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Sexual Dimorphism in the Corpus Callosum: Methodological Considerations in MRI Morphometry

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Studies of sexual dimorphism in the corpus callosum (CC) have employed a variety of methodologies for measurement and normalization but have yielded disparate results. The present work demonstrates how in some cases different manipulations of the same raw data, corresponding to different commonly used methodologies, produce discordant results. Midsagittal CC area was measured from magnetic resonance images (MRIs) of 137 young normal volunteers. Three strategies intended to normalize for average differences in brain size between the sexes, as well as five different normalization variables, were contrasted and evaluated. The stereotaxic method normalizes for intersubject differences in overall brain size by scaling MRIs into a standardized space. The ratio method uses one of five different indices of brain size and divides it into CC area. The covariate method uses one of the indices as a covariate in statistical analyses. Male subjects show significantly larger absolute total area, as well as anterior third and posterior midbody. However, in two of three normalization strategies, namely the stereotaxic and ratio methods, females show relatively larger total area, anterior midbody, and splenium. The covariate method did not show any significant differences at the 0.05 level. Results suggest that different approaches to normalization and analysis are not necessarily equivalent and interchangeable.

INTRODUCTION

The corpus callosum (CC) has been the focus of intense research and debate in the last decades, especially in what concerns putative relationships between its morphology and various aspects of cerebral function. Its functional importance as the main interhemispheric commissure of the human brain has been well established (Sperry, 1968) and continues to be elucidated (e.g., Clarke and Zaidel, 1994). More recently, though, began speculation that the CC might show global and local morphological trends in populations of interest, many of them showing particular pathology such as schizophrenia (Lewine *et al.*, 1991; DeLisi *et al.*, 1995; David, 1992), dyslexia (Filipek, 1995; Hynd *et al.*, 1995), and Alzheimer's disease (Hampel *et al.*, 1998). Some of the more contentious findings have been not in pathological populations, but rather concern claims for possible sexual dimorphism in the CC of the normal population as a cerebral correlate to sex-related differences in functional lateralization (DeLisi *et al.*, 1989; Peters, 1988; Kertesz *et al.*, 1987).

de Lacoste-Utamsing and Holloway (1982) were the first to present postmortem findings suggesting morphological sex differences in the human CC. They found splenial width and area to be absolutely larger in females and total area larger relative to brain size in females. Their pattern of results, however, especially in what concerns differences in absolute measures, have rarely been replicated (Holloway *et al.*, 1993; Holloway and de Lacoste, 1986; Clarke *et al.*, 1989; Allen *et al.*, 1991). Even the direction of non-significant absolute size differences in the CC is not consistent, with some studies showing larger measures in males (e.g., Witelson, 1985; Kertesz *et al.*, 1987; Demeter *et al.*, 1988), others in females (e.g., Holloway and deLacoste, 1986; Byne *et al.*, 1988; Deneberg *et al.*, 1991). What is more often found are relatively larger female callosal measures, that is, measures where an index of overall brain size has been used to normalize for an average sex difference in brain size. Despite this, reported differences are not consistent either in magnitude, statistical significance or location within the CC (Holloway *et al.*, 1993).

This particular area of investigation, it can safely be said, is still very contentious and confused, due in large part to inconsistent methodology (Holloway *et al.*, 1993; Allen *et al.*, 1991). As have done Constant and Ruther (1996), we can nonetheless glean a few broad categories of methodological difficulties. We shall discuss three: sampling, measurement and normalization.

Sampling

One important problem in corpus callosum work undertaken thus far, almost certainly contributing to the

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disparity of results, is the heterogeneity of most subject samples, be they from postmortem or *in vivo* studies. Subjects are often selected for little more than being free of neurological pathology, usually as would be macroscopically obvious from the visual inspection of a brain tissue specimen (e.g., Holloway and deLacoste, 1986) or magnetic resonance (MR) images (e.g., Allen *et al.*, 1991). In other words, they are often unselected for either age or handedness, both of which are suspected as possible confounds, the first because there are changes in brain size with age that are not especially well understood (including possible age by sex interactions; Witelson, 1989; Allen *et al.*, 1991), the second because of its suspected relationship to cerebral asymmetry and, in turn, CC size (Ratcliff *et al.*, 1980; Steinmetz *et al.*, 1991; Witelson, 1989).

This being the case, and given the natural anatomical variability that exists in the human brain (Collins and Evans, 1997), it is imperative that as homogenous a sample as possible be established for the examination of possible sexual dimorphism. Related to the above, of course, is sample size. From the studies conducted thus far, it would seem that the putative sex differences are not robust and that, even under the best of conditions, fairly large sample sizes are needed if any effect of sex is to be revealed. Most postmortem studies have dealt with very small samples (e.g., de Lacoste and Holloway, 1982, $n = 14$; Holloway and de Lacoste, 1986, $n = 16$) and even *in vivo* studies typically have relatively small numbers once subgroups of interest have been created from a larger total sample (Steinmetz *et al.*, 1995; Kertesz *et al.*, 1987).

