Sex Differences in Anatomic Measures of Interhemispheric Connectivity: Correlations with Cognition in Women but not Men

A robust sex difference in the splenium of the corpus callosum, reflecting greater interhemispheric connectivity in women, was observed on magnetic resonance images from 114 individuals. In addition, bulbosity of the corpus callosum correlated with better cognitive performance in women but not in men, indicating that the degree of interhemispheric connectivity has different implications for men and women. These findings were based on a new image analysis technique which allows investigation of local variability in brain morphology.

Introduction
Evidence from several sources suggests that men have greater hemispheric specialization for both verbal and nonverbal abilities and that women are more likely to have bilateral representation of function (Harshman and Remington, 1976; McGlone, 1980; Kimura and Harshman, 1984). Supporting evidence comes from the severity and nature of cognitive deficits following stroke or other brain lesions (Lansdell, 1962; McGlone, 1980) and some experimental studies using dichotic listening techniques to selectively present material to a single hemisphere (Harshman and Remington, 1976; Bryden, 1979; Kimura and Harshman, 1984). Hypothesized sex differences in hemispheric specialization have also been supported using functional magnetic resonance (MR) imaging. While women showed bilateral inferior frontal activation during the performance of a language task involving phonological processing, men had unilateral left hemispheric activation (Shaywitz et al., 1995).

Findings of sex differences in hemispheric specialization have motivated numerous investigations of the morphology of the corpus callosum, the major white matter tract connecting the two hemispheres. Larger callosal size has been associated with greater interhemispheric connectivity (Abotiz et al., 1992). Moreover, it has been hypothesized that morphological sex differences in callosal size might facilitate greater interhemispheric communication in women, which may be important in achieving higher performance when there is bilateral representation of cognitive functioning. Investigations of sex differences in corpus callosum size have yielded mixed results (Bishop and Wahlsten, 1997). DeLacoste-Utamsing and Holloway (1982) reported from autopsy data that the splenium, the posterior section of the corpus callosum, is more bulbous in women than men. This finding has been replicated in some results (Bishop and Wahlsten, 1997). DeLacoste-Utamsing and Holloway’s autopsy data (DeLacoste-Utamsing and Holloway, 1982); (ii) age is negatively correlated with corpus callosum size in both men and women; (iii) corpus callosum size is associated with better performance on cognitive tests for women but not men. As it has been argued that women have greater bilateral representation of function, larger callosal size would facilitate interhemispheric transfer and cognitive performance in women but would not influence performance in men who demonstrate greater hemispheric specialization for a variety of functions. Our results strongly support the hypotheses that there are sex and age differences in corpus callosum morphology and that callosal size is associated with better cognitive performance in women but not men.

Materials and Methods

Subjects
Subjects are non-demented participants in the Baltimore Longitudinal Study of Aging (BLSA) (Shack et al., 1984), who are between 56 and 85 years of age and have enrolled in the longitudinal neuroimaging component of the study. Means ages (±SD) for men and women were 70.9 ± 7.6 and 69.4 ± 8.0 respectively. As is typical of BLSA participants, both men and women are highly educated with 16.6 ± 2.8 years and 15.8 ± 2.3 years of education respectively. Only right-handed participants were included in these analyses. Sixteen of the 114 subjects were included in a report describing the development of our image analysis method (Davatzikos et al., 1996). The data reported are those collected during the first imaging evaluation. At initial assessment, the neuroimaging participants were free of diagnosed central nervous system and severe cardiac disease. Informed consent was obtained from all participants.

MR Image Acquisition
MR images were acquired on a GE Signa 1.5 T scanner using a three-dimensional (3D) volumetric spoiled grass (SPGR) acquisition in the axial plane. Imaging parameters were: T₁ = 35, T₂ = 5, flip angle = 45°, matrix = 256 × 256, field of view = 24 cm, 1 excitation. This protocol yields 124 contiguous sections of 0.94 × 0.94 mm resolution in plane and slice thickness of 1.5 mm.
Image Processing and Analysis

Images were reconstructed as 3D volumes, and midsagittal sections of voxel dimensions 0.94 × 0.94 × 1.5 mm were used for the analysis. To extract the midsagittal section, images were first adjusted interactively for head tilt and rotation, using the coronal and axial views respectively. Images were then reoriented so that the horizontal plane was parallel to the line passing through the anterior and posterior commissures. The slice containing the anterior and posterior commissures was identified by an experienced technologist, who interactively changed the angle (pitch) until both commissures were visible on a reoriented horizontal section. After reorienting the images, a 1.5 mm section centered about the sagittal midline was extracted for quantification.

