Division of the corpus callosum into subregions

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Abstract

Various attempts have been made to subdivide the corpus callosum (CC) into anatomically and functionally distinct subareas. A promising current approach is the use of factor analytic techniques in conjunction with traced MRI images. The traced images are divided into 99 percentile slices, where the widths of the percentile slices are used as variables that are entered into the analysis (Denenberg, Kertesz, & Cowell, 1991). Studies that use this technique agree broadly between 6 and 7 factors, but available factor solutions contain inconsistencies and large gaps, which arise when many of the percentile slices do not load appreciably on any of the factors. The present study uses a larger number of brains (N = 184), all normalized, and some methodological refinements in the analysis of the traced MRI images of the CC. A stable 7 factor solution was found, and the factor structure for males and females was very similar. © 2002 Elsevier Science (USA). All rights reserved.

1. Introduction

The corpus callosum (CC) at the midsagittal level is visually distinct in brain images and lends itself well to quantitative measurement. Advances in relating function to callosal regions require a better subdivision of callosal architecture than is available now. Several formal approaches to subdivide the corpus callosum have been attempted, and these are summarized in Thompson, Narr, Blanton, and Toga (in press). Some approaches are based on purely geometrical solutions. For instance, Clarke and Zaidel (1994) and Witelson (1989) proposed a subdivision of the CC that was based on geometrical schemes. However, a potentially more interesting method has been developed by Denenberg and colleagues (Denenberg et al., 1991). Their method is based on a factor analytic approach that attempts to establish subregions of the CC based on statistically defined internal cohesiveness. Denenberg et al. subdivided the CC into 99 percentile slices and then used widths of the CC defined by these percentile slices in a principal components analysis. The factor structure defined by Denenberg et al. (1991) yields seven factors, albeit with major gaps for percentile widths that do not load significantly on any of the factors. We believe that this approach is promising and further exploration is desirable. Our study differs in two ways from previous
studies that used factoring techniques. First, we used a larger number of subjects than studied before, and, second, we used brain images that had been normalized, under the assumption that normalization allows some degree of control for overall differences in brain size of individual subjects. However, the emphasize here is on “some degree” because it is evident that normalization procedures do not solve all problems of the relation between brain size and a two-dimensional measure such as the mid-sagittal section of the corpus callosum (Bermudez & Zatorre, 2001).

2. Method
2.1. Participants

The brain scans of 184 healthy subjects, average age 27.1 (SD 9.0) years, were used for this analysis. Of these, 97 were males and 87 were females.

2.2. Scan procedures

In vivo magnetic resonance morphometry was performed using a 1.5 T magnet (Siemens Magnetom SP, Erlangen, Germany) and a circularly polarized head coil. After parallel alignment of the interhemispheric plane of the brain with the sagittal plane of imaging, a strongly $T_1$—weighted gradient echo pulse sequence (fast low-angle shot) with the following technical factors was applied: 40 ms repetition time, 5 ms echo time, 40° flip angle, one excitation, 25 cm field of view, $256 \times 256$ matrix, and 128 sagittal slices with 1.17 mm single slice thickness. These images were transferred to ANALYZE-format for post-processing. Post-processing of these brain volume data was performed within SPM99 (http://www.fil.ion.ucl.ac.uk/spm) if not otherwise noted. Each anatomical scan was realigned and normalized into a standardized space using a standard template provided by the Montreal Neurological Institute (Evans et al., 1993). Normalization was performed using only linear transformations. Following normalization, the images were segmented. The segmentation procedure in SPM99 utilizes a modified mixture model cluster analysis technique to partition the brain into gray matter, white matter, CSF, and scalp. In brief, the technique operates by comparing each brain voxel with voxels from a set of similarly normalized prior probability images. The probability images specify the likelihood that each voxel belongs to one of the aforementioned tissue classes. The actual assignment of voxels to a particular tissue class is then determined iteratively depending on the mean and variance of the developing tissue cluster for the brain being analyzed. From the white matter images midsagittal slices were used for manually tracing the corpus callosum using an in-house software tool.

