The Relationship between Corpus Callosum Size and Forebrain Volume

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Using high-resolution *in vivo* magnetic resonance morphometry we measured forebrain volume (FBV), midsagittal size of the corpus callosum (CC) and four CC subareas in 120 young and healthy adults (49 women, 71 men). We found moderate linear and quadratic correlations, indicating that the CC and all CC subareas increase with FBV both in men and women (multiple r^2 ranging from 0.10 to 0.28). Allometric equations revealed that these increases were less than proportional to FBV (r^2 ranging from 0.02 to 0.30). Absolute CC measurements, as well as CC subareas relative to total CC or FBV (the latter measures termed the CC ratios), were further analyzed with regard to possible effects of handedness, gender, or handedness by gender interaction. Contrary to previous reports, left-handers did not show larger CC measurements compared to right-handers. The only apparent influence of gender was on the CC ratios, which were larger in women. However, smaller brains had larger CC ratios which were mainly independent of gender, a result of the less than proportional increase of callosal size with FBV. We suggest that the previously described gender differences in CC anatomy may be better explained by an underlying effect of brain size, with larger brains having relatively smaller callosa. This lends empirical support to the hypothesis that brain size may be an important factor influencing interhemispheric connectivity and lateralization.

Introduction

Individual differences in the corpus callosum (CC), and their possible implications regarding interhemispheric connectivity, have been a matter of long-standing dispute. The first post-mortem reports of sex differences in CC shape or size suggested that women may have a wider and more bulbous splenium than men (de Lacoste-Utamsing and Holloway, 1982), and that even the overall size of the CC may be absolutely larger in women (Holloway and de Lacoste 1986). The majority of follow-up studies failed to replicate these results (Weber and Weis, 1986; Kertesz *et al.*, 1987; Oppenheim *et al.*, 1987; Byne *et al.*, 1988; Demeter *et al.*, 1988; Clarke *et al.*, 1989; Hayakawa *et al.*, 1989; Weis *et al.*, 1989; Elster *et al.*, 1990; Going and Dixson, 1990; Emory *et al.*, 1991; Allen *et al.*, 1991; Habib *et al.*, 1991; Aboitiz *et al.*, 1992a; Steinmetz *et al.*, 1992; Holloway *et al.*, 1993; Pujol *et al.*, 1993; Clarke and Zaidel, 1994; Johnson *et al.*, 1994; Pozzilli *et al.*, 1994; Rauch and Jinkins, 1994; Steinmetz *et al.*, 1995). Nevertheless, most authors found a larger *relative* CC in women (i.e. CC relative to brain or skull size), or larger *relative* posterior portions of the CC in women (i.e. splenium or isthmus relative to total CC) (Kertesz *et al.*, 1987; Reinarz *et al.*, 1988; Clarke *et al.*, 1989; Witelson, 1989; de Lacoste *et al.*, 1990; Elster *et al.*, 1990; Allen *et al.*, 1991; Habib *et al.*, 1991; Steinmetz *et al.*, 1992; Holloway *et al.*, 1993; Clarke and Zaidel, 1994; Johnson *et al.*, 1994; Steinmetz *et al.*, 1995). The common interpretation of this sexual dimorphism has been that it reflects increased interhemispheric connectivity due to increased female ambilaterality, especially for temporoparietal cognitive functions (McGlone, 1980).

Witelson was the first to suggest that hand preference, interacting with gender, might also affect CC morphology (Witelson, 1985, 1989; Witelson and Goldsmith, 1991). In her post-mortem studies, non-consistently right-handed men showed larger total CC areas than consistently right-handed men or women. This again suggested a relationship between laterality and callosal size, at least in men. Subsequent *in vivo* imaging studies, however, revealed equivocal results. Whereas some investigators replicated the findings of Witelson and co-workers for absolute and relative CC subarea measurements (Habib *et al.*, 1991; Denenberg *et al.*, 1991; Cowell *et al.*, 1993; Clarke and Zaidel, 1994), others could not confirm significant influences of handedness (Nasrallah *et al.*, 1986; Kertesz *et al.*, 1987; O'Kusky *et al.*, 1988; Reinarz *et al.*, 1988; Steinmetz *et al.*, 1992, 1995).

Using whole-brain *in vivo* magnetic resonance morphometry we were among those previously describing gender differences in the CC. In a study of 120 normal young adults, we found a moderate linear correlation between forebrain volume (FBV) and total midsagittal CC area. In addition, the total midsagittal CC measurement was significantly larger in women after linear adjustment for variation attributable to FBV (Steinmetz *et al.*, 1995). The present analysis conducted in the same sample intended to answer the following additional questions: (i) Does callosal size increase out of proportion, in proportion, or less than proportional to FBV? (ii) Do FBV-adjusted measurements of the CC (termed CC ratios) correlate with FBV [a problem related to (i)]? (iii) Does the relation between callosal size and FBV differ for anterior or posterior CC subareas? (iv) Is there a true influence of gender? Regarding the latter question we hypothesized that brain size may have been a confounding factor in previous studies of gender differences in the CC.

Methods

Subjects

The subjects were recruited through announcements in a medical school specifically calling for participation in a study relating to cerebral anatomical correlates of gender and handedness. One hundred and twenty consecutive persons reporting no birth complication, neurological or psychiatric illness, learning disability, failure in elementary school or claustrophobia were investigated (49 women and 71 men). Their ages ranged from 18 to 45 years (mean age ± SD, 25.7 ± 4.7 years; Table 1). There was no overlap between these subjects and a previous cohort of 52 normal adults (Steinmetz *et al.*, 1992).

