Fiber composition of the human corpus callosum

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The densities of fibers of different sizes were calculated in ten regions of the corpus callosum of twenty human brains (ten females, ten males). Light microscopic examination revealed a consistent pattern of regional differentiation of fiber types in the corpus callosum. Thin fibers are most dense in the anterior corpus callosum (genu) and decrease in density posteriorly towards the posterior midbody, where they reach a minimum. Towards the posterior corpus callosum (splenium), the density of thin fibers increases again, but in the posterior pole of the callosum the density decreases locally. Large-diameter fibers show a pattern complementary to that of thin fibers, having a peak of density in the posterior midbody and a local increase of density in the posterior pole of the corpus callosum. Across subjects, the overall density of callosal fibers had no significant correlation with callosal area and an increased callosal area indicated an increased total number of fibers crossing through. Considering different fiber sizes, this was only true for small diameter fibers, whose large majority is believed to interconnect association cortex. No sex differences in fiber composition of the corpus callosum were found.

INTRODUCTION

The corpus callosum seems to exhibit a topographic representation of the different cortical areas, and there appears to be an associated functional specialization of different callosal segments. Histological differentiation of callosal regions consistent with these findings has been reported. This indicates that multiple overlapping and function-specific pathways of interhemispheric communication may make up the corpus callosum, each having its own transfer properties based on fiber composition.

Despite the potential importance of determining the regional fiber composition of the human corpus callosum, no detailed study of this structure has been performed in humans until now. The only two extant reports are made by Tomasch, which lacks sufficient detail, and a preliminary study in the posterior corpus callosum made by us, both using light microscopy. The current understanding of the fiber composition of the callosum is therefore limited to our report and to an electron microscopic study in the macaque, suggesting that higher order processing areas in the cortex (formerly called 'association' areas) tend to project to the corpus callosum through small and mid-diameter axons (smaller than 2 nm in diameter), while large axons (larger than 1 /m) and gigantic axons (larger than 3 /m) tend to be concentrated in the callosal regions connecting visual and somatosensory cortices. We have interpreted this as a consequence of the role of callosal axons in the fusion of the sensory midline in primary and secondary sensory areas. Large and gigantic fibers would be especially suitable for performing this task in virtue of their higher conduction velocities.

Additionally, there have been several recent reports concerning individual differences in the morphometry of the human corpus callosum. Besides the controversial nature of the findings, a major question concerns the significance of gross anatomical differences in the corpus callosum. Some authors suggest that a larger callosal area indicates a larger number of fibers crossing through and hence a better capacity for interhemispheric transfer. However, it
is possible that an increase in callosal area is associated with a decrease in fiber density, resulting from an increased fiber diameter or an increased interfiber distance. A recent study in the macaque reported a significant negative correlation between fiber density and callosal area. This indicates a decrease in fiber density as callosal area increases, which may result in a lack of correspondence between callosal area and total number of fibers. (Note that in this study the sample size was relatively small: n = 8.) It is therefore important to establish whether callosal area can be used as an estimator of differences in fiber composition in humans.

The present report is a thorough light microscopic analysis of the regional fiber composition of the human corpus callosum. We characterized the different callosal segments in terms of the densities of fibers of different sizes, and studied the relation between individual variations in callosal area and the total numbers of fibers in this structure.

MATERIALS AND METHODS

Brains were obtained from the Institute of Pathology, Hospital San Juan de Dios, University of Chile, Santiago, Chile. Twenty brains (ten males, ten females), aged 25-68 years (mean±S.D. in females: 43±14.8; in males: 46±10.1), of individuals who died from non-neurological disease were examined. No more than 12 h after death, brains were extracted from the skull and immersed in 20% buffered formalin for 1 day. After this, brains were visually inspected on the surface for gross morphological abnormalities.

The corpus callosum of these subjects was sagittally sectioned at the midline and a 5-mm-thick strip of the whole callosum was obtained from this medial section. The callosums were immersed in 10% buffered formalin for at least 3 weeks.

Histological procedure

The fixed callosum was divided into three equal portions according to maximal straight length (the genu, midbody, and isthmus) and the splenium (Fig. 1A). The callosal pieces extracted were embedded in paraffin and sectioned at 5 Mm in the sagittal plane. Alternate sections were stained with the Holmes (for neurofibils) and the Loyez (for myelin sheaths) methods (Fig. 2).