2.3. Analysis

A software tool (called CALLMEA), based on MATLAB software version 5.2 (MathWorks, http://www.mathworks.com) incorporated in SPM99 (http://www.fil.ion.bpmf.ac.uk/spm), was written to compute the percentile widths. Figs. 1a and b show the essentials of the measurement. The central aspect of the Denenberg et al. method is that the CC is divided into 99 percentile slices, drawn across the CC. The crucial step is the placement of the two points of origin of the line that is drawn through the CC in such a way that the sum of percentile slices around that line forms a minimum. Denenberg et al. defined the optimal placement of the line as that line around which the sum of the 99 percentile widths formed a minimum. Denenberg et al. arrived at this line by trial and error. The contribution of the CALLMEA program is that the points for the placement of the optimal line can be determined by
automatic calculation. To this end, two points are placed at the genu and two at the splenium, as shown in Fig. 1a. The placement is arbitrary but is done so that a reasonable number of points of origin of lines can be drawn between the reference points. The program allows a choice as to how many points are placed equidistant from two reference points on the segment of the callosum tracing that lies between the two reference points at each end of the line. Following this, lines are computed that lie between each of the points at the genu and splenium and the sum of percentile widths is calculated for each of the lines. Fig. 1a gives an example of the placement of the four points. Fig. 1b shows the best line that results when all possible lines are drawn between 16 points spaced equidistantly between the points shown in Fig. 1a. We chose 16 points empirically because the use of a larger number of points did not bring about significant changes in the sum of percentile widths around the best possible line. The CALLMEA program computes the percentile widths for all 256 (16 × 16) lines that are drawn between each of the 16 points at one end of the CC to each of the 16 points at the other, and chooses the line around which the sum of percentile widths forms a minimum. The percentile widths that fall on this best possible line form the raw data on which the factor analyses are based. Also shown are a number of other variables, such as the origins of the best possible line at the splenium [T(S)] and genu [T(G)], various length and width measures and a measure of the “height” of the, L(r). All other procedures, including the use of five tracings for each CC, and the operational definition of when one of the tracings differed sufficiently from the others to be discarded followed the Denenberg et al. (1991) description.

3. Results and discussion

3.1. Factor structure

The CALLMEA program provides 99 percentile widths for each CC. These widths were used to perform a principal components analysis, where the widths were
used as variables, as described by Denenberg et al. (1991). In any such analysis, the number of variables should not be too large in relation to the number of cases and this is why Denenberg et al. performed three different analyses, staggering the widths, so that the first analysis was done for the variables W1, W4, W7..., W97, the second for the variables W2, W5, W8...W98, and the third for W3, W6, W9...,W99. The factor loadings for all of the variables were then combined in the presentation of the factor structure. Following established custom, width lines were considered to load on a factor if their factor loadings exceeded .600. Factors were defined by Eigenvalues exceeding 1.00.

Table 1 shows the results of the Denenberg et al. (1991) and three other data analyses (from Table 1 in Cowell, Kertesz, & Denenberg, 1993). It can be seen that (a) there is a relatively good consistency between the studies, (b) there are between 6 and 7 factors and (c) there are large gaps in terms of the factor loadings of the percentile widths. For example, in the first study listed, that by Cowell et al., none of the percentile widths between the 47th to the 55th percentile reached the cut-off value of .600 on any of the factors. All of these studies used the method described above, where the three separate analyses were combined. Table 2 shows the results from our study. Column A shows the factor structure obtained by using the method employed by Denenberg et al. (1991), where three analyses were carried out separately (W1, W4, W7..., W97 /W2, W5, W8...W98, and W3, W6, W9...,W99) with subsequent combination of the results. With this solution, 92% of the variance was accounted for. Individually, the seven factors accounted for 17.9, 15.8, 13.6, 13.4, 12.4, 11.0,

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<td>91–99, 2, 3</td>
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</table>

Note the large gaps in the first three studies.

aData from Allen, Richey, Chai, and Gorski (1991) and Kertesz, Polk, Howell, and Black (1987).

The numbers denote percentile widths. For instance, in the first study (Cowell et al.) Widths 4–15 load on a single factor. Note that in all of the factor solutions, there are large gaps in the mid-range where widths do not have any significant loadings.

