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Corpus callosal area differences and gender dimorphism in neuroleptic-naïve, recent-onset schizophrenia and healthy control subjects

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Abstract

The study of corpus callosal morphometry is important to unravel the underlying connectivity disturbance in schizophrenia. We studied the corpus callosal area in schizophrenia subjects compared to healthy subjects, while controlling for several confounders that could affect morphometric measures of the corpus callosum (CC). Areas of the whole CC and its sub-regions obtained by two geometric partitioning schemes were studied in 23 right-handed neuroleptic-naïve, recent-onset, schizophrenia patients and compared with 23 right-handed age-, sex- and education-matched healthy subjects. The patients did not differ from controls in whole CC area. On tripartite division of the CC, the area of the anterior sub-region was significantly higher in patients compared to controls. On radial division into 5 sub-regions, the anterior truncus area was significantly higher in patients compared to controls. There was a significant effect of gender (F>M) on the area measures; however there was no significant diagnosis*gender effect. Age, age of onset, duration of illness and psychopathology ratings did not show any significant correlations with whole CC area and area of CC sub-regions. The finding of increased area of the anterior truncus that possibly comprises white fibres connecting the temporal association cortices could be indicative of an "abnormal functional hyperconnection" involving these regions in positive symptom schizophrenia. Additionally, the finding of females having larger areas of the whole CC and of the anterior and middle sub-regions could reflect a "normal hyperconnection" underlying increased ambilaterality in females.

Keywords: Corpus callosum; Disconnection; Schizophrenia; Partitioning; Neuroleptic-naïve

1. Introduction

Schizophrenia is conceptualized as a disorder wherein aberrant cortico-cortical connectivity might play an important role (McGuire and Frith, 1996). Inter-hemispheric connectivity disturbances in schizophrenia are reflected as loss of normal asymmetry resulting in reduced laterality (DeLisi et al., 1997; Gruzelier, 1999). The corpus callosum (CC), being the largest mass of connecting white fibres in the brain has therefore evoked much interest among schizophrenia researchers. The majority of CC fibres constitute homotopic interconnections between association cortices of the hemispheres (Aboitiz et al., 1992). Callosal connections between

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homologous prefrontal and temporoparietal association areas are largely comprised of poorly myelinated, small-caliber (<2 nm diameter) and slowly conducting fibres, whereas those connecting the primary and secondary sensorimotor areas comprise fast-conducting highly myelinated large-caliber fibres (>3 μm diameter) (Aboitiz et al., 1992).

Following the initial report of increased thickness of the CC in post-mortem brains of schizophrenia patients by Rosenthal and Bigelow (1972) and the subsequent MRI replication of this finding by Nasrallah et al. (1986), the morphometry of CC has been extensively studied in schizophrenia. Shenton et al. (2001), reviewing morphometric studies in schizophrenia, pointed out that among 27 MRI studies of the CC, 17 (63%) reported positive findings while 10 (37%) reported negative findings. The findings across studies have been largely inconsistent, with whole CC areas being reported variously as increased (Nasrallah et al., 1986; Jacobsen et al., 1997), decreased (Woodruff et al., 1993) or not different (Uematsu and Kaiya, 1988; Gunther et al., 1991) between schizophrenia subjects and healthy controls.

Partitioning the CC and comparing the morphometry of the sub-regions between schizophrenia patients and controls has the potential to reveal more subtle morphological abnormalities (Jacobsen et al., 1997). Different partitioning schemes have been proposed for partitioning the CC into 3 to 7 sub-regions (de Lacoste et al., 1985; Witelson, 1989; Woodruff et al., 1993; Rumsey et al., 1996; Jancke et al., 1997; Bachmann et al., 2003; Teipel et al., 2004; Keshavan et al., 2007). Amongst these, the most popular method has been the Witelson (1989)'s scheme, which divides the CC into 7 sub-divisions using criteria based on the results of experimental work with monkeys and humans. However, none of the above partitioning schemes, including that by Witelson (1989), exactly mirrors the texture of the CC at the cellular level (Hofer and Frahm, 2006). Nevertheless, structural MRI (sMRI)-based geometric partitioning schemes continue to be widely used for studying the morphometry of the CC in relation to clinical and other biological variables, owing to the ease of studying the morphometry of CC that these techniques provide. We therefore, chose to geometrically partition the CC into 3 [anterior (rostrum + genu + anterior mid-body), mid-body and posterior (isthmus + splenium) (Aboitiz and Montiel, 2003)] and 5 [(rostrum and genu, anterior truncus, middle truncus, posterior truncus and isthmus and splenium) (Hampel e al., 1998)] equidistant sub-regions for group comparisons.

