doi:10.1093/brain/awh618 *Brain* (2005), **128,** 2578–2587

Object working memory deficits predicted by early brain injury and development in the preterm infant

Lianne J. Woodward,[1] Jamie O. Edgin,[1] Deanne Thompson[2] and Terrie E. Inder[3]

[1]University of Canterbury and Van der Veer Institute for Parkinson's and Brain Research, New Zealand, [2]Howard Florey Institute, University of Melbourne and [3]Murdoch Childrens' Research Institute, Howard Florey Institute, University of Melbourne, Australia

Correspondence to: Lianne Woodward, Child Development Research Group, University of Canterbury, Private Bag 4800, Christchurch, New Zealand
E-mail: lianne.woodward@canterbury.ac.nz

Children born preterm and of very low birth weight are at increased risk of learning difficulties and educational under-achievement. However, little is known about the specific neuropsychological problems facing these children or their neurological basis. Using prospective longitudinal data from a regional cohort of 92 preterm and 103 full-term children, this study examined relations between term MRI measures of cerebral injury and structural brain development and children's subsequent performance on an object working memory task at the age of 2 years. Results revealed clear between-group differences, with preterm children having greater difficulty encoding new information in working memory than term control children. Within the preterm group, task performance at the age of 2 years was related to both qualitative MRI measures of white matter (WM) injury and quantitative measures of total and regional brain volumes assessed at term equivalent. Bilateral reductions in total tissue volumes (%region) of the following cerebral regions were specifically related to subsequent working memory performance: dorsolateral prefrontal cortex, sensorimotor, parietooccipital and premotor. Associations between total cerebral tissue volumes at term (adjusted and unadjusted for intracranial volume) persisted even after the effects of WM injury were taken into account. This suggests that early disturbance in cerebral development may have an independent adverse impact on later working memory function in the preterm infant. These findings add to our understanding of the neuropathological pathways associated with later executive dysfunction in the very preterm infant.

Keywords: MRI; preterm; working memory; white matter injury; brain development

Abbreviations: AB = A-not-B; DPFC = dorsal prefrontal Cortex; MDI = mental development index; MSML = multisearch multilocation; PDI = physical development index; PM = premotor; WM = white matter

Received February 1, 2005. Revised June 3, 2005. Accepted July 19, 2005. Advance Access publication September 8, 2005

Introduction

The ability to form mental representations of objects and to remember where they are located is a key aspect of everyday functioning and an important predictor of children's educational progress (Gathercole, 1999). Consequently, there has been considerable interest in the development of working memory and the neurological structures that subserve this process (Kaldy and Sigala, 2004).

One of the most widely used paradigms for assessing the development of object working memory during infancy is the A-not-B (AB) search task (Piaget, 1954). This task consists of two stages and requires an infant to both remember an object's location (Diamond, 1990; Espy *et al.*, 1999) and to inhibit a previously learned response (Espy *et al.*, 1999, 2001). Tasks based on this paradigm have been used extensively with term born infants and toddlers to assess the effects of different experimental manipulations on performance (Marcovitch and Zelazo, 1999; Bremner and Bryant, 2001), as well as to help specify the neurological substrates underlying the development of working memory and related executive abilities (Diamond, 1990; Bell and Fox, 1992; Baird *et al.*, 2002).

One group of children that has attracted attention from researchers interested in both memory processes assessed by the AB task, as well as the effects of early brain development on later outcomes are children born very preterm (≤32 weeks gestation). Clear evidence shows that these children are at high risk of later neurodevelopmental difficulties, including both cerebral palsy (5–15%) and significant learning disability (30–50%) (Marlow *et al.*, 2005). Preterm children have also been shown to be characterized by impaired working memory throughout childhood (Rose and Feldman, 1996; Ross *et al.*, 1996; Luciana *et al.*, 1999; Isaacs *et al.*, 2000; Vicari *et al.*, 2004). Working memory refers to the process of holding task relevant information in mind for brief intervals to then use this information to guide future actions (Gioia *et al.*, 2001). Impairment in the development of this important cognitive skill has been shown to contribute significantly to preterm children's later risks of global intellectual and academic difficulties at school (Rose *et al.*, 1992; Wolke and Meyer, 1999).

To date, the neural mechanisms responsible for these memory impairments in children born preterm have not been specified (McQuillen and Ferriero, 2004). However, existing research does suggest at least two potential neuropathological substrates. These include (i) preterm children's elevated risk of specific forms of cerebral injury, including intraventricular haemorrhage and periventricular leukomalacia (Olsen *et al.*, 1997; Volpe, 1998), and (ii) the possibility of altered or delayed cerebral development as a consequence of both preterm birth and white matter (WM) injury (Huppi *et al.*, 1996; Peterson *et al.*, 2000; Inder *et al.*, 2003, 2005). The extent to which clinically important memory impairments observed in children born very preterm are related to cerebral WM injury and/or altered structural cerebral development has not been established. To address this issue, this study draws on prospective longitudinal data from a regional cohort of very preterm infants to

(i) compare the performance of very preterm and full-term control children at the age of 2 years (corrected for prematurity) on an age appropriate variant of the AB task known as the multisearch multilocation (MSML) search task (Zelazo *et al.*, 1998).
(ii) examine within the very preterm group, associations between MSML task performance at the age of 2 years and (a) perinatal and social background factors correlated with prematurity; (b) WM injury severity assessed using qualitative MRI methods, and (c) cerebral structural development assessed using quantitative MRI measures of total and regional brain volumes at term equivalent.

Methods

Subjects

Two groups of children were included in this study. The first group consisted of a regional cohort of 100 children born very preterm (≤32 weeks gestation) and very low birth weight (<1500 g) who were consecutively admitted to a level III Neonatal Intensive Care Unit at Christchurch Women's Hospital, New Zealand over 2 years (1998–2000). Infants with congenital abnormalities and whose parents did not speak English were excluded. Over the recruitment period, 119 preterm infants were eligible for inclusion in the study. Of these, 10 died before term, 4 failed to be recruited and 5 declined to participate. Excluding deaths, 92% of all eligible infants were recruited. These infants (47 male : 45 female) had a mean gestational age of 27.9 (±2.4) weeks and a mean birth weight of 1088 (±315.2) g. All infants in the preterm group were assessed throughout the perinatal period, at term, 1 and 2 years using a combination of measures, including MRI, medical records, parent interviews and clinical assessments of physical and cognitive functioning. Retention at age 2 years was 93%, with three preterm infants lost to follow-up, three deaths, and one child living abroad.

