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Preterm infant hippocampal volumes correlate with later working memory deficits

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Children born preterm exhibit working memory deficits. These deficits may be associated with structural brain changes observed in the neonatal period. In this study, the relationship between neonatal regional brain volumes and working memory deficits at age 2 years were investigated, with a particular interest in the dorsolateral prefrontal cortex, parietal cortex and the hippocampus. While the eligible sample consisted of 227 very preterm children who were born at the Royal Women's Hospital, Melbourne prior to 30 weeks gestation or weighing < 1250 g, 156 children had complete data sets. Neonatal magnetic resonance images of the brain were obtained at term equivalent age and subsequently parcellated into eight sub-regions, while the hippocampus was manually segmented. The relationship between brain volumes for these regions and performance on a working memory task (delayed alternation) at 2 years of age was examined. Very preterm children who perseverated on the working memory task had significantly smaller hippocampal volumes than very preterm children who exhibited intact working memory, even after adjusting for relevant perinatal, sociodemographic and developmental factors. Preterm children appear to have altered hippocampal volumes by discharge from hospital which may have a lasting impact on working memory function.

Keywords: prematurity; extremely low birth weight; working memory; hippocampus; magnetic resonance imaging

Abbreviations: ALTD = alternated; BSID-II = Bayley Scales of Infant Development-II; FAIL = failed training; GA = gestational age; MDI = mental developmental index; PERS = perseverated; TBV = total brain volume; TEA = term equivalent age; WMI = white matter injury

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Introduction

Preterm children are at increased risk of a range of cognitive and learning problems (Anderson and Doyle, 2003). One of these cognitive domains is working memory, which refers to the ability to retain and manipulate information mentally over a short period of time. Numerous studies have reported working memory deficits in preterm survivors at preschool age (Vicari *et al.*, 2004; Woodward *et al.*, 2005), school-age (Luciana *et al.*, 1999) and adolescence (Bhutta *et al.*, 2002). In addition, working memory deficits have also been linked to language delay (Caravale *et al.*, 2005) and academic problems including poor mathematical (Espy *et al.*, 2004), reading and spelling skills (Downie *et al.*, 2005; Sansavini *et al.*, 2007).

Though a substantial body of work has focused on elucidating the neural basis for working memory deficits, there is more limited understanding in relation to this during brain development, particularly in 'at-risk' populations. In adults, working memory functions have often been associated with activity in the prefrontal and parietal cortices (Goldman-Rakic, 1987, 1998; Fuster, 1997; Cabeza and Nyberg, 2000). However, more recent work indicates that working memory processes are subserved by a network of regions not simply limited to these regions (Ranganath and D'Esposito, 2005). One model proposes a system for visual working memory relying on the inferior temporal cortex via top–down feedback from neocortical areas in the prefrontal and medial temporal cortex, as well as the hippocampus

(Ranganath *et al.*, 2004; Ranganath, 2006). Consistent with this model, increasing evidence suggests that the hippocampus may play a significant role in working memory (Olson *et al.*, 2006; Piekema *et al.*, 2006), in addition to its more traditional role in long-term memory. Additionally, the neural substrates of working memory may differ in children and some suggest they may rely less on core working memory regions, such as the dorsolateral prefrontal and parietal cortices (Scherf *et al.*, 2006; Bunge and Wright, 2007). Kaldy and Sigala (2004) suggest that a system involving the temporal cortex, thalamic and hippocampal structures can account for visual working memory in the infant brain, while core working memory regions such as the dorsolateral prefrontal and parietal cortices become increasingly involved with development.

In preterm infants, the hippocampus has been shown to be an area of specific vulnerability (Abernethy *et al.*, 2002). Reductions in hippocampal volumes have been reported when preterm children are compared with term controls and persist even when total brain volume (TBV) is taken into account (Peterson *et al.*, 2000). Additional evidence from our group shows that some adverse perinatal events such as white matter injury (WMI) and postnatal steroid exposure lead to volumetric reductions in the hippocampus by term equivalent age (TEA) (Thompson *et al.*, 2008). In preterm adolescents, reduced hippocampal volumes have been associated with memory deficits (Isaacs *et al.*, 2000; Gimenez *et al.*, 2004).