Measurement

The methodology of typical postmortem CC studies has the advantages of being able to measure the structure directly, which allows for unambiguous delineation of the midsagittal CC area, and being able to take a brain weight or cranial capacity, often used for purposes of normalization for overall brain size differences. The disadvantages, though, are many. For example, upon death there is swelling in some areas due to the absorption of cerebral spinal fluid (Appel and Appel, 1942) and generalized shrinkage due to cell death (Rauch and Jinkins, 1994), each with its own course, and death to fixation times vary within and between studies. Also, there are difficulties associated with fixation agents. Formalin fixation is thought to cause brain tissue to fluctuate in weight by no more than 5% (Witelson and Goldsmith, 1991), but this effect varies with time and it is not known whether it is uniform between brains and throughout different tissues or different cytoarchitectonic areas of the same tissue (Constant and Ruther, 1996).

Studies making use of *in vivo* magnetic resonance imaging (MRI) have also been technically limited in a number of ways. Although they avoid many of the difficulties particular to postmortem studies, they must contend with others such as partial volume effects and limited image resolution which make difficult the unambiguous delineation of the CC (Clarke and Zaidel, 1994; Constant and Ruther, 1996). Peters *et al.* (2000) have shown how these difficulties can impact estimates of volume and area. Many have obtained measurements by digitizing images from MRI hard copies and then delineating the CC with the help of software or have photographed hard copies to slides for projection and tracing (e.g., Clarke and Zaidel, 1994; Kertesz *et al.*, 1987; Constant and Ruther, 1996; Moffat *et al.*, 1998). There are several points in these methods that are susceptible to error, all of which contribute to reducing signal strength. Other studies that have delineated the CC from the reconstructed MR image still in digital form have relied on very basic and limited software provided with the MR scanner (e.g., Rauch and Jinkins, 1994). None seem to have used real-time verification of contiguous sagittal slices to either side of midsagittal to disambiguate confusion due to noncallosal structures (such as that due to fornix and septum pellucidum visual encroachment onto the CC), image artifacts, and partial volume effects.

A problem experienced in both postmortem and *in vivo* studies is that of assuring consistent orientation of a specimen in the plane of measurement. In the vast majority of studies, any efforts applied to ensuring orthogonal alignment of structures of interest to the plane of measurement have been entirely manual and subjective. It has been shown by Rauch and Jinkins (1996) how this can indeed be of critical importance.

Normalization

One aspect of CC research that has surely been a source of conceptual confusion and contributed to inconsistent results and interpretation is that of normalization or standardization for overall brain size. Seeing as the focus on sexual dimorphism in the CC has been for the most part about the size of the structure rather than its shape, and because, on average, male brains are larger than female brains, brain size is the first and most evident confound one must deal with when examining sex differences. Yet it seems that most studies reporting on sexual dimorphism have not attempted to normalize for overall brain size, sometimes citing low correlations between the normalization variable (such as brain volume) and CC area (e.g., Deneberg *et al.*, 1991; Demeter *et al.*, 1988). When normalization has been applied, strategies have included using any of a number of different indices of brain size (such as brain weight, forebrain volume, cranial capacity, or cross-sectional cerebral area) in a couple of different ways, such as in the creation of simple ratios, where one of these is divided into CC area (e.g., Jäncke *et al.*, 1997),

or as covariates in covariate corrected statistics (e.g., Witelson, 1985). Apart from interpretive difficulties associated with ratios of various sorts, it is still in question whether or not any of these indices are reliably correlated to CC size, seeing as correlational results have varied from $r = 0.022$ (Kertesz *et al.*, 1987) to $r = 0.51$ (Witelson, 1985). If the relationship between CC size and overall brain size were unreliable then, indeed, there would be no need to normalize. Minimally, we would be introducing an unnecessary amount of noise into our measurements by using poorly correlated variables for the purposes of normalization.

Let us discuss one additional difficulty encountered when using a volume to normalize for brain size differences. It is a problem that has been recognized for some time now (Holloway and de Lacoste, 1986) and was alluded to in a more quantitative way by Jäncke *et al.* (1997). It centers on the nonisometric, geometric relationship between an area and a volume. Indices derived for purposes of CC size comparison between the sexes often divide CC area or subarea (2-dimensional) by a forebrain volume, brain weight, or cranial capacity (3-dimensional). Because of the incommensurate increase in the volume of an object over the cross-sectional area of that object, the value of this ratio is reduced disproportionately as head size increases, regardless of sex. Seeing as most larger headed individuals will be male, and assuming a reasonably good correlation between CC midsagittal area and brain size, there exists the risk of introducing a systematic bias in favour of the smaller headed females. In an attempt to correct for the area/volume relationship, some studies have raised the volume measurement to the power of 2/3 (e.g., Holloway and de Lacoste, 1986; Holloway *et al.*, 1993). Barring this correction, the only way to avoid this basic geometric problem when using a ratio strategy is to have the normalization variable also be 2-dimensional, in other words, an area (such as a sagittal cerebral area).