Table 2

<table>
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<tr>
<th>Present study, Aa</th>
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<th>Present study C</th>
<th>Present study D</th>
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<td>95–99, 1, 2, 3, 4</td>
<td>94–99, 1, 2, 3, 4</td>
<td>95–99, 1, 2, 3, 4</td>
</tr>
</tbody>
</table>

a For A, three separate analyses for 1, 4, 7...97 and 2, 5, 8......98 and 3, 6, 9......99 were performed and the results combined as in Denenberg et al. (1991).
b For B, all 99 percentile widths were included in a single analysis.
c In some cases, percentile widths bordering two factor regions loaded significantly on both.
and 7.8% of the variance. Column B shows the same type of procedure, but carried out by entering all widths (from W1 to W99) in a single analysis. It can be seen that the factor structure for A and B is very similar. It is likely that this approach is workable because of the relatively large number of brains used. These analyses were done with a varimax rotation. Column C shows the procedure as in Column B, but done with an oblique rotation. Oblique rotations were used in all of the studies shown in Table 1 and Column C shows that the difference between the present and the other students is not due to the use of a different method of factor rotation. The 7 factorsolution in Column C is quite similar to the solutions in A and B but there are some gaps that do not exist in the other solutions (i.e., widths 17, 53, and 54 do not reach a .600 loading on any of the factors).

Finally, the separate analyses for males and females are shown in Columns D and E of Table 2, indicating a similar factor structure for both sexes. This supports the procedure by Denenberg et al., who combined the data from males and females for their analysis. Figs. 2 and 3 show the percentile widths for males and females. A horizontal reference line is drawn in to show the very slight differences in widths; females show slightly greater widths in the splenium while the converse is true for the genu. However, these differences are small. Fig. 4 shows the percentile widths for sexes combined, with lines drawn in to delineate the regions that load on different factors. The first and last region (beginning of the genu and end of the splenium) load on the same factor.

### 3.2. Sex and handedness effects

The literature on sex differences in the CC is by no means in agreement (cf. Bermudez & Zatorre, 2001; Bishop & Wahlsten, 1997; Cowell, Allen, Zalatimo,
Fig. 3. The CC profile for all male subjects. It can be seen that the profiles are very similar. The horizontal reference line suggests a slightly greater width of the splenium relative to the genu for females with the converse pattern for males.

Fig. 4. The CC profile collapsed over sex, because of the essentially very similar profiles. Data from this figure are used for the computations in the following figures. The vertical lines indicate the boundaries of regions in which percentile widths load together on a factor.
& Denenberg, 1992; Davatzikos & Resnick, 1998; Driesen & Raz, 1995; Going & Dixson, 1990; Habib et al., 1991; Holloway, Andersen, Defendini, & Harper, 1993; Jäncke, Preis, & Steinmetz, 1999, 1997; Pozzilli et al., 1994; Sullivan, Rosenbloom, Desmond, & Pfefferbaum, 2001; Thompson et al., in press; Witelson, 1989). A complication in evaluating main effects of sex is that some studies report interactions of sex with age, with handedness, and with “part of the callosum”. Our own data on this issue do not clarify the picture. Figs. 2 and 3 show that the shapes of the male and female callosa are quite similar. However, Denenberg et al. (1991) suggested that average widths in the region of the isthmus were somewhat greater for males than females. In defining the isthmus, Denenberg et al. (1991) counted the percentile widths in the region defined as isthmus by Witelson (Witelson, 1989). However, both the Denenberg et al. data (1991, Table 3) and our own data (Figs. 2–4) show quite clearly that the narrowing of the callosum occurs in a shorter band than indicated by the range of percentile widths from 65 to 85, and that the widths beyond percentile 75 can be considered to be part of the splenium. Using the Denenberg et al. definition of the isthmus, we performed an ANOVA for the factors of Sex × Handedness, for those individuals for whom handedness data were available. No significant differences were observed. However, when using a more restricted region that corresponds more exactly to the isthmus (widths 57–74), a significant, albeit weak (only 3% of the variance in callosum width accounted for by the variable Sex, statistical power = .517), sex difference was observed ($F(1, 126) = 4.1$, $p < .045$). The literature also suggests (Davatzikos & Resnick, 1998; Thompson et al., in press) that the splenium of the female corpus callosum is somewhat more bulbous than that of males. Of course, it should be recalled that agreement on sex differences in subregions of the corpus callosum is by no means universal (Holloway et al., 1993). Our data do not provide any statistically significant evidence on this point. However, it is worth noting that Figs. 2 and 3 show the percentile widths in females to be relatively greater for the splenium than for the genu while the converse is true for females. The fact that no significant interaction between region and sex was obtained suggests that if an effect can be found it will be very small in magnitude, and will require a great number of subjects to be demonstrated.

