A Comparison of Sex Differences Revealed by Two Partitioning Schemes of the Human Corpus Callosum

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We compared two schemes of partitioning the human corpus callosum, both of which purport to divide corpus callosum axons into functionally distinct subregions. Witelson’s (1989) scheme was based largely on data obtained from *rhesus macaques*. In contrast, Hofer and Frahm’s (2006) scheme was based on data from humans using Diffusion Tensor Imaging (DTI) and as such should more accurately partition the human corpus callosum into functional units. Sex differences in the human isthmus have been reported using the Witelson scheme. Examining Hofer and Frahm’s scheme may reveal sexual dimorphisms in axon bundles that are allegedly more functionally homogeneous. In this study, we employed both schemes independently on human corpora callosa and compared results. Analysis of data from the Witelson’s scheme revealed that the isthmus area was larger in males than in females. This difference was also observed when corrected for overall brain size. No sex differences were found in the corpus callosum subregions as defined by the Hofer and Frahm scheme. Witelson’s scheme designated some axons as part of the isthmus that the Hofer and Frahm scheme designated as part of splenium. However, an examination of this disputed region revealed no sex difference, so we were unable to attribute the differential outcomes to this set of axons. We also examined the possibility that there was proportionately more variance in measurements of the isthmus when using the Hofer and Frahm scheme relative to the Witelson scheme, but the two schemes generated similar coefficients of variation. Re-examination of both schemes revealed that neither consistently partitioned the corpus callosum into functionally homogeneous regions as defined by individual DTI data. Partitioning the corpus callosum may be best accomplished using an individual’s unique DTI data rather than using any general scheme for all individuals.

Abbreviations: DTI - Diffusion Tensor Imaging; ANCOVA - Analysis of Covariance
Keywords: Diffusion Tensor Imaging; Isthmus; Hemispheric Connectivity; human brain; Sex Differences; Sexually Dimorphic

Introduction

The human corpus callosum is a large, curved bundle of axons projecting from one cerebral hemisphere to the other, thus allowing inter-hemispheric communication. The human corpus callosum can be partitioned according to numerous schemes, though studies have conventionally used the scheme proposed by Witelson (1989), which divides the length of the corpus callosum by creating a partition at the midpoint, a second and third partition at one-third and two-thirds of the entire corpus callosum length from the anterior side, and a final partition at one-fifth of the entire corpus callosum length from the posterior side. Witelson’s partitioning scheme is primarily based on lesion and autoradiography data obtained by analyzing rhesus monkey brains (Witelson, 1989).

According to Witelson, the genu, rostrum, and rostral body of the corpus callosum, which accounts for one-third of the entire corpus callosum area, are thought to include fibers projecting to and from the prefrontal cortex, premotor, primary motor, and supplementary motor cortices, respectively. The genu composes the rounded anterior end of the human corpus callosum and is directly adjacent to both the rostral body and rostrum. The rostral body is anatomically superior to the pointed rostrum. The anterior midbody and posterior midbody make up one-third
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of the entire corpus callosum area and are posterior to the rostral body. Fibers originating in the motor cortex are thought to pass through the corpus callosum in the anterior midbody, whereas somesthetic and posterior parietal fibers are thought to pass through in the posterior midbody. The final one-third of the corpus callosum area in the most posterior region is the relatively thin isthmus and the thicker, bulb-shaped splenium. The isthmus consists of projections between the left and right hemisphere's superior temporal and posterior parietal cortices, whereas the splenium consists of projections between the left and right occipital, and inferior temporal, cortices in Witelson's scheme (see Figure 1).

More recently, a scheme based on DTI data, a magnetic resonance imaging technique, has been proposed (Hofer and Frahm, 2006). Hofer and Frahm distinguished corpus callosum subregions based on the fibers projecting to different regions of the cortex. Their primary rationale for proposing this new scheme stems from the location of callosal fiber bundles as revealed by DTI, which allows researchers to construct a model of hemispheric connectivity in the human brain based on the anisotropic diffusion of water molecules in white matter.

