The size and fibre composition of the corpus callosum with respect to gender and schizophrenia: a post-mortem study

J. Robin Highley, Margaret M. Esiri, Brendan McDonald, Mario Cortina-Borja, Brian M. Herron and Timothy J. Crow

Summary
In this study the cross-sectional area (in \( n = 14 \) female controls, 15 male controls, 11 female patients with schizophrenia, 15 male patients with schizophrenia) and fibre composition (in \( n = 11 \) female controls, 10 male controls, 10 female patients with schizophrenia, 10 male patients with schizophrenia) of the corpus callosum in post-mortem control and schizophrenic brains was examined. A gender × diagnosis interaction (\( P < 0.005 \)) was seen in the density of axons in all regions of the corpus callosum except the posterior midbody and splenium. Amongst controls, females had greater density than males; in patients with schizophrenia this difference was reversed. A reduction in the total number of fibres in all regions of the corpus callosum except the rostrum was observed in female schizophrenic patients (\( P = 0.006 \); when controlling for brain weight, \( P = 0.053 \)). A trend towards a reduced cross-sectional area of the corpus callosum was seen in schizophrenia (\( P = 0.098 \)); however, this is likely to be no more than a reflection of an overall reduction in brain size. With age, all subregions of the corpus callosum except the rostrum showed a significant reduction in cross-sectional area (\( P = 0.018 \)) and total fibre number (\( P = 0.002 \)). These findings suggest that in schizophrenia there is a subtle and gender-dependent alteration in the forebrain commissures that may relate to the deviations in asymmetry seen in other studies, but the precise anatomical explanation remains obscure.

Keywords: corpus callosum; fibre; axon; gender; schizophrenia

Introduction
Given the evidence for an alteration of brain asymmetry in schizophrenia (Crow et al., 1989; Crow, 1993; Bilder et al., 1994; Falkai et al., 1995a, b; DeLisi et al., 1997), there is good reason to suspect that there may also be alterations in inter-hemispheric connectivity. Accordingly, many workers have examined the principal inter-hemispheric commissure, the corpus callosum, in schizophrenia.

Rosenthal and Bigelow (1972) measured the average thickness of the middle portion of the corpus callosum (i.e. excluding the genu and splenium) on post-mortem brains. They found an increase in thickness in the schizophrenic cases. Bigelow et al. (1983) replicated this finding when comparing brains from patients with schizophrenia with brains from patients who had suffered from other psychiatric and neurological diseases. Since these two studies, the majority of work on corpus callosum has been performed with MRI.

On callosal width in schizophrenia, various studies have shown (i) no difference between patients and controls (Brown et al., 1986; Smith et al., 1987; Kelsoe et al., 1988; Uematsu and Kaiya, 1988; Casanova et al., 1990; Young et al., 1991); (ii) an increase in schizophrenia (Nasrallah et al., 1986; Machiyama et al., 1987); (iii) a decrease in schizophrenia (Woodruff et al., 1993); (iv) a decrease in female but not male patients (Hauser et al., 1989); (v) a gender × diagnosis interaction, such that females showed an increase, whereas males showed a decrease (Raine et al., 1990). Close examination of these studies provides no real clue as to the reason for the discrepancies.

The anterior–posterior length of the corpus callosum has
also been examined. In the majority of such studies no difference between controls and schizophrenics was found (Rossi et al., 1988b; Uematsu and Kaiya, 1988; Hauser et al., 1989; Casanova et al., 1990; Raine et al., 1990; Colombo et al., 1994); however, in one study an increase (Mathew et al., 1985), and in another a decrease, in length in schizophrenic men but not women was found (Nasrallah et al., 1986). Again, there appear to be no immediately obvious differences between the studies which can account for the discrepancies.

Many researchers have examined the cross-sectional area of the corpus callosum in the mid-sagittal plane. Most have found no significant alteration in schizophrenia (Machiyama et al., 1987; Kelsoe et al., 1988; Rossi et al., 1988b; Uematsu and Kaiya, 1988; Hauser et al., 1989; Stratta et al., 1989; Casanova et al., 1990; Lewine et al., 1990; Colombo et al., 1994). One study showed an increase in callosal area (Rossi et al., 1988a), one study showed an increase in the area of the anterior third of the corpus callosum but no change in the area of the middle and posterior thirds (Uematsu and Kaiya, 1988), two studies showed a decrease in schizophrenic males but not females (Nasrallah et al., 1986; Woodruff et al., 1993), and one study showed a reduction in females, but not males (Hoff et al., 1994).

