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Corpus callosum size and very preterm birth: relationship to neuropsychological outcome

Chiara Nosarti, Teresa M. Rushe, Peter W. R. Woodruff, Ann L. Stewart, Larry Rifkin and Robin M. Murray

Division of Psychological Medicine, Section of General Psychiatry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK

Correspondence to: Chiara Nosarti, PhD, Division of Psychological Medicine, Section of General Psychiatry, PO Box 63, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK
E-mail: c.nosarti@iop.kcl.ac.uk.

Summary

Thinning of the corpus callosum (CC) is often observed in individuals who were born very preterm. Damage to the CC during neurodevelopment may be associated with poor neuropsychological performance. This study aimed to explore any evidence of CC pathology in adolescents aged 14–15 years who were born very preterm, and to investigate the relationship between CC areas and verbal skills. Seventy-two individuals born before 33 weeks of gestation and 51 age- and sex-matched full-term controls received structural MRI and neuropsychological assessment. Total CC area in very preterm adolescents was 7.5% smaller than in controls, after adjusting for total white matter volume (P = 0.015). The absolute size of callosal subregions differed between preterm and full-term adolescents: preterm individuals had a 14.7% decrease in posterior ($P < 0.0001$) and an 11.6% decrease in mid-posterior CC quarters ($P = 0.029$). Preterm individuals who had experienced periventricular haemorrhage and ventricular dilatation in the neonatal period showed the greatest decrease in CC area. In very preterm boys only, verbal IQ and verbal fluency scores were positively associated with total mid-sagittal CC size and mid-posterior surface area. These results suggest that very preterm birth adversely affects the development of the CC, particularly its posterior quarter, and this impairs verbal skills in boys.

Keywords: corpus callosum; MRI; preterm; verbal fluency

Abbreviations: ANCOVA = analysis of covariance; CC = corpus callosum; DIL = ventricular dilatation; LSD = least significant difference; MANCOVA = multivariate analysis of covariance; PVH = periventricular haemorrhage; WISC-R = Wechsler Intelligence Scale for Children—Revised.

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Introduction

One of the most common brain abnormalities in individuals born very preterm is thinning of the corpus callosum (CC), particularly of the posterior body (splenium) (Cooke and Abernethy, 1999; Stewart *et al*., 1999; Peterson *et al*., 2000). Such injury may be partly explained by the vulnerability of the developing CC to hypoxic–ischaemic damage and haemorrhage, possibly due to the intrinsic vulnerability of immature oligodendrocytes (Back *et al*., 2001).

Studies of subjects with agenesis of the CC or commissurotomy have demonstrated that the CC plays an important role in establishing hemispheric lateralization of function (de Guise *et al*., 1999). Damage to the CC during development has been found to be associated with poor neurological outcome and neuropsychological performance (Sauerwein and Lassonde, 1994). Furthermore, Kirkbride and colleagues, who investigated a cohort of very preterm (<33 weeks gestation) individuals at 8 years of age, found that although their mean full-scale IQ was well within the normal range, half the subjects showed evidence of poor inter-hemispheric transfer of cognitive information (Kirkbride *et al*., 1994*a*). The authors speculated, but had no proof, that such deficits were due to damage to the posterior third of the CC, which lies adjacent to the periventricular region (Kirkbride *et al*., 1994*b*). A functional MRI (fMRI) study with a subset of individuals who also took part in this study used visual and auditory tasks and showed that the very preterm subjects with damaged CCs (as qualitatively rated by two neuroradiologists) had significantly different neuronal activation patterns compared with a control group, and a group

of preterm adolescents without brain damage (Santhouse *et al*., 2002). These findings suggested a plasticity of function compensating for early damage to the CC.

We now present quantitative MRI measurements of the mid-sagittal surface area of the CC and its four subregions in the same cohort of preterm subjects as studied by Kirkbride *et al*. (1994*a*), at a point when the subjects had reached adolescence. We hypothesize that decreased area of the CC in preterm individuals will persist into adolescence, and that the most affected area will be its posterior part. We predict that cognitive function, which was measured at the time of assessment at age 14–15 and at age 8 years, will be related to decreased area of the CC. We hypothesize that decreased CC area will be related to poorer performance on tasks involving language, as language has been shown to be the main cognitive deficit in adolescence in our sample of very preterm individuals (Rushe *et al*., 2001).

Methods

Study population

In 1979–1980, 109 infants were born before 33 weeks of gestation and admitted consecutively to the Neonatal Unit of University College Hospital, London, survived and were discharged. All of these individuals had ultrasonographic imaging daily for the first 4 days, at 1 week, and weekly until discharge from hospital. Of this cohort, four died within 24 months; the remaining 105 were enrolled for long-term follow-up (Stewart *et al*., 1999). Prospective assessments of the neurological and cognitive development of these children were carried out at 1 and 4 years of corrected age and 8 years of age (Costello *et al*., 1988; Roth *et al*., 1993). At 14–15 years, 103 (98%) individuals were traced. Of the 92 living in the UK, 76 (83%) agreed to attend for assessment. MRI scanning was carried out on 72 individuals. Qualitative and quantitative MRI results and their relationship to neurodevelopmental outcome in adolescence have been published in separate papers (Stewart *et al*., 1999; Nosarti *et al*., 2002).

A normal gestation (38–42 weeks) control group of 47 infants delivered at University College Hospital in 1979–1980 had been enrolled to act as age-matched controls for assessments made on the cohort at 4 years of age. An attempt was made to contact all those individuals who were living in the UK (45) at 14–15 years; 22 agreed to have an MRI, although one refused on the day (Stewart *et al*., 1999). Thirty other full-term individuals matched for age, who were recruited through advertisements in the press, were also studied. The two control groups differed only in terms of parental socio-economic status [$\chi^2(4) = 17.9$, $P < 0.001$]. None of the controls had ultrasonographic imaging during the neonatal period.