In the stained slides, the genu and midbody were further segmented into three equal portions according to maximal straight length (G1, G2, G3, and B1, B2, B3, respectively: Fig. 1A). The posterior third was divided into the isthmus (the area between the posterior 1/3 and the posterior 1/5) and the splenium (the posterior 1/5). The splenium was subdivided into three equal portions according to maximal straight length (S1, S2, S3: Fig. 1A). This partitioning method, based on straight length, has been compared morphometrically with other methods that consider the curvature of the corpus callosum. No differences have been found between the two procedures.

Fibers were counted in every callosal pon using both stains, in the specific loci shown in Fig. 1A. With the exception of the isthmus, where two loci were used to count fibers, each of the subdivisions had only one counting locus situated in the middle of the field (indicated by a dot in Fig. 1A). In the anterior two thirds of the callosum, 6 different loci equally distant from one another were selected to make the fiber counts.

In the posterior third, two loci were located in the isthmus (Fig. 1A): note that the densities reported for the isthmus represent an average of these two loci. Special emphasis was put on this segment since it apparently includes fibers connecting the posterior language areas. Three other counting loci were located in the posterior fifth (the splenium: Fig. 1A). The counting loci were located on a curved line equidistant to the dorsal and the ventral borders of the corpus callosums. In each of these loci, three different fields were used to count fibers, with a grid divided into 100 squares. The fields were within a 1-mm radius circle with center on the selected locus.

This sampling procedure excluded fibers located close to the dorsal and ventral borders of the corpus callosum, especially in the most bulbous regions (genu and splenium). We determined whether these 'peripherally' located fibers in the genu and the splenium differed from more 'centrally' located fibers (as specified in the loci of Fig. 1A) in terms of fiber types and in the relation between fiber density and callosal area. For that purpose, 'peripheral' fibers located 1.5-2 Mm. from the dorsal and ventral callosal surfaces in both the genu and the isthmus were analyzed. We counted fibers in 4 different loci (two superior, two inferior) in each of these segments in 8 subjects (4 females, 4 males). Our results were in every respect similar to those obtained by counting fibers in the 'central' loci specified in Fig. 1A.

With the Holmes stain (Fig. 2A), every distinguishable fiber (larger than 0.4 Mm in diameter, as seen in the stained material) was counted. With this stain, a total magnification of 1.850x was used, and the area of each counting field was 54x54 Mm. With the Loyez stain (Fig. 2B), separate counts were made of myelinated fibers larger than 1 Mm. of fibers larger than 3 Mm. and of fibers larger than 5 Mm in internal diameter. With this stain, counts were made at a total magnification of 1.000x. and the area of each counting field was 100x 100 Mm. Fiber diameters were calculated directly from the stained material. Fibers larger than 1 Mm. included the smallest fibers we could see with a clear myelin sheath: fibers target than 3 Mm were those with an internal diameter larger than 1/3 of the length of each of the smallest squares of the grid, and fibers larger than 5 Mm had an internal diameter larger than 1/2 of the length of the grid's squares.

Substantial shrinkage after fixing and embedding the tissue in paraffin constitutes a problem. This shrinkage was calculated to approximate 65% of the whole tissue area in all callosal regions. Therefore, the fiber diameters observed were actually smaller than their true size. In order to calculate fiber velocities of conduction, the fiber diameters calculated under light microscopy need to be corrected in terms of the shrinkage factor. From the calculated shrinkage factor, fibers 0.4 Mm in observed diameter correspond to a true diameter of 0.6 Mm. Similarly, fibers 1 Mm, 3 Mm and 5 Mm in observed diameter correspond to true diameters of 1.5 Mm, 4.6 MHi and 7.5 Ml respectively. In the text, we will refer to the fiber categories by reference to the observed diameter. When referring to true diameters, we will use the terms 'true' or 'corrected' diameter. Additionally, there is a limit to light-microscopic resolution at about 0.4 Mm. Accordingly, the population of fibers below this diameter could not be taken into account with this method. This number was estimated by means of an electron microscopic analysis of one brain (see below).