In a meta-analysis of the literature on mid-sagittal callosal area, Woodruff et al. (1995) concluded that, overall, there is evidence for a small but significant reduction in this area in schizophrenia. As there is some evidence that there are sex differences in the anatomy of this structure (Driesen and Raz, 1995) it is unfortunate that gender was not included in the analysis.

It appears that there is a reduction in overall brain size in schizophrenia (Ward et al., 1996) and it is important to assess whether the reduction in callosal size is merely a reflection of a change in brain size, or whether it is peculiar to the structure. In a few studies this issue either has been addressed by examining the ratio of the area of the corpus callosum to the area of the medial surface of the hemispheres, or by performing analyses whilst covarying for an index of brain structure. In a few studies this issue either has been addressed by examining the ratio of the area of the corpus callosum to the area of the medial surface of the hemispheres, or by performing analyses whilst covarying for an index of brain size. Of such studies, in three no difference (Smith et al., 1987; Uematsu and Kaiya, 1988; Hauser et al., 1989) and in four a reduction (Rossi et al., 1988b; Stratta et al., 1989; Woodruff et al., 1993; Hoff et al., 1994) in patients with schizophrenia compared with controls was reported.

Overall, it appears that there is a small decrease in the size of the corpus callosum in schizophrenia. The small size of this difference between schizophrenic and control individuals is likely to explain the lack of differences found by most authors. Any positive findings obtained with small subject numbers could be due to chance differences in sampling. The effect of gender has been relatively overlooked. Both gender and brain size may affect callosal anatomy and should thus be considered.

It is possible that a greater understanding of the effect of schizophrenia on the corpus callosum may be gleaned from microscopy. In only three studies has this been attempted:

(i) Nasrallah et al. (1983) examined histological sections, stained for nerve fibres, taken from anterior and posterior regions of the corpus callosum. No differences were observed between the controls ($n = 11$) and the schizophrenic patients ($n = 18$) in the density of fibres; however, the researchers reported considerable difficulty in delineating the fibres and questioned the reliability of their measures. Gender was not examined.

(ii) Machiyama et al. (1987) carried out a post-mortem investigation of the corpus callosum in five schizophrenic and seven control brains. They also found no significant difference between the two groups in the total number of fibres, although the statistical power of this study is limited.

(iii) Casanova et al. (1989) studied the density of fibres in the corpus callosum in controls ($n = 13$) and schizophrenic patients ($n = 11$) and found no evidence for any difference between the two groups. There were only three female patients in the sample.

In summary, in these studies small sample sizes were used, a few restricted regions of the corpus callosum were used, other neuropathology was not screened for and gender was not taken into consideration.

The work presented here addresses these issues. Specifically, data are presented on the cross-sectional area and fibre composition of the corpus callosum (divided into nine subregions). Gender has been taken into account, as has overall brain size, where relevant. In addition, all cases have been screened for other psycho- and neuropathology.

On the basis of previous studies reviewed above, most notably that of Woodruff et al. (1995), it was expected that we should find little or no alteration in the cross-sectional area of the corpus callosum in schizophrenia.

In schizophrenia there is a reduction in the brain asymmetries seen in controls (Crow et al., 1989, 1992; Falkai et al., 1992, 1995a; Bilder et al., 1994; DeLisi et al., 1997). Further, there is evidence to suggest that there is an inverse relationship between the degree of asymmetry seen in the brain, and measures of inter-hemispheric connectivity, such that more asymmetrical structures show a lesser degree of interconnectivity (Witelson and Goldsmith, 1991; Aboitiz et al., 1992a, b; Ringo et al., 1994). Accordingly, the working hypothesis at the time the measures were being made was that there would be an increase in total fibre number, and thus in fibre density, in schizophrenia. Subsequent investigations on this set of brains (Highley et al., 1998) have demonstrated a more complex pattern of asymmetry alterations in schizophrenia. Had these data been available prior to the study, other hypotheses might have been formulated.