Ethical approval for the study was obtained from Joint University College London/University College Hospital Committee on Ethics of Human Research, and the Joint Medical Ethical Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery. Written informed consent for the assessment, including MRI, was obtained from an accompanying parent, and verbal consent was obtained from the cases and controls. The cohort members who were not available for investigation at age 14–15 years did not differ from those studied in terms of birth weight, gestational age at birth, sex ratio, mode of delivery, condition at birth, the need for mechanical ventilation or neonatal cranial ultrasonographic findings; nor did they differ in neurodevelopmental status at 1, 4 or 8 years (Stewart *et al*., 1999).

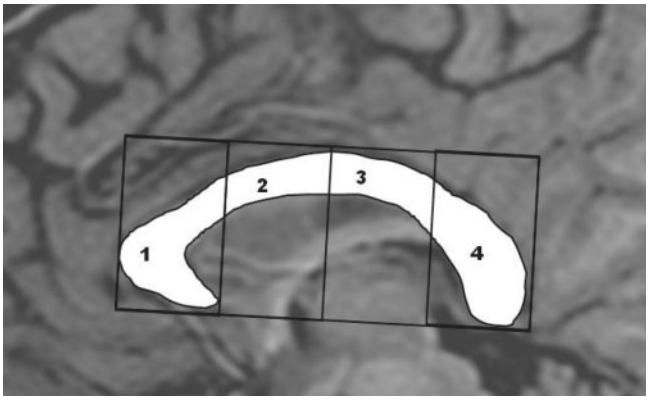

Fig. 1 Example of technique used to measure the mid-sagittal surface area of the CC and its division in quarters (1 = anterior; 2 = mid-anterior; 3 = mid-posterior; 4 = posterior).

MRI

A 1.5 T GE Signa Horizon machine (GE Medical Systems, Milwaukee, WI) was used to obtain the following sequences: sagittal T2-weighted fast spin-echo, 27 × 4 mm contiguous slices [(repetition time) TR 2500 ms, echo time (TE) 85 ms]; axial T2-weighted double-echo fast spin-echo, 28 × 5 mm contiguous slices (TR 2900 ms, TE 19 and 95 ms); three-dimensional T1-weighted gradient-echo sequence that allowed reconstruction in any plane of 124 1.5 mm slices (TR 35 ms, TE 5 ms, flip angle 35°).

Structural brain measurements were rated blind to group affiliation using the image analysis software Analyze™, Biomedical Imaging Resource, Mayo Foundation (Robb, 1990). The area of the CC was measured on the mid-sagittal slice. The CC was subdivided into four regions by drawing lines perpendicular to its antero-posterior length as described by Woodruff *et al*. (1993). An example of this method is shown in Fig. 1.

Inter-rater reliability, performed on six randomly selected independent ratings of total CC area and its subdivisions, was 0.99 ($P < 0.0001$). Intra-rater reliability performed on total CC area measurements for 10 ratings was 0.98 ($P < 0.0001$). Intra-rater reliability performed on all subregions of the CC for the same 10 ratings was 0.94 ($P < 0.0001$) for the anterior CC area, 0.87 ($P < 0.001$) for the mid-anterior CC area, 0.98 ($P < 0.0001$) for the mid-posterior area and 0.99 ($P < 0.0001$) for the posterior area. All reliability analyses were performed using Pearson's correlation, two-tailed.

Cognitive assessments

Cognitive variables were selected to test specific *a priori* hypotheses about the data, i.e. CC size would be associated with scores on language tasks, as language was shown to be the main cognitive deficit in adolescence in our sample of very preterm individuals (Rushe *et al*., 2001). They included: the Wechsler Intelligence Scale for Children—Revised (WISC-R) (Wechsler, 1981) which had been administered at age 8 years. Cognitive measures administered at age 14–15 years (Rushe *et al*., 2001) comprised the Schonnel Graded Reading and Spelling Tests (Schonnel and Schonnel, 1960), and a measure of verbal fluency; this Controlled Oral Word Association Test (Benton and Hamsher, 1976; Spreen and Strauss, 1991) consists of the 'FAS' test (three trials of word production, where participants are requested to produce words beginning with a given letter, i.e. F, A and S, within

a specific time frame); the Animal Trial, where participants are required to produce as many names of animals as they can within a time frame; and the Object Trial, where participants are requested to produce the name of objects.

Statistical analysis

Data were analysed with SPSS 10.0.7 (SPSS, Chicago, IL). All pairings of neonatal characteristics, anthropometric and socio-demographic variables were analysed with χ^2 or univariate analysis of variance, as appropriate. The 95% confidence intervals (CIs) were calculated for magnitude. Differences in mid-sagittal surface areas of the CC in mm^2 between preterm adolescents and controls were assessed by univariate analysis of covariance (ANCOVA) defined by two between-subject group factors [group (preterm and full-term subjects) and gender (males and females)], adjusting for white matter volume and ventricular size. Differences in the size of CC quarters between preterm adolescents and controls were assessed by multivariate analysis of covariance (MANCOVA) defined by two between-subject group factors [group (preterm and full-term subjects) and gender (males and females)] and four within-subjects variables, i.e. anterior, mid-anterior, mid-posterior and posterior quarters, adjusting for total mid-sagittal surface area of the CC and ventricular size. For the model, we used a type III sum of squares, which calculates the sums of squares of an effect as the sums of squares adjusted for any other effects that do not contain the effect investigated and orthogonal to any effects (if any) that may contain it. As there is some evidence that the size of the CC may be affected by hand dominance (Witelson, 1985, 1989), we explored the relationship between CC surface area and handedness in a subset of the sample, for whom data were available at age 8 and/or 14 years; we therefore carried out a MANCOVA, defined by two between-subject group factors [group (preterm and full-term subjects) and handedness (right and non-right)] and a four-level (anterior quarter of the CC, mid-anterior quarter of the CC, mid-posterior quarter of the CC and posterior quarter of the CC) within-subject factor. ANCOVAs were also used to explore differences in total mid-sagittal CC and its subdivisions in preterm subjects according to neonatal ultrasound classification (Stewart *et al*., 1987): uncomplicated periventricular haemorrhage (PVH), PVH and ventricular dilatation (PVH + DIL) and normal ultrasound results. These categories formed a three-level between-subject group factor, while white matter volume for total mid-sagittal CC area and total mid-sagittal CC area for CC quarters were used as covariates As comparisons were made between more than two groups, we used the Fisher protected least significant difference (LSD) test to compare *post hoc* each individual group.