Inferences of possible functional significance for gross anatomical differences that may be observed in the CC must be predicated upon consistent and valid morphometry and analysis. That lack of true replication and convergence in the CC sexual dimorphism literature has made it difficult to ascertain progress toward this goal. This study had three primary objectives: the first was to present novel methods for the inspection of possible sexual dimorphism, methods that introduce less variability and are free of some important interpretive difficulties encountered with more common approaches. The second was to replicate some of the most popular approaches undertaken in the CC sexual dimorphism literature under rigorous and homogenous conditions so as to evaluate their validity and utility and perhaps provide perspective on existing findings. The third was to create a probabilistic map of the CC. Briefly, we obtained both native (absolute) and stereotaxic midsagittal areas for the corpus callosum as well as native forebrain volumes and areas in a large group of normal subjects. Measures from the native MRI space, which preserve absolute differences in size between subjects, allow us to compare to existing literature and assess some of its strengths and weaknesses. In contrast, stereotaxic measures, collected from MRIs registered into a standardized space, allow for direct comparison of CC areas between the sexes without the need for circuitous normalization strategies. Given the present, slight preponderance of evidence in the literature, it was expected that females would show a larger relative corpus callosum size, at least for the posterior fifth of the structure (splenium).

METHODS

Subjects

Subjects were 137 young, normal volunteers (78 male, 59 female, averaging 24.6 years of age $\pm$ 4.8 SD) whose MRIs were acquired as part of the International Consortium for Brain Mapping (ICBM) project. They were right-handed as determined by a handedness questionnaire.

Image Acquisition and Processing

The MRI data sets were comprised of one T1-weighted (TR = 18 ms, TE = 10 ms, $1 \times 1 \times 1$ mm voxels), two T2-weighted (TR = 3.3 s, TE = 120 ms, 1×1 mm in plane, 2-mm-thick slices, 1 mm offset), and two proton density images (TR = 3.3 s, TE = 34 ms, 1×1 mm in plane, 2-mm-thick, 1 mm offset). Images were registered to each other and to a Talairach-like stereotaxic space during an algorithmic, 9-parameter registration process (Collins *et al.*, 1994), resampled to $181 \times 217 \times 181$ slices at $1 \times 1 \times 1$ mm resolution and nonuniformity corrected (Sled *et al.*, 1998). Tissue classified images, where every voxel of a volume is classified into one of four categories (outside the head, cerebral spinal fluid, grey matter, white matter), were created by an artificial neural network classifier using the three scan types and a 170-point training set as input (50 points for each of CSF, grey matter and white matter, and 20 points for background; Zijdenbos *et al.*, 1996; Kollokian, 1996; Zijdenbos *et al.*, 1998).

Area and Volume Determinations

The CC was segmented manually from T1 images with the use of Display (MacDonald *et al.*, 1994), a 3-D interactive image viewing and segmentation application running on SGI workstations. Native space or absolute values were obtained by reversing the appropriate dimensions of scaling recovered during the ste-

reotaxic transformation. A MATLAB (Mathworks Inc., Sherborn, MA) algorithm was used to obtain CC subarea measurements according to Witelson-like criteria (Witelson, 1989; Fig. 3). Interrater reliability for CC labeling of 32 subjects was $r = 0.95$.

Native forebrain volume (FBV) was obtained from tissue classified volumes by counting grey and white matter 1 mm^3 voxels in the forebrain, forebrain being defined as all grey and white matter excluding the cerebellum and all structures below the thalamus. As in the case of native CC areas, native values were obtained by reversing the scaling applied during the stereotaxic transformation. Sagittal, coronal, and horizontal cerebral areas were obtained in a similar manner from single slices at stereotaxic Talairach coordinates (Talairach and Tournoux, 1988) $x = -10$ and $x = 10$ (left and right slices averaged), $y = -20$, and $z = 15$, respectively.