**Material and methods**

**Subjects**

All of the brains had been fixed by suspension from the basilar artery in 10% formalin solution for several months.
in a number of different centres around the UK. Clinical notes were assessed by a psychiatrist (T.J.C. or Dr Stephen J. Cooper of The Queen’s University of Belfast) to ensure that the control brains were free of psychopathology, and that there was clear evidence that the schizophrenic cases conformed to Diagnostic Statistical Manual IV (American Psychiatric Association, 1994) criteria for schizophrenia or schizoaffective disorder. Note was also taken of the age of disease onset, lifetime history of neuroleptic medication (little, average or much), mode of death (cardiovascular, respiratory, other) and death to brain fixation interval.

All brains were confirmed as being free of significant neuropathology (including Alzheimer’s disease, Parkinson’s disease or stroke) by a neuropathologist (B.McD. or M.M.E.) who was blind to diagnostic category (schizophrenia or control), using the CERAD criteria (Mirra et al., 1991). The control brains were selected from a pool of brains which had been prospectively collected from individuals who died without a history in life of neuropsychiatric disorder and for whom the next of kin had consented for tissues to be used for medical research. The control cases used were selected with cerebrum weight entered as a covariate.

Terminology and callosal subdivision

For the purpose of this study, the corpus callosum was divided into nine regions of interest in a manner similar to that of Aboitiz et al. (1992a, b), and Witelson (1985, 1989). The maximum anterior–posterior length (l) of the corpus callosum was identified; the corpus callosum was then divided by lines perpendicular to this length, at various points along it, as shown in Fig. 1. A dividing line was made at the level of the most anterior point inside the ‘crook’ of the genu, and another dividing line was made from this point, parallel to the maximum anterior–posterior line, through the genu. This yielded nine regions of interest, the names given to which are shown in Fig. 1.

For each corpus callosum region, data on the following variables were obtained: cross-sectional area in the median sagittal plane, density of fibres and total number of fibres present.

As there is evidence for a reduction in the overall size of the schizophrenic brain (see Introduction), where a decrease in some measure of corpus callosal anatomy was found in patients with schizophrenia, the statistical tests were repeated with cerebrum weight entered as a covariate.

Cross-sectional area measurement

To measure the area of the corpus callosum subregions, the brain was bisected in the midplane. The medial surface was then photographed such that the plane of focus was parallel to the plane of the medial face of the hemisphere, and a ruler was in the field of view so that the scale of the image could be ascertained. The photographs were projected on to a sheet of paper and the outline of the callosus traced. The subregions

### Table 1 The sample sizes, cerebrum weight, mode of death, hospital of origin, post-mortem delay and age at death of cases used in this study

<table>
<thead>
<tr>
<th></th>
<th>Cross-sectional area</th>
<th>Fibre composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Schizophrenic</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Number of cases</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Mean age at death (years)</td>
<td>73.8*</td>
<td>67.2</td>
</tr>
<tr>
<td>Mode of death (cardiovascular/respiratory/other)</td>
<td>9/1/4‡</td>
<td>12/3/0</td>
</tr>
<tr>
<td>Hospital of origin (Belfast/Oxford/Runwell)</td>
<td>0/11/3</td>
<td>0/13/2</td>
</tr>
<tr>
<td>Mean death—post-mortem delay</td>
<td>43.4³</td>
<td>41.9</td>
</tr>
<tr>
<td>Mean cerebrum weight (kg)</td>
<td>0.971§</td>
<td>1.129</td>
</tr>
</tbody>
</table>

* Females significantly older than males (P = 0.035). † Two female patients unknown. ‡ No intergroup differences (all P > 0.50). § Females have significantly lighter brains than males (P < 0.0005); trend towards schizophrenics having smaller brains (P = 0.098); trend towards gender × diagnosis interaction (P = 0.099). This is interpreted as evidence for female schizophrenics showing lighter brains than female controls, with male schizophrenics showing a much less marked change. †† Females have signifcantly lighter brains than males (P = 0.062). † Two female patients unknown. ** No intergroup differences (all P > 0.40). †† Females have significantly lighter brains than males (P < 0.0005); trend towards schizophrenics having smaller brains (P = 0.068); significant gender × diagnosis interaction (P = 0.018). Again, this is interpreted as evidence for female schizophrenics showing lighter brains than female controls, with male schizophrenics showing a much less marked change.
J. R. Highley et al.