Fiber counts were performed twice and then averaged. These duplicate counts correlated highly with each other (r > 0.90), and the standard deviations of the three values at each locus per subject were usually below 5% of the mean, excepting counts in the anterior genu (G1, Fig. 1A) where the standard deviation could reach up to 10% of the mean. Most likely, this was a consequence of the high density of fibers in this region, which interfered with the clarity of the image and made fiber counting more difficult. Two observers performed counts in a subsample of the material, and the correlation between the resulting values was high (r > 0.90).

To determine the total number of fibers per callosal segment, the calculated area of each segment (i.e., G1, G2, etc.) was multiplied by the average fiber density of the respective counting loci. The mean density of fibers in larger regions (such as the genu, comprising G1+G2+G3: see Fig. 1A) was found by adding the total number of fibers in G1, G2 and G3 and then dividing by the summed area of G1+G2+G3.
Electron microscopy

One hour after death, blocks from the genu, anterior body, mid-body, posterior body and splenium of the corpus callosum of a single male individual (age 41) were extracted. 'Posterior body' indicates a region that includes the posterior midbody (B3 in Fig. 1A) and the isthmus. This is to be distinguished from the posterior midbody itself. More precise identification of the topographic location of the analyzed blocks was not possible. The tissue blocks were fixed in 5% buffered glutaraldehyde, postfixed in 1% buffered osmic acid, and embedded in Epon, as described by Swadlow et al. 42. Thick sections were stained with Toluidine blue for preliminary examination area.

TABLE I

<table>
<thead>
<tr>
<th>Fiber types</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibers &gt; 0.4 ( \mu m )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Holmes stain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>3.717X10^5</td>
<td>2.828 x 10^6</td>
</tr>
<tr>
<td>Females</td>
<td>3.731 X10^5</td>
<td>2.664 x 10^6</td>
</tr>
<tr>
<td>Males</td>
<td>3.700 X10^5</td>
<td>3.122 X10^6</td>
</tr>
<tr>
<td>Fibers &gt; 1 ( \mu m )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Loyez stain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>8.705 X10^4</td>
<td>1.884 X10^6</td>
</tr>
<tr>
<td>Females</td>
<td>8.348 X10^4</td>
<td>1.935 X10^6</td>
</tr>
<tr>
<td>Males</td>
<td>9.063 X10^4</td>
<td>1.862 X10^6</td>
</tr>
<tr>
<td>Fibers &gt; 3 ( \mu m )</td>
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<td></td>
</tr>
<tr>
<td>(Loyez stain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>383</td>
<td>187</td>
</tr>
<tr>
<td>Females</td>
<td>397</td>
<td>193</td>
</tr>
<tr>
<td>Males</td>
<td>370</td>
<td>190</td>
</tr>
<tr>
<td>Fibers &gt; 5 ( \mu m )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Loyez stain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>81</td>
<td>46</td>
</tr>
<tr>
<td>Females</td>
<td>82</td>
<td>46</td>
</tr>
<tr>
<td>Males</td>
<td>81</td>
<td>46</td>
</tr>
</tbody>
</table>
examination and for determining the sagittal plane of sectioning. Thin sections were stained with lead citrate and uranyl acetate for electron microscopy. The sections were photographed at magnifications of 2,500-3,000x, and a straight horizontal line was drawn across each picture. One hundred consecutive fibers touching the line were counted and measured in each of three micrographs from

Fig. 2. A: photomicrographs of the anterior midbody (left) and the posterior midbody (right), as seen with the Holmes stain. Dots represent distinct fibers. B: photomicrographs of the anterior midbody (left) and the posterior midbody (right), as seen with the Loyez stain. In this stain, myelin sheaths are stained and fibers are seen as empty circles. In both stains it is clear that in the posterior midbody fibers are less dense and tend to be larger than in the anterior part. Bar - 10 Mm.
different fields within each region. Three hundred fibers were thereby counted and measured in each callosal region. In this material, fiber densities, histograms of fiber sizes and numbers of non-myelinated fibers were calculated. Fiber diameter was calculated as the minimal distance between two inner myelinated sheaths in a fiber section. This procedure prevented distortion due to oblique sectioning of the fibers.

Unfortunately, it was not possible to obtain more material from this subject to provide controls for the Holmes and the Loyez techniques, or to determine the total callosal area.

**Statistical methods**

All variables passed the standard tests of normality. Comparisons between groups were made using two-way analyses of variance, and Pearson product moment correlations were used for testing the correspondence between variables.

