Asymmetry of the Uncinate Fasciculus: A Post-mortem Study of Normal Subjects and Patients with Schizophrenia

The uncinate fasciculus interconnects the anterior temporal and inferior frontal lobes. The temporal lobes show a number of anatomical asymmetries, some of which are altered in schizophrenia. This study was performed to assess the size and symmetry of the uncinate fasciculus in normal subjects and in patients with the disorder. The area, fibre density and total fibre number of left and right uncinate fasciculi were estimated using stereological methods in 21 control subjects and 17 schizophrenics. The uncinate fasciculus was found to be asymmetrical in both sexes, being 27% larger and containing 33% more fibres in the right than the left hemisphere. Of the 25 brains from which both hemispheres were available, the size asymmetry was seen in 20 subjects and the greater number of fibres in 21 subjects. There was no significant effect of schizophrenia upon the uncinate fasciculus, nor interactions of diagnosis with side or sex. We conclude that the uncinate fasciculus is larger in the right hemisphere, perhaps indicating greater right-sided fronto-temporal connectivity. The unchanged size of the fasciculus in schizophrenia contrasts with commissural tracts, which are affected in the left hemisphere, perhaps indicating greater right-sided fronto-temporal connectivity. The unchanged size of the fasciculus in schizophrenia contrasts with commissural tracts, which are affected in the left hemisphere, perhaps indicating greater right-sided fronto-temporal connectivity.

Introduction

The uncinate fasciculus is the major fibre tract connecting the inferior frontal and anterior temporal lobes (Klingler and Gloor, 1960; Petrides and Pandya, 1988; Ungerleider et al., 1989; Ebeling and von Cramon, 1992). It also carries cholinergic fibres from the basal nucleus of Meynert innervating these cortical regions (Seldén et al., 1998).

The most comprehensive descriptions have been made in non-human primates. Petrides and Pandya (Petrides and Pandya, 1988) found three fibre projection systems linking the superior temporal and frontal lobes in the macaque, the most anterior being labelled the uncinate fasciculus. Ungerleider and colleagues (Ungerleider et al., 1989) used the same term, but it appears to correspond to the intermediate fasciculus of Petrides and Pandya (Petrides and Pandya, 1988). The most detailed investigation of the human uncinate fasciculus was by Ebeling and von Cramon (Ebeling and von Cramon, 1992), who extended observations and applied fibre dissection techniques developed earlier (Klingler and Gloor, 1960). They described how the uncinate fasciculus ‘originates like a fan from the anterior three temporal convolutions (area 20, 38) in front of the temporal horn and the cortical nuclei of amygdala (area 28, 34, 36). All fibres unify in the anterior temporal stem in the deep white matter of the second temporal gyrus in front of and at the level of the inferior horn. The fibre bundles form a solid tract while running upward over the lateral nuclei of the amygdala towards the limen insulae. It is a solid bundle of fibres between 5 and 7 mm in width, and 2 and 5 mm in height. ‘The fasciculus terminates in the frontal lobes in the gyrus rectus (area 11), the medial, retro-orbital cortex (area 12) and sub-callosal area (area 25). The neuropathology of schizophrenia remains obscure, but increasing evidence indicates that it involves a structural as well as functional alteration in cortical connectivity (McGuire and Frith, 1996; Harrison, 1999; Lewis and Lieberman, 2000; Selemoin, 2001). Abnormalities of inter-hemispheric pathways are part of this pathology (Woodruff et al., 1995; Highley et al., 1999a,b), perhaps related to the lateralized changes and altered cerebral asymmetry which have been reported in schizophrenia (Holinger et al., 2000; Sommer et al., 2001) and which are posited to be central to its understanding (Crow et al., 1989; Pearlson et al., 1996; Crow, 1997, 2000). Altered fronto-temporal pathways may be another circuitry component affected in schizophrenia, although this has only been shown indirectly (Weinberger et al., 1992; Frith et al., 1995; Deakin et al., 1997; Woodruf et al., 1997; Meyer-Lindenberg et al., 2001; Sigmundsson et al., 2001). Since the temporal and, to a lesser extent, the frontal lobes are anatomically asymmetrical (Geschwind and Levitsky, 1968; Galaburda et al., 1978; Kertesz et al., 1990; Highley et al., 1999c; Galuske et al., 2000; McDonald et al., 2000; Watkins et al., 2001), there may be an interaction in schizophrenia between involvement of fronto-temporal connections and left–right differences. Given these various considerations, we have investigated whether the uncinate fasciculus is asymmetrical in the human brain and whether the fasciculus is altered in the disorder.