The subdivisions of the corpus callosum.

Fig. 1 The subdivisions of the corpus callosum.

described above were then marked out, and the corpus callosum outlines were traced over with a black pen in order to darken them. The resulting traces were digitized using a Umax ‘flat bed’ scanner and an Apple Macintosh microcomputer. The digitized images were then examined using the program NIH Image in order to calculate the cross-sectional area in the midplane of the subregions of the callosum.

On a random selection of brains (n = 15), the photography and measurement procedure was repeated a second time in order to calculate a test–retest reliability. For each region two tests were performed, and the results of these tests can be seen in Table 2. First, a matched pairs t-test was performed to ensure that the two sets of measures had comparable means. Secondly, a linear regression was performed. A low value of P for the regression corresponds to a significant relationship between the two sets of measures. Further, this analysis yielded a B statistic corresponding to the gradient of the regression line of the first measure versus the second measure. A value of 1.0 is optimal, corresponding to a one-to-one relationship between the two. A one-sample t-test was used to ascertain whether there was a significant difference between the value of B and 1.0; the results of this test can also be seen in Table 2.

All area measures demonstrated adequate reliability, with the possible exception of the measures of the rostrum. For this measure there was a significant difference between the two sets of measures, as demonstrated by the t-test (P = 0.023); this corresponded to a 5.8% difference between the two. However, the regression analysis demonstrated a significant relationship between the two measures (P < 0.00005) and a satisfactory gradient of the regression line (B = 1.0314), which was not significantly different from 1.0 (P = 0.5755).

Corpus callosum dissection and histological preparation

After the photography described above, a Vernier calliper was used to measure the maximum anterior–posterior length of the corpus callosum, and to determine the boundaries of the nine regions of interest, which were then cut from each other in situ using a scalpel. The resulting blocks were then dissected out, and embedded in paraffin wax. From these, a series of 5 µm thick sections were cut, in a sagittal plane, and stained with the Palmgren silver stain for nerve fibres, using a protocol similar to that described by Bancroft and Stevens (1990).

Image capturing, and measurement of fibre density and number

The Palmgren stained sections were examined under an Olympus BX50 microscope with a ×100 oil immersion objective. Colour digital images from the section were captured using a colour video camera mounted on the microscope, and a Silicon Graphics Indy computer. A black and white reproduction of such an image of the corpus callosum is shown in Fig. 2. It should be noted that whereas in Fig. 2 differentiation between glial cells and large fibres is difficult, in the colour images this is considerably easier.

Images were captured from a grid pattern of points that covered the entirety of the section. From a pilot study it was determined that 20 images per region would give a reasonable estimate of the fibre density. The spacing of the grid pattern of images was adjusted to give approximately this number. If excess images were obtained, then images from regular intervals throughout the series (say every fifth image) were discarded. The remainder were then displayed on the computer screen using the software package ‘xv’ at an
Schizophrenia, gender and the corpus callosum

Table 2 Reliability of area measures of the corpus callosum subregions

<table>
<thead>
<tr>
<th>Subregion</th>
<th>Mean difference (%)</th>
<th>P of difference</th>
<th>B of regression</th>
<th>P of regression</th>
<th>P of difference between B and 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rostrum area</td>
<td>5.83</td>
<td>0.023</td>
<td>1.0314</td>
<td>&lt;0.00005</td>
<td>0.5755</td>
</tr>
<tr>
<td>Inferior genu area</td>
<td>1.66</td>
<td>0.324</td>
<td>1.0381</td>
<td>&lt;0.00005</td>
<td>0.5496</td>
</tr>
<tr>
<td>Superior genu area</td>
<td>2.63</td>
<td>0.259</td>
<td>0.8759</td>
<td>&lt;0.00005</td>
<td>0.2582</td>
</tr>
<tr>
<td>Posterior genu area</td>
<td>0.84</td>
<td>0.605</td>
<td>0.9650</td>
<td>&lt;0.00005</td>
<td>0.5773</td>
</tr>
<tr>
<td>Anterior midbody area</td>
<td>0.62</td>
<td>0.536</td>
<td>1.0528</td>
<td>&lt;0.00005</td>
<td>0.3649</td>
</tr>
<tr>
<td>Middle midbody area</td>
<td>1.48</td>
<td>0.280</td>
<td>0.9844</td>
<td>&lt;0.00005</td>
<td>0.8335</td>
</tr>
<tr>
<td>Posterior midbody area</td>
<td>2.45</td>
<td>0.181</td>
<td>0.9848</td>
<td>&lt;0.00005</td>
<td>0.8640</td>
</tr>
<tr>
<td>Isthmus area</td>
<td>0.98</td>
<td>0.531</td>
<td>0.9099</td>
<td>&lt;0.00005</td>
<td>0.0983</td>
</tr>
<tr>
<td>Splenium area</td>
<td>0.65</td>
<td>0.385</td>
<td>0.9530</td>
<td>&lt;0.00005</td>
<td>0.2416</td>
</tr>
</tbody>
</table>