Partial correlation controlling for white matter volume was used to study the relationships between perinatal variables and CC area, and partial correlation controlling for mid-sagittal surface area of the CC was used to investigate the relationships between perinatal variables and CC subregions. Finally, the relationships between cognitive variables and CC subregions were investigated between subject groups and within subject groups, by gender, using stepwise linear regression models.

Results

Six MRI scans performed on preterm subjects could not be analysed due to movement or signal artefact. Two further scans were only partly analysed due to contrast problems. All MRI scans performed on controls were rated quantitatively. Therefore, scans from 66 preterm and 51 full-term individuals were analysed. The socio-demographic characteristics of the two groups are shown in Table 1. Subjects and controls did not differ statistically in terms of gender distribution [$\chi^2(1) = 1.89$, $P > 0.05$], handedness [$\chi^2(1) = 1.19$, $P > 0.05$] or socio-demographic distribution (Great Britain Office of Population Censuses and Surveys, 1980) at the time of assessment [$\chi^2(5) = 7.19$, $P > 0.05$]. There were no differences in age [$F(116) = 0.59$, $P > 0.05$] or weight between the two comparison groups, after controlling for gender, at the time of assessment [$F(106) = 0.51$, $P > 0.05$]. Preterm adolescents and controls did, however, differ slightly in height, after controlling for gender [$F(106) = 4.49$, $P = 0.036$], the preterm being smaller than the full-term subjects.

Preterm birth and gender

We observed a statistically significant difference in absolute mid-sagittal callosal size between preterm individuals and controls [$F(116) = 6.98$, $P = 0.009$], and a non-significant difference between genders [$F(116) = 0.17$, $P > 0.05$], as well as a non-significant interaction between group and gender

Table 1 *Socio-demographic characteristics for the preterm and the control groups*

Variable	Cases ($n = 66$)	Controls ($n = 51$)
Neonatal characteristics		
Mean (SD; 95% CI) birth weight (g)	1303.47 (280.90; 1234.42–1372.52)	3590.48 (413.42; 3402.29–3778.66)
Mean (SD; 95% CI) gestation at birth (weeks)	29.70 (1.81; 29.25–31.14)	39.90 (0.83; 39.53–40.28)
Females/males	33/33	19/32
Right/non-right handedness	52/13 (of 65)	25/3 (of 28)
% Parental social class		
I–II	24	24
III	16	8
IV–VI	21	11
Anthropometric data at 14–15 years		
Mean (SD; 95% CI)		
Height (cm)*	163.23 (8.57; 161.02–165.45)	166.91 (7.70; 164.65–169.18)
Weight (kg)	55.08 (12.25; 51.92–58.25)	54.76 (12.99; 50.95–58.58)
Age (years)	14.95 (0.42; 14.84–15.05)	14.87 (0.63; 14.70–15.05)

*$P < 0.05$.

[$F(116) = 0.02$, $P > 0.05$]. We then used a MANCOVA to investigate group by gender differences in the size of CC quarters. We observed a statistically significant main effect for group [based on Wilks' λ, $F(110) = 5.76$, $P < 0.001$] and for gender [$F(110) = 2.74$, $P = 0.032$], but a non-significant effect for group by gender [$F(110) = 1.25$, $P > 0.05$]. A non-significant interaction suggests that the effect of gender was constant across groups. The univariate *F* tests suggested that the main effect for group was accounted for by the reduced size of the posterior CC quarter [$F(116) = 17.90$, $P < 0.0001$] and mid-posterior CC quarter [$F(116) = 4.89$, $P = 0.029$]. Figure 2 shows an example of thinning of the CC in a preterm subject, with particular involvement of the posterior part, and an example of a healthy CC in a control subject. None of the other CC sections differed significantly between groups. Univariate *F* tests also suggested a significant effect for gender only on the size of the mid-posterior CC quarter [$F(116) = 6.30$, $P = 0.014$], where girls had larger areas than boys (Table 2).

As CC size positively and significantly correlated with total white matter volume in both preterm individuals ($r = 0.32$, $P = 0.011$) and controls ($r = 0.50$, $P < 0001$), we performed the same analyses controlling for total white matter volume. However, overall white matter volume did not differ statistically between the study groups. For more details of results concerning other quantitative MRI volumetry, please refer to Nosarti *et al*. (2002). Controlling for white matter volume, we continued to observe a statistically significant difference in total mid-sagittal callosal surface area between preterm individuals and controls [$F(109) = 6.17$, $P = 0.015$], and a non-significant difference between genders [$F(109) = 0.64$, $P > 0.05$], as well as non-significant interaction between group and gender [$F(109) = 0.05$, $P > 0.05$]. CC surface area was also investigated adjusting for ventricular size, as previous research with the same cohort highlighted >40% difference in ventricular size between preterm individuals and controls (Nosarti *et al*., 2002). When ventricular size was added to the model (confounders: white matter volume and ventricular size), total CC area did not differ between groups [$F(109) = 2.76$, $P > 0.05$], genders [$F(109) = 0.52$, $P > 0.05$] and group by gender [$F(109) = 0.08$, $P > 0.05$].