At term equivalent (40 weeks gestation), all preterm infants underwent an MRI scan. Qualitative ratings of WM injury were possible for 99 infants. However, quantitative volumetric MR post-acquisition analysis was reliably completed for only 76 infants, with the remaining 24 infants having limitations in image analysis including motion artefact and MRI intensity errors which limited registration and tissue segmentation. Examination of the effects of this data loss on the representativeness of the sample failed to detect any significant ($P < 0.05$) differences between infants included and excluded from the analysis due to imaging problems on measures of gender, gestational age, birth weight, cerebral injury, or illness severity.

In addition to the above group of very preterm children, a comparison group of 103 (56 male : 47 female) full-term children was recruited at 2 years of age. These children were identified from hospital birth records ($n = 7200$ total births) by alternately selecting for each preterm child, the second previous or next child of the same gender in the delivery schedule. Infants with congenital abnormalities and from non-English speaking families were excluded. The recruitment response from potential term comparison families was 62%. Reasons for non-participation were as follows: untraced (47%), moved overseas (12.5%), refused (12.5%), agreed but not seen within the assessment window (24 months + 2 weeks) owing to illness or family circumstances (28%). These infants (56 male : 47 female) had a mean gestational age of 39.6 (±1.1) weeks and a mean birth weight of 3596.8 (±401.6) g. Although no term MRI data were available for these children, they were subject to the same 2-year assessment protocol as children in the preterm group.

Measures

Object working memory and developmental assessment (2 years corrected)

Within 2 weeks of their second birthday (corrected for prematurity), all study children underwent an ethics approved, comprehensive neurodevelopmental assessment. This assessment included the MSML task and the Bayley Scales of Infant Development (BSID-II; Bayley, 1993 #180). The BSID-II provides a standardized measure of infant cognitive and psychomotor development.

The primary measure of object working memory was a 10 min three-step MSML task based on Zelazo *et al.*'s (1998) four-step procedure. For this study, one task step (pulling out the search tray) was omitted given pilot data showing that preterm children experienced greater motor difficulties with this step than full-term children. The testing apparatus is shown in Fig. 1 and consisted of a 55 cm × 60 cm × 30 cm wooden box with a removable front panel and a side door through which the experimenter could hide an M&M sweet. Behind the opaque removable panel was a transparent

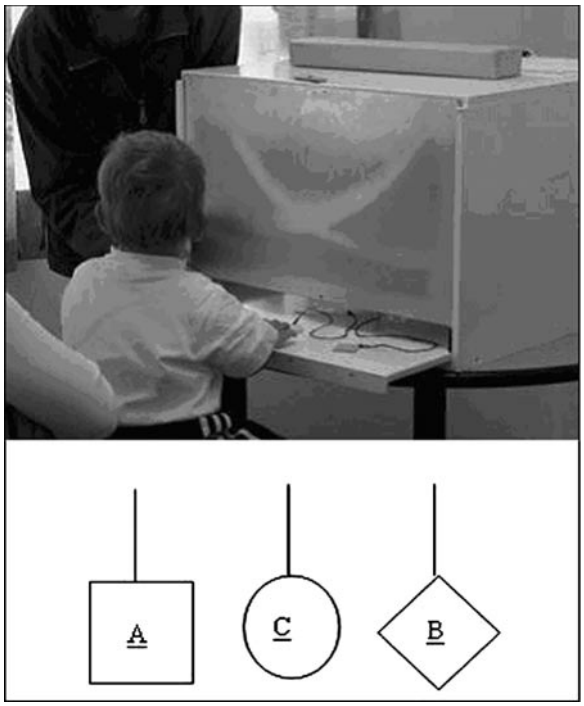

Fig. 1 Three-step MSML task apparatus and stimuli used during experimental trials. During the pre-switch, the M&M was hidden at location A until the child correctly found it on three consecutive trials. Then, if the child succeeded on the pre-switch, the M&M was hidden at location B. Location C served as a distracter and the M&M was never hidden there.

plexiglass window through which children could observe the hiding procedure. Below this window was a narrow gap that allowed for stimulus objects to be presented to the child, but did not allow direct retrieval of the sweet. In addition, a covered foam barrier was also used to conceal stimuli. As illustrated in Fig. 1, the hiding stimuli consisted of four clear plastic bags each attached to a coloured wooden shape by a long piece of string (one training stimulus, three experimental stimuli). An M&M was placed into one bag and then placed at the back of the box, with the attached shape being placed on the testing tray below the plexiglass window. Children sat on their mother's knee during the task. Mothers were asked not to provide their child with any prompts or cues.

The testing procedure involved three phases: (i) training, (ii) pre-switch and (iii) post-switch. During training, the child was taught the three steps needed to retrieve a reward: remove the barrier; select the shape; pull the shape to retrieve the sweet. The experimenter modelled each step and then asked the child 'Can you find the lolly (M&M)?'. Correct retrieval was praised and rewarded with the M&M. The training ended when the child independently executed the retrieval sequence without assistance.

Following successful training, pre-switch trials commenced using three new novel stimuli. For each pre-switch trial, the experimenter placed an M&M into the transparent bag at A, held up the bag and its corresponding symbol and said 'I am hiding the lolly in this one'. She then pointed to the other two symbols and their attached bags at locations C (middle) and B (right side) and for each, said 'the lolly is not in this one'. Immediately following the lowering of the opaque door and placement of the foam barrier over the shapes by the experimenter, each trial was commenced with the child being encouraged to search for the M&M. Each trial ended when the child had removed the foam barrier and pulled one of the strings. Pre-switch trials were repeated until a child reached a criterion of three consecutively correct trials. A trial was abandoned if a child failed to respond within 30 s. Testing was also stopped if a child failed to respond repeatedly (>2 times) or if the criterion was not reached within eight trials.

For all children who reached the pre-switch criterion, post-switch trials were initiated. These trials were identical to pre-switch, but with the M&M being visibly hidden at a different (B) location. Post-switch trials were repeated until the child found the M&M, failed repeatedly (on three consecutive trials), lost interest, or the number of trials exceeded five.

Several measures of task performance were recorded. These included the duration of training, number of pre-switch trials to first correct retrieval, number of pre-switch trials discontinued or repeated, achievement of pre-switch criterion and the search location for post-switch trials. On the basis of their task performance, children were classified into four groups. The first group consisted of children who were unable to achieve the pre-switch criterion of three consecutively correct trials. The second group included children who passed the pre-switch criterion but made a non-perseverative error (searched at a never used location) on their first post-switch trial. These children learnt the pre-switch rule for location but were unable to use this representation to guide their post-switch response. The third group consisted of children who achieved the pre-switch criterion, but searched perseveratively at post-switch; thus they were unable to override their previously learned search behaviour. The final group consisted of children who searched successfully at post-switch. Children who progressed further through the task and were more successful also tended to have higher mental development index (MDI) scores [$F(3, 82) = 3.13$, $P = 0.03$] supporting the validity of these groups.