Finally, it is of interest to note that no significant effects of handedness were found even though our data contained 126 subjects with handedness information as opposed to the 52 subjects in the Denenberg et al. study. However, we are not certain as to the optimal classification of handedness in the context of callosal measures. Unlike other studies, we also had a hand motor performance measure to assess handedness, which allowed independent validation of the handedness classification. The measure is a composite performance measure across several tasks (Jäncke,

<table>
<thead>
<tr>
<th>Grouping of factor widths</th>
<th>Average of the five studies</th>
<th>Present study$^a$</th>
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<tr>
<td>3.6–17.2</td>
<td>4–17 (12.4)</td>
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<td>19.0–41.0</td>
<td>18–37 (17.9)</td>
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<tr>
<td>43.5–58.0</td>
<td>38–54 (15.8)</td>
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<tr>
<td>56.0–66.4</td>
<td>55–66 (11.0)</td>
<td></td>
</tr>
<tr>
<td>69.0–82.6</td>
<td>67–79 (13.4)</td>
<td></td>
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<tr>
<td>86.0–92.0</td>
<td>80–93 (13.6)</td>
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</tr>
<tr>
<td>93.2–99.0$^b$</td>
<td>94–99, 1, 2, 3, 4 (7.9)</td>
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$^a$Values from analysis A in Table 2. Figure in brackets is the amount of variance accounted for as determined by the factor solution.

$^b$Several of these studies also had common loadings of percentile widths 2 and 3 on this factor, ignored in averaging.
The performance measure relates significantly to both handedness classification. If handedness is defined in terms of righthanders vs. nonrighthanders, a significant performance difference emerges \( F = 80.1 \ (p < .0001) \). If handedness is defined in terms of lefthanders, individuals with mixed handedness and righthanders, there also is a significant group difference \( (F = 84.4, \ p < .0001) \). However, in terms of effect size, the former definition of handedness accounts for 38.5% of the variance in hand performance, while the latter accounts for 57.1% of the variance. The classification of individuals in terms of righthanders and nonrighthanders varies greatly as a function of the hand preference questions, and can yield a prevalence of almost no righthanders to more than 90% righthanders (Peters, 1992). In view of this situation, it is unclear what “handedness” means in the quest for a relation between handedness and corpus callosum parameters. The reason for the uncertainty in the literature about the relation between CC parameters and handedness most likely lies not only in procedural differences, but also in different ways of defining handedness (Cowell et al., 1993; Habib et al., 1991; Hopper, Patel, Cann, Wilcox, & Schaeffer, 1994; Steinmetz et al., 1992).

3.3. Correlation between regional width and overall CC surface area

Correlations between individual widths and the overall CC surface area were also computed. The reasoning was that a correlation profile extending over the entire CC would indicate those regions that show greater variation in terms of their contribution to the overall size of the CC, and would therefore show lower correlation coefficients. The correlation profiles shown in Fig. 5 show that such an analysis has some promise. The figure shows the pattern of correlation coefficients for each of the percentile widths with the total CC, averaged over all of the brain scans available in

![Correlation of each percentile width with the total surface area of the cc](image)

Fig. 5. Shows Pearson Product Moment correlation coefficients that were computed between each percentile width and the overall surface area of the CC; it can be seen that the correlation patterns relate to the factor boundaries.
the study. Superimposed are the factor boundaries of the factors shown in Table 4. In comparing Figs. 4 and 5, it can be seen that the factor boundaries in Fig. 5 appear to fall across meaningful boundaries. In performing such an analysis, a natural concern is the fact that each of the variables contributes its value to the entire surface area value for the CC, and is thus part of the whole. However, because each of the widths contributes, on average, only about 1% to the total, this was not perceived to be a significant problem.