Previous studies that divided the CC into 3 to 5 sub-regions found increased thickness in the middle and anterior but not the posterior, parts of the callosal body (Nasrallah et al., 1986), larger anterior third region (Uematsu and Kaiya, 1988), smaller mid-anterior and mid-posterior quarters (Woodruff et al., 1993), or no regional difference (Hauser et al., 1989) in schizophrenia subjects compared to healthy controls.

The possible reasons for the inconsistent findings of whole CC area and that of the sub-regions in schizophrenia subjects include confounding effects of gender (Witelson, 1989; de Lacoste et al., 1990; Holloway et al., 1993; Highley et al., 1999), handedness (Witelson and Goldsmith, 1991; Preuss et al., 2002), age (Johnson et al., 1994; Woodruff et al., 1995; Keshavan et al., 2007), chronicity of illness (Woodruff et al., 1993; Tibbo et al., 1998), heterogeneity in symptom profiles of the samples studied (Gunther et al., 1991; Tibbo et al., 1998) and medication status along with differences in the CC partitioning schemes employed by the different studies as mentioned above. With a view to controlling for these confounders, we aimed at studying the CC morphometry in neuroleptic-naïve, recent-onset, schizophrenia (NRS) subjects in comparison to age-, sex- and education-matched healthy subjects (HS). Only right-handed subjects were included in the study. We measured the whole CC area as well as the areas of sub-regions derived by semi-automated partitioning of the mid-sagittal CC area respectively into 3 and 5 equidistant partitions. We hypothesized that whole CC areas will not be different between NRS patients and HS, whereas significant differences in area would emerge in the CC sub-regions, that connect cortical regions implicated in recent-onset schizophrenia.

2. Methods

The study was carried out at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore India, with due approval from the NIMHANS Ethics Committee, thus conforming to the ethical standards laid down in the 1964 Declaration of Helskinki. Written informed consent was obtained from all the subjects (and their legally qualified representatives in case of patients) prior to enrollment into the study.

2.1. Subjects

2.1.1. Patients

Twenty three right-handed NRS patients were recruited into the study by purposive sampling, from those who attended the outpatient services of NIMHANS. The inclusion criteria were: a DSM-IV diagnosis of schizophrenia or schizophreniform disorder,

right-handedness [as assessed by modified Annett's inventory (1976)], duration from onset of illness ≤5 years and age between 17 and 50 years. The diagnosis of schizophrenia or schizophreniform disorder was arrived at using criteria from the Diagnostic and Statistical Manual for Mental Disorders-Fourth edition (DSM-IV) based on the consensus of a research psychiatrist who conducted a semi-structured interview and a trained research assistant who used the Mini International Neuropsychiatric Interview (MINI) Plus (Sheehan et al., 1998). The exclusion criteria included previous exposure to psychotropic drugs; significant suicidal or homicidal risk or other disruptive behaviour which warranted immediate interventions and history of ECT within the previous 6 months. The baseline severity of schizophrenia psychopathology was evaluated using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) for all subjects by one trained rater (V.A.) who had established good inter-rater reliability with another trained rater (B.R.) [Intra-Class correlation Coefficient (ICC) for: PANSS positive symptom subscale=0.87; negative symptom subscale=0.92; general psychopathology subscale=0.85; total score=0.84]. Overall clinical status was assessed using Clinical Global Impression-Severity (CGI-S) (Guy, 1976). The psychopathology and CGI-S ratings were carried out during the initial 1 week following recruitment, with the psychopathology during the previous week being considered for rating. In a majority of patients, who were co-operative for psychopathology assessment on the day of recruitment itself, the PANSS ratings were completed in the untreated state; however, in the remaining patients, psychopathology ratings could only be completed following initiation of treatment, but within one week of recruitment.

2.1.2. Healthy subjects

Twenty three age-, sex- and education-matched right-handed healthy individuals were recruited by word of mouth. These subjects, predominantly constituted by hospital staff and attendants of hospitalized patients, were interviewed by a trained research assistant using MINI-Plus and ascertained to have no present or past Axis I or II psychiatric disorders; in addition, there was no history of psychotic disorders in their first degree relatives, as ascertained using an unstructured clinical interview of the recruited subjects. Further, these subjects did not have any medical/neurological condition requiring regular medications; current exposure to psychotropic medications was also ruled out.

The socio-demographic and clinical characteristics of the subjects are shown in Table 1. The patients and controls were matched for gender, age and educational status. All the patients had a rating of ≥3 on delusions and or hallucinatory behavior on PANSS.