MRI procedure

All very preterm infants underwent a 30-min MRI scan at term (39–41 weeks gestation). Prior to imaging, infants were fed, wrapped and placed unsedated in a Vac Fix bean bag. MRI was performed with a 1.5 T General Electric Signa System (GE-Medical Systems, Milwaukee, WI, USA). For the acquisition of primary MRI data, two different imaging modes were applied. These included (i) a 3D Fourier transform spoiled gradient recalled (SPGR) sequence (1.5 mm coronal slices, flip angle 45°, repetition time 35 ms, echo time 5 ms, field of view 18 cm, matrix 256 × 256); and (ii) a double echo (proton density and T2 weighted)-spin echo sequence (DE) (3 mm axial slices, repetition time 3000 ms, echo times 36 and 162 ms, field of view 18 cm, matrix 256 × 256, interleaved acquisition). The voxel (volume of the pixel) dimensions for the SPGR acquisition were $0.7 \times 0.7 \times 1.5$ mm^3 and for the spin echo acquisition were $0.7 \times 0.7 \times 3$ mm^3, respectively.

Imaging analysis

Images were analysed using both qualitative and quantitative methods. Qualitative structural analysis involved the scoring of representative images for the presence and severity of WM abnormality. WM abnormality was graded using five 3-point scales assessing: (i) the nature and extent of WM signal abnormality; (ii) periventricular WM volume loss; (iii) the presence of any cystic abnormalities; (iv) ventricular dilatation; and (v) thinning of the corpus callosum (Inder *et al.*, 2003; Woodward *et al.*, 2004). Independent

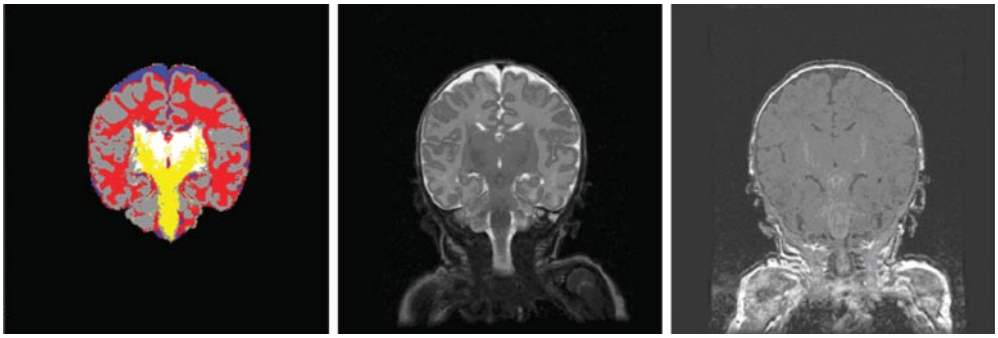

Fig. 2 Post-acquisition segmentation atlas with the segmented image (*left*), T2 weighted coronal MR image (*middle*) and T1 weighted SPGR image (*right*) which are all coregistered. In the segmented map image cortical grey matter is shown in grey, myelinated white matter in yellow, unmyelinated white matter in red, deep nuclear grey matter in white and cerebrospinal fluid in blue colours.

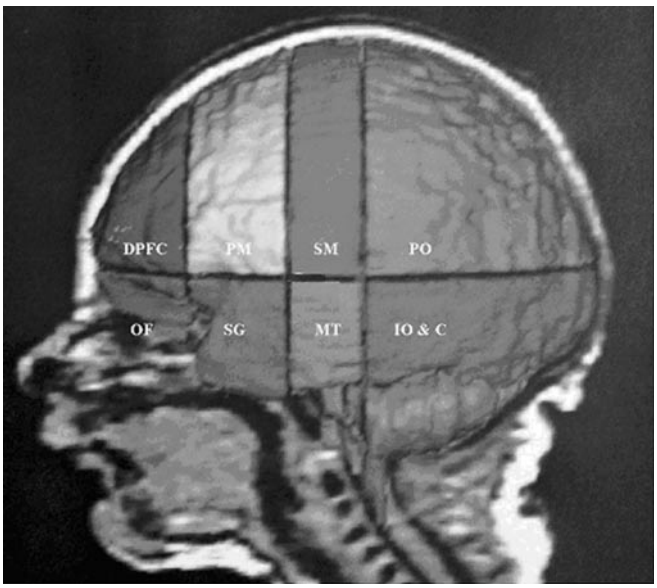

Fig. 3 Cerebral subregions from parcellation (left hemisphere). Subregions: dorsal prefrontal (DPFC), oribitofrontal (OF), premotor (PM), subgenual (SG), sensorimotor (SM), midtemporal (MT), parietooccipital (PO), and inferior occipital and cerebellum (IO&C).

scoring by a blinded paediatric neuroradiologist and neurologist showed high interrater agreement (95%), with consensus ratings given to discrepant cases. Further scoring details are provided in Inder *et al.* (2003).

For the quantitative volumetric analysis, post-acquisition processing was done using a sequence of image processing algorithms to segment each of the MRI slices into five separate tissue classes: cortical grey matter, deep nuclear grey matter, myelinated WM, unmyelinated WM and cerebrospinal fluid (Fig. 2) (Peterson *et al.*, 2003; Inder *et al.*, 2005). Results for each tissue type were reported in terms of absolute tissue volumes (mm^3) and as a relative percentage of the intracranial cavity (%ICV). The total intracranial cavity was then parcellated into eight subregions for each hemisphere using a combination of three coronal planes and one axial plane (Peterson *et al.*, 2003). These parcellated subregions are shown in Fig. 3 and included the dorsal prefrontal (DPFC), oribitofrontal (OF), premotor (PM), subgenual (SG) (e.g. anterior cingulate), sensorimotor (SM), midtemporal (MT), parietooccipital (PO), and inferior occipital cortex and cerebellum (IO&C). Examination of associations between each subregion and outcome were analysed using the tissue volume adjusted for the total volume of the subregion.