This technique is particularly useful when it comes to performing tractography on the human corpus callosum, since it is entirely white matter. One limitation of DTI is that the model assumes that there is only one orientation of all the tracts going through a given voxel and, as such, it can be difficult to identify all of the fibers in a given voxel (Qiu et al., 2012). This limitation is not a huge concern when it comes to the corpus callosum, however, because the axons generally do travel in the same orientation through the corpus callosum from one hemisphere to the other.

In the Hofer and Frahm scheme, one-sixth of the corpus callosum area on the anterior end incorporates the genu, rostrum, and rostral body and is designated as including fibers originating in the prefrontal cortex. The rest of the anterior half of the corpus callosum consists of premotor and supplementary motor projections through the anterior midbody. The posterior midbody, which is thought to include projections from the motor cortices, is the only subsection that has not been altered from Witelson’s classification.

Figure 1. The corpus callosum is partitioned as such by the Witelson scheme. The letters correspond to each subregion created as a result of using the scheme: A: genu, rostrum and rostral body, making up 1/3 of the total area. B: anterior midbody, making up 1/6 of the total area. C: posterior midbody, making up 1/6 of the total area. D: Isthmus, making up 2/15 of the total area. E: splenium, making up 1/5 of the total area.
The isthmus and splenium are also altered in Hofer and Frahm’s scheme relative to Witelson’s. Hofer and Frahm have expanded the region devoted to the splenium and reduced the area corresponding to the isthmus. In Witelson’s scheme, the splenium accounts for one-fifth of the corpus callosum area on the posterior end but in Hofer and Frahm’s scheme, it comprises one-fourth of the corpus callosum area on the posterior end. The isthmus is thought to include sensory (presumably somatosensory) projections and the splenium includes parietal, occipital, and temporal interhemispheric projections.

Witelson’s scheme has revealed a sex difference in the isthmus (Witelson, 1989). Some studies reported a larger isthmus in consistently right-handed females relative to right-handed males (Witelson, 1989; Steinmetz et al., 1992; Poltana et al., 2001), and others reported a larger isthmus in right-handed men relative to right-handed women (Tuncer et al., 2005; Luders et al., 2006). Still others suggested that sex differences depend on the normalization scheme, or method of standardization used to compare study subjects employed (Bermudez and Zatorre, 2001).

The Witelson scheme has been used in studies that examine sexual dimorphism in the human corpus callosum since its inception (Clarke and Zaidel, 1994; Steinmetz et al., 1992), but the Hofer and Frahm has not been used in investigations of sex differences in any corpus callosum subsection, even though the divisions should contain more functionally homogeneous fibers due to its basis in DTI data. The goal of this study is to provide a comparison of these schemes using the same MRI images of human brains and look for sex differences in the subregions of the corpus callosum as defined by each partitioning scheme.

Materials and Methods

The subjects of this experiment, who were originally recruited by Dr. Russell A. Poldrack, included thirty human males and thirty human females with ages ranging from 19 to 39 years. We used Tiff images constructed from data collected with a 3T Siemens Allegra scanner, using a magnetization prepared rapid-acquisition gradient echo (MPRage; FOV = 256, 71 sagittal slices, slice thickness 1 mm, TR = 2000 ms, TE = 2.1 ms, matrix 192 x 192) for each subject. These images were used in accordance with UCLA IRB exemption #11-000712.

Data analysis

One midsagittal slice for each brain was selected as best for analysis. The analyzed slices were chosen from a pool of sagittal slices by selecting the slice with a prominent fornix and an easily outlined corpus callosum that was not closely bordered by arteries, particularly by the cerebral
arteries intruding upon the image of the genu and rostrum. All measurements were to the nearest millimeter squared. The corpus callosum area was measured. In doing so, the fornix was excluded from the total subsectional corpus callosum areas by assuming a straight line across the inferior aspect of the corpus callosum immediately superior to the fornix. Any adjacent arteries were removed by finding the artery width, assuming the width is constant, and excluding it. As a measure of cortex size, the area of the midsagittal cerebral cortex was determined for each subject. When tracing the area of the midsagittal cortex, all cerebral spinal fluid cisterns were excluded. The posterior region of the cortex near the cerebellum was traced just above the straight sinus at the cortex.

A line defining the longest rostral-caudal extent of the corpus callosum was drawn and determined to the nearest hundredth of a millimeter. The Witelson scheme was applied to partition the corpus callosum. The area of each subregion, denoted by letter in Figure 1, was traced. (see Figure 1).