This study explores the association between brain volumes at TEA and subsequent performance on a task of visual working memory at 2 years of age (delayed alternation) in a large representative cohort of very preterm children. Given that the areas associated with working memory in preterm infants remain unclear, we undertook an exploratory study with a particular focus on regions that have been implicated across different developmental stages, including the dorsal prefrontal cortex, parietal cortex and the hippocampus. We hypothesized that altered development of these regions in the neonatal period, reflected by reductions in volumetric measurements at TEA, would predict later visual working memory deficits in this cohort.

Methods

Subjects

Very preterm infants with gestational age (GA) at birth <30 weeks and/or birth weight <1250 g surviving to TEA were recruited from the Royal Women's Hospital, Melbourne, Australia. During the recruitment period of July 2001 and December 2003, 348 infants were eligible for recruitment and 230 families of these infants consented to the study. Informed parental consent was obtained in compliance with approved ethical guidelines of the Royal Women's Hospital and in accordance with the Declaration of Helsinki. Three infants were later diagnosed with a congenital abnormality and were excluded from analyses, leaving an overall sample of 227. The mean GA for the sample was 27.4 weeks (range: 22–32) and the mean birth weight was 957 g (range: 414–1580).

Magnetic resonance imaging

Scanning took place within a 1.5 Tesla General Electric Signa MRI scanner (Milwaukee, WI, USA). Of those infants recruited 207 were able to be scanned within the TEA range (38–42 weeks corrected GA). In order to minimize motion artifacts, infants were fed and swaddled, placed in a vacuum fixation bean bag, outfitted with earphones and scanned while sleeping. No sedation was administered.

Images were acquired, applying two different sequences: 3D T1 spoiled gradient recalled (1.2 mm coronal slices; flip angle 45°; repetition time 35 ms; echo time 9 ms; field of view $21 \times 15\ cm^2$; matrix 256×192) and T2 dual echo fast spin echo sequences with interleaved acquisition (2 mm coronal; repetition time 4000 ms; echo time 60/160 ms; field of view $22 \times 16\ cm^2$; matrix 256×192, interpolated 512×512).

Images were analysed qualitatively for WMI as described previously (Inder *et al.*, 2003; Woodward *et al.*, 2006).

Quantitative volumetric MR analysis

Analysis was undertaken on Sun Microsystems workstations (Palo Alto, CA, USA). The brain tissue was segmented into total tissue, cerebrospinal fluid (CSF), cortical grey matter (CGM) and deep nuclear grey matter (DNGM), myelinated white matter (MWM) and unmyelinated white matter (unMWM) according to previously described criteria (Warfield, 1996; Warfield *et al.*, 2000; Thompson *et al.*, 2007). The TBV was measured by creating a brain versus non-brain tissue mask on the T_1-weighted image. TBV included all the GM, WM and CSF within the skull. For the regional comparisons, the brain image was parcellated into 16 regions according to previously described and validated criteria (Peterson *et al.*, 2000; Peterson and Ment, 2001; Thompson *et al.*, 2007) with measurements for each parcel including total brain and CSF volumes.

Hippocampal segmentation

The hippocampus was manually outlined in the coronal view on the combined raw T_2-weighted and proton density weighted image volumes as previously described (Thompson *et al.*, 2008). In general, anatomical boundaries followed the approach of Watson *et al.* (1992).

2-year follow-up assessment

Social risk index

Social risk was assessed using a 12-point index comprising six aspects of social status including family structure, education of primary caregiver, occupation of primary income earner, employment status of primary income earner, language spoken at home and maternal age at birth (Roberts *et al.*, 2008). Low scores represent low risk while high scores represent high risk.

General cognitive development

At 2 years corrected age, infants were assessed for developmental delay using the mental developmental index (MDI) from the Bayley Scales of Infant Development-II (BSID-II) (Bayley, 1993). MDI scores were also classified as significantly delayed (>2 SD below normative mean), mildly delayed (between 1 and 2 SD below normative mean), within normal limits and accelerated performance (>1 SD above the normative mean).

Working memory

Delayed alternation was chosen as a measure of working memory, inhibition and set shifting. Delayed alternation is based on delayed response tasks that have been used within the neuroscience literature (Diamond, 1990) and have been adapted for use with very young children (Espy *et al.*, 1999, 2001, 2002, 2004) including those with known conditions affecting prefrontal functioning such as phenylketonuria (Diamond *et al.*, 1997) and prenatal cocaine exposure (Espy *et al.*, 1999).