Methods and Materials

Subjects Studied

The clinical and pathological details of the subjects studied have been described previously (Highley et al., 1999a). Some brains from the series were not included in the present study because of damage to the temporal stem during processing. We also omitted the four leucotomized patients. The demographic details are summarized in Table 1. Autopsy consent from next-of-kin or permission of the coroner (medical examiner) was obtained for each subject. Brains were coded at the time of autopsy and the study was performed blind to knowledge of diagnosis and sex. Both hemispheres were available for 16 of the controls and nine of the patients. All brains were formally examined by a neuropathologist to exclude focal and neurodegenerative abnormalities. After fixation, the temporal and occipital lobes were separated from each hemisphere and coronally sliced at 5 mm thickness. The slices were embedded in paraffin and a 16 mm thick section was cut from each and stained with the Palmgren silver stain for nerve fibres (Bancroft and Stevens, 1990).

Definition and Delineation of the Uncinate Fasciculus

Using a Wild dissecting microscope, the stained fibres of the uncinate fasciculus could be seen running in the plane of the tissue section. A line running across the fasciculus, normal to the orientation of the fibres, was drawn from the deepest cortex of the circular sulcus. This line, henceforth referred to as the transection line, was drawn on each slide on which the uncinate fasciculus was to be studied and defined the plane through the fasciculus in which measurements would be made. The borders were drawn as follows. The posterior limit was the level at which Heschl’s gyrus meets the circular sulcus between the insula and superior temporal cortex; anteriorly, the limit was the temporal cortex on the...
superior surface of the temporal pole. The lateral limit of the transection line was the cortex lining the deepest part of the circular sulcus. The medial limit was formed, from anterior to posterior, by: the cortex on the superior surface of the temporal lobe and anterior to the amygdala; the fibres of the amygdalo-temporal fasciculus; and, finally, the antero-posterior orientated fibres of the inferior longitudinal fasciculus. This definition corresponds to the uncinate fasciculus as described by Petrides and Pandya (Petrides and Pandya, 1988), together with a small contribution from the middle fronto-temporal fascicle; it subsumes the uncinate fasciculus as described by Ebeling and von Cramon (Ebeling and von Cramon, 1992).

The delineation of the uncinate fasciculus as described above is shown schematically in Figure 1a and representative sections are shown in Figure 2a–c.

Measurements of the Uncinate Fasciculus

Measurements were made using an Olympus BX50 microscope. Slides were orientated such that the transection line ran parallel to the x-axis of the stage (Fig. 3). The uncinate fasciculus was examined at 1 mm points along the transection line on each slide. The first point studied on each transection line was located a random distance <1 mm from the edge of the uncinate fasciculus. The stage was then moved so that the fasciculus could be studied 1 mm further along the transection line. This was repeated until the end of the line was reached, at which time the next section in the series was studied (Fig. 1b,c). The number of such points gives an estimate of the length of the transection line on each slide. These length estimates, when summated and multiplied by the inter-slice distance, give an estimate of the cross-sectional area of the structure.

The density of fibres in the uncinate fasciculus was measured as follows. At each study point, the microscope was focused near the upper surface of the tissue section using a ×100 objective and the image viewed in real time on a computer monitor via a JVC TK-1070E video camera at a total magnification of 3500. Fibres were counted using a simplified version of the optical dissector (Gundersen et al., 1988; Howard and Reed, 1998). The video image depicted the fibres running vertically up and down the screen (Fig. 2d). A horizontal probe line was superimposed over this image at right angles to the fibre orientation and parallel to the transection line. This probe line corresponded to a length of 3.57 µm (see Fig. 5a). The microscope stage was then raised up, pushing the plane of focus and the probe line down through the section a depth of 5 µm, and fibres counted according to the following rules. (i) Fibres that were in focus at the outset were not counted. (ii) Fibres that came into focus and crossed the probe line were counted. (iii) Fibres that came into focus and touched the right-most tip were counted, irrespective of whether or not they were seen to cross the probe line. (iv) Fibres that came into focus, but touched the left-most tip of the probe line were not counted, irrespective of whether or not they were seen to cross the probe line. The effect of this procedure is to generate a flat, rectangular counting frame, orientated normal to the plane of the section and parallel to the transection line (Fig. 3b).