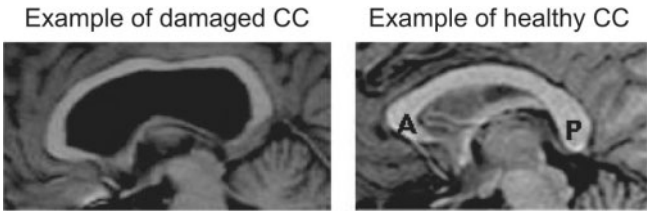

Fig. 2 Examples of thinning of the CC in an individual born very preterm and healthy CC in a control subject. Thinning of the posterior region in the damaged CC example is particularly noticeable (A = anterior; P = posterior).

We then used a MANCOVA, after controlling for mid-sagittal CC area, to investigate group by gender differences in the size of CC quarters. We observed a statistically significant main effect for group [based on Wilks' λ, $F(109) = 3.86$, $P = 0.006$] and for gender [$F(109) = 3.01$, $P = 0.021$], but a non-significant effect for group by gender [$F(109) = 1.83$, $P > 0.05$]. The univariate *F* tests suggested that the main effect for group was accounted for by the reduced size of the posterior CC quarter [$F(116) = 11.51$, $P = 0.001$] and the increased size of the anterior CC quarter [$F(116) = 11.61$, $P = 0.001$]. None of the other CC sections differed significantly between groups (after controlling for mid-sagittal CC area). Univariate *F* tests also suggested a significant effect for gender only on the size of the mid-posterior CC quarter [$F(116) = 9.58$, $P = 0.002$], where girls had larger areas than boys (Table 3). When ventricular size was added to the model in univariate analyses (confounders: total CC area and ventricular size), the results remained unaltered and showed only reduced size of the posterior CC quarter [$F(111) = 5.33$, $P = 0.023$] and increased size of the anterior CC quarter [$F(111) = 4.59$, $P = 0.034$] in preterm adolescents. Girls had larger mid-sagittal CC areas than boys [$F(111) = 9.16$, $P = 0.003$].

As the literature suggests that the CC may be affected by hand dominance (Witelson, 1985, 1989), we investigated callosal size by handedness in a subgroup of preterm adolescents and controls, for whom these data were available. Results of an ANCOVA controlling for white matter volume revealed a non-significant difference for handedness [$F(88) = 0.17$,

Table 2 *Observed mean, SE and 95% CI for total and subregional mid-sagittal areas of the CC in mm² for the preterm and the control groups*

Variable	Cases ($n = 66$)			Controls ($n = 51$)		
	All	Males	Females	All	Males	Females
Total mid-sagittal area	390.75 (71.43; 373.19–408.31)	392.45 (67.04; 368.68–416.23)	389.05 (76.56; 361.90–416.19)	424.24 (63.27; 406.44–442.03)	421.52 (56.00; 401.33–441.71)	428.80 (75.39; 392.47–465.14)
Anterior quarter	137.05 (28.67; 129.99–144.09)	137.45 (25.85; 128.29–146.62)	136.64 (31.64; 125.42–147.86)	135.86 (21.07; 129.94–141.79)	138.84 (18.13; 132.30–145.37)	130.86 (25.00; 118.80–142.91)
Mid-anterior quarter	65.72 (15.80; 61.83–69.60)	68.37 (15.87; 62.75–74.00)	63.07 (15.52; 57.56–68.57)	71.34 (18.63; 66.10–76.58)	70.45 (16.84; 64.38–76.53)	72.83 (21.71; 62.36–83.29)
Mid-posterior quarter	42.61 (15.26; 38.86–46.37)	38.60 (15.04; 33.26–43.92)	46.64 (14.62; 41.45–51.82)	48.18 (15.82; 43.73–52.63)	45.77 (16.25; 39.91–51.63)	52.22 (14.59; 45.19–59.26)
Posterior quarter	128.90 (30.26; 121.46–136.34)	128.93 (29.97; 118.31–139.56)	128.86 (31.02; 117.86–139.86)	151.10 (24.41; 144.24–157.97)	149.98 (22.68; 141.80–158.15)	153.00 (27.63; 139.68–166.32)

Table 3 *Estimated marginal means, SE and 95% CI with covariates held at their mean value for total mid-sagittal and subregional CC areas in mm² for the preterm and the control groups*

Variable	Cases (n = 66)			Controls (n = 51)		
	All	Males	Females	All	Males	Females
Total mid-sagittal area [+]	393.30 (8.10; 377.25–409.35)*	389.53 (11.54; 366.63–412.43)	397.07 (11.40; 374.46–419.69)	425.03 (9.88; 405.43–444.62)	418.51 (11.57; 395.58–441.45)	431.54 (16.07; 399.67–463.40)
Anterior quarter[†]	141.07 (2.19; 136.72–145.41)***	141.13 (3.09; 135.01–147.24)	141.01 (3.08; 134.91–147.10)	129.39 (2.59; 124.26–134.52)	134.38 (3.13; 128.18–140.59)	124.40 (4.07; 116.33–132.46)
Mid-anterior quarter[†]	68.50 (1.38; 65.76–71.24)	71.48 (1.95; 67.62–75.33)	65.52 (1.94; 61.68–69.37)	67.87 (1.64; 64.63–71.11)	67.37 (1.98; 63.46–71.29)	68.36 (2.57; 63.27–73.45)
Mid-posterior quarter[†]	44.88 (1.36; 42.18–47.57)	41.12 (1.91; 37.32–44.91)**	48.64 (1.91; 44.86–52.42)	45.92 (1.61; 42.74–49.11)	43.26 (1.94; 39.41–47.11)**	48.58 (2.53; 43.58–53.59)
Posterior quarter[†]	133.61 (2.11; 129.49–137.87)***	134.27 (2.98; 128.38–140.17)	133.01 (2.97; 127.21–138.97)	144.99 (2.50; 140.04–149.94)	144.68 (3.02; 138.69–150.66)	145.31 (3.93; 137.53–153.09)