Handedness Measurement

Hand preference was determined by observing each subject's performance of 12 unimanual and bimanual tasks taken from Annett's handedness questionnaire (Annett, 1970, 1994). Participants using the right or left hand for all tasks (with 'either' preferences being acceptable for unscrewing a jar lid, holding the top of a shovel, dealing cards or

holding the top of a broom) were classified as consistent right-handers (n = 54) or consistent left-handers (n = 28) respectively. All other subjects were defined as mixed-handers (n = 38), irrespective of writing hand. As a cross-validation, left- and right-hand motor skill was measured with the Hand Dominance Test (HDT) (Steingrüber, 1971). This paper-and-pencil test consists of three dexterity tasks (tracing lines, dotting circles, tapping on squares) each to be performed with maximum speed and precision over 15 s. Laterality coefficients (R–L)/(R+L) were calculated. Because the performances of the three HDT subtests correlated strongly (Pearson correlations ranging from 0.81. to 0.91), a total HDT score was calculated. Consistent right-handers showed positive total scores (mean ± SD: 0.14 ± 0.06), consistent left-handers showed negative total scores (–0.23 ± 0.42), and the scores of mixed-handers were centered around zero (–0.01 ± 0.12). Thus, the handedness classification according to the Annett questionnaire (hand preference) was cross-validated by the measurements of hand skill, a point that deserves mention here because both measurements have been used in previous studies. In the following we will only refer to the hand preference measure because this has been most commonly used (Nasrallah *et al.*, 1986; Witelson, 1989; Habib *et al.*, 1991; Witelson and Goldsmith, 1991; Steinmetz *et al.*, 1992, 1995).

In Vivo Magnetic Resonance Morphometry

This was performed using a 1.5 T magnet (Siemens Magnetom SP, Erlangen, Germany) and a circularly polarized head coil. After parallel alignment of the interhemispheric plane of the brain with the sagittal plane of imaging, a strongly T_1-weighted gradient echo pulse sequence (fast low-angle shot) with the following technical factors was applied: 40 ms repetition time, 5 ms echo time, 40° flip angle, one excitation, 25 cm field of view, 256 × 256 matrix, 128 sagittal slices with 1.17 mm single slice thickness. The midsagittal image was selected for segmentation of the CC on an off-line workstation (Steinmetz *et al.*, 1992). The total midsagittal CC area was subdivided into four subareas according to the principles illustrated in Figure 1 (CC subareas: anterior third, middle third, isthmus, splenium). The intercommissural line was used for the definition of maximum CC length. For the present sample, the interobserver reliabilities calculated for CC subarea measurements according to the formula of Bartko and Carpenter (1976) ranged from 0.81 to 0.93. All CC measurements were performed by a blinded investigator.

FBV was measured by means of brain image segmentation, i.e. a stepwise, interactively controlled procedure which removes all tissue and fluid not corresponding to brain gray or white matter from each image slice (Huang *et al.*, 1993; Steinmetz *et al.*, 1995). The volume of all remaining image voxels was summed. In order to obtain FBV, the hindbrain was removed by a cut-off line spanning from the base of the mamillary bodies to the upper margin of the posterior commissure. All brain volume measurements were performed by a blinded investigator.

Data Analysis

Three different CC measurements were analyzed: (i) absolute CC area and subareas; (ii) CC subareas relative to total CC; (iii) CC ratios (i.e. CC area and subareas relative to FBV). The CC ratios were computed as CC (sub)area/FBV (dimension: mm^2/l). Because there was no unique relationship between CC measurements and FBV for men or women (linear, quadratic or diverging slopes), it was impossible to calculate analyses of covariances with FBV as covariate. We will not present transformed brain size measurements as done by Holloway and co-workers ($FBV^{0.6666}$) (Holloway and de Lacoste, 1986; Holloway *et al.*, 1993) since this score is unit-less and may be difficult to interpret. The present results were not significantly altered by using transformed FBV measurements ($FBV^{0.6666}$) instead of untransformed values for calculating the CC ratios.

Linear and curvilinear associations between FBV and CC measurements were examined with hierarchical polynomial regression analyses, i.e. higher-order trends were tested for the predictive increments they afforded over and above lower-order trends. For instance, the significance of a quadratic function was tested by evaluating the increment in the multiple r^2 value it produced beyond that obtained when a linear function was used.

Gender effects were also evaluated using hierarchical polynomial regression models that included a dummy-coded vector representing gender. Gender differences in the slopes of these regressions were analyzed by significance testing of the increment in the multiple r^2 value afforded by the addition of a product term including gender (i.e. gender × FBV, gender × FBV^2, gender × FBV^3). Gender differences in the intercepts of these regressions, examined when the slopes were determined to be equivalent for men and women, were assessed by testing of the increment in the multiple r^2 value afforded by the addition of the dummy-coded gender variable to the linear and higher-order terms in the regression model. In addition, we report r^2 values for each meaningful effect describing the variance accounted for. Prior to these analyses, the necessary assumptions for applying multiple regressions (i.e. approximately normal distribution of the residuals, homoscedasticity) were confirmed for the present dataset. A full description of the statistical model has been given by Pedhazur (1982).

To further analyze possible allometric covariation, power functions were calculated explaining the relationships between brain size and callosal size as

$$\text{CC} = \text{constant} \times \text{FBV}^{\text{exponent}} \quad (1)$$

$$\text{CC ratio} = \text{constant} \times \text{FBV}^{\text{exponent}} \quad (2)$$

For further statistical evaluation, FBV and the CC measurements were transformed logarithmically resulting in the following allometric equations:

$$\log \text{CC} = \log \text{constant} + b \times \log \text{FBV} \quad (3)$$

$$\log \text{CC ratio} = \log \text{constant} + b \times \log \text{FBV} \quad (4)$$

where b is the slope of the regression line.