Analyses

We intended to contrast and evaluate three different normalization strategies, each purporting to remove the variance associated with gross intersubject differences in brain size. These will be referred to as the stereotaxic method, the ratio method, and the covariate method. The first involves applying three scaling factors, one for each spatial dimension, to each MRI volume during a linear, nine-parameter registration into a standardized stereotaxic space (Collins *et al.*, 1994). The stereotaxic CC areas collected in this method can simply be submitted to analysis of variance without further manipulation. The second method divides a normalization variable (an index of brain size, either native FBV, $FBV^{2/3}$, sagittal area, coronal area, or horizontal area) into native CC areas to create ratios intended to reflect the relative relationship of CC area to brain size. These ratios are then submitted to analysis of variance. The last method uses one of the aforementioned indices of overall brain size as a covariate in covariate statistical analyses of native CC areas. To summarize then, we are contrasting three different normalization methods and five different indices of brain size for use in the ratio and covariate methods.

Statistical Probability Anatomy Maps (SPAMs)

The stereotaxic CC label volumes were averaged so that for each *x, y, z* coordinate there exists a value ranging from 0 to 1 describing the probability of there being CC at that particular location for this collection of data sets (Penhune *et al.*, 1996). This sort of voxel-by-voxel average of the same structure label over a number of subjects has several uses, including offering a highly graphical, probabilistic description of shape, location, and size in a group of subjects.

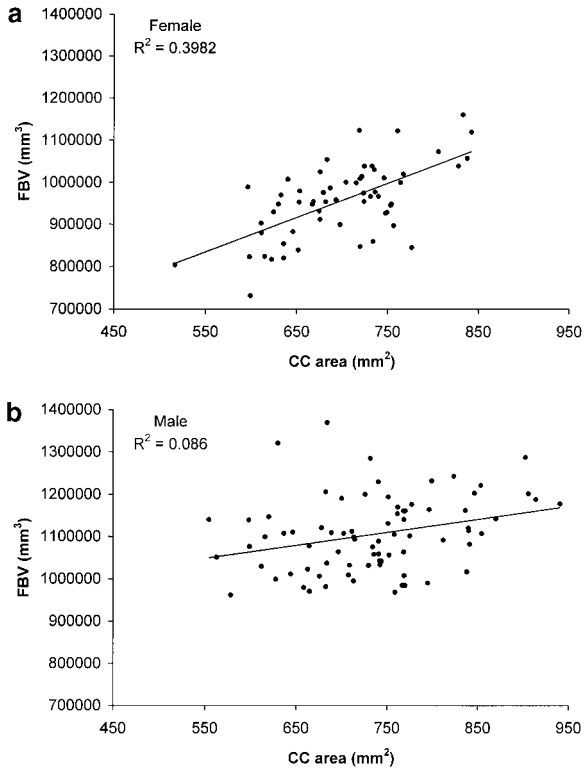

FIG. 1. Scattergrams showing the correlation between total CC area and FBV in (a) females and (b) males.

RESULTS

Descriptives

The average forebrain volume for females was 956980.6 mm^3 (87661.2 SD) and 1105347.5 mm^3 (88357.9 SD) for males ($F = 95.4$, $P < 0.001$). FBV was significantly correlated with total CC area for the group ($r = 0.457$, $P < 0.001$, $n = 137$). However, it would seem that FBV accounts for a significantly larger proportion of the variance in females ($n = 59$, $r^2 = 0.398$, $P < 0.001$; Fig. 1a) than it does in males ($n = 78$, $r^2 = 0.086$, $P = 0.009$; Fig. 1b), with a Fisher (Z) = 2.50 ($P < 0.01$). Correlations of total callosal area to FBV and other indices of brain size are presented in Table 1.

Native vs Stereotaxic Space

When native space (absolute) CC area is compared between the sexes, the total and all subareas are larger in males with the total ($F = 7.29$, $P = 0.008$), anterior third ($F = 11.36$, $P = 0.001$), and posterior midbody ($F = 4.95$, $P = .028$) reaching significance (Fig. 2a and Table 2). Absolute differences in the anterior midbody, isthmus, and splenium were all nonsignificant ($P > 0.1$). CC areas from the stereotaxic space show a complete reversal of trend with all areas being larger in

TABLE 1

Correlations of Total CC Area to Brain Size Indices

	FBV	$FBV^{2/3}$	Sagittal area	Coronal area	Horizontal area
All subjects (n = 137)	0.457**	0.460**	0.534**	0.297**	0.312**
Females (n = 59)	0.631**	0.630**	0.594**	0.514**	0.366**
Males (n = 78)	0.293**	0.296**	0.446**	0.025	0.145

* Significant at the 0.05 level.
** Significant at the 0.01 level.

females and the total (F = 6.16, P = 0.014), anterior midbody (F = 7.36, P = 0.008), and splenium (F = 9.89, P = 0.006) reaching significance (Fig. 2b and Table 2). Stereotaxic differences in the anterior third, posterior midbody, and isthmus were nonsignificant ($P > 0.1$).