**RESULTS**

**Light microscopy**

**Regional differentiation of the corpus callosum**

Tables I and II show the average axon densities and the average total numbers of callosal axons, respectively, for the entire sample, females and males, by fiber type. Fibers larger than 1 μm, 3 μm and 5 μm represented about 20%, 0.1% and 0.02% of the total fibers counted (larger than 0.4 μm), respectively. There were no significant sex differences in fiber densities of any type or in total number of fibers, either in the whole callosum or in each of the callosal segments (F,18 < 3.8, /> 0.05). However, a slightly larger number of fibers larger than 0.4 μm and 1 μm were found in males, while in females the numbers of fibers larger than 3 μm and 5 μm were slightly larger (see Table II).

In this sample, age did not decrease the numbers of callosal fibers (-0.1 < r < 0.3, P > 0.05. n = 10), presumably because the subjects’ ages were not too advanced.

As seen in the Holmes stain (fibers larger than 0.4 μm), fiber density tended to decrease from the genu to the posterior midbody (B3 in Fig. 1A), and to increase again when entering the splenium (Figs. IB and 2A). In the most posterior splenial region (S3), fiber density tended to decrease again (Fig. IB; previously reported by us in a smaller sample).

As seen in the Loyez stain, the densities of fibers larger than 1 μm and larger than 5 μm showed a pattern exactly inverse to that found in the Holmes stained material (Figs. IB and 2B). The region of highest density of large fibers was the posterior midbody (B3. Fig. 1A), and density decreased both anteriorly and posteriorly from there, excepting the posterior splenium (S3), where the density increased again. The increase in density of large fibers in the posterior splenium was reported before for a smaller sample.

**Relations between fiber density and callosal area**

No significant correlations between the mean fiber densities and total callosal area were found for any fiber type (Table III). These findings indicate that no decrease in fiber density occurs as callosal size increases. Consequently, a larger callosal area may indicate that there are more fibers crossing through. The same results were found in every callosal segment (r > -0.23, /> 0.05, n = 20) and remained the same when separating the sexes.

**Electron microscopy**

As seen in electron microscopy (Fig. 3), myelinated fiber sizes ranged from 0.2 to more than 10 μm in diameter. The proportional distribution of different fiber sizes varied according to the callosal region. For example, fibers between 0.2-1 μm represented about 72% of the total fiber population in the genu, while in the posterior body (i.e., posterior midbody (B3) and isthmus. Fig. 1A; see Materials and Methods) they represented only about 45% of the total (Fig. 4). The posterior part of the callosum (splenium) and the anterior and midbody represented patterns intermediate between the genu and the posterior body. Gigantic fibers (larger than 3 μm in diameter) were more abun-
Fig. 4. Histograms of fiber size frequencies in different regions of the corpus callosum, as seen in electron micrographs of a single subject. 'Anterior body' represents a region including the posterior genu and the anterior midbody (G3 and 81 in Fig. 1A). 'Posterior body' represents a region including the posterior midbody (B3) and the isthmus in Fig. 1A.

Diameter (μm)

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENU</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>ANT. BODY</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>MID BODY</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>POST. BODY</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>SPLENIUM</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Regional differentiation: fibers larger than 0.4 μm
The average total number of callosal axons detected here with light microscopy (1.6 x 10^8 axons) is slightly below that reported by Tomasch (1.8 x 10^8 axons). The regional distribution of fiber sizes seen in light microscopy is generally consistent with previous findings. As seen in Fig. 1, thin fibers are most dense in the genu and the mid-splenium (S2), and least dense in the posterior midbody (B3). In these cortical regions, processing time may be long enough to make the delay produced by interhemispheric transfer through smaller diameter fibers irrelevant. Alternatively, the delays may permit cascade processing, where computation in separate hemispheric modules can be time-staggered. La Mantia and Rakic speculate that fine-caliber callosal axons may transmit a more tonic influence, as opposed to a more phasic type transmitted by large-diameter axons.

Function of fibers larger than 3 μm and 5 nm
These fibers tend to be concentrated in the midbody of the callosum, with a peak in the posterior midbody (B3 in Fig. 1), and they also show a smaller peak in the posterior splenium (S3 in Fig. 1). This pattern is clearly complementary to the distribution of thin fibers (Fig. 1).