Statistical analysis

Data analysis was conducted in two stages. In the first stage, between-group differences in MSML task performance at the age of 2 years were tested using one-way analysis of variance for continuously distributed variables and the χ^2-test of independence for dichotomous variables. In the second stage, within the very preterm group only, relations between the quality of children's MSML task performance and a series of antecedent factors were examined using either one-way analysis of variance with tests for linear trend, or the Mantel Haenszel χ^2-test of linear association. Antecedent factors examined included: (i) perinatal and social background factors; (ii) WM injury severity; and (iii) volumetric measures of cerebral development. Finally, to examine relations between perinatal and neurological factors in predicting outcome, linear regression models were fitted to the data. Model fitting was conducted using methods of forwards and backwards variable selection to identify the best fitting and most parsimonious model.

Results

Clinical characteristics

The preterm and term control children, as expected, differed with respect to gestational age ($P < 0.0001$), birth weight ($P < 0.0001$) and multiple births (preterm 34.8% versus term 1.9%, $P < 0.0001$). The gender distribution was similar for the two groups (male: preterm 51.1% versus term 54.4%, $P < 0.60$). Only six children in the preterm group were treated with post-natal steroids. Qualitative MRI evaluations for preterm infants seen at 2 years showed that 24.2% were characterized by no WM abnormality, 57.1% by mild and 18.7% by moderate to severe WM abnormalities (15.4% non-cystic, 3.3% cystic).

At the age of 2 years, clear differences were evident between preterm and term control children on standardized measures of cognitive and psychomotor development. On the MDI of the Bayley, 47.8% of preterm compared with 78.7% of control children scored in the normal to accelerated range (MDI score > 84). A further 40.2% of preterm and 17.5% of control

Table 1 Performance of very preterm and full-term children on training and pre-switch trials of the MSML search task at the age of 2 years

Measure	Very preterm (n = 92)	Full term (n = 103)	F/χ^2	P
Training trials				
Mean (SD) duration of training (s)	110.86 (76.80)	87.11 (47.35)	6.91	<0.01
Pre-switch trials				
Mean (SD) *N* trials to first correct retrieval	2.06 (1.47)	1.75 (0.62)	2.49	<0.12
Mean (SD) *N* trials needed to be discontinued or repeated	0.51 (1.18)	0.17 (0.62)	2.38	<0.05
Children unable to reach pre-switch criterion (%)	26.1	12.6	5.73	<0.05

children were mildly delayed and 12.0 and 3.9%, respectively, showed serious cognitive delay (MDI < 70 or 2 SD below mean). On the physical development index (PDI), 68.5% of preterm compared to 90.3% of control children scored in the normal to accelerated range (PDI score > 84). A further 27.2% of preterm and 7.8% of control children were mildly delayed and 4.3 and 1.9%, respectively, had serious motor delay (PDI < 70 or 2 SD below mean). These findings indicate that whilst very preterm infants were characterized by significantly ($P < 0.001$) poorer mental and motor performance than full-term infants, rates of moderate to severe disability, especially motor disability, were relatively modest in this regionally representative cohort.

MSML task performance of children born preterm and full term

Training and pre-switch

Table 1 compares the performance of preterm and full-term children on the training and pre-switch trials of the MSML task. During training, preterm children required significantly ($P < 0.01$) longer time to learn and independently complete the retrieval sequence. The greater difficulty experienced by preterm infants on this tasks was also evident during pre-switch trials. Compared with term children, preterm children tended to take longer to correctly retrieve the reward at *A* for the first time ($P < 0.12$), were significantly more likely to need to repeat pre-switch trials ($P < 0.05$), and were twice as likely (26% versus 13%) to fail to reach the pre-switch criterion of three consecutively correct A search trials ($P < 0.05$). These findings suggest that the preterm group took longer to learn the retrieval sequence, and once having successfully performed the sequence, required more pre-switch trials in which to encode and reliably retrieve the reward from the A hiding location.

Post-switch

Table 2 shows the performance of preterm and full-term children on the first post-switch trial of the MSML search task. Shown in this table are the proportions of children (i) searching correctly at B, (ii) searching incorrectly at A (perseverative error), (iii) searching incorrectly at C (non-perseverative error), and (iv) being unable to reach the criterion to enter post-switch. Post-switch search behaviour of children in the preterm group was significantly different from the search behaviour of full-term infants [$\chi^2(3) = 8.44$, $P < 0.05$], with full-term children being 1.4 times more likely to search correctly at B than preterm children. Examination of the types of errors committed showed that preterm children were nearly twice as likely as term children to make a non-perseverative error (searching at neither A or B), whereas full-term children tended to be more likely to commit the more common and expected, perseverative error (searching at the original A hiding location).

Table 2 Proportion of very preterm and full-term infants making correct (B), perseverative (A), or non-perseverative (C) search responses at post-switch

Measure	Very preterm (n = 92)	Full term (n = 103)
Correct switch (B)	28.3 (26/92)	39.8 (41/103)
Perseverative (A)	32.6 (30/92)	39.8 (41/103)
Non-perseverative (C)	14.1 (13/92)	7.8 (8/103)
Failed to reach post-switch trial	25.0 (23/92)	12.6 (13/103)

$\chi^2(3) = 8.44$; $P < 0.05$.

Relationship between perinatal and social background factors and later MSML task performance

Perinatal and social background factors that might help explain the impaired MSML task performance of very preterm infants were examined in Table 3. There was a trend for two perinatal factors, maternal fever and proven sepsis in the infant at delivery (both of which were highly correlated, $r = 0.73$), to be associated with preterm children's later performance on the MSML task ($P = 0.06$). Further analysis showed that the relationship between maternal fever and child outcome was mediated by WM injury. However, associations between infant sepsis and outcome persisted after the effects of WM injury were taken into account ($P < 0.05$). Finally, there was a trend for gestational age to be associated with outcome, with increasing immaturity tending to be associated with poorer task performance ($P = 0.10$). No other perinatal or social background factors were associated with later task performance.