For the current study, the delayed alternation task was administered in a similar manner to Espy *et al.* (2002) with extra training trials administered in order to promote the childrens' understanding of the task requirements. The apparatus consisted of two identical blue cups and an opaque red screen. The task required children to remember the location of a reward, maintain this information over a delay and then use the information to guide future responses (i.e. working memory). Children also had to inhibit a reinforced tendency to search for a reward at its most recent hiding location. The initial side of hiding was randomized and the testing procedures involved 3–8 training trials and 12 experimental trials, taking between 5 and 10 min to administer. Rewards included candy, stamps or small toys and were changed whenever a child appeared to be losing interest in order to maximize the child's persistence and facilitate task completion.

In the first trial, the reward was hidden underneath one of the two identical blue cups positioned to the left and right of the child's midline. This took place behind the opaque red screen, so that the child could not see the side of hiding. In experimental trials the screen was removed after a 3-s delay and the child was encouraged to find the reward. For subsequent trials the reward continued to be hidden behind a screen, but the side of hiding changed according to whether or not the child had correctly found the reward on the most recent trial. If the child found the reward, the side of hiding was switched without warning to the other cup for the next trial. If the child did not correctly retrieve the reward, the side of hiding remained the same until correct retrieval occurred. Therefore in order to achieve the maximum number of correct retrievals ($n = 12$) children needed to alternate their searching between the left and right cups for successive trials. A perseverative error was defined as searching for the reward under the same side as the most recent trial instead of alternating searching between the two cups.

During the training phase the screen was not used and the reward was hidden in view of the child. The purpose of the training phase was to teach children the behavioural sequence of searching for a reward under a cup and alternating their searches between the two cups. Training continued until the child had met a criterion of three consecutive correct trials. Training stopped if after eight trials a child was unable to meet this criterion. Reasons for not meeting criterion included failing to respond to a trial, repeatedly responding incorrectly or refusing to participate (e.g. getting out of chair, temper tantrums, impulsivity). Following successful training, experimental trials were administered. Experimental trials were discontinued in instances where children refused to continue, failed to respond or would not remain seated.

While this measure is multi-dimensional, in this study we were particularly interested in working memory deficits and, as such, performance was categorized according to the presence or absence of perseverative behaviour. Non-perseverative errors were also recorded during administration; however, these are likely to reflect disinhibition rather than poor working memory and therefore are not reported. Perseverative behaviour was defined using similar criteria to Woodward *et al.* (2005) and children were classified into one of three groups: (i) 'Failed Training' (FAIL), consisted of children who failed the training trials and those who were unable to persist with (at least six) experimental trials; (ii) 'Perseverated' (PERS), consisted of children who exhibited working memory deficits, that is, they were able to pass training and persist with at least six experimental trials but made three or more consecutive perseverative errors; (iii) 'Alternated' (ALTD), consisted of children who were able to alternate their search and committed two or fewer errors in a row.

Statistical analyses

Differences in perinatal, social and developmental characteristics between the three groups (FAIL, PERS and ALTD) were assessed using analysis of variance (ANOVA) for continuous variables and Pearson's chi-square statistic for categorical variables. ANOVA was also used to examine group differences for total and regional brain volumes. Subsequent analyses using analysis of covariance (ANCOVA) were used to adjust first for TBV, and second for TBV and relevant perinatal, social and developmental variables. The perinatal variables used as covariates in these analyses included moderate/severe WMI, administration of antenatal corticosteroids, administration of postnatal corticosteroids, small for GA, necrotizing enterocolitis (NEC) and bronchopulmonary dysplasia (BPD), as we have previously shown these risk factors to be associated with brain development in very preterm infants (Inder *et al.*, 2005; Thompson *et al.*, 2007, 2008). In particular, WMI was included as a covariate in light of results from previous studies which indicate that its presence is associated with reductions in cerebral volumes and that it is the main perinatal factor associated with reductions in preterm hippocampal volumes (Inder *et al.*, 2005; Shah *et al.*, 2006; Thompson *et al.*, 2007, 2008). Social risk was also included as a covariate as it is well established that social-environmental factors (e.g. maternal education, employment status) are strongly associated with cognitive development. In order to control for general cognitive development, MDI was included as a covariate. *Post hoc* analyses involved pairwise comparisons.