In their recent study on the macaque callosum, La Mantia and Rakic have proposed that the 'gigantic fibers in the callosal midbody correspond to primary and secondary somatosensory areas. These cortical areas project through the middle portion of the callosal midbody (B2 in Fig. 1). In our human material, large fibers in the middle portion of the midbody (B2. Fig. 1)
may connect primary and secondary somatosensory (and perhaps also motor) areas. Their role may be to fuse the two representations of the body in the midline.

The present analysis indicates that in humans, the region with largest callosal fibers is the posterior midbody (B3, Figs. 1 and 2), a region presumably devoid of somatosensory fibers in the human, as well as in the macaque. Note that Fig. 9 of La Mantia and Rakic indicates that in the macaque, high proportions of large-diameter axons are also found in the posterior midbody and in part of the isthmus.

One possibility is that the gigantic fibers in the posterior midbody of the human (and the macaque) connect primary and secondary auditory areas. These cortical regions are mapped somewhere between the posterior midbody and isthmus of the callosum of the human and the macaque. If these areas are mapped anterior to the perisylvian areas, conserving the topography, then they may interconnect through segment B3.

We suggest that the large callosal fibers connecting auditory regions may contribute to the production of an auditory map in space, so as to augment the auditory representation which is already bilateral in each hemisphere. Perhaps the timing of the cellular responses in each hemisphere is important for localizing sounds in space. It would be interesting to investigate whether callosal auditory fibers are especially large in echolocating species such as bats or dolphins.

Another region with a high proportion of large fibers is the posterior splenium. We have suggested that these fibers serve to fuse the two hemirepresentations of the visual field. Perhaps gigantic visual axons contribute to perceptual processes such as stereopsis. Again, it would be interesting to determine if binocularity affects the size of visual callosal axons in different species. It is interesting that a large fraction (70% in the rabbit) of the fibers connecting primary and secondary visual areas produce inhibitory postsynaptic potentials in the contralateral side, presumably by exciting inhibitory interneurons. This may imply that a large proportion of the gigantic callosal axons exert an inhibitory effect in the opposite hemisphere.

In summary, the regional distribution of thin and large-diameter fibers have complementary patterns. Thin fibers are densest in the genu and mid splenium (S2), callosal regions that connect prefrontal and higher-order sensory areas. Large-diameter fibers tend to be more dense in regions connecting primary and secondary somatosensory, auditory and visual areas (regions B2 and B3 of the midbody, and S3 of the Plenium, respectively). The callosal region with highest density of large-diameter fibers in humans seems to connect the auditory areas instead of the somatosensory or visual areas, as was reported in the macaque.

However, the presence of gigantic fibers in the midbody is unlikely to be the direct cause of the reduction in overall fiber density. From the anterior genu (G1 in Fig. 1) to the posterior midbody (B3 in Fig. 1) overall fiber density decreases from approximately 400,000 to 300,000 fibers/mm², i.e., a decrease of 25%. This indicates that in segment B3, 25% of the area is either left without fibers or is occupied by larger diameter fibers. If the presence of large and gigantic fibers in the posterior midbody is the cause of the decrease in overall density, the area occupied by them should be about 25% of the total area. We estimated that the additional area occupied by the increased diameter of gigantic axons is presumably less than 5% and thus does not account for the differences in density between low-density and high-density regions. Calculating an average fiber diameter of about 4.5 μm for the population of fibers that are larger than 3 μm in diameter, the average axon would occupy an area of 15.9 mm². The density of fibers > 3 μm is about 910 fibers/mm² in the posterior midbody (Fig. 1B). Therefore, this axon population occupies an area of about 15,000 μm² in each mm². Considering the myelin sheath and lowering the minimal diameter of these large fibers to 2 μm, the area occupied by these axons in each mm² may be increased to 50,000 μm². This is only 5% of the total area in the posterior midbody. (For the sake of simplicity we assumed that in G1 there are no fibers > 3 μm.) Therefore, increased interfiber distance may better account for the overall decrease in fiber density towards the posterior midbody.

A tentative explanation of the above finding is that the increase in interfiber space results from the process of terminal retraction that takes place in the perinatal period, a process which is more extensive in callosal regions connecting primary and secondary sensory areas. Another possibility is that there is increased oligodendroglial proliferation associated with larger diameter fibers, to accommodate the larger myelin requirement.