The number of axons counted in this way generates an estimate of the density of axons in the uncinate fasciculus in terms of number of fibres

### Table 1 Details of subjects studied

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Schizophrenia³</th>
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<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Number of cases</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Age (years)³²</td>
<td>74.5 (12.5)</td>
<td>70.0 (14.1)</td>
<td>70.0 (17.2)</td>
<td>58.3 (15.2)</td>
</tr>
<tr>
<td>Post-mortem delay (h)³²</td>
<td>40.1 (19.0)</td>
<td>39.9 (29.9)</td>
<td>40.8 (39.9)</td>
<td>37.7 (27.6)</td>
</tr>
<tr>
<td>Fixation time (years)³²</td>
<td>2.3 (1.2)</td>
<td>2.1 (1.5)</td>
<td>5.1 (2.0)</td>
<td>3.5 (1.6)</td>
</tr>
<tr>
<td>Hospital of origin</td>
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<td>0/8/2</td>
<td>5/1/2</td>
<td>3/3/3</td>
</tr>
<tr>
<td>Cause of death</td>
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<td>8/2/0</td>
<td>1/2/4</td>
<td>5/0/3</td>
</tr>
<tr>
<td>Neuroleptic medication</td>
<td>NA</td>
<td>NA</td>
<td>1/4/3</td>
<td>0/5/4</td>
</tr>
</tbody>
</table>

Values are mean (SD).

Diagnosed according to DSM-IV criteria (American Psychiatric Association, 1994).

No significant inter-group differences [all F(1,34) = 2.85, P = 0.101].

No significant inter-group differences [all F(1,33) = 0.03, P = 0.865].

Difference between diagnostic groups [F(1,33) = 16.93, P < 0.0005], no other differences [all F(1,33) ≤ 3.03, P = 0.091].

Significant inter-group difference [χ² = 19.75, d.f. = 6, P = 0.003].

Significant inter-group difference [χ² = 12.87, d.f. = 6, P = 0.045].

No sex difference in patient group [χ² = 1.20, d.f. = 6, P = 0.549].
The method of cross-sectional area estimation has been detailed above. Multiplication of the estimates of fibre density by cross-sectional area gave an estimate of total fibre number.

Statistical Analysis
Each of the measures (area, fibre density, fibre number) was assessed by analysis of variance (ANOVA) using SAS for Macintosh v. 6.12. The strategy for statistical analysis reflected the fact that one or other uncinate fasciculus was not available to measure in some individuals. Firstly, two ANOVAs, one for each side, were performed with sex and diagnosis as between-subjects factors. Two further ANOVAs were performed to investigate the effects of side and its interactions with sex and diagnosis as between-subjects factors. The first ANOVA treated side as a within-subjects factor, the second treated side as a between-subjects factor. The former, repeated-measures, design has greater sensitivity for side effects and interactions in that proportion of the cohort where data from both sides are available. It is, however, restricted to this subset, whereas the between-subjects design is not.

Results
Quality of Measurements
The quality of the measurements made was assessed by calculation of the observed coefficient of error or $OCE$ (Gundersen, 1984; Pakkenberg and Gundersen, 1997). The $OCE$ was 0.153 for the area measurements, 0.066 for the density measurements and 0.178 for the total fibre number measurements. $OCE$ is a measure of the variance between individuals which is due to the inaccuracy of the measurement tool and allows this to be separated from the variance which is due to true inter-individual variation in the parameter being measured. Where the latter proportion is $>50\%$, the measurement can be deemed to be satisfactory. For cross-sectional area, true inter-individual variance accounted for 78.4 and 72.2\% of the observed relative variance on the left and right, respectively. For the fibre density measurements, the proportions were 87.6 and 76.9\%, and for the total fibre number estimates the proportions were 81.0 and 65.7\%. On this basis, all measurements can be deemed to be of sufficient accuracy.

Effects of side, sex and schizophrenia on the size and fibre content of the uncinate fasciculus
The results for cross-sectional area, fibre density and fibre number in the left and right uncinate fasciculi of normal subjects and patients with schizophrenia are summarized in Table 2.