[+]Evaluated at covariates which appeared in the model: white matter volume = 466.72 mm³. [†]Evaluated at covariates which appeared in the model: CC total area = 405.35 mm². *P = 0.05; **P = 0.01; ***P = 0.001.

$P > 0.05$] and a non-significant difference for the interaction between handedness and group [$F(88) = 0.75$, $P > 0.05$].

The distributions of mid-sagittal surface area of the CC, mid-posterior and posterior quarters in mm², for preterm adolescents and controls, are shown in Fig. 3.

Neonatal ultrasound and perinatal variables

Mid-sagittal callosal surface area differences were investigated in relation to the neonatal cerebral ultrasound classification (Stewart *et al*., 1987) which had divided neonates into three groups: those with uncomplicated PVH, those with PVH + DIL and those with normal ultrasound results (see Table 4). None of the preterm adolescents who participated in this study had had ultrasound evidence of periventricular leukomalacia and only one individual had had evidence of cysts following haemorrhagic parenchymal infarction. After controlling for total white matter volume, an ANCOVA revealed a significant difference in total mid-sagittal callosal area between ultrasound groups [$F(62) = 3.67$, $P < 0.05$]. Results of a MANCOVA with the three neonatal cerebral ultrasound groups as between-subject group factors and CC quarters as within-subject factor, controlling for total white matter volume (which was previously found to be associated with ultrasound classification; Nosarti *et al*., 2002), revealed a trend for a main effect of ultrasound classification [based on Wilks' λ, $F(112) = 1.83$, $P = 0.079$]. Univariate F tests revealed group differences in mid-posterior [$F(65) = 3.64$, $P = 0.032$] and posterior quarters [$F(65) = 5.66$, $P = 0.005$]. Specifically, the PVH + DIL group had a total callosal area 14.7% smaller than in the PVH group (Fisher LSD, $P < 0.023$) and 16.9% smaller than in the normal ultrasound group (Fisher LSD, $P < 0.004$). The PVH + DIL group also differed in the surface area of the mid-posterior callosal quarter, which was 32.8% smaller than in the normal ultrasound group (Fisher LSD, $P = 0.010$). Finally, this group differed in the size of the posterior callosal quarter, which was 19.9% smaller than in the PVH group (Fisher LSD, $P = 0.021$) and 23.4% smaller than in the normal ultrasound group (Fisher LSD, $P = 0.001$). Percentage differences evaluated at covariates appeared in the model (i.e. white matter volume).

As it seemed that a history of PVH and DIL continued to affect the size of the CC in adolescence, we performed two MANCOVAs, adjusting for white matter volume, to investigate differences between preterm and full-term adolescents; we first excluded the PVH + DIL group only, and then, secondly, we excluded all individuals with abnormal ultrasound results (PVH and PVH + DIL groups) and compared preterm individuals with normal ultrasound results with controls. When the PVH + DIL group only was excluded, there was not a significant difference in total callosal area between preterm individuals and controls [$F(100) = 2.88$, $P > 0.05$]. The analyses of callosal quarters excluding the PVH + DIL group and controlling for white matter volume suggested a main effect for group [based on Wilk's λ, $F(95) = 3.17$, $P = 0.017$]. The univariate F tests suggested a significantly reduced size of the posterior CC quarter in individuals who were born very preterm [$F(100) = 10.24$, $P = 0.002$].

When all individuals with abnormal ultrasound results were excluded, there was no significant difference in total callosal area between preterm individuals and controls [$F(85) = 1.83$, $P > 0.05$]. The comparison of callosal quarters between preterm individuals with normal ultrasound results and controls revealed a borderline main effect for group [$F(80) = 2.43$, $P = 0.055$]. The univariate F tests suggested a significant reduced size of the posterior CC quarter in individuals who were born very preterm [$F(85) = 6.59$, $P = 0.012$].

We additionally explored possible differences in the posterior callosal quarter between preterm individuals with normal ultrasound results and controls, after accounting for decreases in total CC surface area. Results of an ANCOVA showed that this area was 10.5% smaller in the preterm compared with the control group [$F(92) = 7.74$, $P = 0.007$], even in the absence of any brain injury detected on neonatal ultrasound.

The relationships between perinatal variables, CC area and its subregions were also investigated in individuals who were