**Interhemispheric transfer times**

The estimates of interhemispheric distances (from photographs of brains) are about the same for visual, auditory, somatosensory and perisylvian areas, i.e., between 100 and 130 mm (considering the convoluted fiber paths). We can also estimate the conduction velocities of different myelinated fiber sizes by assuming a linear relation between fiber diameter and conduction velocity, with a proportionality constant of 8.7 mm/ms per μm of axon diameter (Table IV). This has
been calculated in the peripheral nervous system. Conduction velocities of unmyelinated fibers were calculated from Swadlow and Waxman14. Table IV shows the estimated minimal conduction velocities and the longest estimated interhemispheric transmission times for the different fiber types studied.

Using evoked potentials, the interhemispheric transmission times in the occipital lobe have been calculated to be 13 msec28 or 26 msec30. According to Table IV, the interhemispheric delay measured with evoked potentials would fit nicely the population of myelinated fibers between 0.6 and 1.5 μm in true diameter (fibers that are included in the Holmes counts but not in the Loyez counts). In behavioral experiments, the shortest interhemispheric transmission times have been calculated to be around 3 ms for simple motor reaction time tasks, and hemispheric differences can be as long as 300 ms48, though they usually are not longer than 45 ms48. Table IV shows that the shortest transfer times would have to be produced by the gigantic fibers (true diameters larger than 4.6 Mm).

Further, interhemispheric transfer times of less than 45 ms might be too short for unmyelinated fibers, but can be accommodated by small-diameter myelinated fibers (that tend to connect association cortex and transfer cognitive rather than sensory information). Still, relatively small behavioral laterality effects (around 45 ms) may not necessarily reflect interhemispheric exchange of cognitive information through small myelinated fibers, but rather inherent differences in speed of hemispheric computations. Unmyelinated fibers may play only a subsidiary role in interhemispheric exchange (they are relatively few), particularly in the integration of slow and complex control processes.

In this context, it is interesting to mention the work of Grafstein16, who found that unmyelinated callosal fibers produce an evoked potential of longer latency and of opposite sign than myelinated fibers. One role of unmyelinated fibers may perhaps be thought of as an aftereffect of the excitation or inhibition produced by faster, myelinated fibers, thereby modulating the temporal dimension of the interhemispheric stimulus. As mentioned, La Mantia and Rakic7 suggest that, in general, fine-diameter axons may exert a tonic (as opposed to phasic) influence on the contralateral side.

### Relations between density, area and total fibers

The negative correlation between fiber density and callosal area reported by La Mantia and Rakic7 was not detected in this sample (Table III). This was true for all the fiber populations studied and for every callosal segment investigated.

As described in Materials and Methods, our sampling procedure consisted of counting fibers in the region equidistant from the superior and inferior edges of the corpus callosum. Fibers close to the superior and inferior edges were not included. By contrast, La Mantia and Rakic7 counted fibers in the whole vertical extension of the callosum. Could this sampling difference account for the discrepant findings? If fibers closer to the dorsal and ventral edges of the callsum have a strong, negative correlation with callosal area, a decrease in overall fiber density with increasing callosal area would be a reasonable possibility. In a subsample of 8 brains, we found no significant correlations between fiber density in these locations and segment area (-0.56 < r < 0.61; P > 0.05, n = 8; see Materials and Methods). Therefore, our suggestion of a lack of correlation between fiber density and callosal area remains valid.

As the corpus callosum increases in cross-sectional area, more fibers tend to cross through because density remains, on the average, relatively the same for different callosal sizes. However, this may hold only for the groups of fibers larger than 0.4 and 1 pm since their variances are relatively small. The variances of fibers larger than 3 and 5 μm are sufficiently high (Table I) that a change in callosal size may not significantly affect the total number of fibers. Consequently, the correlation between the total number of callosal fibers of each type (average density x callosal area) and callosal area itself was significant for fibers larger than 0.4 and 1 μm (r > 0.64; P < 0.01; n = 20), but not significant for fibers larger than 3 and 5 Mm (r < 0.27; P > 0.05; n = 20). The one exception to this rule was the isthmus, where number of fibers > 1 Mm correlated positively with isthmus area.