Ratio Method

Ratios created by dividing the native CC area by $FBV^{2/3}$, sagittal area, coronal area, and horizontal area all yielded similar patterns of results to those of the stereotaxic space, with varying levels of significance

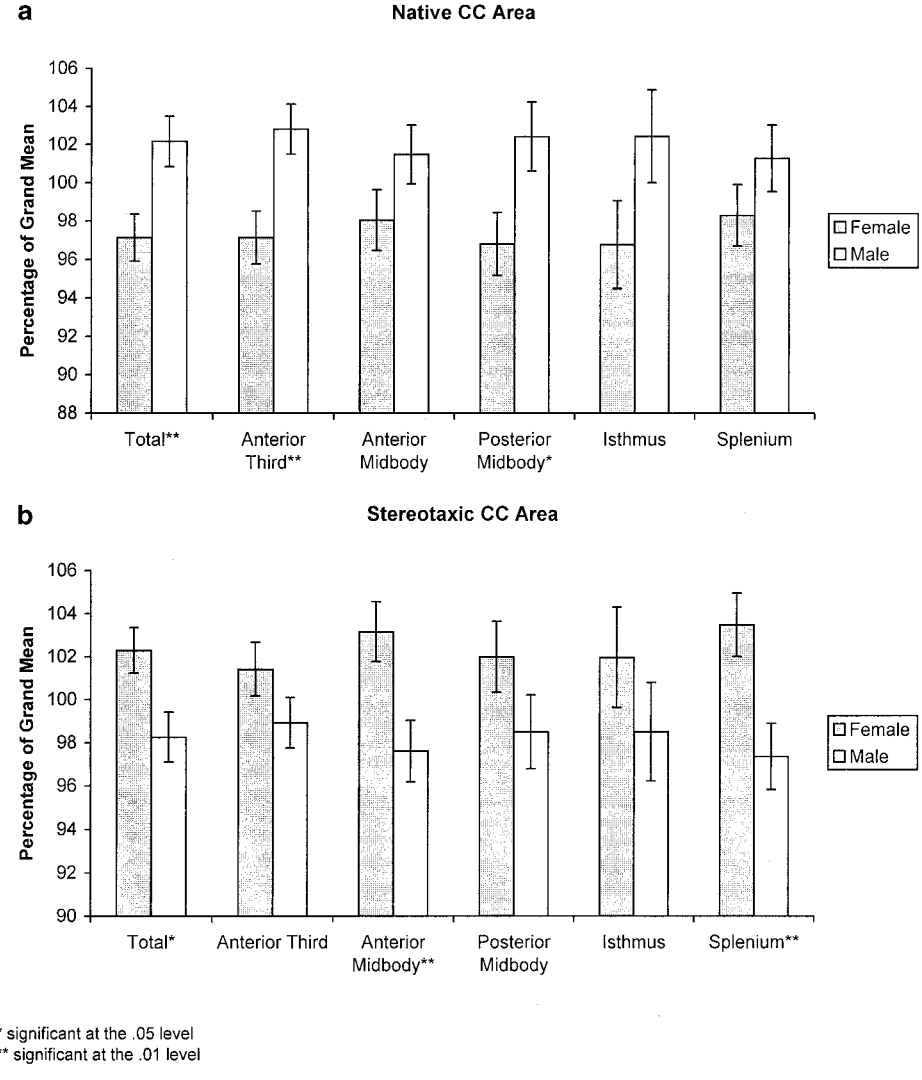

FIG. 2. Differences between the sexes in callosal area expressed as a percentage of the grand mean (± standard error) in (a) native space and (b) stereotaxic space.

TABLE 2

Native and Stereotaxic Space Areas in mm^2 (SD)

	Total	Anterior third	Anterior midbody	Posterior midbody	Isthmus	Splenium
Native measures						
Female (n = 59)	699.41 (±67.61)	283.19 (±31.13)	88.97 (±11.01)	75.72 (±9.82)	65.25 (±11.84)	186.28 (±23.35)
Male (n = 78)	735.55 (±84.33)	302.37 (±34.29)	92.07 (±12.33)	80.11 (±12.51)	69.07 (±14.48)	191.94 (±29.03)
Total (n = 137)	719.99 (±79.36)	294.11 (±34.20)	90.74 (±11.84)	78.22 (±11.59)	67.42 (±13.49)	189.50 (±26.78)
Stereotaxic measures						
Female (n = 59)	886.98 (±70.19)	359.11 (±34.29)	112.77 (±11.72)	96.12 (±11.95)	82.81 (±14.60)	236.18 (±25.81)
Male (n = 78)	851.97 (±89.45)	350.25 (±36.76)	106.69 (±13.85)	92.83 (±14.25)	80.00 (±16.41)	222.20 (±30.90)
Total (n = 137)	867.05 (±83.27)	354.07 (±35.86)	109.31 (±13.28)	94.25 (±13.36)	81.21 (±15.66)	228.22 (±29.55)

(see Table 3). The ratio created by dividing CC area by FBV, on the other hand, showed significantly larger values in the females for total and all subareas (Table 3). Correlations of the various CC ratios to their denominators are all negative and significant for the group at $P < 0.01$ (Table 4).