Results

Due to problems with scan quality, related mostly to imaging movement artefact, the scans for 32 infants were not suitable for volumetric analysis. Of the remaining 195 children, five were not seen at the 2-year follow-up and 34 children had missing data for the delayed alternation task (13—too delayed or impaired; 10—assessed interstate or overseas; 10—refused to participate; 1—assessed at 18 months' corrected age only). Of the total sample of 227 preterm children, complete neonatal imaging and 2-year cognitive data were available for 156 (69%). The characteristics of the total sample and the sample with complete data are presented in Table 1. There were no differences in perinatal characteristics, social risk or general cognitive development for those groups with and without complete data.

Children were classified into three groups according to performance on the delayed alternation task: 52 children

failed to learn or follow task instructions (FAIL), 56 perseverated (PERS) and 48 alternated (ALTD). Of the children in the FAIL group, 37 (71%) were unable to pass the training phase and were not administered the experimental trials and only 2 (4%) were able to persist to complete more than three experimental trials. The sociodemographic and perinatal characteristics of the three groups were similar (Table 2). The groups did, however, differ significantly with regards to the presence of moderate/severe WMI [$\chi^2(2) = 7.0$, $P = 0.03$], with significantly fewer children in the ALTD group exhibiting WMI than the PERS group ($P = 0.008$). Also, the groups differed in terms of general cognitive development (MDI) [$F(2,153) = 12.3$, $P < 0.001$)], with the children in the FAIL group exhibiting greater cognitive delay than children in the PERS ($P = 0.001$) and ALTD ($P < 0.001$) groups. Not surprisingly, given their difficulties understanding the delayed alternation instructions, only 29% ($n = 15$) of children in the FAIL group exhibited age appropriate cognitive development. This is in contrast to 57% ($n = 32$) of the PERS and 69% ($n = 33$) of the ALTD groups (see Fig. 1).

Table 1 Demographic and perinatal characteristics for the participant sample

	Total sample $N = 227$	Retained sample $N = 156$
Male	116 (51)	82 (53)
GA[a]—mean (range)	27.4 (22–32)	27.5 (22–32)
Birthweight (g)—mean (range)	958 (414–1580)	954 (414–1395)
SGA[b]	21 (9)	16 (10)
Multiples[c]	95 (42)	67 (43)
Antenatal corticosteroids	201 (89)	135 (87)
Postnatal corticosteroids	21 (9)	13 (8)
Bronchopulmonary dysplasia[d]	77 (34)	54 (35)
Sepsis	101 (45)	69 (44)
NEC	25 (11)	16 (10)
Grade III/IV IVH	9 (4)	6 (4)
Cystic PVL	12 (5)	5 (3)
WMI mod/severe	37 (16)	27 (17)
Social risk index—mean (95% CI)	2.6 (2.2–2.9)	2.6 (2.2–3.0)
BSID-II MDI—mean (95% CI)	83.4 (80.8–86.1)	84.4 (81.6–87.1)

All data presented as counts (%), except where indicated—mean (SD).
[a]GA in completed weeks.
[b]Z-score >2 SD below mean weight for GA.
[c]Multiples refers to twin or triplet births.
[d]Required oxygen at 36 weeks GA.
SGA = small for GA; NEC = necrotizing enterocolitis; IVH = intraventricular haemorrhage; PVL = periventricular leukomalacia.

Overall brain volumes

The three delayed alternation groups had similar total brain, total tissue, CGM, DNGM and unMWM volumes (Table 3). A significant group difference was noted for CSF [$F(2,153) = 3.1$, $P = 0.05$], with the PERS group found to have more CSF than the ALTD group ($P = 0.02$); however this effect was no longer significant after adjusting for TBV. A significant group difference was also noted for MWM [$F(2,153) = 3.6$, $P = 0.03$]. The PERS group had more MWM than the FAIL ($P = 0.02$) and ALTD ($P = 0.02$) groups, and this remained significant after adjusting for TBV [$F(2,152) = 3.2$, $P = 0.04$; PERS > FAIL: $P = 0.02$; PERS > ALTD: $P = 0.05$]. When adjusting for relevant perinatal, social and developmental variables the difference between the PERS and FAIL groups with regards to MWM volume remained significant [$F(2,152) = 3.2$, $P = 0.04$;