The logarithmic forms are more suitable for calculating confidence intervals and gender differences with conventional statistics software. Therefore, only results based on equations (3) and (4) will be presented in the following. The slopes (b) of equations (3) and (4) are identical to the exponents in equations (1) and (2). The slopes are the main messages (*allometric signals*) carried by the regression lines. They are used to detect proportionality or deviations from proportionality. For instance, if the slope in equation (3) is <1, callosal size increases with FBV, but less than proportional to FBV. If the slope in equation (3) is >1, callosal size also increases with FBV, but out of proportion to FBV. In addition, the slopes are useful to evaluate whether the CC/FBV relationship follows a simple geometrical rule. According to this rule, the size of a cross-sectional area of a three-dimensional object does not increase proportionally to the volume of this object, but only to the two-thirds power of the volume. If a surface/volume relationship follows this geometrical rule the exponents or slopes are 0.67 in equations (1) or (3), and –0.33 in equations (2) or (4) (Schmidt-Nielsen 1984). In this case smaller bodies have, relative to their volumes, larger cross-sectional

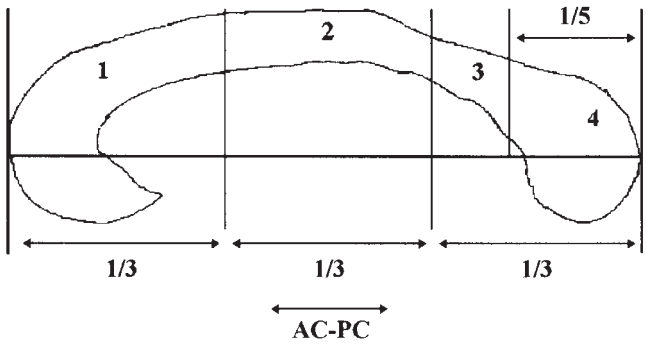

Figure 1. Anatomical subdivision of the corpus callosum (CC) used in the present study: parallel to the bicommissural line (AC–PC) the maximum anterior–posterior length of the CC is determined and divided into four subareas as demonstrated (1, anterior third; 2, middle third; 3, isthmus; 4, splenium).

Table 1
Means and SDs for age, total brain volume (TBV), forebrain volume (FBV), body height (BH) and hand motor performance score (HDT) for the three handedness groups (CRH, CLH: consistent right- or left-handers; MH: mixed handers)

	CRH	MH	CLH	Total
n				
Women	19	15	15	49
Men	35	23	13	71
Total	54	38	28	120
Age (years)				
Women	28.2 ± 4.8	25.4 ± 4.1	25.7 ± 5.5	26.3 ± 4.9
Men	27.5 ± 5.6	25.1 ± 3.8	22.2 ± 2.7	25.3 ± 4.6
Total	27.8 ± 5.2	25.2 ± 3.9	24.0 ± 4.7	25.7 ± 4.7
TBV (l)				
Women	1.15 ± 0.13	1.11 ± 0.10	1.13 ± 0.10	1.12 ± 0.11
Men	1.25 ± 0.13	1.23 ± 0.10	1.26 ± 0.11	1.24 ± 0.11
Total	1.21 ± 0.14	1.19 ± 0.12	1.19 ± 0.12	1.20 ± 0.12
FBV (l)				
Women	0.99 ± 0.11	0.98 ± 0.10	1.00 ± 0.10	0.99 ± 0.10
Men	1.08 ± 0.14	1.08 ± 0.09	1.11 ± 0.10	1.08 ± 0.11
Total	1.04 ± 0.13	1.04 ± 0.11	1.05 ± 0.11	1.04 ± 0.11
BH (cm)				
Women	172 ± 4.4	169 ± 6.0	169 ± 7.1	170 ± 6.0
Men	182 ± 6.5	180 ± 6.3	180 ± 6.2	181 ± 6.3
Total	178 ± 7.4	176 ± 8.2	174 ± 8.8	176 ± 8.2
HDT				
Women	0.15 ± 0.06	–0.04 ± 0.13	–0.15 ± 0.06	–0.02 ± 0.15
Men	0.13 ± 0.05	0.01 ± 0.12	–0.32 ± 0.61	–0.02 ± 0.31
Total	0.14 ± 0.06	–0.01 ± 0.12	–0.23 ± 0.42	–0.02 ± 0.26

surfaces than larger bodies of the same shape. In order to test whether the CC/FBV relation deviates from proportionality, the slopes of equation (3) were tested for deviation from 1 because a slope of 1 indicates exact proportionality [$(b - 1)/SD_b$, according to Sachs (1984)]. In case of deviation from proportionality we evaluated whether the slopes were similar to those expected if the CC/FBV relation followed the geometrical rule by comparing the slope of interest with a hypothetical slope derived from the rule (0.67 or –0.33).

In order to test handedness and gender effects as well as handedness and gender interactions on the CC measurements, two-way ANOVAs were computed. Since cell frequencies were disproportional, resulting in a non-orthogonal design, the 'experimental' model for calculating *F*-values was applied (Appelbaum and Cramer, 1974). Homogeneity of variances was tested for each dependent variable applying the Bartlett–Box test (Winer, 1962). If not otherwise mentioned, homogeneity of variances was confirmed for each dependent variable. Additionally, the *effect size* was computed because it is not only important how probable an effect is, but also how large. Effect size was calculated in terms of the variance accounted for (Pedhazur, 1982). For instance, an effect size of 0.10 (conventionally entitled as ETA^2) for the gender factor would state that 10% of the observed variance in the dependent variable is due to the variable gender.

A significance level of $P < 0.05$ was chosen. All statistical analyses were performed using the SPSS for Windows software package, version 6.0.

Results

Analysis of Baseline Variables

Mean total brain volume was 1.120 l (SD 0.110) for women, and 1.240 l (SD 0.110) for men. Regressing total brain volume on FBV and on hindbrain volume revealed that 95% of total brain volume variability was determined by FBV variability. Owing to this, and to the fact that all callosal fibers originate in the forebrain, it was justified to use FBV for the following analyses.