Table 1
Socio-demographic and clinical characteristics of the study samples

Socio-demographic and illness characteristics	Subjects (N=46)	
	Schizophrenia	Controls
	[n=23]	[n=23]
Gender		
Male	10 (43.5%)	10 (43.5%)
Female	13 (56.5%)	13 (56.5%)
Age (years)	30.13 (SD=5.79); Range: 20–40	30.13 (SD=5.05); Range: 20–39
Education		
Illiterate/primary education	11	11
Secondary/pre-university	09	09
Graduate/post-graduate	03	03
Diagnosis		
Paranoid schizophrenia	08	NA
Undifferentiated schizophrenia	11	
Schizophreniform disorder	04	
Age of onset of illness (years)	28.96 (SD=5.44); Range: 20–40	
Duration of illness (weeks)	69.96 (SD=63.35); Range: 6–260 weeks	
PANSS positive total score	24.61 (4.49)	
PANSS negative total score	25.04 (6.33)	
PANSS global psychopathology total score	46.48 (8.98)	
PANSS total score	96.13 (15.46)	
CGI score	5.00 (0.74)	

2.2. Image acquisition and pre-processing

MR scans were acquired on a Siemens MAGNETOM Vision 1.5 Tesla MRI machine at NIMHANS using an MPRAGE sequence (TR=9.7 ms, TE=4 ms, flip angle=10°, section thickness=1.25 mm, field of view=256×256 mm, matrix=256×256, number of acquisitions=3, number of slices=128, orientation=sagittal, scanning time=6 min, 36 s) that acquires 1.25 mm×1 mm×1 mm voxels across the entire cranium. The MR scans were then reoriented to transverse plane and interpolated in to 0.5 mm×0.5 mm×0.5 mm isotropic voxels using trilinear interpolation. The scans were preprocessed and rotated into AC-PC co-ordinates using Mayo 3D brain Atlas module of Analyze™ 7.0 software (Robb et al., 1989; Robb and Hanson, 1991), as previously described in John et al. (2007).

2.3. Corpus callosum measurements

Outlining of the whole CC as well as grid and radial partitioning were performed using Analyze™ (Robb et al., 1989) on the mid-sagittal section. We referred to the guidelines provided by Woodruff et al. (1993) and Talairach and Tourneaux (1993) for identifying the mid-sagittal section of the CC, on which morphometric measurements were made. One expert rater and a trained rater (J.P.J. and M.S.K. respectively) independently identified the mid-sagittal section, selecting the slice which best satisfied both or at least one of the above sets of identifying criteria. Since the CC area measurement is made only on one slice per MRI, the data are vulnerable to errors resulting from inconsistencies in the choice of the "best mid-sagittal slice" (McCarley et al., 1999). Combination of both the above sets of criteria resulted in a highly reliable identification of the mid-sagittal slice (Intra-class Correlation Coefficient: standardized item alpha = 0.99).

Manual tracing of the CC and generation of the object map on the mid-sagittal section were performed using the 'Edit' function of Analyze™ (Fig. 1). The CC measurements included the whole CC area, areas of the three individual segments generated by the tripartite division as well as areas of the 5 sub-regions generated by the radial division. Partitioning into 3 and 5 sub-regions respectively was performed on the whole CC object map using the "region-of-interest (ROI)" module of Analyze™. For tripartite division, the "grid" option was used to divide the object (whole CC) vertically into 3 equal partitions (CC-I to CC-III), which were saved as anterior, middle and posterior sub-regions respectively and their areas generated (Fig. 1). For radial division into 5 sub-regions, the whole CC object was radially divided into 5 equal partitions (CC1-CC5). The origin of the radial lines corresponded to the co-ordinates of the mid-point of the base of the minimum enclosing rectangle (MER) encompassing the CC (Details of the radial division given in Appendix A). These 5 equal partitions were saved as rostrum and genu (CC-1), anterior truncus (CC-2), middle truncus (CC-3), posterior truncus (CC-4), and isthmus and splenium (CC-5) (Fig. 1) and their areas were generated.