Table 3 Relationship between perinatal and social background factors assessed at term equivalent and children's performance on the MSML task at age 2

	Post-switch group					
	Correct switch (B) (n = 26)	Perseverative (A) (n = 30)	Non-perseverative (C) (n = 13)	Failed to reach post-switch (n = 23)	F	P
Child perinatal factors						
Mean (SD) gestation	28.42 (2.18)	28.10 (2.20)	27.46 (2.82)	27.43 (2.50)	2.63	>0.10
Mean (SD) birth weight	1117.31 (230.68)	1094.33 (317.71)	1121.85 (348.66)	1027.57 (381.61)	0.80	>0.30
% Male	46.2	43.3	76.9	52.2	0.88	>0.30
% Singletons	57.7	60.0	76.9	73.9	2.05	>0.15
% Intrauterine growth restriction*	3.8	10.0	0.0	13.0	3.72	>0.20
Mean (SD) CRIB score≠	2.04 (2.14)	2.70 (2.81)	3.31 (2.53)	3.27 (3.68)	2.57	>0.10
% Maternal fever >38°C	3.8	7.6	0.0	23.1	7.49	0.06
% Proven sepsis in infant	23.1	26.7	23.1	39.1	3.49	0.06
% Intraventricular haemorrhage grade3/4**	3.8	6.7	7.7	0.0	0.73	>0.30
% Bronchopulmonary dysplasia (oxygen requirement at 36 weeks)	23.1	30.0	38.5	36.4	1.19	>0.20
Social background factors						
Mean (SD) maternal age	30.38 (5.10)	31.20 (4.79)	28.0 (5.46)	32.00 (5.90)	0.32	>0.50
% Mother no formal educational qualifications	34.6	30.0	61.5	43.5	1.29	>0.20
% Un-/semi-skilled socioeconomic status^	30.8	33.3	38.5	47.8	1.65	>0.15
% Family income < $25 000	39.1	30.8	13.3	26.9	1.76	>0.15

*IUGR—Z-score > 2 SD below weight for gestational age. ≠Critical risk index for babies. **IVH based on Papile classification. ^Socioeconomic status defined according to Elley and Irving (2003).

Relationship between early cerebral injury and development and later MSML task performance

The relationship between children's performance on the MSML task at the age of 2 years and term qualitative and quantitative MRI measures is shown in Table 4. In terms of WM abnormality, findings showed that with increasing injury severity, there was a tendency for fewer children to successfully complete the task by searching correctly at post-switch ($P < 0.05$). Specifically, infants classified as having moderate–severe WM abnormalities on term MRI had the lowest rate of task completion and also exhibited a high rate (42%) of atypical searching behaviour at post-switch. Although the small number of infants in the moderate–severe group with cystic abnormalities precluded separate analysis of these infants, visual examination of performance profiles of children with and without cystic injury revealed similar task achievement rates across both groups (54% non-cystic, 50% cystic).

In terms of relations between task performance and earlier quantitative measures of total cerebral tissue volumes, the results in Table 4 show a significant linear association between cerebrospinal fluid volume at term and later task performance ($P < 0.05$). Increasing volumes of cerebrospinal fluid (unadjusted and adjusted for ICV) at term were associated with decreasing task performance on the MSML task 2 years later. Consistent with this was the significant association between the proportion of the intracranial cavity occupied by brain tissue at term and subsequent task performance at the age of 2 years ($P = 0.02$). No significant associations were found between task performance and total cerebral volumes of cortical grey matter ($P < 0.95$), myelinated WM ($P < 0.95$), unmyelinated WM ($P < 0.30$) or deep nuclear grey matter ($P < 0.75$).

To explore the extent to which total cerebral tissue volumes in specific subregions previously linked with children's performance on an AB task might be particularly predictive of child outcome, associations between total tissues volumes as a proportion of the parcel were evaluated for each of the parcellated subregions. This analysis showed that MSML task performance was related to the proportion of cerebral tissue in the left DPFC ($P = 0.06$, $r^2 = 0.05$), right DPFC ($P = 0.09$, $r^2 = 0.04$), left PM ($P < 0.05$, $r^2 = 0.06$), right PM ($P < 0.05$, $r^2 = 0.06$), left SM ($P < 0.05$, $r^2 = 0.07$), right SM ($P < 0.01$, $r^2 = 0.09$), left PO ($P < 0.05$, $r^2 = 0.08$) and right PO ($P < 0.01$, $r^2 = 0.11$) subregions.

Finally, to examine the extent to which WM injury and altered cerebral development made unique contributions to later memory function, regression analysis was used. Results showed that both volumetric measures of cerebral development and the qualitative measure of white injury severity were predictive ($P < 0.10$) of later outcome, with the key independent predictors of MSML task performance at the age of 2 years being gender ($B = 0.52$, $P = 0.04$), WM injury ($B = -0.36$, $P = 0.09$) and percentage of total tissue volume ($B = 0.06$, $P = 0.07$). No interactive relationships were found between these risk factors.

Discussion

This study draws on prospective longitudinal data from an unselected cohort of preterm infants to examine relations

Table 4 Relationship between term MRI measures and MSML task performance at age 2

	Post-switch group					
	Correct switch (B)	Perseverative (A)	Non-perseverative (C)	Failed to reach post-switch	F/χ^2	*P*
White matter injury	(*n* = 26)	(*n* = 30)	(*n* = 12)	(*n* = 23)		
% No injury	38.5	23.3	0.0	21.7		
% Mild injury	53.8	63.3	58.3	52.2		
% Moderate/severe injury	7.7	13.3	41.7	26.1	13.86	<0.05
Tissue volumes (mm^3)	(*n* = 21)	(*n* = 26)	(*n* = 11)	(*n* = 18)		
Cortical grey matter						
Mean (SD) absolute volume (mm^3)	175.97 (37.51)	179.32 (32.92)	185.33 (27.25)	175.70 (48.60)	0.01	<0.95
% ICV	39.11 (7.59)	39.25 (5.90)	40.53 (4.55)	38.35 (7.70)	0.04	<0.85
Deep nuclear grey matter						
Mean (SD) absolute volume (mm^3)	13.15 (3.77)	11.94 (3.39)	13.65 (6.30)	12.24 (3.72)	0.10	<0.75
% ICV	2.94 (.88)	2.60 (.61)	2.95 (1.35)	2.73 (.85)	0.15	<0.70
Myelinated white matter						
Mean (SD) absolute volume (mm^3)	13.39 (5.61)	12.56 (5.56)	14.62 (6.29)	13.00 (5.20)	0.01	<0.95
% ICV	2.96 (1.15)	2.73 (1.07)	3.23 (1.50)	2.87 (.99)	0.01	<0.95
Unmyelinated white matter						
Mean (SD) absolute volume (mm^3)	208.48 (29.25)	214.26 (31.95)	198.71 (31.66)	201.45 (31.57)	1.20	<0.30
% ICV	46.50 (6.44)	47.01 (5.10)	43.40 (5.31)	44.70 (6.62)	1.97	0.17
Cerebrospinal fluid						
Mean (SD) absolute volume (mm^3)	38.01 (16.43)	38.95 (20.11)	45.98 (19.01)	52.44 (28.28)	5.33	<0.05
% ICV	8.48 (3.64)	8.41 (4.14)	9.89 (3.39)	11.35 (5.28)	5.51	<0.05
Total cerebral tissue						
Mean (SD) absolute volume (mm^3)	410.99 (31.80)	418.08 (50.47)	412.31 (42.18)	402.38 (58.80)	0.45	<0.55
% ICV	91.52 (3.64)	91.59 (4.14)	90.11 (3.39)	88.65 (5.28)	5.51	<0.05

