Gender effects on callosal thickness in scaled and unscaled space

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Some empirical data suggest that sexual dimorphisms in callosal morphology exist, but findings are not consistently replicated across laboratories. We applied novel computational surface-based methods to encode callosal thickness at high spatial resolution. We further examined whether callosal thickness and related gender effects are influenced by brain size adjustments achieved through data scaling. Significant gender differences were absent in scaled data, and women showed no regional thickness increases compared with men (in either scaled or unscaled data). In unscaled data, men exhibited significantly greater callosal thickness in a number of regions that may be attributable to larger brain dimensions in men. Alternatively, given their regional specificity, the observed differences in unscaled callosal thickness may contribute to gender-specific cognition and behavior. *NeuroReport* 17:1103–1106 © 2006 Lippincott Williams & Wilkins.

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Introduction
Unprecedented findings in the early 1980s indicating a larger and more bulbous callosal splenium in women [1] were followed by a considerable number of morphometric examinations of gender effects on the size and shape of the corpus callosum (CC). Although various observations suggest that sexual dimorphisms in callosal morphology exist, the findings have not been consistently replicated across laboratories [2]. For example, discrepancies exist concerning the affected callosal region (e.g. splenium [1,3–5], isthmus [6–8], genu [9,10], total CC [3,4,11,12]), and with respect to the direction of the gender effect. Moreover, a number of studies failed to detect any significant differences in callosal morphology between men and women. Study-specific criteria for subject selection as well as for callosal measurements (e.g. definitions of subdivisions or adjustments for individual brain volumes) may account for some discrepancies in results.

The present study was designed to shed further light on the presence and direction of gender effects on callosal morphology where analyses were not dependent on previously employed parcellation schemes [6,7,13] or cluster generation methods [14,15] that generate predefined callosal regions. For this purpose, we applied a recently published anatomical surface modeling approach that allows the mapping of callosal thickness across the whole callosal surface at high spatial resolution [16]. In order to clarify whether callosal measurements and related gender effects are affected by brain size adjustments, we conducted callosal thickness analyses using (a) imaging data that were corrected for differences in head alignment only and (b) imaging data that were also corrected for differences in brain size.

Materials and methods
Subjects
We analyzed a well-matched sample of 60 right-handed healthy subjects (30 women, 30 men), selected from a database of high-resolution anatomical magnetic resonance images acquired at the Center for Neuroscientific Innovation and Technology (ZENIT), Magdeburg (Germany). Young adults with a relatively narrow age range (women: 24.32 ± 4.35 years; men: 25.45 ± 4.72 years) were included so as to minimize the influences of age and possible interactions of age with gender on callosal fiber presence and possibly callosal morphology [17]. Participants were volunteers and included university students from different fields, with handedness determined by self-reports of the hand preference. All participants gave informed consent according to institutional guidelines (Ethics Committee of the University of Magdeburg).

Magnetic resonance imaging acquisition
Images were obtained on a 1.5-T magnetic resonance imaging system (General Electric, Waukesha, Wisconsin, USA) using a T1-weighted spoiled gradient echo pulse sequence with the following parameters: TR=24 ms,

TE=8 ms, 30° flip angle, FOV=250×250 mm^2, matrix size=$256 \times 256 \times 124$, voxel size=$0.98 \times 0.98 \times 1.5$ mm^3.

Image preprocessing

Image preprocessing and callosal thickness measurements were performed as described previously [16]. Briefly, image volumes were corrected for differences in head alignment by placing them into the standard coordinate system of the ICBM-305 average brain [18] using six-parameter rigid-body transformations [19], hereafter referred to as unscaled data. In a parallel analysis of the same data set, we corrected image volumes for differences in brain size by converting them into the dimensions of the ICBM-305 average brain using 12-parameter transformations, hereafter referred to as scaled data. In both unscaled and scaled data, one rater who was blind to gender delineated the CC in midsagittal sections, which were defined by identifying the interhemispheric fissure. For inter-rater reliability, two independent investigators (E.L. and K.N.) contoured the CC in six different randomly selected brains achieving intraclass correlation coefficients of $r=0.99$.

Callosal thickness measurement

As illustrated previously [16], scaled and unscaled callosal outlines were automatically divided into upper and lower callosal sections (the points of separation are visible in Fig. 1). Both upper and lower sections were redigitized, resulting in 100 equidistant surface points per section. By calculating the spatial mean from homologous surface points representing the upper and lower callosal sections, we created a new callosal section (medial line). Finally, we calculated and averaged the distances from each of the 100 upper and lower surface points to the 100 surface points representing the medial line. These averaged distances are projected as color-coded values onto the callosal surface model and represent half the actual callosal thickness at a given spatial location (Fig. 1, top panel). A systematic overview of the basic steps in the measurement of callosal thickness is provided elsewhere [16].