Table 2 Comparison of demographic and perinatal characteristics of groups

	FAIL $N = 52$	PERS $N = 56$	ALTD $N = 48$	χ^2/F	P
Male	33 (64)	29 (52)	20 (42)	4.8	0.09
GA—mean (SD)	27.5 (1.9)	27.5 (2.1)	27.3 (2.0)	0.2	0.85
GA at scan—mean (SD)	40.4 (1.3)	40.2 (1.5)	40.2 (2.2)	0.3	0.73
Birthweight (g)—mean (SD)	944 (219)	968 (210)	946 (220)	0.2	0.81
Moderate social risk	34 (65)	33 (59)	29 (60)	0.5	0.77
SGA	9 (12)	5 (9)	5 (10)	0.2	0.90
Multiples	23 (44)	29 (52)	15 (31)	4.5	0.11
Antenatal corticosteroids	47 (90)	49 (88)	39 (81)	4.0	0.41
Postnatal corticosteroids	5 (10)	5 (9)	3 (6)	0.4	0.81
Bronchopulmonary dysplasia	20 (39)	17 (30)	17 (35)	2.9	0.57
Sepsis	28 (54)	22 (39)	19 (40)	2.9	0.23
NEC	3 (6)	2 (4)	3 (6)	1.6	0.82
IVH—Grade III/IV	3 (6)	2 (4)	1 (2)	0.9	0.63
Cystic PVL	1 (2)	2 (4)	2 (4)	0.4	0.80
WMI- mod/severe	10 (19)	14 (26)	3 (6)	7.0*	0.03
BSID-II MDI—mean (SD)	76 (17)	86 (15)	92 (17)	12.3**	<0.001

All data presented as counts (%), except where indicated—mean (SD).
*$P \leq 0.05$, **$P < 0.001$.
SGA = small for GA; NEC = necrotizing enterocolitis; IVH = intraventricular haemorrhage; PVL = periventricular leukomalacia.

PERS > FAIL: $P = 0.02$], but the difference between the PERS and ALTD groups failed to reach statistical significance.

Regional brain volumes

No differences between the three delayed alternation groups were found for any of the eight parcellated regions for either hemisphere, including the parietal-occipital and dorsolateral prefrontal regions (Table 4). However, small group differences were found for both the right and left hippocampi [right: $F(2,153) = 2.7$, $P = 0.07$; left: $F(2,153) = 2.9$, $P = 0.06$], and these effects became significant after adjusting for TBV [right: $F(2,152) = 5.8$, $P = 0.004$; left: $F(2,152) = 6.1$, $P = 0.003$], and relevant perinatal, social and developmental variables [right: $F(2,140) = 5.0$, $P = 0.008$; left: $F(2,140) = 4.9$, $P = 0.009$]. *Post hoc* analyses revealed that hippocampal volumes for the PERS group were reduced in comparison to the ALTD group (right: $P = 0.03$; left: $P = 0.03$), and remained so after controlling for TBV and relevant perinatal, social and developmental factors (right: $P = 0.02$, left: $P = 0.006$). The hippocampi for the PERS group also tended to be smaller than the FAIL group (right: $P = 0.08$; left: $P = 0.08$), and this difference reached statistical significance after controlling for TBV and relevant perinatal, social and developmental variables (right: $P = 0.02$; left: $P = 0.01$). Interestingly, hippocampal volume correlated weakly with MDI prior to (right: $r = 0.118$, $P > 0.05$; left: $r = 0.140$, $P > 0.05$) and after adjustment for TBV and relevant perinatal variables (right: $r = -0.02$, $P > 0.05$; left: $r = 0.02$, $P > 0.05$). Thus, hippocampal volume in the neonatal period is not strongly associated with early cognitive development.

