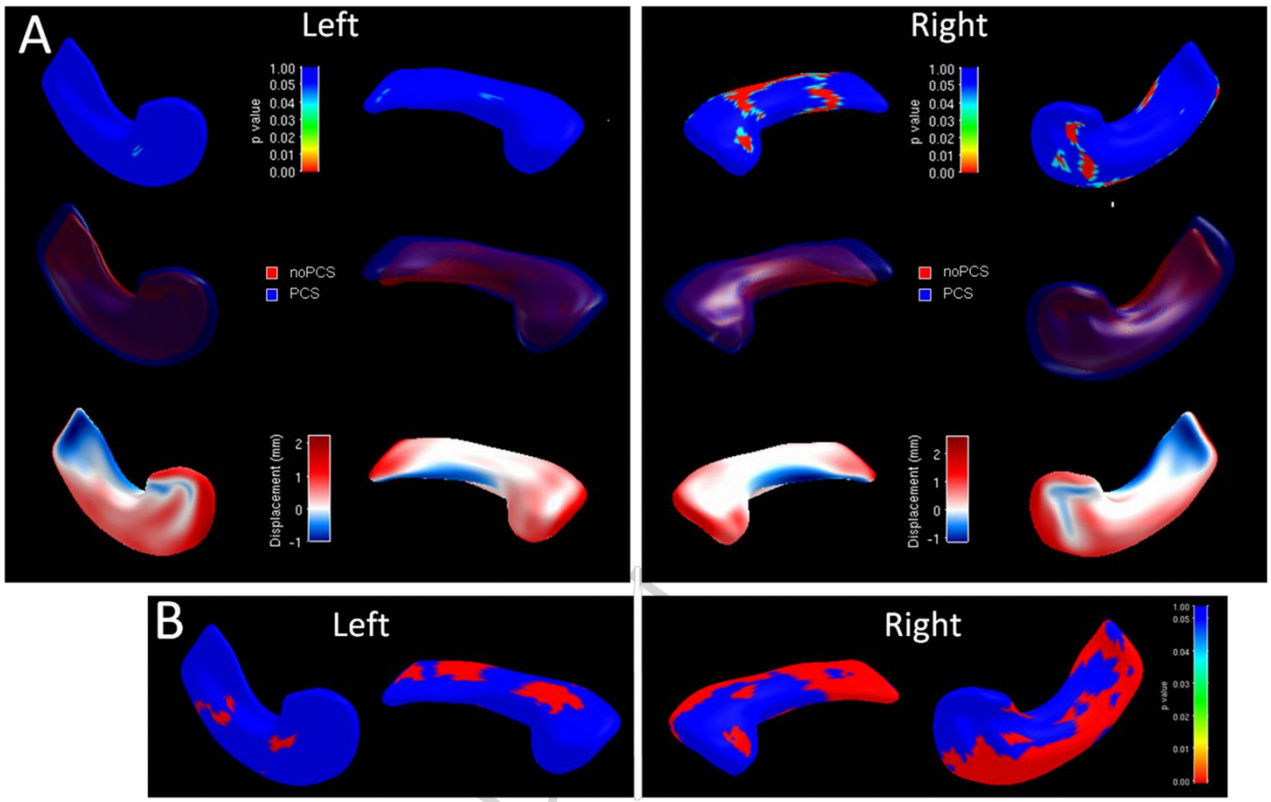

Figure 3

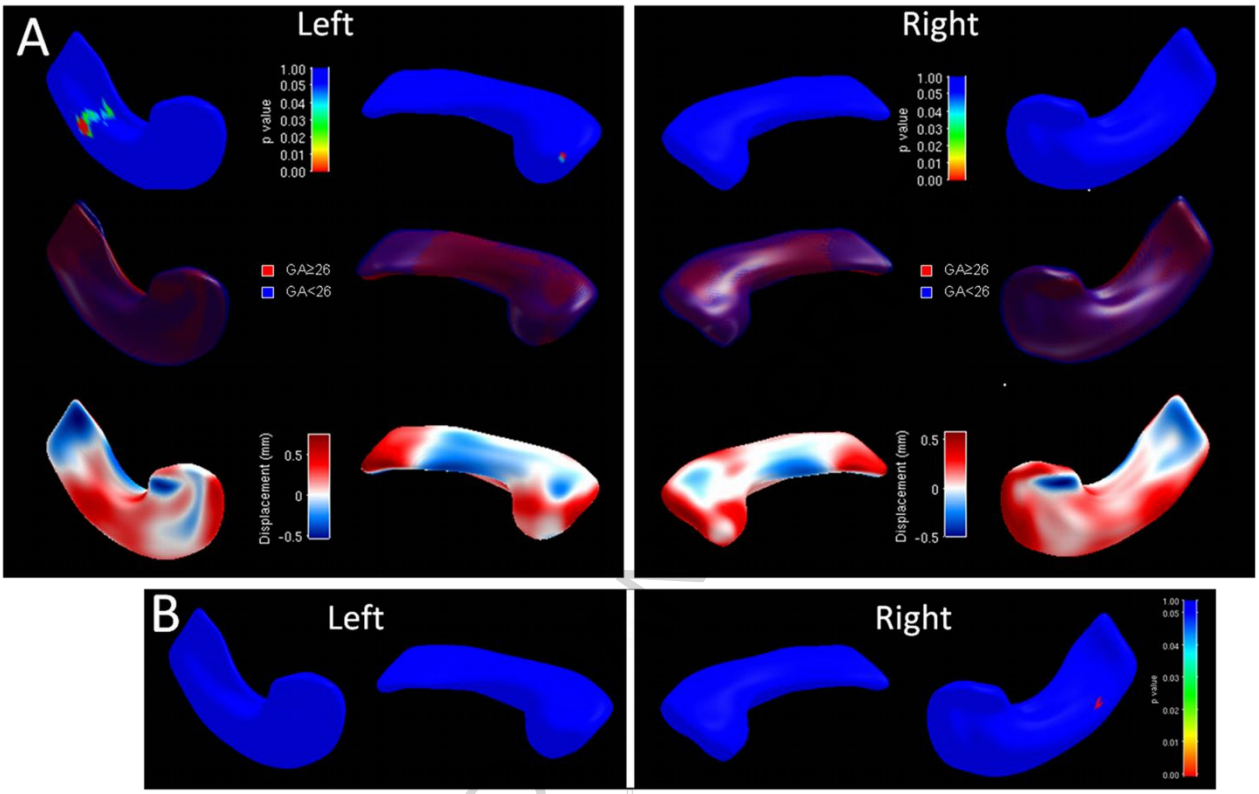

Figure 4

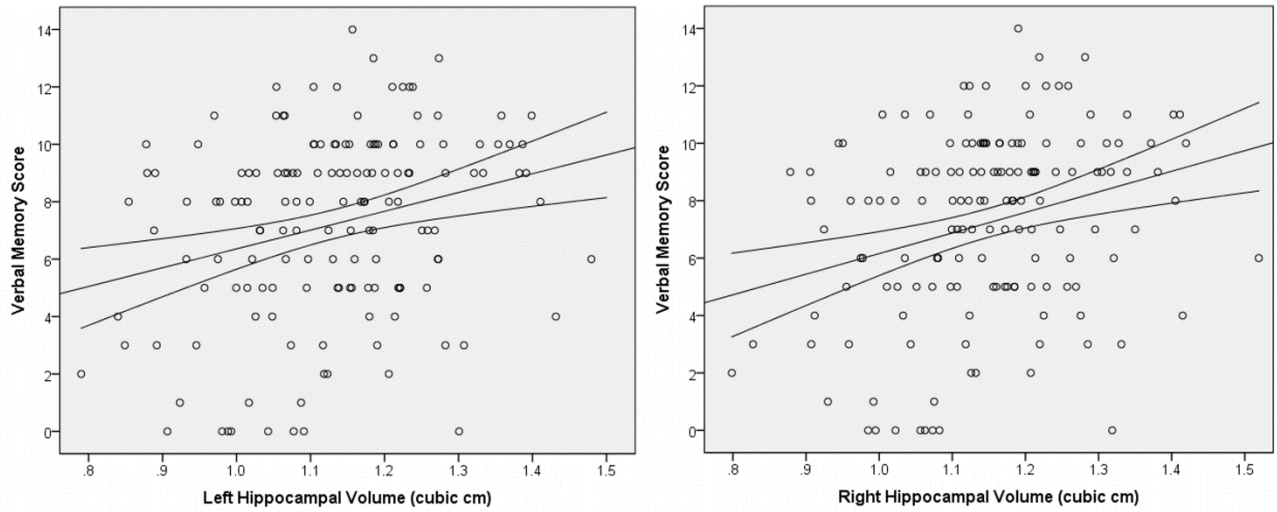

Figure 5

Table 1. Perinatal and demographic characteristics of the total cohort

Characteristic	Very preterm n=184	Full-term n=32	*p*
Gestational age at birth (weeks) – mean (SD)	27.6 (1.9)	39.0 (1.2)	**<0.0005**
Gestational age <26 weeks – n (%)	34 (19)	0 (0)	**0.008**
Gestational age at MRI (weeks) – mean (SD)	40.1 (1.1)	40.5 (1.0)	0.07
Birthweight, g – mean (SD)	964 (219)	3289 (504)	**<0.0005**
Male – n (%)	93 (51)	18 (56)	0.6
Bronchopulmonary dysplasia[1]– n (%)	61 (33)	0 (0)	**<0.0005**
Intrauterine growth restriction[2] – n (%)	20 (11)	1 (3)	0.2
Necrotising enterocolitis – n (%)	19 (10)	0 (0)	0.07
Sepsis – n (%)	79 (43)	1 (3)	**<0.0005**
Indomethacin therapy – n (%)	63 (34)	0 (0)	**<0.0005**
Postnatal steroid therapy[3] – n (%)	14 (8)	0 (0)	0.1
White matter injury (grade III/IV) - n (%)	39 (21)	0 (0)	**0.004**
Intraventricular hemorrhage (grade III/IV) - n (%)	7 (4)	0 (0)	0.3