The reliability (standardized item alpha) of the CC area measurements carried out independently by the raters (J.P.J. and M.S.K.) ranged from 0.89 for the whole CC area to 0.99 for the anterior sub-region obtained

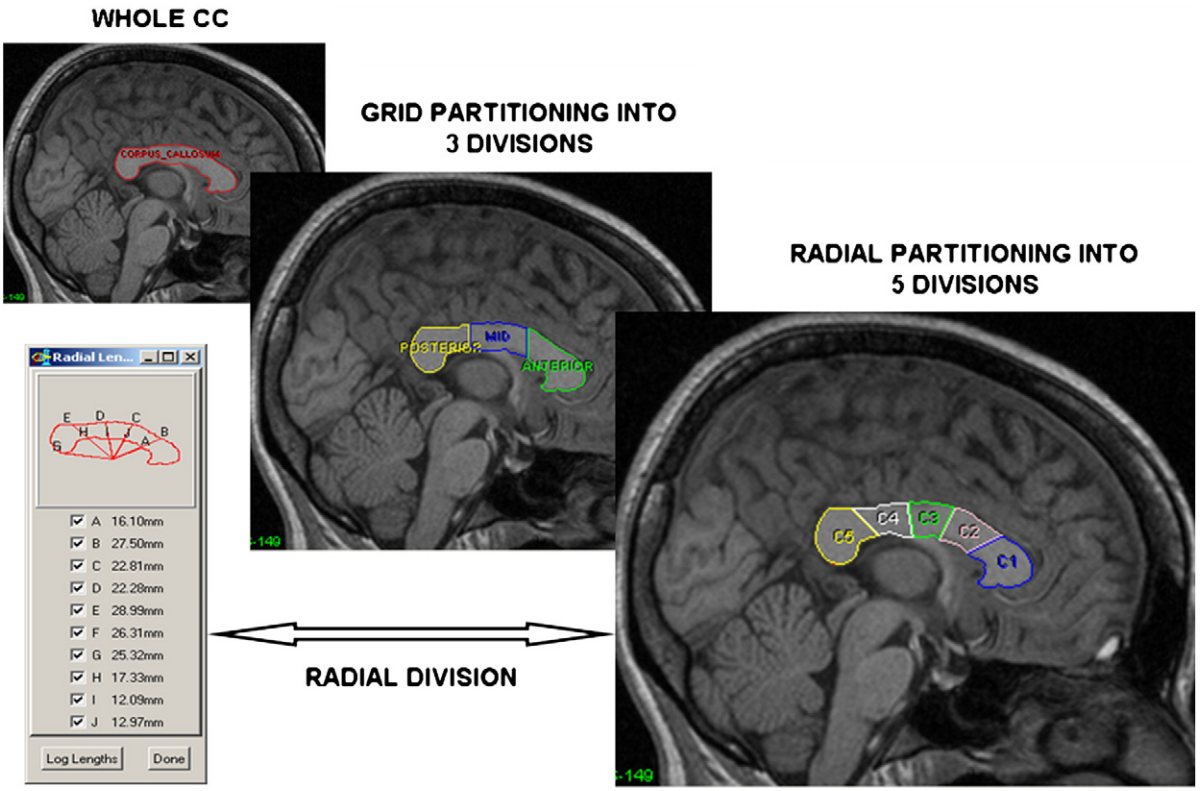

Fig. 1. Corpus callosum partitioning schemes (intended for reproduction in black and white).

using tripartite division. Subsequent to establishing satisfactory reliability of the measurements, outlining of the CC and partitioning into sub-regions on the mid-sagittal section as well as estimation of areal measures were performed by the same trained rater (M.S.K.) on all the 46 MRIs. This rater was blind to the group status, socio-demographic data, psychopathology ratings, and the research hypotheses while performing the MRI analysis and measurements. Forty one out of the 46 brains fulfilled both the Woodruff et al. (1993)'s and Talairach and Tourneaux (1993)'s criteria for identifying the mid-sagittal section. The mid-sagittal section fully satisfying at least one of the criteria sets was selected in the remaining 5 brains. No significant difference in coronal tilt was noted between MRIs of patients and controls.

Total brain volume (TBV) and intracranial volume (ICV) were estimated using the VBM2 module of SPM2 (www.fil.ion.ucl.ac.uk/spm/). Reliability of TBV and ICV estimates using SPM2 and Analyze™-based manual segmentation methods was estimated on 3 test brains (standardized item alpha = 0.99) (John et al., 2007). The TBV was not significantly different between NRS and HS [HS = 986.58 mm^2 (SD = 109.95); NRS = 973.99 mm^2 (SD = 147.19); F = 1.685; $p < 0.202$], as assessed using General Linear Model (GLM) Analysis of Co-variance (ANCOVA) with diagnosis and gender as fixed factors and age and ICV as co-variates.