eventual magnification of approximately ×4600. A stereology counting frame which corresponded to 25 × 25 µm was placed over the image and the number of fibres within the frame was counted according to stereological rules (Gundersen et al., 1988). During processing, embedding and staining, the tissue shrinks. A shrinkage factor was therefore calculated. From this shrinkage factor and the density counts described above, the density of fibres in the callosal subregions was calculated.

On a random selection of 20 images, the counts of the fibre density were repeated by a second experimenter (M.M.E.) in order to assess inter-rater reliability. A paired samples t-test demonstrated that there was no significant difference between the mean of the two observers’ sets of measures of fibre density (P = 0.410). Secondly, a regression analysis demonstrated a significant relationship between the two sets of measures (P < 0.00005). The regression line showed a satisfactory gradient which was not significantly different from 1.0 (B = 1.0380, P = 0.686).

From the density and cross-sectional area measurements, an estimate was made of the total number of fibres passing through the different regions of the corpus callosum.

Results

Cross-sectional area

A bar chart of the mean values of the mean cross-sectional area of each of the callosal subregions is shown in Fig. 3. The data for all the measures were standardized producing a Z score. A principal components analysis was then performed on these Z scores. The reason for standardizing the data was that if one region had a large variance due to having values over a large range, and another region had variance of considerably less numerical value, the variance in the former would swamp that of the latter. The result of this would be that the factors generated are disproportionately representative of variation in the former measure as opposed to the latter. Standardizing the measures eliminated this bias. The factors were then entered into a MANOVA (multivariate analysis of variance) as dependent variables with gender and diagnosis as independent variables and age as a covariate.

The principal components analysis yielded two factors, accounting for a total of 75.6% of the variance. Factor 1 corresponded to the area of all callosal regions with the exception of the rostrum. Factor 2 corresponded to the area of the rostrum. The derived factors and their coefficients for each subregion are presented in Table 3.

The regression analysis revealed an effect of age on factor 1 (P = 0.018), but not factor 2 (P = 0.339). This implies that the area in each callosal region except the rostrum decreases with increasing age.

The MANOVA revealed no effect of gender (P = 0.248), a trend towards a diagnosis effect (P = 0.098) and no interaction between the two (P = 0.207). A pair of post hoc ANOVAs (analysis of variance) indicated that this diagnosis trend was due to a trend towards an effect on factor 1 (all regions bar rostrum, P = 0.098, schizophrenics smaller), but not factor 2 (rostrum, P = 0.132). This ANOVA for factor 1 was repeated with cerebrum weight as well as age as a covariate, and in this analysis, the diagnosis trend was not significant (P = 0.374). This implies that the trend towards a callosal reduction in schizophrenia is merely a reflection of an overall brain weight reduction.

Fig. 2 The corpus callosum stained for nerve fibres (bar = 50 µm).
Thus, in summary, with increasing age, there is a decrease in the cross-sectional area of all callosal regions with the exception of the rostrum. There is a slight trend towards a reduction of the cross-sectional area in all regions except the rostrum in schizophrenia. This, however, is likely to be no more than a reflection of an overall decrease in brain size in schizophrenia.

**Fibre density**

A bar chart of the mean values of the fibre densities of each of the callosal subregions is shown in Fig. 4.