between structural cerebral development at term equivalent and later cognitive functioning, with a specific focus on the development of object working memory. To date, much of the research concerned with brain–behaviour relationships in the preterm infant has been based on small and/or selected samples of older children (Isaacs *et al.*, 2000, 2001; Peterson *et al.*, 2000; Nosarti *et al.*, 2002, 2004; Kesler *et al.*, 2004). However, several recent studies have valuably extended this work by showing that disturbances in neuroanatomical development are evident by term equivalent and that these changes are predictive of later cognitive outcome, thus more clearly specifying the timing of cerebral alterations (Ajayi-Obe *et al.*, 2000; Peterson *et al.*, 2003; Inder *et al.*, 2005). However, virtually all of these studies have relied on global measures of cognitive function (Allin *et al.*, 2001; Peterson *et al.*, 2003; Isaacs *et al.*, 2004; Nosarti *et al.*, 2004) and/or global measures of cerebral abnormality, such as total brain volume (Ajayi-Obe *et al.*, 2000). The present study with its unselected cohort of preterm infants and its inclusion of both qualitative injury and volumetric measures of neuroanatomical structure, in conjunction with a well established infant neuropsychological paradigm, further advances the understanding of the role of both cerebral injury and development in the evolution of the neuropsychological difficulties associated with prematurity. The major findings and implications of this study are reviewed below.

Our study demonstrates clear differences between preterm and full-term infants as early as 2 years, with preterm infants showing impaired performance across a range of task measures. Specifically, during initial task training, they took longer to learn and independently complete a novel retrieval sequence than term control children. During pre-switch, they had higher rates of abandoned trials and were twice as likely to be unable to achieve the pre-switch criterion of three consecutively correct retrievals. These findings suggest that preterm children had greater difficulty learning a new behavioural sequence. In addition, amongst those who successfully proceeded to post-switch, fewer children in the preterm group were able to flexibly respond to the changed hiding contingency by searching correctly at the new, visibly demonstrated (B) location. An examination of the types of errors committed at post-switch by both groups further revealed that preterm children were almost twice as likely as term children to make a non-perseverative error (searching at neither A nor B). In contrast, full-term children more often tended to commit the developmentally common and expected perseverative error (searching at the original A hiding location). Searching at A generally implies that an infant has a memory store of the original hiding location, but experiences difficulty either updating this information with the new location or inhibiting a previously learned response. In contrast, searching at C (the never used hiding location) tends to suggest difficulties associated with the encoding and retention of a location memory store. The higher frequency of this latter error amongst children in the preterm group, in addition to performance differences during training and pre-switch trials

provides converging evidence to suggest that preterm children experienced greater difficulties in the encoding of new information in working memory, and importantly, that these difficulties were already present and detectable by the age of 2 years.

These findings are consistent with previous research showing impaired spatial and object working memory abilities in infants and older preterm children (Rose *et al.*, 1992, 2001; Luciana *et al.*, 1999; Espy *et al.*, 2002; Vicari *et al.*, 2004). For example, Ross *et al.* (1992) have shown that by 10 months, preterm infants (<32 weeks) with and without ultrasound evidence of subependymal or mild intraventricular haemorrhage perform less well than healthy term infants on an AB task. Luciana *et al.* (1999) also report similar deficits in school aged preterm children, with these deficits being specific to spatial working memory and not spatial recognition memory.

Given the central role of working memory in a range of cognitive processes including learning, planning, problem solving and language development, these impairments are likely to impact significantly on these children's later learning, educational and social progress. Potential interventions that may help these children with the executive challenges of school and everyday life include the provision of task scaffolding, practice and behavioural support to aid learning, as well as the teaching of metacognitive strategies, such as rehearsal.

Study findings also offer useful insights into the potential neuropathological pathways that place preterm infants at risk of later working memory deficits (McCormick, 1997; McQuillen and Ferriero, 2004). First, consistent with previous research linking early WM injury to later adverse cognitive and educational outcomes (Olsen *et al.*, 1997), there was clear evidence to show that WM injury during the perinatal period is a major contributing factor in the development of later object working memory difficulties. Preterm infants without significant MRI defined WM abnormality were six times more likely to complete the MSML task than infants with such WM abnormality. However, later working memory difficulties were not unique to infants with moderate–severe WM abnormalities but were also shared to a lesser extent by infants with mild WM pathology. This may, in part, reflect limitations in qualitative evaluations based on conventional MRI. With newer techniques, such as diffusion weighted imaging, quantitative evaluations of the microstructural integrity of the WM and visualization of fibre tract connectivity will be possible. Such techniques are likely to add greatly to our understanding of the relationship between alterations in WM and specific learning impairments, such as object working memory.

These findings do, however, highlight the importance of regional connections for memory function (Alexander and Stuss, 2000; Johnson *et al.*, 2002; Olesen *et al.*, 2003). They also raise the possibility that disconnection or damage to central WM tracts (Huppi *et al.*, 2001) could either directly or indirectly impact on the development and organization of neural cells involved in memory function (Olsen *et al.*, 1997; Inder *et al.*, 1999). Further research should help define the mechanism by which WM injury impacts axonal and neural development, as well as the potential role of these neuropathological processes in explaining associations between WM injury and later working memory.

A second major finding of this study was the significant association between quantitative MRI measures of total cerebral tissue volume (adjusted and unadjusted for ICV) and MSML task performance. Although other studies have reported a similar association between total brain tissue volume at term and general cognition (Ajayi-Obe *et al.*, 2000), our findings extend this work by demonstrating that this association remains even after the effects of WM injury severity are taken into account. In the preterm infant, structural cerebral alterations, and particularly disturbances in cerebral growth (Ajayi-Obe *et al.*, 2000; Inder *et al.*, 2005) appear to impact on later neuropsychological function, independent of qualitative WM injury during the perinatal period. This may help to explain why substantial numbers of preterm children exhibit intellectual and cognitive difficulties even in the absence of clinically defined WM pathology.

To help define further the specific areas of regional disturbance that might be related to later working memory function, the quality of children's performance at the age of 2 years was examined in relation to total percentage cerebral tissue volumes across eight parcellated subregions. Results from this exploratory analysis concur with Peterson *et al.* (2003) in supporting the importance of SM and PO regions for later cognitive function. In addition, we also found that reductions in cerebral tissue volumes within the left and right DPF cortices, as well as PM regions, were associated with children's later object search performance. This is in agreement with animal neuroscience and human neurodevelopmental research showing a clear link between dorsolateral prefrontal cortical function and AB task performance (Diamond and Goldman-Rakic, 1989; Bell and Fox, 1992; Baird *et al.*, 2002).