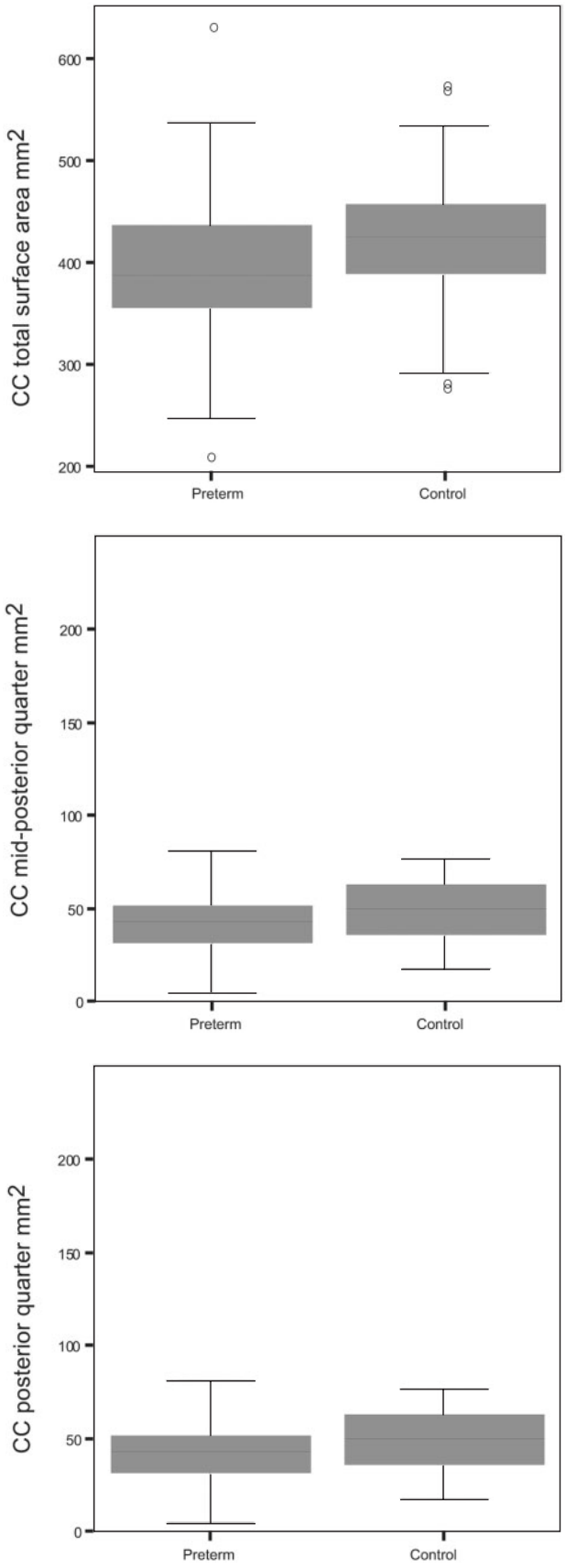

Fig. 3 Distributions of mid-sagittal surface area of the CC, mid-posterior and posterior quarters, by subject group.

born very preterm. Further partial correlation controlling for total white matter and whole brain volume showed no significant association between CC size and gestational age ($r = 0.18$, $P = 0.15$) and individuals' weight at birth ($r = 0.24$, $P = 0.36$). CC subregions also did not significantly correlate with perinatal variables.

Neuropsychological outcome

Finally, the relationships between cognitive variables, CC area and its subregions were investigated, using stepwise linear regression analyses. For a first model, independent variables were: mid-sagittal CC area, ventricular size, white matter volume and full-scale IQ (when not using subcomponents of IQ score as a dependent variable). For a second model, independent variables were the four CC quarters and the variables used in the first model. Results of linear regression analyses are displayed in Table 5.

No statistically significant associations were observed in controls between cognitive variables and the mid-sagittal area of the CC or its subdivisions. However, in individuals who were born very preterm, scores on the verbal fluency test showed a significant relationship to total mid-sagittal callosal area and to the size of the posterior CC quarter. WISC-R verbal IQ scores demonstrated a significant relationship to the size of the mid-posterior CC quarter (see Fig. 4). Schonnel reading and spelling scores were not significantly associated with CC size.

The statistically significant associations between cognitive variables and CC area and its four subdivisions were investigated further by gender with linear regression analyses using the same independent variables described in the previous paragraph, as anatomical gender differences in terms of CC size were noted in this sample. Verbal fluency scores were significantly associated with total CC area and the size of the mid-posterior CC quarter in preterm boys only. WISC-R verbal IQ scores were also associated with the surface area of the mid-posterior CC quarter in preterm boys only.

Discussion

Our study demonstrated that the total surface area of the CC in very preterm adolescents was 7.5% smaller than in controls, after accounting for total white matter volume. Our results also indicated differences in absolute size of callosal subregions between preterm and full-term adolescents: a 14.7% decrease in the posterior and an 11.6% decrease in the mid-posterior CC quarters.

When we controlled for overall mid-sagittal CC surface area, we still noted a 7.9% decrease in the posterior CC quarter; furthermore, we observed that the anterior quarter was 8.3% larger in adolescents who were born very preterm compared with controls.

The reduction in posterior CC surface area in our study group is in line with results of previous MRI studies (Cooke and Abernethy, 1999; Stewart *et al*., 1999; Peterson *et al*., 2000). A recent diffusion tensor imaging study with preterm

Table 4 *Quantitative MRI measurements of the CC by neonatal ultrasound classifications in preterm individuals*

Variable mean (SD; 95% CI)	Normal ultrasound (n = 41)	Uncomplicated PVH (n = 15)	PVH + DIL (n = 9)
Total mid-sagittal area	404.62 (69.66; 385.69–427.99)	392.40 (63.56; 356.12–425.55)	326.75 (66.59; 273.49–364.98)*,†
Anterior quarter	138.95 (26.63; 131.41–148.92)	136.65 (32.68; 121.43–150.16)	128.50 (29.82; 105.47–143.33)
Mid-anterior quarter	67.10 (13.82; 62.86–72.45)	69.90 (15.46; 61.64–77.37)	53.75 (21.89; 41.51–62.24)+,†
Mid-posterior quarter	45.88 (14.50; 41.06–50.40)	41.10 (17.06; 33.54–48.86)	31.25 (10.88; 21.65–41.84)
Posterior quarter	135.27 (28.88; 126.91–144.85)	128.40 (19.51; 113.25–142.69)	100.25 (38.04; 78.81–117.60)*,†

*PVH + DIL versus PVH, $P < 0.05$; +PVH + DIL versus PVH, $P < 0.01$; †PVH + DIL versus normal ultrasound, $P < 0.01$.