Covariate Method

For none of the indices of brain size submitted as covariates (either FBV, $FBV^{2/3}$, sagittal area, coronal area, or horizontal area) were any of the CC areas significantly different between the sexes. Callosal subsections show a pattern similar to the other methods when either FBV or $FBV^{2/3}$ is used as a covariate in that both the anterior midbody and splenium approach significance (none better than $P = 0.06$). For the other indices of brain size, the pattern of results evident in the other methods breaks down completely. The assumption of homogeneity of regression slopes between the sexes necessary for covariate analysis is violated in the case of the regression of coronal area onto total CC area. Therefore, strictly speaking, we could not proceed with this type of analysis for this variable.

Probability Maps

The map for the group as a whole shows that the location, size and shape of the CC is highly variable, although less so than cortical structures such as Heschl's gyrus and the Planum Temporale (Penhune *et al.*, 1996; Westbury *et al.*, 1999). Visual inspection of Fig. 3 reveals that few areas reach $P = 1$. For instance, approximately 17% of voxels in the map lie between 90 and 100% probability. Differences between the sexes are evident from the subtractions of the male SPAM from the female SPAM and vice-versa, where each x, y, z coordinate in one map is subtracted from the same coordinate in the other. In particular, there is an apparent shift downwards in position of the splenium in males as compared to females.

DISCUSSION

All three normalization methods contrasted in this study are purported to be conceptually equivalent in that they are intended to remove the variance in CC area measurements associated with global brain size which, given our significant correlations between FBV and CC area, we consider to be a real confound. Despite this, only 2 of 3 methods show concordant results. The stereotaxic and ratio methods yielded a similar pattern of results (with the exception of ratios created with FBV, most likely because of the geometric problem discussed in the introduction), but this pattern was not evident in the covariate method. Furthermore, we verified that in the case of coronal area used as a covariate it was inappropriate to proceed with such an analysis seeing as the assumption of homogeneity of slopes had been violated. The principal results from the two methods that do concur are those of a larger anterior midbody and splenium, as well as total area, in females relative to brain size.

TABLE 3

Significance Levels of Sex Differences for the Different Indices of Brain Size Used in the Ratio Method

	P values					
	Total	Anterior third	Anterior midbody	Posterior midbody	Isthmus	Splenium
FBV	<0.001	<0.001	<0.001	0.001	0.011	<0.001
$FBV^{2/3}$	0.007	0.091	0.004	0.111	0.251	0.003
Sagittal area	0.043	0.331	0.013	0.238	0.403	0.016
Coronal area	0.054	0.268	0.024	0.244	0.385	0.018
Horizontal area	0.017	0.128	0.006	0.134	0.264	0.007

TABLE 4

Correlation of CC Ratios to Their Denominators

	FBV	$FBV^{2/3}$	Sagittal area	Coronal area	Horizontal area
CC/FBV	−0.532**	—	—	—	—
$CC/FBV^{2/3}$		−0.226**	—	—	—
CC/Sagittal area			−0.263**	—	—
CC/Coronal area				−0.432**	—
CC/Horizontal area					−0.391**

* Significant at the 0.05 level.
** Significant at the 0.01 level.

Even though FBV is highly significantly larger in males than in females, in addition to total CC area only two subsections of the CC are significantly resolved as absolutely larger in males in the native space. Therefore, as we might expect, once a normalization for overall brain size is applied, those subsections which are not significantly different in native space become significantly larger in females relative to brain size (with the exception of the isthmus, which is not different between the sexes either before or after normalization; Fig. 2). We see this in both the stereotaxic and ratio methods.

Two admonitions stem from our work: first, in our data, using an uncorrected brain volume (FBV) to normalize for overall brain size in the ratio method clearly biased results, exaggerating the female advantage in relative CC size and suggesting that this approach may be misleading. Second, it may be that the relationship between CC area and brain size is somewhat divergent between the sexes, in which case one must take care to verify the homogeneity of slopes when intending to use an index of brain size as a normalization variable in an analysis of covariance. Most studies that have used the covariate method for removing variance associated with brain size have not reported separate trends for males and females. To our knowledge, only Jäncke *et al.* (1997) report a verification of this basic ANCOVA assumption and, as in the case of coronal area in this work, find that the linear trends are different between the sexes, thereby precluding traditional covariate analyses. Also, as brain size seems to be less relevant to CC size in males, we should consider that for any transformation applied to CC area values for the purposes of normalization, be it the application of scaling factors as in the stereotaxic method or the creation of a ratio with an index of brain size (e.g., $FBV^{2/3}$) as in the ratio method, more irrelevant information is being introduced into the male sample than the female sample. In the case of covariate analysis, an average, compromise regression slope is being used to represent both sexes.