2.4. Statistical analysis

Descriptive statistics were expressed as mean and standard deviation (SD) of the whole CC area as well as the areas of the sub-regions (Table 2). All the CC area measures including whole CC area, areas of the tripartite sub-divisions as well as the areas of the 5 sub-regions derived by radial division were normally distributed in both HS and NRS subjects, as assessed using Shapiro–Wilks test of normality. However, the TBV in NRS subjects contained two outliers, while the TBV in HS was normally distributed. For the TBV to be used as a co-variate in the parametric statistics mentioned below, the two outliers were omitted from analysis to ensure that the data satisfied basic assumptions for conducting the parametric tests.

Whole CC area was compared between NRS subjects and HS by GLM ANCOVA with diagnosis (NRS vs HS) and gender as fixed factors and age and TBV as co-variates. Areas of the CC sub-regions obtained by geometric partitioning were compared between NRS subjects and HS using GLM Multivariate Analysis of Co-variance (MANCOVA) with diagnosis and gender as fixed factors and age and TBV as co-variates. Subsequently, post-hoc univariate ANCOVA was carried out individually for areas of each sub-region. Similarly, the areas of the sub-regions were compared across genders in both NRS and HS groups using GLM ANCOVA with gender as fixed factor and age and TBV as co-variates. Correlation between age, psychopathology ratings and CC areas were carried out by calculating the partial correlation co-efficient with TBV as co-variate. The level of statistical significance was set *a priori* at $p < 0.05$.

3. Results

There was no significant difference in whole CC area between NRS subjects and HS (Table 2). There was a significant effect of gender (females > males) on whole CC area ($F = 18.774$; $p < 0.000$). GLM MANCOVA with the areas of the 3 CC sub-regions (CC-I, CC-II and CC-III) derived by tripartite division as dependent variables, diagnosis and gender as fixed factors and age and TBV as co-variates, however,

Table 2
Comparison of whole CC area and areas of sub-regions between schizophrenia subjects and healthy subjects

	Mean area (mm^2) (SD)		Group		Gender		Group × gender	
	Schizophrenia	Control	*F*	Significance	*F*	Significance	*F*	Significance
	(n = 23)	(n = 23)						
Whole CC	663.33 (73.82)	662.88 (77.59)	0.612	0.439	18.774	.000	.081	.777
CC-I (anterior)	274.07 (30.50)	259.88 (36.92)	7.027	.012	16.231	.000	.045	.834
CC-II (middle)	153.50 (25.62)	150.57 (24.62)	1.133	.294	15.822	.000	.261	.613
CC-III (posterior)	235.76 (30.51)	252.43 (29.27)	2.952	.094	6.157	.018	.650	.425
CC-1 (rostrum and genu)	194.51 (20.58)	193.73 (24.50)	.608	.440	13.313	.001	.015	.904
CC-2 (anterior truncus)	111.00 (16.00)	100.09 (18.42)	14.807	.000	18.828	.000	.039	.844
CC-3 (middle truncus)	91.80 (15.41)	87.89 (18.16)	1.800	.188	11.831	.001	.196	.660
CC-4 (posterior truncus)	92.49 (21.04)	99.04 (18.98)	.973	.330	6.178	.017	.587	.448
CC-5 (isthmus and splenium)	173.01 (20.94)	181.79 (22.40)	.926	.342	3.105	.086	.171	.681

revealed significant group differences [$F(3,36)=5.369$; $p<0.004$]. There was a significant effect of gender on areas of the tripartite sub-regions [$F(3,36)=6.903$; $p<0.001$], while there was no significant diagnosis * gender effect [$F(3,36)=0.603$; $p<0.617$]. Similarly, GLM MANCOVA with the areas of the 5 CC sub-regions derived by radial division (CC-1-CC-5) as dependent variables, revealed significant group differences [$F(5,34)=4.916$; $p<0.002$]. There was a significant effect of gender on areas of the radial sub-divisions [$F(5,34)=4.175$; $p<0.005$], while there was no significant diagnosis * gender effect [$F(5,34)=0.520$; $p<0.759$]. Post-hoc univariate GLM ANCOVA revealed significantly higher areas of the CC-I of the tripartite division and the CC-2 of the radial division in NRS subjects compared to HS. The comparison of the whole CC area and those of the sub-regions derived by geometric partitioning between NRS subjects and HS are given in Table 2. Scatter plots depicting the distribution of areas of CC-I and CC-2 are shown in Fig. 2. The gender effect was significant for all the areal measurements except that of isthmus and splenium ($p<0.086$).