The fibre density data from the callosal subregions were entered into a principal components analysis. Three factors were generated, explaining a total of 79.4% of the variance. Factor 1 corresponded to the fibre density in all areas except the splenium, and a slightly lesser contribution from the rostrum and posterior midbody. Factor 2 corresponded to the splenium. Factor 3 corresponded to the rostrum and posterior midbody, such that increasing values of this factor indicated increasing densities in the rostrum, and decreasing densities in the posterior midbody. The derived factors and their coefficients for each subregion are presented in Table 4.

These factors were entered into a MANOVA with age as a covariate and gender and diagnosis as factors.

The regression analysis for age revealed that there was a trend towards an effect on factor 1 ($P = 0.057$, density reduces with age), but no effect of age on either of the other factors ($P = 0.804$ for factor 2; $P = 0.976$ for factor 3). The MANOVA demonstrated no main effect of diagnosis ($P = 0.211$), no main effect of gender ($P = 0.721$) but a gender $\times$ diagnosis interaction ($P = 0.015$). Subsequent post hoc ANOVAs revealed that the interaction was due to an effect on factor 1 ($P = 0.005$), as opposed to factor 2 ($P = 0.560$) or factor 3 ($P = 0.161$). This interaction survived Bonferroni correction. The post hoc ANOVA for factor 1 was repeated with cerebrum weight as a covariate, and the gender $\times$ diagnosis interaction remained robust ($P = 0.010$), suggesting that it could not be attributed to differences in overall brain size.

As can be seen from Fig. 4, this pattern of results corresponded to the female controls having a greater fibre density than the male controls, with this difference reversed in schizophrenia. This pattern was seen in all callosal areas with the exception of the posterior midbody, and was present in only attenuated form in the splenium. The fact that the rostrum shows the interaction effect, but not the posterior

### Table 3 The factor loadings for callosal area factors 1 and 2, and the percentage of variance contributed by each factor

<table>
<thead>
<tr>
<th>Subregion</th>
<th>Factor 1 (60.5% of variance)</th>
<th>Factor 2 (15.2% of variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rostrum</td>
<td>0.07462</td>
<td>0.96199</td>
</tr>
<tr>
<td>Inferior genu</td>
<td>0.68418</td>
<td>0.11274</td>
</tr>
<tr>
<td>Superior genu</td>
<td>0.81856</td>
<td>-0.05440</td>
</tr>
<tr>
<td>Posterior genu</td>
<td>0.73177</td>
<td>0.48621</td>
</tr>
<tr>
<td>Anterior midbody</td>
<td>0.94176</td>
<td>0.07319</td>
</tr>
<tr>
<td>Middle midbody</td>
<td>0.85799</td>
<td>-0.20584</td>
</tr>
<tr>
<td>Posterior midbody</td>
<td>0.84435</td>
<td>-0.36750</td>
</tr>
<tr>
<td>Isthmus</td>
<td>0.82716</td>
<td>-0.06015</td>
</tr>
<tr>
<td>Splenium</td>
<td>0.86115</td>
<td>0.00877</td>
</tr>
</tbody>
</table>

Factor 1 = area of all callosal regions except the rostrum. Factor 2 = area of the rostrum.
midbody is reflected by the fact that the rostrum contributes to factor 3 to a lesser extent than the posterior midbody.

In summary, with age, there was a trend towards a reduction in fibre density in all regions with the exception of the posterior midbody and splenium. Further, for the controls, in all callosal regions (with the exception of the posterior midbody and splenium), the females had a greater fibre density than males. This pattern was reversed in schizophrenia. This effect was not influenced by brain weight.

**Total number of fibres**

A bar chart of the mean number of fibres passing through each of the callosal subregions is shown in Fig. 5.

The principal components analysis generated two factors accounting for a total of 79.0% of the variance. Factor 1 corresponded to all callosal regions bar the rostrum. Factor 2 corresponded to the fibre number of the rostrum alone. The derived factors and their coefficients for each subregion are presented in Table 5.

The regression analysis of the effect of age on these factors demonstrated a significant effect on factor 1 ($P = 0.002$, fibre number reducing with age), but not on factor 2 ($P = 0.284$).

The MANOVA suggested no main effect of gender ($P = 0.508$) and no main effect of diagnosis ($P = 0.233$); however, a trend towards a gender × diagnosis interaction was indicated ($P = 0.063$).