Therefore, the present findings suggest that cross-sectional callosal area may be used to estimate the total number of the very abundant, relatively small fibers (which, for the most part, connect higher-order

### Table IV

<table>
<thead>
<tr>
<th>Fiber group</th>
<th>Corrected diameter μm&gt;</th>
<th>Velocity  Imm /ms</th>
<th>Delay Imm</th>
<th>Interhemispheric delay times (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmyelinated</td>
<td>0.1-1</td>
<td>0.3-3</td>
<td>50-433</td>
<td></td>
</tr>
<tr>
<td>0.4 μm</td>
<td>0.6</td>
<td>5.2</td>
<td>19.2-24.9</td>
<td></td>
</tr>
<tr>
<td>1 μm</td>
<td>1.5</td>
<td>13.1</td>
<td>7.6-9.9</td>
<td></td>
</tr>
<tr>
<td>3 μm</td>
<td>4.6</td>
<td>40</td>
<td>2.5-3.2</td>
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</tr>
<tr>
<td>5 μm</td>
<td>11</td>
<td>67</td>
<td>1.5-1.9</td>
<td></td>
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</table>

As the corpus callosum increases in cross-sectional area, more fibers will tend to cross through because density remains, on the average, relatively the same for different callosal sizes. However, this may hold only for the groups of fibers larger than 0.4 and 1 μm since their variances are relatively small. The variances of fibers larger than 3 and 5 μm are sufficiently high (Table I) that a change in callosal size may not significantly affect the total number of fibers. Consequently, the correlation between the total number of callosal fibers of each type (average density x callosal area) and callosal area itself was significant for fibers larger than 0.4 and 1 μm (r > 0.64; P < 0.01; n = 20), but not significant for fibers larger than 3 and 5 Mm (r < 0.27; P > 0.05; n = 20). The one exception to this rule was the isthmus, where number of fibers > 1 Mm correlated positively with isthmus area. Therefore, the present findings suggest that cross-sectional callosal area may be used to estimate the total number of the very abundant, relatively small fibers (which, for the most part, connect higher-order...
processing cortices). Callosal area is less useful in estimating the number of scarce, large fibers (larger than 3 \( \mu m \), which tend to be concentrated in regions connecting sensory areas).

Our results agree with Tomasch\(^4\), who, despite having only three human subjects, did not find a relation between callosal area and fiber density. A more recent study on callosal fibers\(^5\) also suggests that in humans, a larger callosal area indicates that there is an increased number of fibers.

**Sex differences**

No sex differences were found in the fiber composition of the corpus callosum. This result is interesting, considering the robust difference in brain weight between the sexes. One possibility is that, as discussed by Peters\(^35\), sex differences in brain weight relate to cortical areas that receive and send projections to the sensory and motor surfaces, and not to the association areas, which may comprise the bulk of the callosal projection. It is still unclear whether the larger brain of males (and presumably their larger cortex) includes a larger number of cortical neurons. From cross-species comparisons, it is known that the number of neurons increases at a slower rate than the size of the brain\(^24\). Haug\(^20\) reported that cell density is smaller in the larger cortex of human males than in the smaller cortex of females, resulting in a similar number of neurons in the two sexes. If this is the case, it would not be surprising that there are no sex differences in the number of callosal fibers, since similar numbers of neurons would project through the callosum.

**Electron microscopy**

**Estimation of total callosal fibers**

The mean fiber density (about 38 fibers/100 \( \mu m^2 \)) calculated from our electron micrographs falls within the range observed in the macaque by la Mantia and Rakic\(^7\), although it is lower than the mean obtained by them (mean: 76.62 fibers/100 \( \mu m^2 \), with a threefold range of variation). When the minimal diameter observed in the Holmes stain (0.4 \( \mu m \) corrected to a value of 0.6 \( \mu m \)) was matched to the frequency histograms of fiber diameters as seen in electron microscopy, it was found that approximately 20% of the fibers were not detected under light microscopy (see Fig. 4). This yields about 2 x 10^7 fibers in the whole corpus callosum.