Subsequently, the corpus callosum subregions were measured using the Hofer and Frahm Scheme. The area of each subregion, denoted by letter in Figure 2, was traced (see Figure 2). The fractional anisotropy maps provided by Hofer and Frahm, which displayed unique fiber bundles connecting homotopic regions of the cortex, were also examined for sex differences. Each unique fiber bundle was identified by color, signifying different homotopic connections.

Statistical analyses

Raw data values for total and subsectional corpus callosum areas as defined by each scheme were compared for sex differences using t-tests. Further, values were normalized into proportional ratios by dividing a given region by its total corpus callosum area. We also normalized each subregion area by dividing it by the corresponding midsagittal cortex area as an index of total brain size. The resulting proportional and normalized values for males and females were also compared using t-tests. The data within each partitioning scheme were additionally subjected to analyses of covariance, which controlled for overall brain size, as indexed by midsagittal cortex size, before comparisons between sexes were made within subregions of a given scheme.

Sex differences in fiber bundle area for the eight subjects of the Hofer and Frahm study were analyzed using a two-tailed t-test.

Results

Sexual dimorphisms in corpus callosum subregions for each scheme

Analyzing the raw data for each subregion of the Witelson scheme revealed a statistically significant sex difference in midsagittal isthmus size, with males being larger, \( t(58) = 2.10; p < 0.05 \). No other subregions showed a sex difference using the Witelson scheme. The unadjusted average size of each subregion as defined by the Witelson scheme is presented in Figure 1. The Hofer and Frahm scheme revealed no statistically significant sex differences in any region including the isthmus, \( t(58) = 1.43; p = 0.16 \) (see Figure 3). The unadjusted average size of each subregion, as defined by the Hofer and Frahm Scheme, is presented in Figure 3.

![Figure 3: Unadjusted sex comparison of midsagittal isthmus area for each scheme.](image-url)
normalized by dividing the subregion areas by the area of the midsagittal cortex, a sex difference in the midsagittal isthmus area was maintained in the Witelson scheme, $t(58) = 1.978; p = 0.05$. The Hofer and Frahm scheme again yielded no sex differences in any subregion with this correction (all $p > 0.05$). The sexual dimorphism in the isthmus area was still present after an ANCOVA that corrected for brain size (midsagittal cortex area) in the Witelson scheme, $F(1,57) = 4.45; p < 0.05$, but again midsagittal isthmus area was not statistically significant in the Hofer and Frahm scheme when corrected for brain size using an ANCOVA ($p > 0.05$).

There were no sex differences in average midsagittal cortex area ($p > 0.60$), nor were there any sex differences in average corpus callosum area ($p > 0.20$). When the raw data were normalized by dividing the subregion areas by the area of the corpus callosum, no sex difference in isthmus (Witelson: $t(58) = 1.71; p > 0.09$, Hofer and Frahm: $t(58) = 0.80; p > .42$) or any other subregion was revealed in either scheme (all $p > .40$).

**Each Scheme generated similar coefficients of variation**

To understand the discrepancy in results between these two partitioning schemes, we first examined the possibility that there was proportionately more variance in measurements of the isthmus when using the Hofer and Frahm scheme relative to the Witelson scheme. The two schemes, however, generated similar coefficients of variation (see Table 1). Thus, our failure to find a sex difference in the isthmus with the Hofer and Frahm scheme is probably not due to proportionately more variability in this scheme’s measurements.

<table>
<thead>
<tr>
<th>Partitioning scheme</th>
<th>Sex</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIT</td>
<td>Male</td>
<td>0.245924</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.20088</td>
</tr>
<tr>
<td>H&amp;F</td>
<td>Male</td>
<td>0.271596</td>
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<tr>
<td></td>
<td>Female</td>
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Table 1: Table of coefficients of variation for the isthmus of each scheme. Wit corresponds to the Witelson scheme values and H&F corresponds to the Hofer and Frahm scheme values. Values are presented separately for males and females.