Despite the fact that the stereotaxic and ratio methods show essentially the same pattern of results, we believe the stereotaxic method to be superior for a number of reasons, among them: (1) the automated registration into stereotaxic space used here (Collins *et al.*, 1994), with its linear rescaling of volumes, is a more direct way of dealing with gross brain size differences; (2) the translation and rotation parameters of the registration ensure consistent orthogonal orientation of the specimen in the plane of measurement and specification of the midsagittal position ($x = 0$); (3) the error-prone and labor-intensive process of collecting an index of brain size is completely circumvented.

Differences between probability maps of the structure seem to indicate not only a larger female splenium, as reflected in the quantitative measurements, but also a difference in position between the sexes, where the male splenium seems to be inferiorly positioned as compared to the females. These findings agree with those of Oka *et al.* (1999). There is also evidence for the oft encountered description of greater 'bulbosity' in the female CC (e.g., Allen *et al.*, 1991). Overall variability in shape, size, and position of the CC is also evident in that high probability areas make up a relatively small area of the map, highlighting some limitations in the traditional quantification of this structure (Davatzikos *et al.*, 1996). Some studies have labored to overcome these limitations by the use of deformation based methods which describe and account for local variations in shape (e.g., Davatzikos *et al.*, 1998; Thompson *et al.*, 1998). Methods are currently being developed in our lab to make use of the normative information provided by the statistical probability anatomy maps. They include overlaying the probability map on the stereotaxically registered MRI volumes of patients with incomplete callosotomies to estimate the location and quantity of remaining matter and the assessment of possible atrophy in the CC, symptomatic of certain disease states such as Alzheimer's disease (Hampel *et al.*, 1998).

It is clear that our work in no way speaks to function directly and we have sought only to make statements about structure. Let us nonetheless revisit the reasoning underlying the interest in macroscopic morphometry of the CC, for contextual and speculative purposes. Larger callosal areas are typically conjectured to be macroscopic morphological correlates to a lesser degree