Two-way ANOVAs with handedness (three levels: consistent right-handers, consistent left-handers, mixed-handers) and gender (two levels: men, women) as factors, and age, body height and FBV as dependent variables were calculated to examine whether handedness or gender groups differed strongly with respect to these variables. The ANOVAs for age revealed no significant handedness or gender effect, and no significant interaction. As expected, the ANOVAs for FBV and body height

Table 2
Corpus callosum (CC) measurements and forebrain volume (FBV): summary of significant linear and quadratic regressions of absolute CC area measurements and CC ratios on FBV ($P < 0.05$). In cases of significant slope differences between genders the results are given separately for men and women

	Anterior third	Middle third	Isthmus	Splenium	Total CC
CC area					
Men					
r^2_{linear} [a]	–	0.07	–	0.08	–
$r^2_{quadratic}$ [b]	–	0.11	–	0.09	–
Women					
r^2_{linear}	–	0.23	–	0.23	–
$r^2_{quadratic}$	–	–	–	–	–
Total sample					
r^2_{linear}	0.23	–	0.15	–	0.18
$r^2_{quadratic}$	–	–	–	–	–
CC ratio					
Men					
r^2_{linear}	–	0.16	–	0.13	–
$r^2_{quadratic}$	–	0.12	–	0.11	–
Women					
r^2_{linear}	–	0.10	–	0.07	
$r^2_{quadratic}$	–	–	–	–	
Total sample					
r^2_{linear}	0.10	–	–	–	0.21
$r^2_{quadratic}$	–	–	–	–	–

[a] r^2_{linear} indicates the proportion of CC area or CC ratio variance accounted for by FBV as found for the linear trend of the hierarchical polynomial regression analysis.

[b] $r^2_{quadratic}$ indicates the proportion of CC area or CC ratio variance accounted for by FBV as found for the quadratic trend of the hierarchical polynomial regression analysis independent by the linear association.

–, $P \geq 0.05$ indicating no significant linear or quadratic association.

Table 3
Summary of regression analyses of log corpus callosum (CC) area on log forebrain volume (FBV) (allometric equations)

	Women		Men		
	b	r^2	b	r^2	
CC area					
Anterior third	0.73	0.29	0.62	0.21	
Middle third	0.62	0.21	0.35†	0.05	*
Isthmus	0.58	0.11	0.77	0.16	
Splenium	0.64	0.21	0.43	0.08	*
Total CC	0.67	0.30	0.52	0.17	*
CC ratio					
Anterior third	–0.27	0.05	–0.38	0.09	
Middle third	–0.38	0.09	–0.65	0.16	*
Isthmus	–0.42	0.06	–0.23	0.02‡	
Splenium	–0.36	0.08	–0.57	0.12	*
Total CC	–0.33	0.10	–0.48	0.15	*

*Significant gender difference with respect to slopes, $P < 0.05$.

†Trend for difference from 0.67 ($P = 0.065$); all other r^2-values were significant ($P < 0.05$).

‡No significant allometric equation ($P = 0.10$).

r^2, proportion of log CC area measurement and log *CC ratio* variance accounted for by log FBV.

b, regression coefficient of allometric equation (see Methods).

Table 4
Means and SDs for *absolute* midsagittal corpus callosum (CC) area or subareas for consistent right-handers (CRH), mixed handers (MH), consistent left-handers (CLH), women and men (mm^2)

	CRH	MH	CLH	Total
Anterior third				
Women	269.8 ± 38.3	265.8 ± 31.6	255.6 ± 38.2	263.9 ± 35.5
Men	276.2 ± 32.1	264.0 ± 36.6	273.3 ± 36.7	269.7 ± 35.2
Total	273.7 ± 34.3	264.7 ± 34.6	263.8 ± 37.8	267.3 ± 35.3
Middle third				
Women	161.8 ± 21.9	150.6 ± 17.6	153.2 ± 22.1	154.8 ± 20.5
Men	161.9 ± 25.6	146.4 ± 19.2	149.5 ± 21.2	152.0 ± 22.6
Total	161.9 ± 23.9	147.9 ± 18.6	151.5 ± 21.4	153.2 ± 21.8
Isthmus				
Women	66.2 ± 14.7	61.5 ± 9.1	58.9 ± 9.8	62.1 ± 11.4
Men	66.5 ± 9.5	62.5 ± 12.4	64.5 ± 14.1	64.2 ± 11.8
Total	66.4 ± 11.6	62.1 ± 11.3	61.5 ± 12.1	63.3 ± 11.7
Splenium				
Women	186.0 ± 33.9	178.7 ± 20.9	185.9 ± 23.1	183.1 ± 25.8
Men	186.7 ± 30.3	169.8 ± 22.7	177.6 ± 28.8	176.7 ± 27.2
Total	186.4 ± 31.3	172.9 ± 22.3	182.1 ± 25.8	179.3 ± 26.7
Total CC				
Women	683.8 ± 91.9	656.6 ± 70.1	653.6 ± 84.4	664.0 ± 81.0
Men	691.4 ± 83.1	642.6 ± 74.1	664.9 ± 88.5	662.5 ± 81.6
Total	688.4 ± 85.5	647.5 ± 72.4	658.9 ± 84.9	663.1 ± 81.0

revealed highly significant gender effects with larger measurements in men (Table 1). There were no significant handedness effects, and no interactions.

Absolute CC Areas and FBV

Regressing the absolute CC measurements on FBV, we found that total CC, anterior third and isthmus of the CC were strongly linearly related with FBV (Table 2). Men and women did not differ with respect to the slopes or intercepts of these linear regressions. For the middle third and splenium, the slopes of the multiple regressions differed between men and women (both *P* values <0.01). Thus, separate hierarchical polynomial regressions had to be calculated for each gender, including the linear and curvilinear terms. They revealed significant linear relationships between FBV and middle third, as well as between FBV and splenium in women. In men these relationships were linear and quadratic (Table 2). As demonstrated by Figure 2, all CC measurements increased with FBV.

The allometric equations revealed significant relationships between all CC measurements and FBV. These relations were of the similar order of magnitude to the linear regressions (r^2 values between 0.02 and 0.30). For men, the hierarchical polynomial regressions revealed a better fit for the middle third and the splenium of the CC than the allometric equations because the quadratic term determined ~10% of the remaining variance. The slopes of the allometric equations ranged from 0.35 to 0.77 (Table 3) and turned out to be significantly lower than 1, indicating that the absolute CC measurements increased less than proportional to FBV. All slopes were of the order of magnitude expected if the CC/FBV relationship followed the geometrical rule (expected $b = 0.67$). There were significant gender differences in the slopes for the middle third, splenium and total CC. The slopes for men were less steep, indicating smaller increases of CC measurements than in women.