Although previous neuropsychological research has tended to emphasize the role of the dorsolateral prefrontal cortex for working memory function, there is now a growing appreciation that, given the complex and interrelated nature of neural systems, other neural structures with direct connections to the prefrontal cortex, such as the parietal and temporal cortex, anterior cingulate and basal ganglia, may also be critical (Johnson, 2000; Luciana, 2003; Kaldy and Sigala, 2004). Given the changing nature of cerebral development (Herschkowitz, 2000; Johnson *et al.*, 2002) and the relative immaturity of prefrontal neural systems during infancy and early childhood, the notion that other cortical and subcortical structures, in addition to the dorsolateral prefrontal cortex, may also help mediate early executive skills, such as object working memory is compelling and is clearly supported by this study. The link between PM regions, bilaterally, and outcome probably reflects the motor component of this task common to most AB tasks.

One anatomical structure that we were not able to examine in relation to working memory outcome, owing to its minimal

discriminating properties on MRI near term, was the hippocampus. Reductions in hippocampal volumes have been shown in adolescents, born preterm children, to be correlated with impairments in everyday memory function (Isaacs *et al.*, 2000). Given the vulnerability of the hippocampus to neurological insult (Schmidt-Kastner and Freund, 1991) and its accepted role in memory and learning, closer evaluation of this structure earlier in development is merited.

Finally, several limitations of this analysis should be noted. First, despite our comparatively large sample, post-acquisition volumetric analysis was possible for only 77% of scans owing to motion artefact and imaging intensity errors. Although this rate is highly consistent with other MRI studies of term aged infants (Peterson *et al.*, 2003) and there was no systematic bias evident in this data loss, it is nonetheless an important issue that needs to be addressed in future studies. Secondly, it should be acknowledged that the regional volumetric analysis was exploratory and effect sizes were small. Consequently, strong conclusions cannot be drawn and further replication is warranted.

Nonetheless, these findings do highlight potentially important anatomical correlates of later deficits in object working memory and executive function in the preterm infant. They also suggest that the quantity and quality of cerebral connectivity is relevant, and ought to be examined more closely with newer techniques including diffusion tensor MRI. It is also important to note, that disturbances in cerebral development observed on MRI can only be inferred to predict and not to have 'caused' later cognitive deficits, such as those described in this study (Luciana, 2003). To be precise, relationships are much more likely to be bidirectional with developmental disability and delay also, in turn, affecting brain development and function. Early cerebral changes may also impact in as of yet unknown ways on later neurological development (Eslinger *et al.*, 2004). And finally, there is a critical need to examine the mediating and/or moderating influence of environmental enrichment/adversity on developmental outcomes of children born preterm, since such factors have also been shown to make a major contribution to variability in outcome. Given emerging evidence to support the importance of early neuroanatomical changes in predicting outcome, more careful examination of these issues within a dynamic developmental framework is likely to be crucial to developing a better understanding of the aetiological processes underlying the neurodevelopmental challenges facing the preterm infant.

Acknowledgements

We are grateful to Carole Spencer, Michelle VanDyk and the Canterbury Radiology Group for their assistance with MRI and follow-up data, and to Tom Keenan for early helpful discussion and assistance in constructing the MSML test apparatus. Our special thanks to study families for their willingness to share their children's lives with us. This research was funded from grants from the Neurological Foundation of New Zealand, Lottery Grants Board, Canterbury Medical Research Foundation and the Health Research Council of New Zealand.

References

Ajayi-Obe M, Saeed N, Cowan F, Rutherford M, Edwards A. Reduced development of cerebral cortex in extremely preterm infants. Lancet 2000; 356: 1162–3.

Alexander MP, Stuss DT. Disorders of frontal lobe functioning. Semin Neonatol 2000; 20: 427–37.

Allin M, Matsumoto H, Santhouse A, Nosarti C, AlAsady M, Stewart A, et al. Cognitive and motor function and the size of the cerebellum in adolescents born very pre-term. Brain 2001; 124: 60–6.

Baird A, Kagan J, Gaudette T, Walz K, Herslag N, Boas D. Frontal lobe activation during object permanence: data from near-infrared spectroscopy. Neuroimage 2002; 16: 1120–6.

Bell MA, Fox NA. The relations between frontal brain electrical activity and cognitive development during infancy. Child Dev 1992; 63: 1142–63.

Bremner A, Bryant P. The effect of spatial cues on infants' responses in the AB task, with and without a hidden object. Dev Sci 2001; 4: 408–15.

Diamond A. The development and neural bases of memory functions as indexed by the AB and delayed response tasks in human infants and infant monkeys. Ann N Y Acad Sci 1990; 608: 267–317.

Diamond A, Goldman-Rakic P. Comparison of human infants and rhesus monkeys on Piaget's AB task: Evidence for dependence on dorsolateral prefrontal cortex. Exp Brain Res 1989; 74: 24–40.

Elley WB, Irving JC. Revised socio-economic index for New Zealand. N Z J Educ Studies 2003; 38: 3–17.

Eslinger PJ, Flaherty-Craig CV, Benton AL. Developmental outcomes after prefrontal cortex damage. Brain Cogn 2004; 55: 84–103.

Espy KA, Kaufmann PM, McDiarmid MD, Glisky ML. Executive functioning in preschool children: performance on A-not-B and other delayed response format tasks. Brain Cogn 1999; 41: 178–199.

Espy KA, Kaufmann PM, Glisky ML, McDiarmid MD. New procedures to assess executive functions in preschool children. Clin Neuropsychol 2001; 15: 46–58.

Espy KA, Stalets M, McDiarmid M, Senn T, Cwik M, Hamby A. Executive functions in preschool children born preterm: application of cognitive neuroscience paradigms. Neuropsychol Dev Cogn C Child Neuropsychol 2002; 8: 83–92.

Gathercole S. Cognitive approaches to the development of short-term memory. Trends Cogn Sci 1999; 3: 410–19.

Gioia GA, Isquith PK, Guy SC. Assessment of executive functions in children with neurological impairment. In: Simeonsson RJ, Rosenthal SL, editors. Psychological and developmental assessment: children with disabilities and chronic conditions. New York: Guilford Press; 2001. pp. 317–56.