**Species differences**

When comparing these results with the macaque\(^25\)\(^-\)\(^27\)\(^-\)\(^42\), two main differences appear. First, La Mantia and Rakic\(^27\) report that, especially in the region of higher fiber diameters, two equally sized peaks are evident in the histograms of fiber size frequencies: one at 0.6-0.8 \( \mu m \), and the other at approximately 1 \( \mu m \). This second peak appeared more like a shoulder to the first peak in humans (Fig. 4). In other words, small fibers (0.7 \( \mu m \) in diameter) seem to make up a higher proportion of the total fibers in the human than in the macaque. These results are in agreement with the histograms of fiber size frequencies reported by Tomasch\(^43\) under light microscopy. Comparing fiber size frequencies in the callosum of the mouse and the macaque. Jerison\(^25\) presented a figure with a similar pattern: very thin fibers seem to represent a higher proportion of the total in the macaque than in the mouse.

The explanation of this difference may relate to the higher proportion of association areas present in the human brain. As mentioned, it has been suggested\(^27\) that higher-order processing areas tend to be connected through smaller-diameter axons in the corpus callosum than do primary sensory areas. Perhaps the increase in the proportion of small axons reflects the relative growth of the so-called association areas in the human brain.

The diameters of unmyelinated fibers have been reported to be similar across species, and if anything, their average diameter may tend to decrease with increasing brain size\(^6\).

The second difference relates to the upper limit of fiber diameters seen in different species. In the human, we saw fibers up to 15 \( \mu m \) in diameter (corrected size) under light microscopy, while using electron microscopy the largest fiber was about 13 \( \mu m \) in diameter (not shown in the Figures), observed in the midbody. The largest diameter fibers reported by Swadlow et al.\(^42\) in the macaque were of 8 \( \mu m \) (however, they did not specify the callosal region investigated). La Mantia and Rakic\(^27\) make no mention of the maximal axon diameter found in the macaque, but from their pictures, the largest axons may reach 11.4 \( \mu m \). In the cat, the largest diameter callosal fibers connecting sensory-motor cortices are about 6 \( \mu m \), with an occasional fiber larger than 8 \( \mu m \). Waxman and Swadlow\(^44\) report a maximal axon diameter of 185 \( \mu m \) in the splenium of the rabbit. Jerison\(^25\) reports that gigantic fibers are more abundant in the macaque than in the mouse. It thus seems that there is a small population of large and gigantic fibers whose diameter increases with increasing brain size. This group of very large axons may compensate for the increased delay of interhemispheric transfer that occurs in primary sensory regions in species with larger brains. On the other
hand, interhemispheric transfer in association areas is not related to early steps of sensory processing, and the imposed delay of increased interhemispheric distance may be irrelevant when compared to the time of intrins-ic processing.

Regional differentiation

The pattern of regional differentiation seen in the electron microscopy sample agrees with the findings of La Mantia and Rakic\(^\text{1}\)\(^\text{1}\), and with the light microscopy studies presented here. The only additional point is that the most common axon diameter increased steadily from 0.6 \(\mu\text{m}\) in the genu to 1 \(\mu\text{m}\) in the splenium.

However suggestive, the electron microscopy findings reported here must be considered with caution. The results were obtained from only one subject and the conditions of histological fixation in human material are not optimal.

Final conclusions

This study concludes that (i) the regional histological differentiation of the human corpus callosum has similarities and differences with that reported in the macaque. There is a larger proportion of large diameter fibers in callosal regions that interconnect primary sensory (and perhaps motor) areas than in regions that interconnect association and prefrontal areas. In the human, the region with the highest density of large callosal fibers is the posterior midbody, and it is proposed to represent the primary and secondary auditory areas.

(ii) The callosal area may be considered a good estimator of total fibers in the human corpus callosum, but perhaps not of fibers larger than 3 \(\mu\text{m}\) in diameter.

(iii) No sex differences were found in microscopic callosal structure.

(iv) The electron microscopy study suggests that species differences in callosal structure might relate to two parameters: (1) proportions of thin fibers (less than 1 \(\mu\text{m}\) in diameter), and (2) size of gigantic fibers (more than 3 \(\mu\text{m}\) in diameter). Presumably, as brains grow larger, thin fibers may increase in number due to the relative increase in association areas, and gigantic fibers in primary sensory areas may increase in size in order to maintain the continuity of the two sensory hemi-fields at early stages of information processing. However, these latter conclusions need to be confirmed since our observations were made on a single subject.

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