CC Ratios and FBV

In order to examine whether the CC ratios are correlated with FBV, additional hierarchical regression analyses were performed. Regressing the CC ratios on FBV, FBV^2, gender, and the interaction terms including gender and the FBV measures (gender × FBV, gender × FBV^2), we discovered a negative linear association for the CC ratios of the anterior third and total CC, with similar linear regressions (same slope and intercepts) for women and men (Table 2, Fig. 3). For the CC ratios of the middle third and splenium, linear and quadratic relationships emerged for men while only linear regressions were found for women. Only the CC ratio of the isthmus was not strongly related to FBV [isthmus: $r^2_{linear} = 0.04$, $F(1,119) = 2.9$, $P = 0.09$]. The regressions showed that the CC ratios decreased with increasing FBV (Fig. 3).

Fitting the relationships betweeen CC ratios and FBV applying the allometric equations, we found significant covariations (r^2 values between 0.05 and 0.16) with slopes ranging from –0.22 to –0.65, the only exception being a non-significant relation between the CC ratio of the isthmus and FBV (Table 3). The slopes were in the range expected from the geometrical rule (expected $b = -0.33$). They differed significantly between genders for the middle third, splenium and total CC, with steeper slopes in men (Table 3).

CC, Handedness and Gender

In order to test for influences of handedness, gender, or handedness by gender interaction, two-way ANOVAs with handedness (consistent right-handers, consistent left-handers, mixed-handers) and gender (men, women) and the 14 CC measurements as dependent variables were computed (five absolute CC (sub)areas, four CC subareas relative to total CC and five CC ratios) (Tables 4 and 5). Thus, we calculated a total of 14 two-way ANOVAs. For absolute CC area or subareas, and for CC subareas relative to total CC, no significant main effects or interactions between handedness and gender emerged, with one exception. Handedness had an effect on the absolute size of the middle third of the CC [$F(2,114) = 4.95$, $P = 0.009$, $ETA^2 = 0.08$]. Contrasting consistent right-handers with mixed-handers and consistent left-handers, we found that 96% of the variance afforded by the handedness effect (including all three handedness groups) was determined by this contrast [$F(1,114) = 9.53$, $P = 0.003$].

The two-way ANOVAs with gender and handedness as

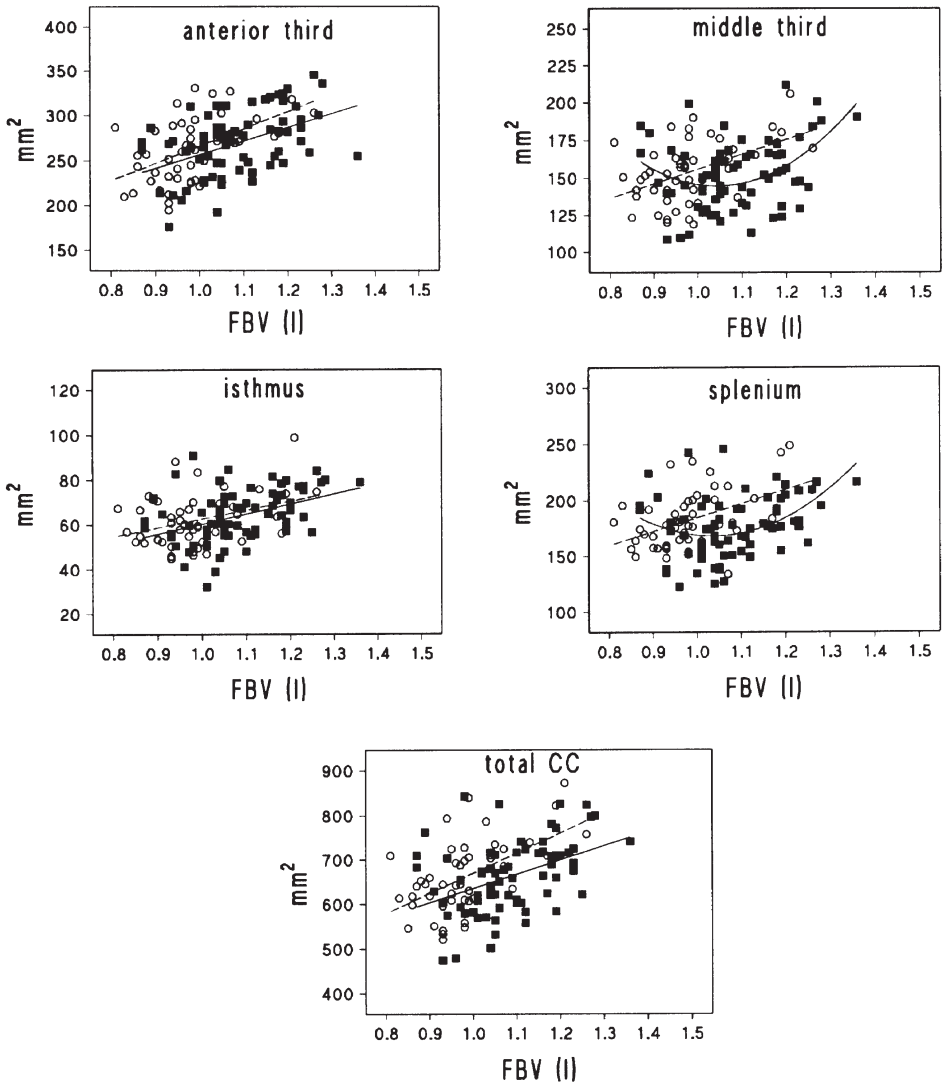

Figure 2. Corpus callosum (CC) areas and forebrain volume (FBV). Unfilled circles indicate women, and filled squares men. Regression slopes for women are dashed.

independent factors and *CC ratios* as dependent variables revealed significant gender effects for total CC and all subareas [anterior third: $F(1,114) = 11.16$, $P = 0.001$, $ETA^2 = 0.09$; middle third: $F(1,114) = 17.7$, $P < 0.001$, $ETA^2 = 0.14$; isthmus: $F(1,114) = 4.4$, $P = 0.03$, $ETA^2 = 0.04$; splenium: $F(1,114) = 20.4$, $P < 0.001$, $ETA^2 = 0.15$; total CC: $F(1,114) = 19.7$, $P < 0.001$, ETA2 = 0.15]. As shown in Table 5 and Figure 4, women exhibited larger CC ratios than men (~7–14% larger). As for the absolute middle third of the CC, the CC ratio for this subarea revealed a relatively strong handedness effect [$F(2,114) = 5.45$, $P = 0.006$, $ETA^2 = 0.09$]. There were no effects of handedness or handedness by gender interaction.