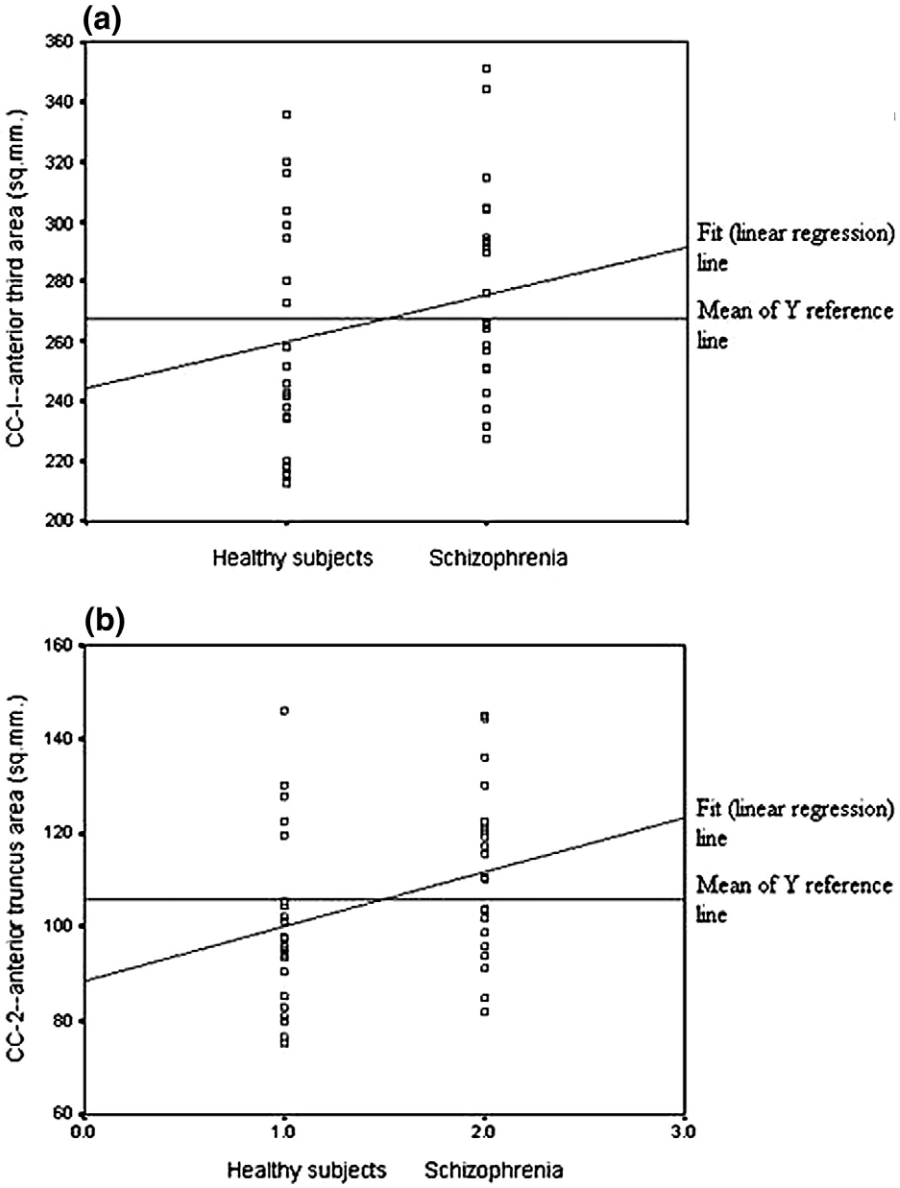

Fig. 2. Scatter plots depicting the distribution of areas of (a) the anterior third (CC-I) and (b) anterior truncus (CC-2) of the corpus callosum in schizophrenia and healthy subjects.

A GLM MANCOVA done separately for NRS subjects and HS with the three CC sub-regions derived by tripartite division as dependent variables, gender as fixed factor and age and TBV as co-variates revealed significant gender differences (females>males) in both HS [$F(3,17)=4.398$; $p<0.018$] and NRS subjects [$F(3,15)=3.587$; $p<0.0391$]. With the radial subdivisions, the GLM MANCOVA did not reach significance for HS [$F(5,15)=2.657$; $p<0.065$] but revealed significant gender differences in NRS subjects [$F(5,13)=3.128$; $p<0.045$]. Planned univariate gender-wise comparison of whole CC area and that of the sub-regions, separately for NRS subjects and HS using GLM ANCOVA with age and TBV as co-variates are given in Table 3. Whole CC area was significantly higher in females in both HS and NRS groups. On tripartite division, females had significantly higher areas of the CC-I and CC-II sub-regions in the HS group, whereas the areas of all three sub-regions were higher in the NRS group. With the radial partitioning, control females had higher areas of CC-1, CC-2 and CC-3, whereas schizophrenia females had higher areas of CC-1 and CC-2.