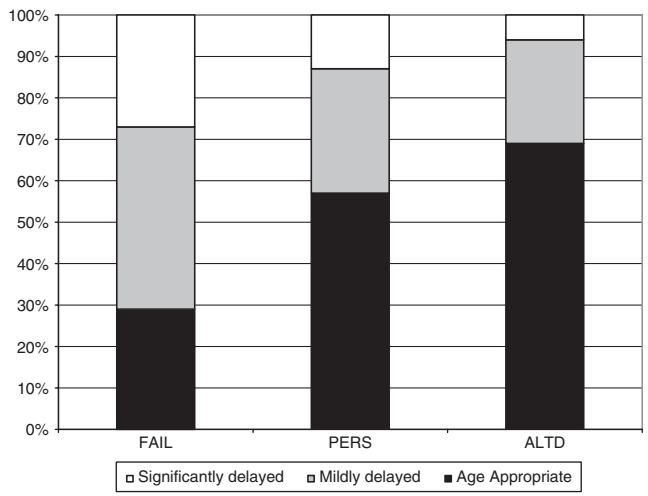

Fig. 1 Cognitive development classification for the three delayed alternation groups according to the BSID-II MDI.

Discussion

The aim of this study was to determine whether working memory deficits in preterm children at 2 years' corrected age are associated with reductions in specific brain regions

Table 4 Group differences in regional brain volumes

Parcellated brain regions (cm^3)—mean (SE)	FAIL $N = 52$	PERS $N = 56$	ALTD $N = 48$
DLPFC			
R	24.5 (0.8)	25.0 (0.8)	24.5 (0.9)
L	21.6 (0.7)	22.4 (0.7)	21.8 (0.7)
OFC			
R	5.6 (0.4)	5.9 (0.4)	5.2 (0.5)
L	5.0 (0.4)	5.0 (0.4)	4.4 (0.4)
Premotor			
R	25.7 (0.8)	25.5 (0.8)	24.8 (0.9)
L	24.1 (0.7)	23.8 (0.7)	23.1 (0.8)
Subgenual			
R	10.1 (0.4)	10.5 (0.4)	9.6 (0.5)
L	9.1 (0.4)	9.7 (0.4)	8.8 (0.4)
Sensorimotor			
R	29.8 (0.9)	29.6 (0.8)	30.0 (0.9)
L	28.9 (0.8)	28.7 (0.8)	28.5 (0.8)
Midtemporal			
R	14.6 (0.5)	14.8 (0.5)	14.7 (0.5)
L	13.8 (0.5)	13.9 (0.5)	14.2 (0.5)
Parieto-occipital			
R	58.7 (1.5)	58.8 (1.4)	57.2 (1.6)
L	60.6 (1.6)	60.9 (1.5)	60.1 (1.6)
Inf. Occipital/cerebellum			
R	35.3 (1.2)	34.6 (1.1)	34.1 (1.2)
L	35.5 (1.3)	34.2 (1.2)	35.5 (1.3)
Manually segmented region (ml)—mean (SE)			
Hippocampus			
R	1.16 (0.02)	1.10 (0.02)	1.18 (0.03)**
L	1.14 (0.03)	1.08 (0.02)	1.16 (0.03)**

**$P < 0.01$.

Table 3 Group differences in regional brain volumes

Brain area (ml)—mean (SE)	Total $N = 156$	FAIL $N = 52$	PERS $N = 56$	ALTD $N = 48$
Cortical grey matter	162.5 (3.4)	160.9 (5.9)	163.8 (5.7)	162.6 (6.2)
Deep nuclear grey matter	13.8 (0.3)	14.2 (0.5)	13.3 (0.5)	14.0 (0.5)
Unmyelinated white matter	215.3 (2.6)	219.2 (4.5)	215.3 (4.4)	211.2 (4.7)
Myelinated white matter	10.1 (0.4)	9.4 (0.6)[a]	11.4 (0.6)[a]	9.4 (0.7)
Subtotal (total tissue)	401.7 (5.0)	403.6 (8.7)	403.9 (8.4)	397.2 (9.1)
Cerebrospinal fluid	46.0 (2.1)	47.5 (3.6)	51.0 (3.4)	38.7 (3.7)
TBV	447.8 (5.6)	451.1 (9.8)	454.9 (9.4)	435.9 (10.1)

[a]$F(2,152) = 3.2$, PERS > FAIL: $P = 0.02$.