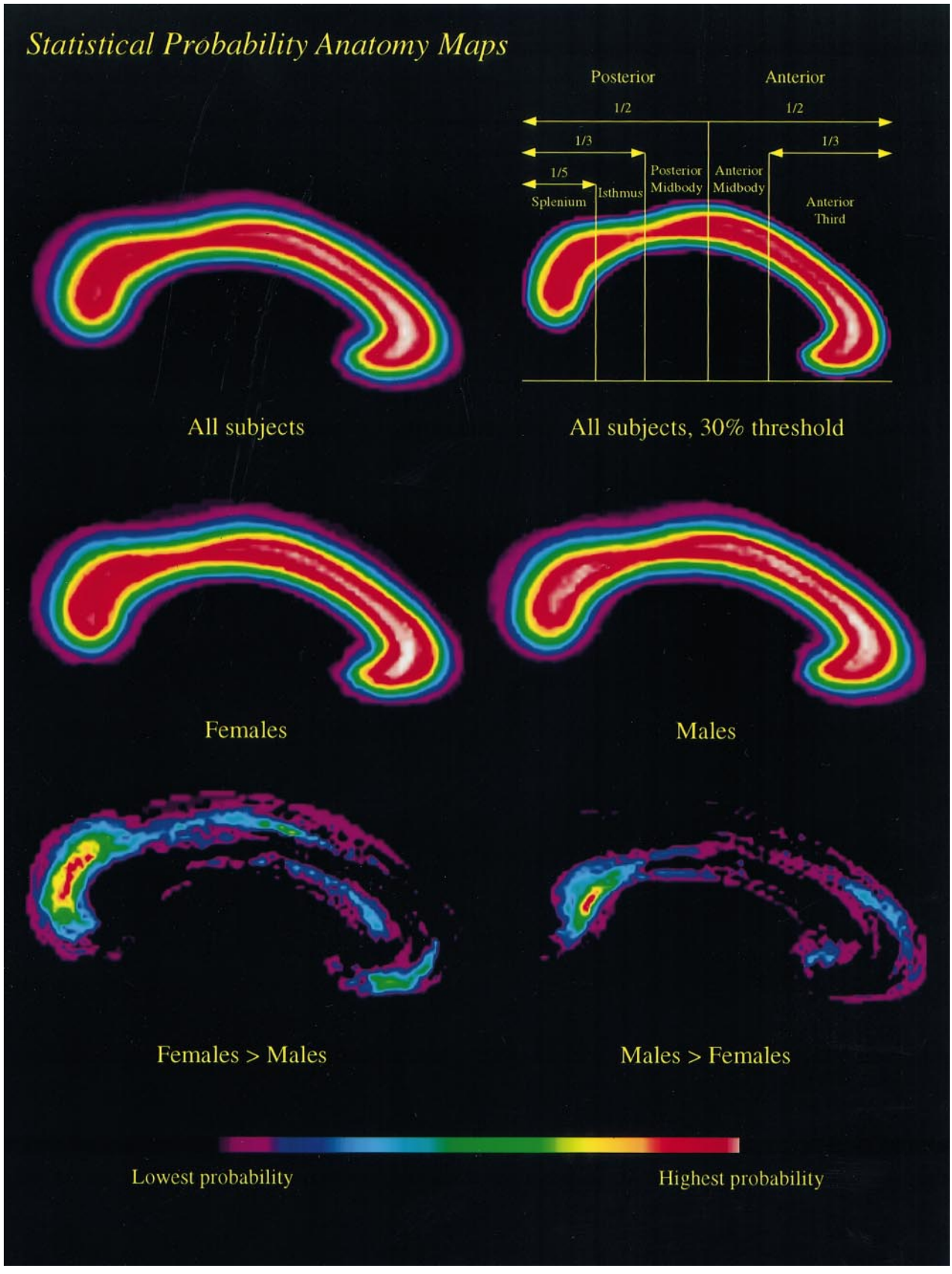

FIG. 3. Statistical anatomical probability maps (SPAMs) of the corpus callosum. Probability values in the SPAMs for All Subjects, Females and Males range from 0 to 1. Values for the Female > Male and Male > Female SPAMs range from 0 to 0.278 and 0 to 0.298, respectively. A 30% thresholded group SPAM is used to illustrate the Witelson-like subdivision criteria (Witelson, 1989).

of lateralization for certain cognitive abilities in females as compared to males. This relative symmetry might then require greater interhemispheric transfer which would, in turn, evince itself as a larger number of callosal fibers or greater myelination of those fibers. The midsagittal CC area is taken as a convenient index of the number of fibers and/or thickness of myelination of these fibers and Aboitiz *et al.* (1992) have shown that midsagittal CC area is correlated to the number of small diameter fibers (3 μm or less, thought to interconnect mainly higher-order cortices). Now, it may be that increasing the number of fibers coursing through the CC of more functionally symmetric brains is the most efficient way of dealing with demands for greater interconnectivity of the hemispheres and that this is what we are in effect observing. On the other hand, as suggested by Ringo *et al.* (1994), it may also be that beyond a certain point this "strategy" becomes unfeasibly taxing, anatomically speaking, and that lateralization of function becomes a more viable solution for decreasing transmission times during time-critical neuronal computations. Our data show that all indices of brain size increase disproportionately to CC area (Table 4), including $FBV^{2/3}$ which has been corrected for the geometric problem discussed in the introduction. Therefore, we agree with Jäncke *et al.* (1997) that there may be a simple head size confound in the CC sexual dimorphism issue, at least in our conceptualization of it, and this is in keeping with the hypothesis of Ringo *et al.* (1994) concerning the evolutionary pressures that might have driven lateralization of function in the brain. It posits that increasing transmission delay between the hemispheres as brain size increases, given a fixed conduction speed, may be the principal factor in the origin of hemispheric specialization.

In our sample, the 30 largest female brain volumes, with an average of 1021066.2 mm^3, correlated to total CC area at $r = 0.61$, whereas the smallest 30 male brain volumes, with a nearly identical average of 1020545.0 mm^3, only correlated with an $r = 0.04$. It does seem as though there is a sex difference in the relative importance of brain size to CC size that is separate from the influence of eventual adult brain size. This is in favor of the position of Ringo and colleagues (1994) for a more abstracted, evolutionary level role for brain size. Brain size may become less relevant to CC size once a more significant degree of functional lateralization is in place, as it is said to be the case for males compared to females. A real sex difference in hemispheric specialization and relative callosal size in a sample like ours today would have had its origin as an evolutionary engineering problem related to overall brain size.

By virtue of the corpus callosum's role in the interhemispheric connection of cortical areas, morphological deviations from normal appear to serve as an index for the presence and progress of numerous neuropathological conditions. Developing a strong method for the quantitative and qualitative description of the CC, therefore, has wide ranging implications, not only in the greater endeavour of anatomical and functional characterization of the brain, but also in immediate applications of clinical relevance. As for the specific issue of sex differences, we believe that the present work provides strong evidence for localized differences in relative size and possibly shape and position of the CC between the sexes.

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Supplementary data and images are available through our World Wide Web site at www.zlab.mcgill.ca.

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