Quantification of regional callosal morphology utilizes a reference template, which is used as a unit, to measure how much each individual’s callosum locally differs in size from the template. This operation is accomplished by elastically adapting the template to the shape of each individual’s corpus callosum (Gee et al., 1993; Miller et al., 1993; Davatzikos et al., 1996). The resulting shrinkage or expansion of the template is quantified at each point by a deformation function, a collection of coefficients measuring how much the template has to be deformed around each point to move into spatial registration with each individual’s callosum. As shown in Figure 1, higher values of the deformation function reflect a higher degree of local stretching of the template and, therefore, a locally more bulbous callosal shape. For these analyses, the template employed was based on the average of eight subjects of a pilot study (Davatzikos et al., 1996) and included 3133 points. The approach to the analysis of the deformation functions is detailed in (Davatzikos et al., 1996); in that paper, we also showed that analysis using the deformation functions is robust to the particular template employed.

To determine the elastic deformation of the template with reference to each individual’s callosum, it is necessary to first define the callosum outline for each individual. The outlines are generated using a semi-automated algorithm for boundary detection (Davatzikos et al., 1996). In this procedure the callosal outlines are first defined interactively by manual tracing and subsequently refined by an active contour algorithm. This algorithm allows the initial outlines to elastically adapt to the callosal edge as defined by the image gradient.

As the deformation functions provide local size estimates with respect to the standard template, size differences between groups are examined by statistical analysis of the deformation functions for each pixel. Area measurements of arbitrary callosal subregions are calculated by integrating the deformation functions across all points within a specified subregion. Regions of interest (ROIs) can be defined either a priori or a posteriori based on the results of the pixel-based comparison. Similarly, associations between cognitive performance and the degree of interhemispheric connectivity are found by correlating the local deformation functions with performance on cognitive tests.

In addition to the callosal measurements, the total volume of brain tissue (gray + white) was computed. Tissues outside the brain were first removed using an automated procedure, followed by manual editing. Brainstem structures inferior to the level of the mammillary bodies and the entire cerebellum were also removed by manual tracing. This approach yields reliable and accurate measures of total brain volume (Goldszal et al., 1998).

Neuropsychological Tests

Correlations with regional callosal size were examined for five neuropsychological tests. Card Rotations (CR) (Wilson et al., 1975) was used to measure two-dimensional spatial rotation and is thought to be sensitive to right parietal function. The dependent measure was the number of correct responses minus the number of incorrect responses. The Boston Naming Test (Kaplan et al., 1983) is a language task that measures object identification and naming, and involves recognition of pictures and language skills. The number of correct responses was the dependent measure. Letter Fluency (Spreen and Benton, 1969) assesses fluent production of words and is associated with frontal, especially left-sided, function. The total number of correct responses for the letters F, A and S was the dependent measure. The Verbal (VRM) and Figural (FRM) Recognition Memory Tests (Golski et al., 1998) are parallel memory tasks which are associated with frontal, temporal and visual association (for figural memory) function (Golski et al., 1996). The dependent measure for each recognition memory test was the sensitivity index, the number of correct ‘yes’ responses or hits adjusted for false positive responses. These five tests were selected from a larger battery, based on an attempt to sample both verbal and nonverbal functions while restricting the total number of variables investigated. An effort was made to include tests likely to tap right as well as left hemispheric functions.

Statistical Analysis

T-Tests were used to examine sex differences in total callosal area, in point-based comparisons between deformation functions, in regional area measurements, and in performance on the neuropsychological measures. Results of the point-based t-tests are presented graphically as images depicting effect sizes. The effect size is an index of the magnitude of the effect and is defined as: (mean deformation function for women – mean deformation function for men)/pooled standard deviation. Associations between callosal size, as measured by the deformation functions, and age were measured by point-wise correlation analysis. Hierarchical regression analysis was performed to examine the contribution of sex to splenial size after accounting for the effects of age and brain volume. Finally, partial correlations, adjusted for age, were computed point-wise, separately for men and women, to examine within-sex associations between callosal size and the neuropsychological measures.

Statistical analysis was performed for either the raw deformation functions or for normalized data adjusted for total callosal area. The raw and normalized data provide information on complementary issues, absolute and relative size. Age effects were examined for both normalized and nonnormalized data. Sex differences were presented for normalized data or relative size only due to the sex difference in total callosal area (described below). Correlation analyses are based on raw data for investigation of within-sex associations between absolute size and neuropsychological performance.

Results

Total Callosal Area

On average, men had larger callosal areas than women: 555.3 ± 90.8 versus 501.0 ± 75.1 mm², t (112 df) = 2.12, P < 0.05, two-tailed. These values are comparable to an average area of 524 mm² reported for a group of older men and women (Doraishwamy et al., 1991).
Sex Differences in Regional Callosal Area

Due to the larger overall brain size and total callosal area in men, it was necessary to control for sex differences in total callosal area for examination of regional size differences. To adjust for variability in total callosal size, the deformation functions were normalized by dividing each deformation function by the individual’s total callosal area.