Table 5 *Results of linear regression analyses with FAS and verbal IQ scores as dependent variables*

	Total CC			Posterior CC			Mid-posterior CC			White matter volume			Ventricular size		
	β	(df)	P	β	(df)	P	β	(df)	P	β	(df)	P	β	(df)	P
FAS total (all)	**0.35**	**(60)**	**0.006***	**0.32**	**(60)**	**0.012***	0.18	(60)	0.21	0.02	(60)	0.88	−0.02	(60)	0.88
FAS total (boys)	**0.44**	**(28)**	**0.017***	0.20	(28)	0.32	**0.45**	**(28)**	**0.015***	0.11	(28)	0.55	0.10	(28)	0.59
Verbal IQ (all)	Below TC			−0.07	(61)	0.62	**0.33**	**(61)**	**0.009***	0.25	(61)	0.84	−0.07	(61)	0.60
Verbal IQ (boys)	Below TC			−0.16	(29)	0.42	**0.48**	**(29)**	**0.013***	0.09	(29)	0.51	0.19	(29)	0.28

Only statistically significant results are shown. Below TC = below tolerance criterion and not entered in equation. With Bonferroni correction, compute P value × number of comparisons (n = 4).

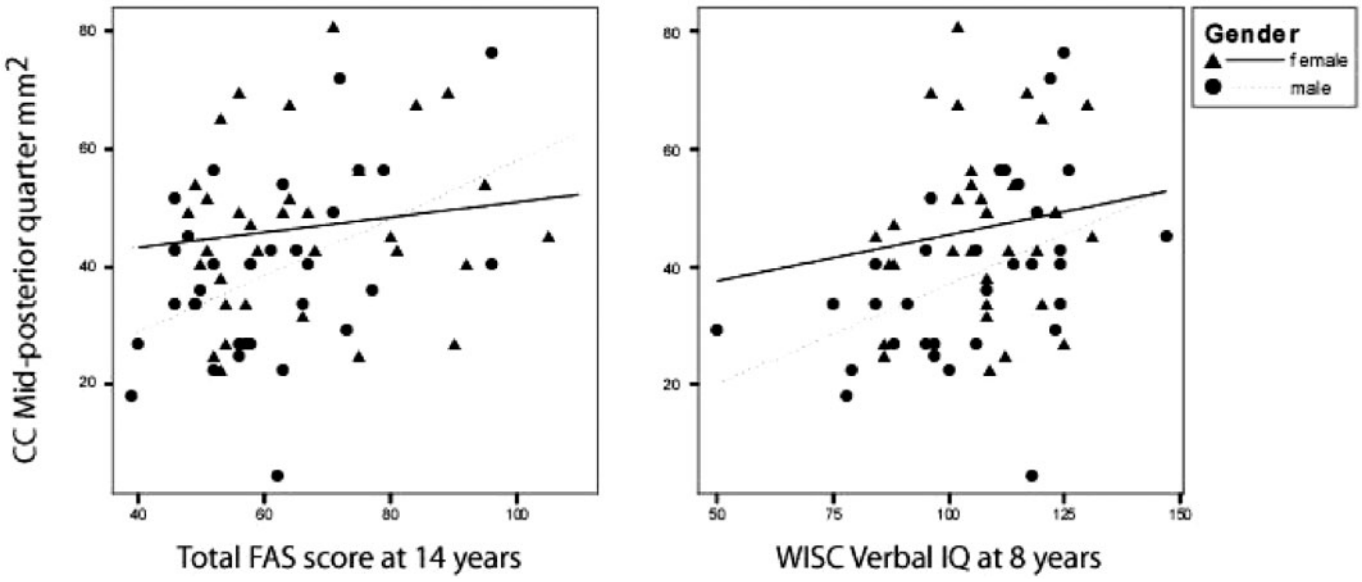

Fig. 4 Relationship between mid-posterior CC surface area and verbal fluency and verbal IQ scores in preterm individuals, by gender.

children at 11 years of age (Nagy *et al*., 2003) also demonstrated damage to the CC in the preterm group compared with controls: poorer white matter integrity was observed predominantly in posterior CC, as reflected by reduced diffusion anisotropy.

In order to understand how very preterm birth may affect the development of the CC, it is important to consider embryological data. The CC originates at 10–11 weeks gestation and first develops rostrally to form the genu. Other parts of the CC, the rostrum (continuous below with the genu) and the splenium, are formed after the trunk is developed (Ramaekers, 1991), and by 16 weeks the shape of the adult CC is recognizable (Cumming, 1970). In its early development, the genu grows faster than the splenium which does not show a rapid growth until after birth (Ramaekers, 1991). Thus, the later development of the splenium and posterior area of the CC may explain why they appear particularly susceptible to damage in the third trimester and perinatal period. Accounting for white matter volume and ventricular size in the analyses provides additional evidence for selective thinning of the posterior part of the CC. This suggests that CC damage is independent of other evaluated brain abnormalities in the studied group (e.g. ventricular enlargement) (Nosarti *et al*., 2002), even though CC and lateral ventricles are adjacent and CC fibres extend to the posterior and inferior

horns of the lateral ventricles. Experimental evidence that hypoxia in the postnatal period affects the normal development of the CC comes from animal studies. Langmeier *et al*. (1989) observed myelination deficits in the CCs of rats which had been exposed to hypoxia: axons were decreased in number, the mean section area of myelinated axons was lower, and the myelin sheets were thinner. The observed increase is the surface area of the anterior CC quarter in preterm adolescents, after adjusting for smaller overall CC size, may be explained by relative sparing from injury of this earlier developing CC area.