Due to the larger size of the isthmus, as defined by the Witelson scheme, it encompasses some axons that are not included in the Hofer and Frahm isthmus (see Figure 4). The sexually dimorphic axons could have been a part of the isthmus as defined by the Witelson scheme, but not part of the isthmus as defined by the Hofer and Frahm scheme. Therefore, we subtracted the isthmus area according to Hofer and Frahm from the area given by the Witelson scheme in each individual and examined this sub-region for a sex difference (see Figure 4). This sub-region was not sexually dimorphic, $t(58) = 1.28; p = 0.21$, however.

**Figure 4: The isthmus as defined by each scheme.** The isthmus as defined by the Witelson scheme, shown in black and grey, makes up 8/60 of the total corpus callosum area, but the Hofer and Frahm isthmus, shown in only black, is 5/60 of the total corpus callosum area. The grey area was examined for sex differences.

Upon applying either scheme to the anisotropic maps of the corpus callosum provided by Hofer and Frahm (Hofer and Frahm, 2006), we discovered that although the Hofer and Frahm scheme did a better job of DTI defined subregions, neither scheme perfectly matched the anisotropic maps across all subjects. The anisotropic maps provided by Hofer and Frahm were examined for sex differences and the midsagittal area of each fiber bundle as defined by DTI was traced for all eight subjects of the Hofer and Frahm study. Only one fiber bundle, the prefrontal lobe axons traversing the anterior-most subregion of the corpus callosum, was sexually dimorphic, with males having a larger area, $t(6) = 2.52; p < 0.05$. No other fiber bundles showed any sexual dimorphism (all $p > 0.12$).

**Discussion**

Our findings regarding sexual dimorphism
in the human corpus callosum are mixed. Applying the Witelson Scheme on all 60 subjects yielded a sexually dimorphic midsagittal isthmus area, which is consistent with some previously published work (Tuncer et al., 2005; Luders et al., 2006). This dimorphism was maintained even after corrections using midsagittal cortex area. Surprisingly, the Hofer and Frahm scheme did not yield any sex differences in unadjusted or adjusted comparisons of midsagittal subregion areas. We examined the possibility that there was proportionately more error associated with our measurements in the Hofer and Frahm scheme because the Hofer and Frahm scheme defines the isthmus as a smaller subregion of the corpus callosum than does Witelson’s. Thus, the proportionately larger error could have concealed differences. Nonetheless, the coefficients of variation for the isthmus were similar across the two schemes, making this explanation unlikely.

Neither Witelson’s nor Hofer and Frahm’s scheme can reconcile systematic corpus callosum partitions with the actual fiber projections in the corpus callosum as defined by DTI. We tried overlaying both the Hofer and Frahm Scheme and the Witelson Scheme onto fractional anisotropy maps provided by Hofer and Frahm. When we applied either scheme to the anisotropic maps, we found that neither scheme perfectly defined subregions that were consistent with the DTI-defined axonal bundles across individuals. This led us to examine Hofer and Frahm’s fractional anisotropy maps themselves, which yielded sex differences in the anterior region of the corpus callosum with males, showing a larger anterior region. In another DTI study of the microstructure of the corpus callosum, this same dimorphism in the anterior regions was upheld (Westerhausen et al., 2011). Witelson also reports an overall sex difference in the rostral regions of the corpus callosum regardless of handedness (Witelson, 1989). Westerhausen et al. (2011) suggest that the functional implications of this dimorphism may be due to greater frontal lobe connectivity in males. According to Hofer and Frahm, this region includes fibers connecting the prefrontal lobes, which agrees with Westerhausen’s suggestion.

Hofer and Frahm justified the creation of a new scheme by using DTI data, which should provide greater accuracy than that of the lesion and autoradiography data, upon which the Witelson scheme is based. The rationale for the Witelson scheme was to partition the fibers into homogeneous functional units. The Hofer and Frahm scheme improves upon the Witelson scheme, in that boundaries are better determined by DTI. Nonetheless, even Hofer and Frahm’s scheme doesn’t completely capture and segregate axons by origin and destination in every individual.

Perhaps using DTI to define the fiber bundles rather than an arbitrary scheme will provide better insight into both sex differences in corpus callosum structure and the functional implications of such differences. Using DTI in future studies will provide researchers with a clearer picture of the location of origin for the fibers projecting between cortical hemispheres, thus enabling them to search for sex differences or even physical, emotional, or behavioral differences associated with fiber bundle areas.

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