Age at assessment (years) – mean (SD)	7.5 (0.2)	7.6 (0.2)	**0.03**

[1] Required Oxygen at 36 weeks gestational age.

[2] Z-score more than 2 standard deviations (SD) below the mean weight for gestational age.

[3] Postnatal dexamethasone, 0.15 mg/kg per day, reducing over 10 days.

Table 2. Relationships between very preterm infant hippocampal volumes (corrected for intracranial volume) and 7 year verbal and visual long-term memory scores.

Outcome	Side	r	Unadjusted β (95% CI)	Adjusted* β (95% CI)
Verbal memory (n=152)	Right	0.28	7.19 (3.26, 11.11), **p<0.0005**	6.76 (2.89, 10.63), **p=0.001**
	Left	0.27	6.59 (2.79, 10.39), **p=0.001**	6.40 (2.68, 10.12), **p=0.001**
Visual memory (n=151)	Right	0.14	1.47 (-0.20, 3.14), p=0.08	0.83 (-0.87, 2.53), p=0.3
	Left	0.19	1.91 (0.32, 3.50), **p=0.02**	1.43 (-0.19, 3.05), p=0.08

r = Correlation coefficient; β = Regression coefficient representing the increase in memory score per 0.1cm^3 increase in hippocampal volume; CI = Confidence Interval.

*Adjusted for age at assessment, gender, and white matter injury.

Research Highlights

- At term equivalent, preterm infants' hippocampi are less infolded than term infants
- Preterm hippocampi are less curved toward midline and less arched superior-inferiorly
- Straighter hippocampi are associated with white matter injury and postnatal steroids
- Infant hippocampal shape is not related to 7 year memory
- Larger infant hippocampal volume is associated with better verbal memory at 7

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Title:
Hippocampal shape variations at term equivalent age in very preterm infants compared with term controls: Perinatal predictors and functional significance at age 7

Date:
2013-04-15

Citation:
Thompson, D. K., Adamson, C., Roberts, G., Faggian, N., Wood, S. J., Warfield, S. K., Doyle, L. W., Anderson, P. J., Egan, G. F. & Inder, T. E. (2013). Hippocampal shape variations at term equivalent age in very preterm infants compared with term controls: Perinatal predictors and functional significance at age 7. NEUROIMAGE, 70, pp.278-287. https://doi.org/10.1016/j.neuroimage.2012.12.053.