Finally, in order to examine whether there was a true gender difference in the CC ratios, and to acccount for slope differences in the allometric equations, we divided our sample into FBV quintiles, with 24 brains per quintile (Table 6). For each quintile *t*-tests were calculated to compare FBV and CC measurements between both genders. There were no significant gender differences, except for the third quintile of brains with a mean FBV of 1.03 l. For this quintile we found a larger total CC area and CC ratio in women. Subsequent analyses revealed that this gender difference was restricted to the middle third and splenium, subareas where gender differences in the slopes of the allometric equations had been found previously (Table 3). However, because this quintile comprised only five female and 19 male brains, a sampling error may well account for the result.

Discussion

Allometric Relationship between Brain and CC Size

Using *in vivo* magnetic resonance morphometry in 120 young and healthy adults, we found a mean ± SD total brain volume of 1.120 ± 0.110 l for women and 1.240 ± 0.110 l for men (Table 1). The only post-mortem study of subjects of similar age with apparently normal brains is the one by Dekaban and Sadowsky (1978). They reported brain weights of 1.30 kg for women and 1.44 kg for men (unfixed post-mortem material, North American subjects between 22 and 30 years of age). Taking into account a specific gravity of fresh post-mortem brain tissue of 1.04–1.09 kg/l (Blinkov and Glezer, 1964, p. 24), and the up to 9% increase

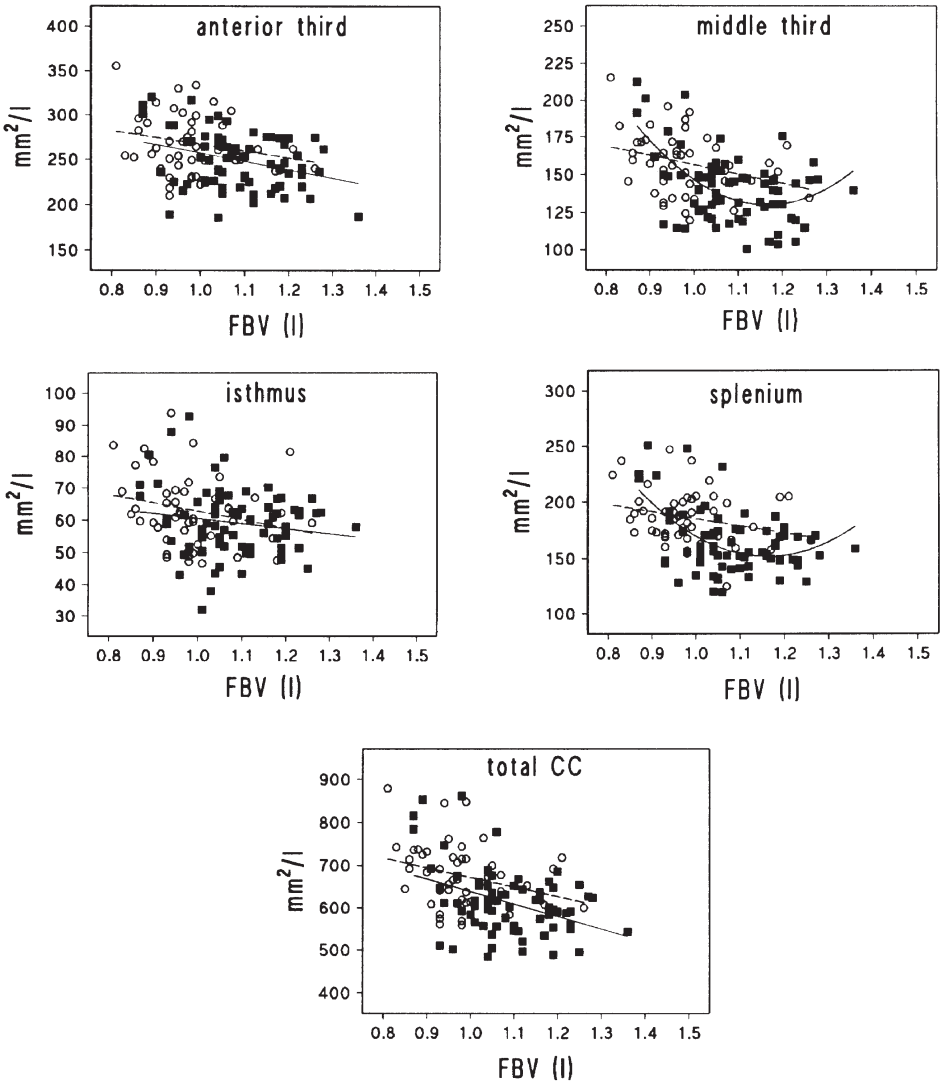

Figure 3. Corpus callosum (CC) ratios and forebrain volume (FBV). Unfilled circles indicate women, and filled squares men. Regression slopes for women are dashed.

in brain volume during the first hours after death (presumably due to the absorption of cerebrospinal fluid) (Appel and Appel, 1942), our measurements obtained in living subjects correspond exactly to what one would expect from these post-mortem data. The validity of our morphometric method is further supported by the close correspondence between our mean total CC area measurements (women, 664 mm^2; men, 663 mm^2) and those obtained post-mortem by Zilles (women, 630 mm^2; men, 620 mm^2) (Zilles, 1972), Witelson (women, 657 mm^2; men, 719 mm^2) (Witelson, 1989), and others (de Lacoste-Utamsing and Holloway, 1982; Weber and Weis, 1986; Going and Dixson, 1990).