In order to elaborate on the gender × diagnosis interaction trend, a separate post hoc ANOVA was performed for each factor. These revealed that the interaction effect was on factor 1 ($P = 0.030$) as opposed to factor 2 ($P = 0.268$); this interaction does not survive a Bonferroni correction, and thus should be considered a trend. Examination of Fig. 5 reveals that this trend is associated with the females, where...
schizophrenics show a decrease in the number of fibres in all regions of the corpus callosum (with the exception of the rostrum) when compared with control individuals. A pair of post hoc ANOVAs looking at the effect of diagnosis on factor 1 separately for the males and the females confirmed this observation (for the females, P = 0.006; for the males, P = 0.442). The analysis for the effect of diagnosis on factor 1 in the females, when repeated using cerebrum weight as a covariate, shows only a trend towards an effect of diagnosis (P = 0.053).

In summary, these data show a reduction in the number of fibres in the female schizophrenics with little change in the males. This effect is reduced to a trend when one controls for brain size as indexed by cerebrum weight. Further, with increasing age, there is a reduction in the total number of fibres in all regions except the rostrum.

**Artefact analysis**

There may be concern that the effects of diagnosis and gender found in this paper were due to differences in age between the groups, in spite of the fact that this factor was accounted for by analysis of covariance. If this were the case, it would mean that the effect of age on callosal anatomy differs between controls and schizophrenics, or between males and females. This was addressed statistically for the two callosal factors which were found to be altered in schizophrenia (fibre density factor 1 and fibre number factor 1). For these factors, Pearson’s correlation coefficient (r) for the relationship between the factor and age was calculated. In addition, a regression analysis was performed with the factor as dependent variable and age as the independent variable, yielding a value for the gradient (B) of the fitted regression line. These calculations were performed separately for the males and females, and controls and schizophrenics. If it were the case that the effect of age on callosal anatomy differs between groups, one would expect to find statistically significant differences between males and females, and controls and schizophrenics in the values of r and B. Neither r nor B showed any effect of either gender or diagnosis when compared by Fisher’s z-test, or t-test, respectively (all P > 0.05).

The two variables which were shown to be affected by schizophrenia in this study (fibre density factor 1 and fibre number factor 1) are shown in Table 5.
The following conclusions can be drawn.

Discussion

The following conclusions can be drawn.

(i) There is a gender × diagnosis interaction on the density of fibres in all callosal regions with the exception of the posterior midbody and splenium. In controls, females have a greater fibre density than males; however, in schizophrenia this difference is reversed. There are no main effects of gender or diagnosis on this measure.

(ii) There is a trend towards a reduction in callosal cross-sectional area in schizophrenia; however, this is probably an effect of a reduction in overall brain size.

(iii) There is a significant reduction in the number of callosal fibres in the female schizophrenics compared with the female controls. No alteration in fibre number appeared in the comparison between male schizophrenics and controls. Caution should be exercised before this is labelled as a significant gender difference, as the gender × diagnosis interaction on fibre number was only a trend after Bonferroni correction. When alterations in brain weight are compensated for, this is reduced to a near significant trend (P = 0.053).

(iv) With increasing age, the corpus callosum cross-sectional area and fibre number are reduced. There is also a trend towards a reduction in the density of fibres in the callosum.

No effect of either hospital of origin, mode of death or neuroleptic medication could be detected in the schizophrenic patients.

The gender × diagnosis interaction on the density of fibres in the majority of corpus callosum regions is the most striking result obtained in this study. It should be noted that although this interaction appears in the majority of the callosal subregions, it does not occur in all of them: the effect is attenuated in the posterior midbody and splenium. On the basis of what is known from primate commissural connectivity, it is probable that these latter regions have in common the fact that they carry fibres between primary sensory cortices (the primary somatosensory and auditory cortices in the case of the posterior midbody, and the primary visual cortex in the case of the splenium; Pandya and Seltzer, 1986; Demeter et al., 1990). Therefore, the sparing of these areas is in keeping with the suggestion that schizophrenia is a disease which principally effects higher order (heteromodal association) brain regions, as opposed to more primary areas (Pearson et al., 1996).