at TEA, in particular dorsolateral prefrontal, parietal and hippocampal structures. Using the delayed alternation task, we found that children with working memory deficits (i.e. children in the PERS group) had similar volumes for the eight parcellated regions for each hemisphere when compared with those children who exhibited intact working memory (ALTD group) and children who failed to learn or persist with the task (FAIL group). Thus, contrary to expectation, dorsolateral prefrontal and parietal-occipital volumes at TEA were not associated with performance on the delayed alternation task. However, it has been suggested that frontal and parietal regions become increasingly important as childhood progresses (Klingberg, 2006; Scherf *et al.*, 2006; Bunge and Wright, 2007), which could explain the lack of findings for these areas in our young group. In contrast, we found a robust association between hippocampal volume at TEA and performance on the delayed alternation task. More specifically, children who exhibited working memory deficits had marginally smaller hippocampi than children who exhibited intact working memory and children who failed to learn or persist with the task, and this relationship became more significant when taking into account TBV and a range of perinatal, social and developmental variables. These findings may suggest that visual working memory in young preterm children is strongly related to the early integrity of the hippocampus.

Difficult to explain was the finding that children who failed to learn or persist with the task (FAIL) had similar hippocampi volumes to the children who exhibited intact working memory (ALTD) and larger volumes than the children who PERS. Given the FAIL group displayed significantly higher rates of cognitive delay than the other groups, one may assume that a proportion of this group would also be exhibiting working memory deficits and therefore would have smaller hippocampi than the ALTD group. However, the reasons these children failed to learn or persist with the task was not reduced working memory but include significant cognitive delay, poor comprehension of instructions, non-compliance or inattention. Ideally, alternative working memory measures which are more appropriate for these delayed or non-compliant children would have been administered.

While extensive evidence points to a role for the prefrontal cortex in working memory function (see Curtis and D'Esposito, 2003, for a review), our findings support a growing body of evidence indicating that working memory relies more on a distributed network of brain regions which includes the hippocampus. For example, van Asselen and co-workers (2006) have shown that spatial working memory is affected in stroke patients with bilateral damage to the hippocampus. Results from animal studies support these findings by showing that disruption to hippocampal function can negatively affect working memory abilities (Wang and Cai, 2006; Chan *et al.*, 2007; McHugh *et al.*, 2007). Functional neuroimaging studies demonstrate the involvement of the hippocampus in both visual and verbal working memory tasks (Ranganath and D'Esposito, 2001; Bedwell *et al.*, 2005; Karlsgodt *et al.*, 2005; Nichols *et al.*, 2006; Piekema *et al.*, 2006). Particularly relevant to the present study are the findings of Piekema *et al.* (2006) who reported hippocampal activity when participants were required to maintain object–location associations, suggesting this region plays a role when spatial information has to be maintained online, as is the case for tasks requiring delayed alternation.

In addition to supporting a role for the hippocampus in working memory, our findings highlight the importance of taking into account developmental differences when considering the neuroanatomical substrates of cognitive functions. Though the involvement of the prefrontal cortex would be suspected in a working memory task such as delayed alternation, studies have shown that, in comparison to adults, the pattern of neural involvement in working memory may be distinctly different in infants, young children and adolescents (Ciesielski *et al.*, 2006; Bunge and Wright, 2007) who show more diffuse and atypical patterns of activation (Bell and Wolfe, 2007). Using fMRI, Scherf *et al.* (2006) tested subjects of 8–47 years of age on a visuospatial working memory task and found that children had limited activation in the core working memory regions (e.g. dorsolateral prefrontal cortex and parietal regions) compared with adults. These differences are likely to reflect the protracted development of the frontal lobes, which are known to mature late in comparison to more posterior brain regions and continue to develop into the third decade of life (Giedd *et al.*, 1996; Casey *et al.*, 2000; Conklin *et al.*, 2007). In a review of studies of the neural mechanisms associated with object working memory in the infant brain, Kaldy and Sigala (2004) concluded that children rely less on frontal structures for these tasks and more on earlier maturing posterior structures involving the temporal cortex and including the hippocampus. These findings are consistent with the current study which also highlights a strong link between hippocampal structure at TEA and performance on a working memory task at 2 years of age. As such, in infants, the involvement of the hippocampus in working memory may be seen as a reliance on a compensatory network, which becomes more localized and 'typical' with the maturation of frontal areas during adolescence (Casey *et al.*, 2005; Durston *et al.*, 2006). This reliance on an atypical or compensatory network may additionally be a function of prematurity, which has been shown to affect both structure and function in the brain and could therefore alter the brain circuitry responsible for particular cognitive functions, in this case working memory (Ment and Constable, 2007).