Herschkowitz N. Neurological bases of behavioral development in infancy. Brain Dev 2000; 22: 411–16.

Huppi PS, Schuknecht B, Boesch C, Bossi E, Felblinger J, Fusch C, et al. Structural and neurobehavioral delay in postnatal brain development of preterm infants. Pediatr Res 1996; 39: 895–901.

Huppi PS, Murphy B, Maier SE, Zientara GP, Inder TE, Barnes PD, et al. Microstructural brain development after perinatal cerebral white matter injury assessed by diffusion tensor magnetic resonance imaging. Pediatrics 2001; 107: 445–60.

Inder TE, Huppi PS, Warfield S, Kikinis R, Zientara GP, Barnes PD, et al. Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term. Ann Neurol 1999; 46: 755–60.

Inder TE, Wells SJ, Mogridge N, Spencer C, Volpe J. Defining the nature of the cerebral abnormalities in the premature infant: a qualitative magnetic resonance imaging study. J Pediatr 2003; 143: 171–9.

Inder TE, Warfield S, Wang H, Huppi P, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. Pediatrics 2005; 115: 286–95.

Isaacs EB, Lucas A, Chong WK, Wood SJ, Johnson CL, Marshall C, et al. Hippocampal volume and everyday memory in children of very low birth weight. Pediatr Res 2000; 47: 713–20.

Isaacs EB, Edmonds CJ, Lucas A, Gadian DG. Calculation difficulties in children of very low birthweight. Brain 2001; 124: 1701–7.

Isaacs EB, Edmonds C, Chong W, Lucas A, Morley R, Gadian D. Brain morphometry and IQ measurements in preterm children. Brain 2004; 127: 2595–607.

Johnson MH. Functional brain development in infants: elements of an interactive specialization framework. Child Dev 2000; 71: 75–81.

Johnson MH, Halit H, Grice SJ, Karmiloff-Smith A. Neuroimaging of typical and atypical development: a perspective from multiple levels of analysis. Dev Psychopathol 2002; 14: 521–36.

Kaldy Z, Sigala N. The neural mechanisms of object working memory: what is where in the infant brain? Neurosci Biobehav Rev 2004; 28: 113–21.

Kesler SR, Ment L, Vohr B, Pajot S, Schneider K, Katz KH, et al. Volumetric analysis of regional cerebral development in preterm children. Pediatr Neurol 2004; 31: 318–25.

Luciana M. Cognitive development in children born preterm: implications for theories of brain plasticity following injury. Dev Psychopathol 2003; 15: 1017–47.

Luciana M, Lindeke L, Georgieff M, Mills M, Nelson CA. Neurobehavioral evidence for working memory deficits in school aged children with histories of prematurity. Dev Med Child Neurol 1999; 41: 521–33.

Marcovitch S, Zelazo PD. The A-not-B error: results from a logistical meta-analysis. Child Dev 1999; 70: 1297–313.

Marlow N, Wolke D, Bracewell M, Samara M. Neurologic and developmental disability at six years after extremely preterm birth. N Engl J Med 2005; 352: 9–19.

McCormick MC. The outcome of very low birth weight infants: are we asking the right questions? Pediatrics 1997; 99: 869–76.

McQuillen PS, Ferriero DM. Selective vulnerability in the developing central nervous system. Pediatr Neurol 2004; 30: 227–35.

Nosarti C, Al-Asady MHS, Frangou S, Stewart AL, Rifkin L, Murray RM. Adolescents who were born very preterm have decreased brain volumes. Brain 2002; 125: 1616–23.

Nosarti C, Rushe TM, Woodruff PW, Stewart AL, Rifkin L, Murray RM. Corpus callosum size and very preterm birth: relationship to neuropsychological outcome. Brain 2004; 127: 2080–9.

Olesen P, Nagy Z, Westerberg H, Kingberg T. Combined analysis of DTI and fMRI data reveals a joint maturation of white and grey matter in fronto-parietal network. Brain Res Cogn Brain Res 2003; 18: 48–57.

Olsen P, Paakko E, Vainionpaa L, Pyhtinen J, Jarvelin M-R. Magnetic resonance imaging of periventricular leukomalacia and its clinical correlation in children. Ann Neurol 1997; 41: 754–61.

Peterson BS, Vohr B, Staib LH, Cannistraci CJ, Dolberg A, Schneider KC, et al. Regional brain volume abnormalities and long term cognitive outcome in preterm infants. J Am Med Assoc 2000; 284: 1939–47.

Peterson BS, Anderson AW, Ehrenkranz R, Staib L, Tageldin M, Colson E, et al. Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants. Pediatrics 2003; 111: 939–48.

Piaget J. The construction of reality in the child. New York: Basic Books; 1954.

Rose SA, Feldman JF. Memory and processing speed in preterm children at 11 years: a comparison with full-terms. Child Dev 1996; 67: 2005–21.

Rose SA, Feldman JF, Wallace IF. Infant information processing in relation to six-year cognitive outcomes. Child Dev 1992; 63: 1126–41.

Rose SA, Feldman JF, Janowski JJ. Attention and recognition memory in the 1st year of life: A longitudinal study of preterm and full term infants. Dev Psychol 2001; 37: 135–51.

Ross G, Tesman J, Auld PAM, Nass R. Effects of subependymal and mild intraventricular lesions on visual attention and memory in premature infants. Dev Psychol 1992; 28: 1067–1074.

Ross G, Boatright S, Auld PAM, Nass R. Specific cognitive abilities in 2-year old children with subependymal and mild intraventricular haemorrhage. Brain Cogn 1996; 32: 1–13.

Schmidt-Kastner R, Freund T. Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 1991; 40: 599–636.

Vicari S, Caravale B, Carlesimo G, Casadei A, Allemand F. Spatial working memory deficits in children at ages 3–4 who were low birth weight, preterm infants. Neuropsychology 2004; 18: 673–8.

Volpe JJ. Brain injury in the premature infant: overview of clinical aspects, neuropathology, and pathogenesis. Semin Pediatr Neurol 1998; 5: 135–51.

Wolke D, Meyer R. Cognitive status, language attainment, and prereading skills of 6-year-old very preterm children and their peers: the Bavarian Longitudinal Study. Dev Med Child Neurol 1999; 41: 94–109.

Woodward LJ, Mogridge N, Wells S, Inder T. Can neurological examination predict the presence of cerebral injury in the VLBW infant? Dev Behav Paediatr 2004; 25: 326–34.

Zelazo PD, Reznick JS, Spinazzola J. Representational flexibility and response control in a multistep multilocation search task. Dev Psychol 1998; 34: 203–14.