There were moderate but significant linear and quadratic relationships between all CC (sub)areas and FBV, indicating a relatively homogeneous enlargement of the CC with increasing brain size (Fig. 2). The allometric equations revealed increases of CC size less than proportional to FBV (allometric relationship). Our correlations were similar to the brain–CC correlations in most post-mortem studies (Bean, 1906; Mall, 1909; Byne *et al.*, 1988; Witelson, 1989; Aboitiz *et al.*, 1992a). Nevertheless, the allometric relationship with FBV still explained not more than 30% of the total variability in CC size in our sample, demonstrating that the thickness of this interhemispheric fiber tract is mainly influenced by other factors. The majority of callosal fibers are thought to originate from association cortices and subserve higher-order functions (Innocenti, 1986; Pandya and Seltzer, 1986; LaMantia and Rakic, 1990; Aboitiz *et al.*, 1992b). Thus, as previously hypothesized by Peters (1988), a possible lack of an allometric relationship between the size of the brain and the association cortices could account for at least some of the variation in CC size that remains unexplained by the present findings.

No Relationship between CC, Handedness, or Handedness by Gender Interaction

We could not confirm larger CC measurements in non-consistent right-handers, mixed-handers or consistent left-handers when compared to consistent right-handers. This is in agreement with the majority of studies investigating possible relationships between CC size and handedness (Nasrallah *et al.*, 1986; Kertesz

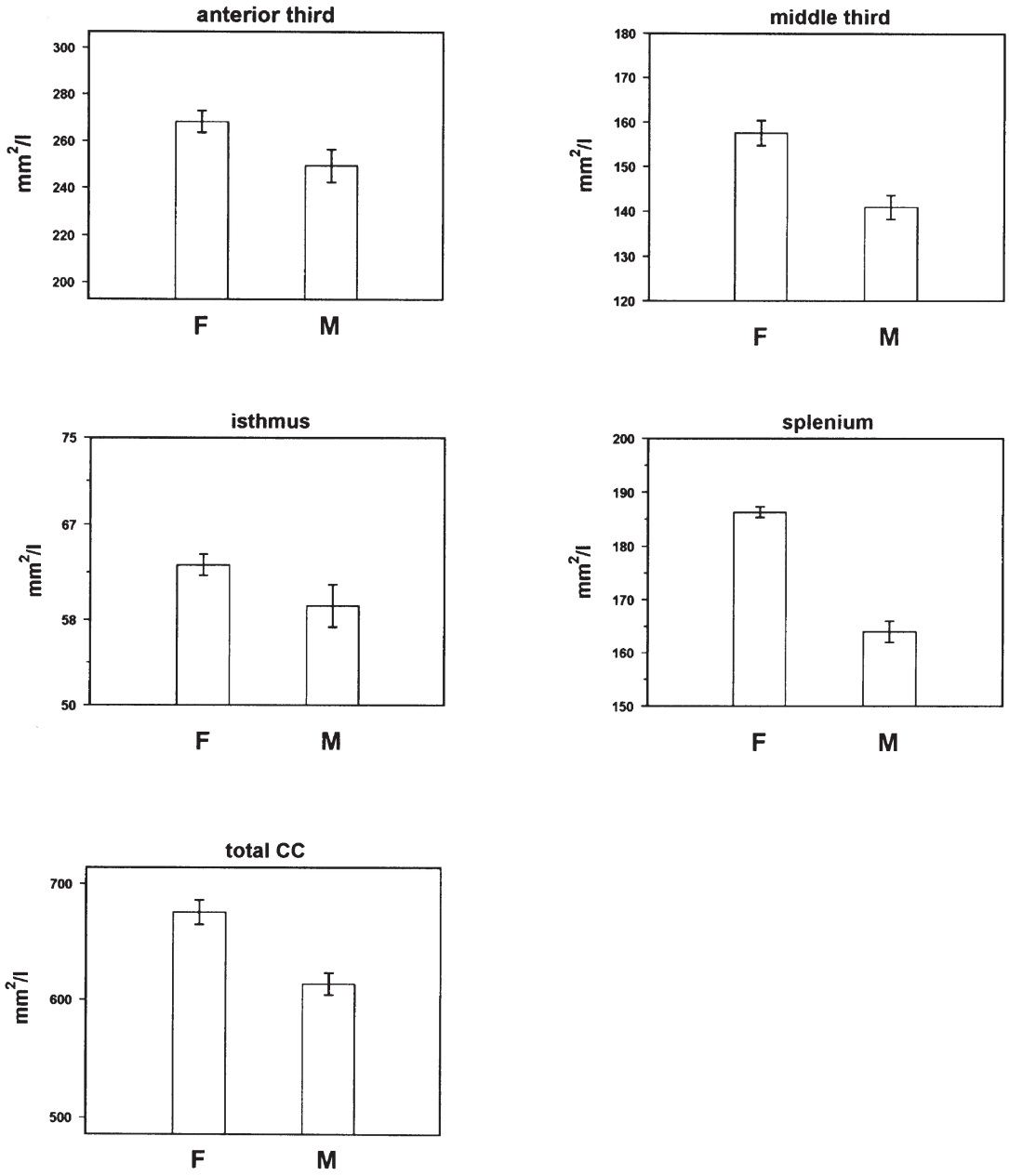

Figure 4. Mean corpus callosum (CC) ratios for women (F) and men (M). Vertical bars indicate 95% confidence intervals.

et al., 1987; O'Kusky *et al.*, 1988; Reinarz *et al.*, 1988; Steinmetz *et al.*, 1992). In our sample, consistent right-handers even showed a larger midbody than other handedness groups, which is in contrast to previous reports of opposite handedness effects (Witelson, 1989; Denenberg *et al.*, 1991; Habib *et al.*, 1991; Cowell *et al.*, 1993; Clarke and Zaidel, 1994). However, it should be mentioned that two of the latter studies (Denenberg *et al.*, 1991; Cowell *et al.*, 1993) were reanalyses of data for which a prior report (Kertesz *et al.*, 1987) had failed to identify a gender or handedness difference, that the post-mortem sample of Witelson (1989) was relatively heterogeneous, and that the effect reported by Clarke and Zaidel (1994) remained small. While our data may add further confusion to this part of the ongoing discussion, it appears fair to say that an influence of handedness on CC size or shape must remain questionable.