The results of this work demonstrate the importance of considering gender when studying schizophrenia. There have been prior reports of brain changes in schizophrenia which have differentially affected the sexes: Vázquez-Barquero et al. (1995) have noted a greater ventricular dilation in female than male schizophrenics. Most relevant to the current study, Hoff et al. (1994) report a reduction in the cross-sectional area of the corpus callosum in female, but not male schizophrenic patients. In a similar manner, in the current study we found the following reductions in the females alone: a non-significant trend towards a decrease in the cross-sectional area, and a significant reduction in the fibre numbers in all callosal regions with the exception of the rostrum. Hoff et al. (1994) report that the reduction which they observe in cross-sectional area in the callosum is preserved even when compensating for alterations in brain weight. This was not the case for the cross-sectional area measures presented here. For the number of fibres, however, the effect was reduced to an almost significant trend (P = 0.053) when controlling for brain weight.

The reduction in total fibre number appears to affect the female schizophrenic patients more markedly than the males. However, it should be noted that the lack of alterations in schizophrenic males, but not females, is not a universal finding. In MRI studies, Nasrallah et al. (1986) have found a reduction in the anterior–posterior length of the corpus callosum in schizophrenic males, but not females, and both Nasrallah et al. (1986) and Woodruff et al. (1993) have found a decrease in callosal cross-sectional area in schizophrenic males but not females.

The demonstration in controls that females have a greater fibre density in the corpus callosum than males is a novel finding. This was not detected by Aboitz et al. (1992a) in their study of the fibre composition of the corpus callosum.
The most likely reason for the discrepancy is that these researchers sampled only from points in the centre of the corpus callosum subregions, whereas in the current study the images were sampled from points evenly distributed over the whole of each subregion.

The gender × diagnosis interaction in fibre density, and the possible female-specific fibre number reduction in schizophrenia should be seen in the light of a similar finding on this set of brains regarding asymmetry in schizophrenia (Highley et al., 1998). Here the curved lengths over the lateral and superior surfaces of the cortex from the frontal and occipital poles to the central sulcus were measured, and a gender × diagnosis interaction on the superior measure of the asymmetry of the frontal lobes was observed. This effect was due to variations in the size of the left frontal lobe. The precise relationship between schizophrenia, gender, asymmetry and interhemispheric connectivity remains elusive. The present finding of marked gender differences in the anatomy of the schizophrenic brain contrasts with a general similarity of clinical picture in the two sexes.

In conclusion, the findings reported here reveal changes in the fibre composition of the corpus callosum that are apparent only when gender is taken into account. Thus, in most areas density of fibre content is increased in males with schizophrenia relative to controls, whereas in females it is decreased. For fibre number there was a decrease that was relatively selective to females. Sex thus has an important influence on inter-hemispheric connectivity in schizophrenia. It must presumably relate to the well established sex difference in age of onset (Penrose, 1991; Hafner et al., 1993) and to the sex difference in pre-morbid precursors of illness (Crow et al., 1995). Gender differences in the gross structure of the corpus callosum have been much discussed and on the basis of a thorough meta-analysis (Bishop and Wahlsten, 1997) can be generally discounted. However, in this study we find that fibre density is greatest in most areas in females than in males. To what physiological function might this relate? We know of only one variable—lateralization—for which there is a sex difference, that may relate both to schizophrenia and to callosal connectivity. In terms of relative hand skill females are more lateralized than males and also have greater verbal ability (Crow et al., 1998). It has been suggested that handedness and the structure (Witelson, 1989) and fibre composition (Aboitiz et al., 1992b) of the corpus callosum are related. A simple view of this relationship—that brains which are more ‘lateralized’ have fewer callosal connexions—will not account for the sex difference that we find, nor in any direct way for the changes in schizophrenia. We nevertheless consider that some formulation—the precise nature of which at present eludes us—of this relationship that takes into account age of onset and rate of hemispheric development may account for the anatomical changes and the impairments of hemispheric communication in schizophrenia.

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References


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Schizophrenia, gender and the corpus callosum


Raine A, Harrison GN, Reynolds GP, Sheard C, Cooper JE, Medley I. Structural and functional characteristics of the corpus callosum in schizophrenics, psychiatric controls, and normal controls. A magnetic resonance imaging and neuropsychological evaluation. Arch Gen Psychiatry 1990; 47: 1060–64.


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