Age, age of onset, duration of illness and psychopathology ratings did not show any significant correlations with whole CC area and area of CC sub-regions.

4. Discussion

Previous morphometric studies of the whole CC and its sub-regions derived using various partitioning schemes in heterogeneous schizophrenia samples have yielded intriguing results. The inconsistencies in the findings that have emerged from previous MRI-based studies have been suggested to be due to the influence of various confounders mentioned earlier, as well as due to the variability in the methods of CC partitioning. We attempted to extend the findings of previous studies by controlling for most of these confounders in a comparative study of the mid-sagittal CC area in a sample of right-handed NRS subjects compared to age, sex and education-matched right-handed HS. All the NRS subjects had ratings of ≥3 on delusions and or hallucinatory behavior on PANSS. In view of the fact that no partitioning scheme exactly mirrors the texture of the CC at the cellular level (Hofer and Frahm, 2006), we chose to estimate the CC areas by employing three progressively finer and overlapping partitioning methods—whole CC area as well as geometric partitioning into 3 and 5 equidistant sub-regions using grid and radial partitioning respectively. Equidistant geometric partitioning in sMRI-based CC morphometric studies would help to ensure uniformity and ease of comparison across studies.

There was no significant difference in whole CC area, controlling for age and TBV, between NRS subjects and HS. This finding is in concordance with many previous reports which found no significant differences in the whole CC area in schizophrenia subjects (Rossell et al., 2001; Goghari et al., 2005). However, many authors have reported smaller whole CC areas in schizophrenia patients (Woodruff et al., 1997; Tibbo et al., 1998; Keshavan et al., 2002; Bachmann et al., 2003). All these studies had significant differences with respect to sample characteristics as compared to the present study. The study by Woodruff et al. (1993) included subjects with right/left/mixed handedness, the study samples were not age-, or sex-matched, and included chronically ill patients (duration of illness=11.3±6.6 years; range=2–28 years), who had been on long-term treatment with neuroleptics. The study by Tibbo et al. (1998) also had similar methodological differences with respect to the sample characteristics compared to the present study. The first-episode, neuroleptic-naïve schizophrenia sample in the study by Keshavan et al. (2007) comprised of

Table 3
Gender-wise comparison of whole CC areas and areas of sub-regions in healthy subjects and schizophrenia patients

	Healthy controls				Schizophrenia			
	Mean area (mm^2)		F	Significance	Mean area (mm^2)		F	Significance
	Males ($n=10$)	Females ($n=13$)			Males ($n=10$)	Females ($n=13$)		
Cc whole	637.22 (90.78)	682.62 (62.32)	9.492	.006	613.44 (42.21)	694.04 (73.23)	10.338	.005
CC-I (anterior)	253.93 (42.82)	264.46 (32.74)	9.689	.006	257.25 (23.19)	284.42 (30.53)	10.220	.005
CC-II (middle)	137.38 (28.25)	160.71 (16.01)	12.711	.002	139.31 (18.73)	162.23 (25.94)	4.771	.043
CC-III (posterior)	245.93 (29.07)	257.44 (29.57)	1.394	.252	216.88 (15.90)	247.38 (31.95)	5.003	.039
CC-1 (rostrum and genu)	185.50 (23.90)	200.44 (23.66)	11.414	.003	181.97 (16.52)	202.23 (19.42)	5.497	.031
CC-2 (anterior truncus)	97.73 (21.50)	101.90 (16.34)	6.234	.022	102.84 (11.32)	116.02 (16.75)	19.781	.000
CC-3 (middle truncus)	79.60 (21.45)	94.27 (12.57)	9.316	.007	82.94 (14.41)	97.25 (13.78)	3.571	.076
CC-4 (posterior truncus)	94.98 (20.15)	102.17 (18.21)	1.912	.183	80.91 (17.03)	99.62 (20.59)	4.259	.055
CC-5 (isthmus and splenium)	179.40 (25.28)	183.63 (20.79)	0.456	.508	164.22 (15.27)	178.42 (22.62)	3.276	.088

patients with a much longer duration of illness (212.09± 280.91 weeks) as compared to the present study (69.96± 63.35 weeks). Further, both the patient and control groups comprised of subjects who differed in their handedness (R/L: patients=23/8; controls=28/3). Bachmann et al. (2003) studied first-episode schizophrenia patients (n=31) compared to healthy controls (n=12); however the sample sizes were unequal and the subjects were not individually matched for age, sex or educational status, and consequently, the gender distribution in the patient and control groups were substantially different.