Gender Difference in the CC Ratio

We found no significant gender differences for absolute CC area or subareas (Table 4), or CC subareas relative to total CC size. However, the *CC ratios* (i.e. CC area measurements relative to FBV) were clearly larger in women (Table 5, Fig. 4). This confirms the results of several post-mortem studies where CC size was adjusted for brain weight (Holloway and de Lacoste, 1986; de Lacoste *et al.*, 1990; Holloway *et al.*, 1993). In contrast to these investigations, however, our data suggest a relatively homogenous increase of the CC ratio along the rostro-caudal

Table 5
Means and SDs for the corpus callosum (CC) ratios (i.e. CC area divided by forebrain volume) for consistent right-handers (CRH), mixed handers (MH), consistent left-handers (CLH), women and men (mm^2/l)

	CRH	MH	CLH	Total
Anterior third				
Women	274.2 ± 35.0	273.3 ± 28.9	256.2 ± 32.1	268.3 ± 32.2
Men	285.8 ± 33.6	244.7 ± 29.1	246.7 ± 30.7	249.7 ± 31.2
Total	264.9 ± 34.5	254.8 ± 31.9	251.8 ± 31.2	257.3 ± 32.8
Middle third				
Women	164.8 ± 22.9	155.0 ± 17.4	153.6 ± 24.8	157.6 ± 19.9
Men	151.9 ± 27.1	136.0 ± 17.1	135.8 ± 24.8	141.1 ± 23.1
Total	157.0 ± 26.0	142.7 ± 19.3	145.4 ± 23.3	147.9 ± 23.3
Isthmus				
Women	67.1 ± 13.5	63.3 ± 9.7	59.3 ± 9.6	63.3 ± 11.2
Men	62.2 ± 8.7	57.8 ± 10.7	58.3 ± 13.2	59.3 ± 10.6
Total	64.2 ± 11.0	59.8 ± 10.6	58.8 ± 11.2	60.9 ± 11.0
Splenium				
Women	189.0 ± 30.2	183.5 ± 16.8	187.1 ± 25.3	186.3 ± 23.8
Men	175.4 ± 34.4	157.5 ± 18.6	161.2 ± 31.8	164.0 ± 27.9
Total	180.8 ± 33.1	166.7 ± 21.8	175.1 ± 31.0	173.1 ± 28.4
Total CC				
Women	695.1 ± 83.0	675.1 ± 62.6	656.2 ± 75.7	675.5 ± 73.4
Men	648.3 ± 92.7	596.1 ± 56.5	601.9 ± 91.4	614.1 ± 79.1
Total	666.8 ± 90.8	623.9 ± 69.5	631.0 ± 86.3	639.1 ± 82.3

Table 6
Means and SDs for forebrain volume (FBV), total corpus callosum (CC) area measurement and the CC ratios (i.e. CC area/FBV) for women and men according to FBV quintile

	Women			Men		
	Mean	SD	*n*	Mean	SD	*n*
FBV quintile 1			17			7
FBV (l)	0.89	39.51		0.90	29.36	
Total CC area (mm^2)	617.01	69.32		633.89	95.30	
CC ratio for total CC (mm^2/l)	694.27	87.41		702.05	122.77	
FBV quintile 2			17			7
FBV (l)	0.97	15.40		0.97	17.46	
Total CC area (mm^2)	656.95	71.37		633.70	115.31	
CC ratio for total CC (mm^2/l)	674.10	73.60		653.03	119.60	
FBV quintile 3			5			19
FBV (l)	1.03	17.67		1.03	17.64	
Total CC area (mm^2)[a]	709.35	64.34		622.73	57.26	
CC ratio for total CC (mm^2/l)[a]	686.02	56.44		603.42	54.92	
FBV quintile 4			5			19
FBV (l)	1.09	25.90		1.10	34.18	
Total CC area (mm^2)	694.05	40.86		667.63	68.96	
CC ratio for total CC (mm^2/l)	637.56	34.44		603.67	63.77	
FBV quintile 5			5			19
FBV (l)	1.20	37.41		1.22	47.93	
Total CC area (mm^2)	718.47	72.85		772.92	69.07	
CC ratio for total CC (mm^2/l)	643.38	57.62		588.34	54.08	

[a]Significant gender difference according to *t*-test for independent samples, *P plain 0.01*.

axis, and no restriction of the apparent sexual dimorphism to posterior portions of the CC, such as isthmus or splenium (Fig. 3).

A More General Effect of Brain Size

Our most important finding is that CC size increases with FBV, but less than proportional to FBV. The allometric equations suggest that the relationship between CC ratio and FBV follows the geometrical rule that a cross-sectional area of a three-dimensional object is scaled non-isometrically to the volume of this object (Schmidt-Nielsen, 1984). The lack of a principle gender difference in this relation implies that small brains exhibit larger CC ratios, irrespective of gender (Tables 3 and 6, Fig. 3). Thus, FBV is the main factor explaining the gender difference suggested by Figure 4.

Based on present anatomical knowledge, a functional interpretation of this inverse relationship between forebrain size and relative callosal size must remain speculative. Let us assume that the packing densities and branching patterns of callosal neurons and axons do not depend on brain size, as suggested by the data of Aboitiz *et al.* (1992b). In this case our study would indicate that the degree of interhemispheric connectedness decreases with increasing human brain size. This would concur with theoretical predictions made by Ringo and co-workers (Ringo, 1991; Ringo *et al.*, 1994). They argued that as brain size is scaled up there must be a fall in interhemispheric connectivity, due to the increasing time constraints of transcallosal conduction delay. Consequently, functionally related neuronal elements would cluster in one hemisphere, so that increasing brain size would be the driving force in the phylogeny of hemispheric specialization. With regard to callosal connectivity, our morphometric data may provide first empirical support of this conjecture. It should be investigated whether findings of apparent gender differences in the asymmetry of higher-order cerebral functions may also be confounded by brain size.

Notes

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