Further evidence regarding the nature of the involvement of the hippocampus in working memory at different stages of development comes from the work investigating the effects of hippocampal damage during early development. Animal models have shown that developmental lesions of the ventral hippocampus impair performance in

working memory tests normally related to functions of the prefrontal cortex (Chambers *et al.*, 1996; Lipska *et al.*, 2002). Conversely, adult hippocampal lesions do not impair performance in the same tasks. This suggests that working memory may be affected only through a unique period of developmental injury to the hippocampus. Early damage to the hippocampus may exert its effect on longer term working memory by creating a functional abnormality of the underlying neural circuitry including the prefrontal cortex (Wood *et al.*, 2003; Tseng *et al.*, 2006). The hippocampus fosters extensive connections with various other brain regions (Rolls, 2000; Thierry *et al.*, 2000). Thus, alterations of this structure early in development may have a secondary impact on other structures through functional networks and anatomic connections.

The contribution of WMI to cognitive functions must also be considered, as it is the primary neuropathology in prematurity (Marin-Padilla, 1997; Back *et al.*, 2001; Counsell *et al.*, 2003; Rees and Inder, 2005; Thompson *et al.*, 2007). WMI in preterm infants has been shown to be linked to impairments in early cognitive development, lower IQ, poor arithmetic abilities and motor function (Peterson, 2003; Woodward *et al.*, 2006; Skranes *et al.*, 2007), and the hippocampus appears to be particularly vulnerable to this type of insult (Thompson *et al.*, 2008). In the current study, however, the impact of altered hippocampal structure on working memory was independent of the effect of WMI, as the association remained robust even after controlling for the effects of WMI. This suggests that changes in the early development of the hippocampus may play a direct pathological role in later cognitive impairments, such as working memory deficits.

This study had some inherent methodological limitations and as such our interpretations of the findings are speculative and largely influenced by functional imaging studies (Kaldy and Sigala, 2004; Piekema *et al.*, 2006). Firstly, assessing 2-year old children is not as reliable as assessing older children given the variability in development, attention, motivation and comprehension for this age group. For example, 33% of our sample failed to learn or persist with the working memory task used in this study. Related to this, few valid and reliable working memory measures are available for this age when these functions are only beginning to emerge (Mahone, 2005). Furthermore, as with all cognitive measures, the delayed alternation task taps multiple cognitive processes, not just working memory, although we categorized performance according to perseverative behaviour, which in this age group is thought to reflect working memory impairment. Follow-up studies using additional measures of working memory at later stages of development are in progress and will assist in understanding the nature and extent of working memory impairment in these children. Another potential limitation is the parcellation technique used for estimating regional brain volumes. While this parcellation technique does not necessarily reflect true anatomical regions, it is the most widely used approach in this age group (Peterson *et al.*, 2000; Woodward *et al.*, 2005; Thompson *et al.*, 2007). Finally, although the data set used here was incomplete for some measures, analyses indicate that the sample was representative of the overall cohort, which constituted a very large group of children studied longitudinally.

Conclusions

The findings from this study suggest that in addition to the more traditional role of the hippocampus in episodic memory function in preterm children (Isaacs *et al.*, 2000), the integrity of this structure in the neonatal period may also contribute to working memory in preterm children as early as 2 years of age. The results of this study have important clinical implications for cognitive development in preterm children. Working memory plays a significant role in many cognitive functions and, in preterm children, may constitute a core deficit. Early disruption of the underlying neural circuitry involved in working memory may subsequently impact on a range of functions, such as language development (Rudner and Ronnberg, 2007), literacy, writing skills and mathematical abilities (Gersten *et al.*, 2005; Gathercole *et al.*, 2006; Lundberg and Sterner, 2006; Andersson, 2007), as well as executive functions, such as planning and organization (Pennington *et al.*, 1996; Proctor *et al.*, 2000). Until now, very little attention has been given to the assessment of working memory functions in prematurity (Woodward *et al.*, 2005); therefore, the present study contributes valuable evidence of its underlying neuroanatomical correlates and vulnerability to working memory deficits in preterm children. Further research is needed to determine the long-term anatomical and functional implications of altered hippocampal volumes and working memory deficits in survivors of preterm birth.

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