Statistical mapping and permutation testing

Gender differences in callosal morphology were assessed by applying independent sample Student's *t*-tests at each distance value. Regions exhibiting significant differences were coded in color and mapped onto the callosal surface model (Fig. 1, bottom panel). Given that statistical tests were made at hundreds of callosal surface points and adjacent data points are highly correlated, permutation testing was employed, to control for multiple comparisons, using a threshold of $P=0.05$. For this purpose, callosal sections were randomly assigned to either male or female groups 100 000 times, while keeping the number of participants in each group the same (i.e. 30 in each) and a new statistical test was performed at each callosal surface point for each random assignment. The number of significant results from these randomizations was then compared with the number of significant results in the true assignment to produce a corrected overall significance value for the uncorrected statistical maps, so that the statistical validity of any detected gender differences was verified even in the presence of correlations in the data.
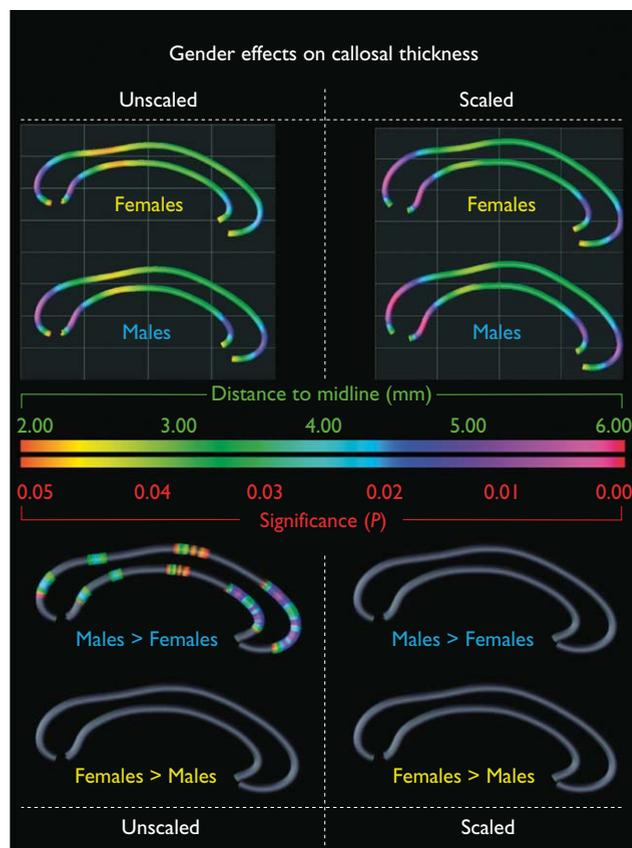

Fig. 1 Gender effects on callosal thickness. The top panel illustrates the averaged distances (mm) from the upper and lower callosal boundaries to the medial line, in men and women. The bottom panel illustrates regions of significant gender differences, with gray indicating no significance. Results shown on the left are mapped in unscaled data (after using six-parameter transformations to correct for head alignment only); findings on the right are mapped in scaled data (after using 12-parameter transformations to correct for individual brain sizes).

Results

Mean callosal thickness

Callosal thicknesses in men and women, both uncorrected (unscaled) and corrected (scaled) for individual brain volumes, are illustrated as color-coded distance maps in the top panel of Fig. 1, where posterior (caudal) callosal regions point to the left and anterior (rostral) regions to the right of the figure. The color bar encodes the averaged distance value (mm) from the upper and lower callosal boundaries to the medial line, with smaller distances (illustrated in orange and yellow) corresponding to a decreased callosal thickness, and larger distance values (depicted in purple and pink) corresponding to an increased callosal thickness.

Although in scaled data CC dimensions were larger overall as a result of registering each image volume to the ICBM-305 template (which is larger than any individual brain volume), the regional patterns of callosal thickness are similar in both scaled and unscaled data, and in men and women. Callosal thickness appears to be largest in posterior callosal regions corresponding to the splenial segment. [Although our analyses were not based on traditional parcellation schemes (e.g. arbitrarily dividing the CC into

segments), for the sake of clarity, we will describe the findings of the present study with respect to CC anatomy by referring to vertical callosal segments used in the modified Witelson scheme [6,7,13]. Here the 'splenium' represents the posterior fifth, the 'isthmus' two-fifteenths, the 'posterior midbody' and 'anterior midbody' each one-sixth, and the 'anterior third' one-third of the callosal area, as described elsewhere [20].] As shown, the averaged distances from the upper and lower callosal boundaries to midline reach up to 6 mm (illustrated in pink, in the scaled data), indicating a maximum callosal thickness of 12 mm. Another relatively thick callosal region can be seen towards the anterior of the CC (illustrated in blue in unscaled data/purple in scaled data) that constitutes a part of the callosal anterior third and includes the callosal rostrum and genu. The CC was thinnest (4 mm) in the vicinity of the posterior midbody and isthmus (illustrated in orange in unscaled data).