To test the hypothesis of sex differences in regional corpus callosum morphology, t-tests were performed point-wise between the normalized deformation functions of men and women. Figure 2a shows the points of the corpus callosum where the deformation functions were significantly greater in women than men ($P < 0.0001$), revealing a relatively larger splenial area in the female group. The effect size is shown as a grayscale image in Figure 2b, and regions with effect sizes larger than 0.75 and 1.00 are highlighted in Figure 2c,d respectively.

To examine the stability of these results, the sample was divided into two subgroups, each including 34 men and 23 women. The two subgroups did not differ significantly in age for either men or women. The deformation analysis was performed for the first subgroup to identify points where the deformation functions differed significantly between men and women. The splenial region defined by these points (shown in Fig. 3) was used to cross-validate this finding on the second subgroup. This region was used to define a single measure of splenial area, which was calculated for each subject in the second subgroup by integrating each subject’s deformation function within the region. Sex differences in the integrated deformation functions for the regions were examined by $t$-test. Consistent with the first group, women in the second subgroup had significantly larger normalized areas than men, $t(55 \, df) = 3.27$, $P = 0.002$, two-tailed.

Age Effects on Regional Callosal Area

As there are conflicting reports regarding sex differences in the relation between age and callosal size, we examined the effects of age on total callosal area and regional corpus callosum morphology by correlation analysis for the sample of 68 men and 46 women. Age was significantly negatively correlated with total callosal area for men and women, $r = -0.45$ and $-0.47$ respectively.
Hierarchical regression analyses were performed to examine the effects of sex on normalized splenial size after accounting for the effects of age and brain volume. The area of the splenial region defined by the point-wise sex differences in the normalized deformation functions for the entire sample was the dependent variable. The first analysis examined the effect of sex after accounting for the effect of age. There were significant effects of age and sex on splenial area, with no significant age by sex interaction. Sex accounted for 19% of the variance in splenial area for men and women, indicating these regions are affected less by age relative to other callosal regions.

The regional specificity of the association with age was examined by point-wise correlation analysis between age and the normalized deformation functions. As shown in Figure 4 (bottom row), age was negatively correlated with relative splenial size in men, indicating that the decreases in corpus callosum size with increasing age were evident throughout most of the structure, with the exception of the anterior and posterior extremes, which showed no association with age (Fig. 4, top row).

The correlation analysis is presented in Table 1. Means and standard deviations for the neuropsychological tests were presented in Table 1. As predicted from a large literature on sex differences in spatial rotation ability (Linn and Petersen, 1985), men obtained higher average scores on the Card Rotations Test. Women, on the other hand, showed a trend toward higher scores on the recognition memory tasks, which reached significance for the Figural Recognition Memory Task. There were no significant sex differences in variance on the neuropsychological measures, with the exception of the Figural Recognition Memory Task on which variance was greater for men ($P < 0.05$).

The association between regional callosal size and cognitive performance was examined by point-wise correlation analysis. Since the correlation analysis was performed within sex, the analysis utilized the deformation functions based on the original rather than the normalized values. Moreover, partial correlations, controlling for the potential confounding effect of age, were employed, as age effects on the area of the corpus callosum and cognition could influence the results.

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The second analysis examined the effect of sex after accounting for the effects of age and brain volume. The effect of sex remained significant and accounted for 4% of the additional variance. Note that this underestimates the contribution of sex, because variation due to sex is confounded with brain volume when brain volume is forced into an earlier step in the analysis. Separate analyses of the effects of brain volume on relative splenial area for men and women reveal that brain volume is significantly associated with splenial size in men ($r = -0.22$) but not women ($r = -0.09$). Thus, sex differences in relative splenial size remain even after accounting for effects of age and brain volume.

**Correlations between Regional Callosal Size and Cognitive Performance**

Means and standard deviations for the neuropsychological tests are presented in Table 1. As predicted from a large literature on sex differences in spatial rotation ability (Linn and Petersen, 1985), men obtained higher average scores on the Card Rotations Test. Women, on the other hand, showed a trend toward higher scores on the recognition memory tasks, which reached significance for the Figural Recognition Memory Task. There were no significant sex differences in variance on the neuropsychological measures, with the exception of the Figural Recognition Memory Task on which variance was greater for men ($P < 0.05$).