For cross-sectional area, the data for the two sides showed no effect or interaction of diagnosis or sex [for the left, all $F(1,28) \leq 0.56$, $P \geq 0.459$; for the right, all $F(1,27) \leq 2.24$, $P \geq 0.146$]. Repeated-measures ANOVA showed a significant effect of side [$F(1,21) = 32.66$, $P = 0.0001$], corresponding to the right being 27\% larger than the left. As illustrated in Figure 4, this reflected a rightward area asymmetry in 20 out of 25 individuals (11/16 controls and 9/9 patients with schizophrenia) in whom both

Figure 2. Delineation of the human uncinate fasciculus. In (a–c), Palmgren-stained coronal sections at three antero-posterior levels show the uncinate fasciculus and surrounding structures. In (d), a higher-power micrograph shows the fibres of the uncinate fasciculus. In (a–c), bar = 1 cm; in (d), bar = 50 µm. A-T, amygdalo-temporal; STG, superior temporal gyrus; TCN, tail of the caudate nucleus.
hemispheres were available; only one subject showed a clear leftward area asymmetry. Repeated-measures ANOVA revealed a trend side × diagnosis interaction \(F(1,21) = 3.52, P = 0.075\) and a trend side × sex interaction \(F(1,21) = 3.30, P = 0.084\), but no side × diagnosis × sex interaction \(F(1,21) = 0.71, P = 0.405\). The between-subjects, general factorial ANOVA similarly demonstrated an effect of side \(F(1,55) = 9.76, P = 0.003\), but did not show any significant interactions involving side \(F(1,55) \leq 0.39, P \geq 0.536\).

For fibre density, the data for the two sides showed no effect or interaction of diagnosis or sex \([F(1,28) = 0.42, P = 0.522\) for the left, and \(F(1,27) = 0.20, P = 0.658\)]. For the two ANOVA investigations of side, this factor showed no significant main effect or interaction \([F(1,21) = 0.69, P = 0.417\) for repeated-measures ANOVA, all \(F(1,55) \leq 0.39, P \geq 0.536\) for general factorial ANOVA].

For fibre number, the data for the two sides showed no effect or interaction of diagnosis or sex \([F(1,28) = 0.90, P = 0.351\) for the left, and \(F(1,27) = 1.71, P = 0.202\)]. An effect of side, corresponding to the right having 33% more fibres than the left, was demonstrated by both the repeated-measures ANOVA \(F(1,21) = 21.20, P = 0.0002\) and the general factorial ANOVA \(F(1,55) = 9.90, P = 0.003\). This reflected a rightward fibre number asymmetry in 21 out of 25 individuals (14/16 controls, and 7/9 patients with schizophrenia) in whom both hemispheres were available (Fig. 5); three subjects had a leftward asymmetry in fibre number. No interaction effects involving side were evident \([F(1,21) \leq 1.27, P \geq 0.273\) for repeated-measures ANOVA, all \(F(1,55) \leq 0.48, P \geq 0.493\) for general factorial ANOVA].

Discussion

This study has two main findings. Firstly, that the human uncinate fasciculus is asymmetrical in ~80% of subjects, being on average 27% larger and containing 33% more fibres than the right in the left hemisphere. Secondly, its size and asymmetry are not demonstrably affected in schizophrenia.

Human quantitative neuroanatomical studies are subject to many limitations and potential sources of variation, such as age, sex, mode of death, post-mortem delay, duration in fixative, etc. Handedness might be another relevant variable. Our sample size was too small to have allowed satisfactory investigation of, or control over, these factors and we do not know the subjects’ handedness. However, no significant correlations with age, post-mortem interval, fixative time, or source of brain were observed (data not shown) and no influence of these factors was found in equivalent measurements of the corpus callosum (Highley et al., 1999a). In any case, the finding of uncinate fasciculus asymmetry was robust when comparing the left and right hemispheres of the same brain (Figs 4 and 5), which overcomes most of these potential confounds. The other main issue concerns the methodology used here to delineate and sample from the uncinate fasciculus, and the extent to which it meets stereological requirements (Gundersen et al., 1988; Tang and Nyengaard, 1997; West, 1999; Benes and Lange, 2001). As far as possible, in terms of the material available and the limitations inherent in tract definition, we applied stereological principles.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Males</th>
<th>Combined</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Schizophrenics</td>
<td>Controls</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Left</td>
<td>81.8 (27.4)</td>
<td>90.5 (34.6)</td>
<td>77.2 (26.8)</td>
</tr>
<tr>
<td>Right</td>
<td>94.7 (32.0)</td>
<td>116.8 (40.7)</td>
<td>102.3 (25.9)</td>
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<tr>
<td>Fibre density (× 10³/mm²)</td>
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<tr>
<td>Left</td>
<td>116.2 (29.2)</td>
<td>120.0 (20.2)</td>
<td>110.7 (16.7)</td>
</tr>
<tr>
<td>Right</td>
<td>125.7 (12.5)</td>
<td>121.5 (24.4)</td>
<td>121.0 (18.2)</td>
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<tr>
<td>Fibre number (× 10⁶)</td>
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</tr>
<tr>
<td>Left</td>
<td>9.62 (3.46)</td>
<td>11.22 (5.58)</td>
<td>8.60 (3.39)</td>
</tr>
<tr>
<td>Right</td>
<td>11.92 (4.26)</td>
<td>14.03 (5.16)</td>
<td>12.15 (2.79)</td>
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</tbody>
</table>