Our study revealed that those preterm individuals who had experienced uncomplicated PVH did not differ in the surface area of their CC compared with their counterparts with normal ultrasound results. Those individuals who experienced PVH + DIL had reduced total CC mid-sagittal areas, particularly posteriorly, compared with both preterm individuals who had experienced uncomplicated PVH and those with normal neonatal ultrasound. We previously reported that the PVH + DIL group also had significantly smaller total white matter volumes in adolescence than individuals with uncomplicated PVH only and preterm subjects with normal ultrasound results (Nosarti *et al*., 2002). This group also had the most severe cases of ventriculomegaly, as well as larger white/grey matter ratios. However, our finding that preterm individuals with normal neonatal ultrasound results had smaller posterior CC areas than full-term controls suggests that not only those individuals who sustained neonatal insult may show CC thinning in adolescence. Conversely, our results suggest that preterm birth, in the absence of obvious neonatal cranial ultrasound abnormalities, directly influences the development of the CC, possibly affecting processes of fibre organization and myelination (Nagy *et al*., 2003). As the number of gestational weeks was not predictive of the size of the CC and its subdivisions, and nor was birth weight, there is indication that crucial processes in the establishment of a normal course of CC development may occur after the 33rd week of gestation. In fact, axons forming major fibre pathways, such as those of the CC, continue to develop throughout childhood and adolescence (Keshavan *et al*., 2002; Keyvani and Schallert, 2002).

Although there is some evidence that the CC, especially the posterior part of the trunk, is dependent on hand dominance and/or sex (DeLacoste-Utamsing and Holloway, 1982; Witelson, 1985; Holloway *et al*., 1993), in our study we found no main effects for handedness in the subgroup of subjects for whom data were available. We observed no gender differences in total mid-sagittal callosal surface in the preterm and control groups. However, in both groups, females had significantly greater mid-posterior CC areas compared with males (15.5% difference in the preterm group and 5.8% in the controls, after accounting for total mid-saggital CC area). Evidence of a gender difference in CC area in healthy controls has been supported by some studies (Elster *et al*., 1990; Holloway *et al*., 1993), but not by others (Weis *et al*., 1989; Giedd *et al*., 1999).

Relationship to neuropsychological function

There is evidence of the involvement of the CC in language processing from neuroimaging data (Duara *et al*., 1991; Rumsey *et al*., 1996), and a possible role for the CC in the pathogenesis of developmental language disorder (DLD) has been hypothesized (Njiokiktjien, 1983; Fabbro *et al*., 2002). A recent study with children and adolescents with prenatal exposure to alcohol found decreased CC area in this group, with specific involvement of the posterior section. Callosal displacement, rather than CC area reduction, was associated with poorer scores on a verbal learning task (Sowell *et al*., 2001). The posterior CC area may be particularly important for speech and language functions, as fibres connecting temporal–parietal–occipital regions of the two hemispheres involved in verbal processing pass through this CC section (de Lacoste *et al*., 1985). Neuropsychological data collected in our preterm cohort at 14–15 years revealed deficits in verbal fluency scores (Rushe *et al*., 2001). However, the same study failed to observe a relationship between any measure of neuropsychological function, including verbal fluency, and qualitative MRI ratings by neuroradiologists (normal, equivocal, abnormal) (Stewart *et al*., 1999). Now we have found that in individuals who were born very preterm, scores on the verbal fluency task were positively associated with total mid-sagittal and posterior CC surface areas, and not overall IQ, even though verbal fluency skills may reflect IQ scores (Joyce *et al*., 1996).

The involvement of the CC in speech and language functions in our study is demonstrated further by the positive association between verbal IQ and the size of the body of the CC (mid-posterior section) in preterm adolescents. Thus, our results substantiate our hypothesis that the size of the CC would be associated with preterm subjects' performance on high-order cognitive processes requiring complicated inter-hemispheric interaction, such as language. They are also in line with the results of Rushe *et al*. (1999) who found that preterm boys with thinning of the CC show differential lateralization of phonological processing.

We found that in preterm boys only, the total mid-sagittal area and the mid-posterior CC quarter were associated with verbal fluency scores. Verbal IQ scores also showed a significant relationship to the size of the mid-posterior CC quarter. These results may be partly explained by gender-associated anatomical differences in our sample, i.e. girls had larger mid-posterior CC quarters compared with boys, even though verbal fluency and IQ scores did not differ between the genders (Rushe *et al*., 2001). Women normally have a better performance than men in linguistic tasks (Kimura, 1987), and males have higher incidence of language-related impairments than females, including reading disability (Flannery *et al*., 2000) and learning difficulties (Steenhuis *et al*., 1993). The results of our study are compatible with the view that males are more lateralized for language than females (Wada *et al*., 1975). If this is the case, language lateralization would be negatively associated with interhemispheric transfer of verbal stimuli. As we found that girls had larger CC areas than boys, our study supports the idea that more symmetric brains

(e.g. functionally less lateralized) may be reflected by a larger CC area which may facilitate interhemispheric transfer (Witelson, 1989).

Conclusion

To conclude, our data demonstrate that very preterm birth adversely affects the development of the CC, particularly its posterior quarter, which is adjacent to the periventricular region, the most common site for damage in the preterm infant. We found that in very preterm individuals, verbal IQ and verbal fluency scores, but not reading age, were positively associated with the size of the posterior half of the CC. Therefore, our findings support the hypothesis that the CC may be involved in some high-order cognitive processes (e.g. speech and language). Our data are compatible with the idea of differences in cerebral lateralization and interhemispheric transfer between the genders, since in preterm boys, but not in preterm girls, the mid-posterior CC area was associated with verbal fluency and verbal IQ scores.

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