Gender differences in callosal thickness

Gender differences in callosal thickness measurements are illustrated as color-coded significance maps in the bottom panel of Fig. 1. The color encodes the P-value, with gray color indicating regions that do not show significant differences between men and women. There was no significant gender effect in scaled data, and no regions (scaled or unscaled) where callosal thickness was larger in women than in men. Men, on the other hand, exhibited significant increases in CC thickness in several CC regions in unscaled data. The most pronounced gender differences (illustrated in pink, purple, and blue shades and mainly depicting significance values between $P<0.01$ and $P<0.03$) were detected in anterior regions that correspond to a large portion of the callosal anterior third. We revealed further regions of increased callosal thickness in men in posterior segments, located in the splenium and isthmus (illustrated mainly in light blue and green with P-values ranging between $P<0.02$ and $P<0.04$). Finally, a callosal mid-region, spanning the border between the anterior and posterior midbody, was significantly thicker in men than in women (illustrated in green, yellow, and orange and corresponding to P-values between 0.03 and 0.05). Results remained significant after employing permutation tests for gender comparisons in unscaled data (callosal top segment: $P \leq 0.0123$, corrected; callosal bottom segment: $P \leq 0.0116$, corrected) indicating that the observed gender differences do not occur by chance.

Discussion

When callosal thickness data were compared in unscaled space (achieved through correcting images for head alignment only, thus without employing brain size corrections), we observed pronounced differences between men and women, with men having significantly thicker callosal areas in a number of regions. The present findings in unscaled space corroborate earlier reports regarding the direction of the gender effect (men > women), and to a certain extent with respect to the regional correspondence [3,9,11,14,21].

When callosal thickness data were compared in scaled space (achieved through correcting images for brain dimensions), we did not observe any significant differences between men and women. Thus, it appears that individual differences in callosal size are largely accounted for by individual brain sizes. Similarly, previous findings indicated

that there were no gender effects after adjustment for total cerebral volume [9,20,22,23].

In general, our findings confirm previous reports based on a meta-analysis of 49 studies published between 1980 and 1992 [2]; the authors conclude that unadjusted CC size is slightly larger in men, with gender effects disappearing when statistically correcting for brain size. Hence, the present analysis also did not reveal any evidence for a larger or more bulbous splenium in female brains, either in scaled or in unscaled data.

Regional specificity of gender effects in unscaled space

Significant gender effects were observed only in portions of the callosal subdivisions previously used as a standard for dividing the CC into compartments roughly on the basis of fiber characteristics (splenium, isthmus, posterior/anterior midbody, and anterior third). For example, gender effects were confined to regions constituting the border between posterior midbody and anterior midbody, but are not found in middle or rear regions of the posterior body, nor in middle or frontal regions of the anterior midbody. Moreover, a large area of the anterior third appears to be thicker in men, albeit that gender effects were absent in the most anterior tip of the rostrum. Similar spatial restrictions apply to findings in the splenium and isthmus. Insufficient statistical power might account for the lack of spatial correspondence between our findings and those using traditional boundaries to segment the CC (e.g. subdivisions defined according to callosal length). That is, even though only some portions showed statistical significance, the male CC might actually be thicker everywhere, which may have been detected if we had analyzed a larger sample of participants. Alternatively, the lack of spatial correspondence with respect to traditional parcellation boundaries might help explain discrepancies in previous findings. If men differ in callosal morphology only for a small portion of a segment, the observation of the gender effect for the whole segment becomes clearly susceptible to study-specific and gender-specific variations in callosal shape and length (which determine the outcomes of the parcellation procedure). In addition, the method used to delineate subdivisions in traditional studies (e.g. straight-line vs. curved-line vs. bent-line vs. radial gravity method etc.) might have further enhanced discrepancies in outcomes across laboratories. The current approach, which captures extremely local characteristics by computing a point-wise indicator for callosal thickness, provides a statistically powerful alternative for analyzing callosal morphology without relying on callosal parcellation. It also provides a multiple comparisons correction approach that safeguards against Type I error while inherently accounting for correlations in morphology between neighboring callosal regions.

Functional significance of gender effects in unscaled space

Given that men, on average, have larger brains than women (a significant effect that was also established for the current sample [24]), it could be argued that the increased unscaled callosal thickness in men has no functional relevance, but is rather a morphological byproduct related to body scale or weight. On the other hand, it appears intriguing that gender differences were not observed as evenly distributed involving the whole surface of the CC to the same degree, but show a regional specificity. Callosal fibers are organized

topographically (e.g. fibers connecting posterior regions travel through the caudal CC), where callosal subdivisions also correspond to functional boundaries. More specifically, the anterior third connects the prefrontal cortices, the anterior and posterior bodies contain projections from motor, somato-sensory, and auditory cortices, while the isthmus and splenium are involved in the transfer of auditory, language, and visual information [25]. If callosal thickness was an indicator of the degree of myelination and/or the number of axons (previous studies revealed a positive correlation between callosal area and small diameter fibers number [13]), cognitive and behavioral consequences owing to alterations in interhemispheric connectivity and information transfer are possible. At this point, however, it remains speculative whether the observed differences in regional callosal thickness contribute to gender-specific cognition and behavior. Future analyses relating individual behavioral measurements to callosal thickness data might help elucidate this issue.

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