Values are mean (SD).

Effect of side \(F(1,55) = 9.76, P < 0.003\).

Effect of side \(F(1,55) = 9.90, P < 0.003\).

Figure 3. The 2-D dissector used to measure fibres in the uncinate fasciculus. For details, see text.
(including being unbiased, with explicit counting criteria and with a satisfactory OCE for all measurements). However, no cortical white matter tract can be unambiguously separated from adjacent white matter, in this instance the more posterior fronto-temporal tracts. Whilst the limitations of our methods and materials mean that the absolute estimates of size and fibre number must be viewed with caution, there seems no obvious artefact by which the observed asymmetry would have arisen.

Several macroscopic and histological asymmetries of the human cerebral cortex are known (Holinger et al., 2000), but to our knowledge this is the first demonstration of a structurally asymmetrical cortico-cortical fibre tract. Its magnitude and prevalence suggest that the result is robust. Although no functional consequence can be assumed, a plausible interpretation is that there may be more extensive fronto-temporal connectivity in the right than the left hemisphere. In turn, this may contribute to the anatomical basis of the relative specialization of the right hemisphere for integrative and global processing, as proposed in various neuropsychological and psychophysiological theories. At present it is unclear whether there are corresponding asymmetries of the particular cortical areas interconnected by the uncinate fasciculus, nor is it apparent how the asymmetry may relate to the normal right-frontal-left-temporo-occipital torque of the brain (Le May, 1977; Kertesz et al., 1990; Bilder et al., 1994).

The finding that the size and asymmetry of the uncinate fasciculus is unchanged in schizophrenia has two main implications. First, it contrasts with studies of inter-hemispheric white matter tracts, which have found differences in their size and/or fibre content. Our own post-mortem studies (in the brain series used here) found significant interactions of diagnosis and gender for the corpus callosum (Highley et al., 1999a) and the anterior commissure (Highley et al., 1999b). In addition, a meta-analysis of magnetic resonance imaging (MRI) studies of the corpus callosum concluded that there is a small but significant area reduction in schizophrenia (Woodruff et al., 1995). These data together imply that there may be involvement of inter- rather than intra-hemispheric connections in the disorder; however, this is a relative rather than an absolute distinction since we also found minor alterations in the fornix (Chance et al., 1999). The second implication concerns the hypothesis that schizophrenia is a disorder of cerebral asymmetry (Crow et al., 1989; Crow, 1997, 2000). The evidence from this brain series is that temporal and occipital lobe asymmetries are reduced in schizophrenia (Highley et al., 1999c; McDonald et al., 2000), but the present data show that these changes are not reflected in altered asymmetry of fronto-temporal pathways as represented by the uncinate fasciculus. Nor are they accompanied by asymmetries in the length of the temporal lobes as defined by the posterior limit of the Sylvian fissure (Highley et al., 1998).

Figure 4. Cross-sectional area of the uncinate fasciculus in controls (circles) and patients with schizophrenia (triangles). Left and right hemisphere measurements from the same individual are joined by solid (men) or dashed (women) lines. The right side is larger than the left, repeated-measures ANOVA \( F(1,21) = 32.66, P = 0.0001 \).

Figure 5. Estimated fibre number of the uncinate fasciculus in controls (circles) and patients with schizophrenia (triangles). Left and right hemisphere measurements from the same individual are joined by solid (men) or dashed (women) lines. There are more fibres in the right than the left uncinate fasciculus, repeated measures ANOVA \( F(1,21) = 21.20, P = 0.0002 \).
References