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The results of the correlation analysis are presented in Figure 6. These maps depict all partial correlations significant at $P \leq 0.05$. The correlation analyses revealed significant positive associations between the deformation functions and performance for all five neuropsychological tests in women, indicating larger callosal size was associated with better performance. In contrast, there were no significant associations between corpus callosum size and performance in men, despite greater sample size and power to detect significant correlations in men. For women, the regions of significant association differed across all five tests, indicating better performance with greater interhemispheric connectivity. Positive associations with performance on selected tests were also observed for the callosal midsection (Card Rotations, Boston Naming and Letter Fluency) and genu (Verbal Recognition Memory and Boston Naming).
Discussion

Our findings provide strong evidence of sex differences in the morphology of the corpus callosum and its relationship to neuropsychological performance in older adults. Since the deformation function measures bulbosity of the corpus callosum, sex differences in the deformation functions indicated a larger and more bulbous splenium in women compared with men. This finding is consistent with DeLacoste and Holloway’s (1982) initial report of greater splenial area in women and with Salat et al.’s (1997) recent report of larger splenial area in older women compared with men. Greater splenial size in women could be due to sex differences in axon number, size or myelination, any of which could facilitate interhemispheric communication. In both the human (Aboitiz, 1992) and monkey (LaMantia and Rakic, 1990) corpus callosum, there are specific patterns of regional variability in axon density, size and degree of myelination.

Positive correlations between the deformation functions and performance in women indicated that greater callosal area was associated with better performance. For all five neuropsychological tasks examined, greater anterior splenial size was correlated with better performance. This callosal region contains projections from the associative parietal and temporal cortices (Aboitiz, 1992), which are likely to be involved in many tasks that assess more complex higher cortical functions. In contrast to the findings for women, splenial size was not related to neuropsychological performance in men. Since greater callosal area is associated with more extensive connections between the two hemispheres (Aboitiz et al., 1992), positive correlations for women suggest that greater interhemispheric connectivity facilitates performance when cognitive processing is more bilateral. On the other hand, the absence of significant associations in men may imply that men show greater lateralization of these functions and the extent of interhemispheric connectivity does not affect their performance.

Other investigators have also reported correlations between callosal size and cognitive performance in women. Hines et al. (1992) studied a group of 20–45 year old women and found a positive association between verbal fluency performance and splenial area. These investigators also reported a negative correlation between the area of the posterior callosum, predominantly containing the splenium, and language lateralization, consistent with our hypothesis that splenial size or interhemispheric connectivity is reduced when cognitive function is more lateralized. While Hines et al. studied women only, Salat et al. (1997) examined both men and women in an older sample of individuals over age 65. They reported significant positive associations between visual memory and the anterior callosum for women but not men. Splenial size in this study was not associated with cognitive performance. It is important to note, however, that both prior investigations were based on arbitrary callosal subdivisions. The splenial region in these studies included the posterior as well as anterior portion of the splenium. Neuroanatomical studies of the anterior and posterior splenial regions in both the human (Aboitiz, 1992) and monkey (LaMantia and Rakic, 1990) corpus callosum indicate differences in the types and distributions of fibers for these subregions. For example, in the monkey anterior splenial region, axon density is of an intermediate level, and there are small, medium and large myelinated axons and an intermediate level of unmyelinated axons. In contrast, the posterior splenial region, which contains projections from primary and secondary visual cortex, has the lowest axon density, all types of myelinated axons and a low percentage of unmyelinated axons. In our analyses, the most consistent effects were restricted to the anterior portion of the splenium. This pattern of findings highlights the regional specificity of our technique, which does not require a priori knowledge of the approximate location and shape of a region of interest, a limitation of traditional ROI-based techniques.

In our sample of older adults, age was negatively correlated with total callosal area in both men and women. Correlations between age and the normalized deformation functions revealed that the anterior portion of the splenium was the callosal region showing the greatest negative association with age. While age is associated with splenial size, it did not account for the observed sex differences. Similarly, the sex difference in relative splenial size could not be explained by variation in brain volume. Jancke et al. (1997) have argued that smaller brain volumes are associated with larger splenial size and that relative splenial size appears larger in women due to their smaller overall brain volume. We found significant effects of sex on splenial size even after adjusting for brain volume. Moreover, the modest negative association between brain volume and relative splenial size observed for men did not hold for women. Thus, effects of brain volume on splenial area may be confounded with those of sex when the relationship is examined in mixed-sex samples.

In summary, we have applied a new approach for quantitation of brain morphology, which allows point-based analysis of the corpus callosum. Applying this technique to a relatively large sample of older adults, we demonstrated a sex difference in splenial morphology and its association with neuropsychological functioning. A limitation of our study is its focus on individuals age 56–85. Since sex differences in corpus callosum morphology may vary over development (Clarke et al., 1989; Cowell et al., 1992), it will be important to apply our quantitative approach to younger samples to assess the generalizability of these findings across the lifespan.

Notes

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