4. Discussion

The first point to be raised concerns the degree of agreement between studies. For greater simplicity of comparison, the groupings of the five studies (values for the present study were from analysis A in Table 2) were averaged. The averaged values can be compared with the present study and the correspondence is relatively good, except for the range of percentile widths that extends from, roughly, width 40 to width 55. There are no two studies that can be said to agree more with each other than with others; each has a range of agreement with another study and also ranges of disagreement. The present study appears to agree quite well with the averages from all five studies, and can therefore be considered to represent a reasonable approximation of width groupings of the CC in non-selected healthy human subjects.

The second point concerns the question of whether the factor groupings map on any functional zones so that, e.g., the region from width 4 to 17 would be functionally different from the regions adjacent to it because they load on a different factor. At this point, there is no clear answer to this question. It might be suggested that in the rat at least—where factor analytic techniques also yield seven factors—there is some relation between the factor structure and function (Berrebi et al., 1988; Fitch, Cowell, Schrott, & Denenberg, 1990). However, the regions in question said to be most sensitive to sex hormones, the genu, and splenium, do not show unambiguous factor boundaries. At this point, the only specific association between a particular callosal area and significant functional/pathological relations has been identified by Thompson et al. (in press) who report a localized reduction specific to the isthmus region in patients with Alzheimer’s disease. Several additional observations suggest that the allocation of functional regions of the corpus callosum, as defined by factoring techniques, will be wrought with difficulties. The first is somewhat trivial, and relates to the fact that in three of the five studies listed in Tables 1 and 2, the very first and the very last percentile widths in the genu and splenium load on the same factor. The most likely interpretation of such a pattern is that these widths cluster together not because of characteristics related to function but rather because of common linear characteristics (such as very short widths). The second observation concerns the relation of callosal zones to cortical areas. Coarse allocations can be made, such that the anterior third of the CC connects anterior frontal cortex, the middle third connects the more primary sensory and motor regions and the final third connects some temporal areas as well as the parietal and the occipital cortex. Nevertheless, both human clinical data (De Lacoste, Kirkpatrick, & Ross, 1985) and anatomical analyses in animals (Pandya & Seltzer, 1986) suggest there is no easy direct mapping of the factor-defined callosal regions and anatomical connections. Another complicating factor relates to connections between heterotypical cortical areas that are made across the CC and the anterior commissure (Di Virgilio, Clarke, Pizzolato, & Schaffner, 1999).

Does this invalidate the factoring approach proposed by Denenberg et al. (1991)? We think not. Whether or not the regions defined by factors or components will ultimately turn out to have some significant relation to function, the idea of computing a standard representation of parts of the corpus callosum is useful. Our data
provide two examples of the potential of this method. First, Figs. 2 and 3 allow a direct and very simple evaluation of the sex differences in callosal topography. The computation of statistical differences between specified groups can be done conveniently on the basis of the percentile widths. Ultimately, the percentile representation will also be useful for the exploration of individual differences in callosal architecture as related to cognitive function. In addition, the percentile widths can also be used as raw data for analyses based on Fourier analyses (Ferrario, Sforza, Serrao, Frattini, & Del Favero, 1996).

Finally, the analysis shown in Fig. 5 indicates that while the factors derived from a component analysis of the percentile widths do not relate directly to callosal width topography, they show a closer relation to the pattern of correlations of individual widths with overall callosal surface area. In this latter case, the percentile width approach can lead towards a direct analysis of the characteristics of subregions of the corpus callosum. One possible interpretation of the regional variability in the magnitude of correlations coefficients between individual percentile widths and total surface area of the CC concerns a distinction between areas that are more similar across individuals vs. areas that show greater differences. Thus, regions that show low correlations indicate greater variability within and between individuals than is the case for regions with higher correlations. Perhaps the regions with greater variability are more open to individual experiential factors that determine the amount of interhemispheric traffic than is the case for areas of lower variability which might indicate a greater degree of genetic predetermination of connections. Of course, more complex interpretations involving individual differences in lateralization of function and their impact on interhemispheric connections (Ringo, Doty, Demeter, & Simard, 1994) can also be entertained.

Acknowledgments

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References