The tripartite (Rumsey et al., 1996; Anstey et al., 2007) and radial divisions (Georgy et al., 1993; Teipel et al., 2004) have been used in previous morphometric studies of the CC in several neuropsychiatric conditions other than schizophrenia. In the present study, multivariate analyses revealed significant differences between NRS and HS for areal measures of sub-regions derived by both partitioning schemes. Post-hoc univariate analysis revealed that NRS subjects had significantly higher areas of CC-I (anterior sub-region) and CC-2 (anterior truncus). This finding of increased area of the anterior CC is in agreement with previous reports of increased area, thickness or width of this region in schizophrenia patients (Nasrallah et al., 1986; Uematsu and Kaiya, 1988; Narr et al., 2000). Larger anterior and posterior CC areas have been reported in childhood-onset schizophrenia (Jacobsen et al., 1997). Moreover, an enlarged whole CC size has been suggested to be associated with positive-symptom, good-prognosis schizophrenia (Gunther et al., 1991). Of the above studies, Nasrallah et al. (1986) divided the maximum antero-posterior length of the CC into 4 equidistant partitions using lines perpendicular to the ventral callosal wall, while Uematsu and Kaiya (1988) sub-divided the CC using the tripartite division. Narr et al. (2000) and Jacobsen et al. (1997) employed the Witelson's (1989) scheme for CC partitioning.

Functional topographic organization of the CC in the human brain is not fully understood. In primates, fibres from frontal cortical areas are shown to traverse the CC through the rostrum and genu, while those from the primary sensorimotor, posterotemporal, parietal and occipital cortical areas pass through the truncus and splenium (Pandya et al., 1971). In humans, fibres from the inferior frontal cortex and anterior inferior parietal regions have been shown to course through the rostrum and genu of the CC, while those from the parieto-occipital junction pass through the posterior truncus and splenium (de Lacoste et al., 1985). The rostrum, genu and the anterior truncus project mainly in the frontal and posterotemporal lobe while the rest of the callosal body and splenium send their fibres to the parietal and occipital cortices, as shown by intra-operative electrophysiologic stimulation of the CC in patients with epilepsy (Tan et al., 1991). Thus, there is no clear consensus regarding the topographical organization of fibres coursing through the human CC. In the present study, the significantly larger area of the anterior sub-region of the CC (CC-I), derived by the tripartite grid partitioning was further localized to the anterior truncus (CC-2) using the radial partitioning into 5 sub-regions. As mentioned in the Introduction, the callosal connections between homologous prefrontal and temporoparietal association areas, comprised of small-caliber (<2 nm diameter) fibres, are most dense in the anterior sub-region of the CC (Aboitiz et al., 1992). Therefore our finding of increased callosal area in the anterior sub-region of the CC may indicate a cortical abnormality in the prefrontal and/or temporoparietal association areas. The absence of significant findings in the CC-1 area derived by radial partitioning, which connects the prefrontal association areas, indicates that the cortical abnormality may be localizable to the temporal association cortex. This finding is in keeping with the consistently reported left superior temporal gyrus (STG) volume reduction in positive symptom schizophrenia (review, Shenton et al., 2001). The finding of increased callosal area in the anterior truncus may indicate "abnormal functional hyperconnection" (David, 1993) involving these regions, in accordance with the notion that schizophrenia is caused by difficulties in filtering and selecting relevant information (Braff and Geyer, 1989). Therefore, the increased area limited to the anterior truncus of the CC may reflect the underlying cortical abnormality localizable to the left STG. This abnormal hyperconnection involving the temporal association cortices might result in reduced laterality underlying the positive symptoms of schizophrenia, as suggested by previous authors (Gruzelier, 1999). However, in view of the fact that all the schizophrenia subjects in our sample had severe psychosis at the time of the study, the possibility that this focal increase in CC area may be state-related also needs to be considered.

Alterations of white matter connectivity have been demonstrated in first-episode schizophrenia using the relatively newer technique of diffusion tensor imaging (DTI) (Federspiel et al., 2006). Moreover, Brambilla et al. (2005) demonstrated a link between positive symptoms and the anterior callosal apparent diffusion co-efficient (ADC) measures, in consonance with the findings of the present study.

Corpus callosal abnormalities are not restricted to schizophrenia, but have been reported in other

psychiatric conditions such as autism (Hardan et al., 2006; Vidal et al., 2006), attention-deficit hyperactivity disorder (Hynd et al., 1991), dyslexia (Rumsey et al., 1996) and bipolar affective disorder (Coffman et al., 1990). A variety of neurological conditions (review, Georgy et al., 1993) including multiple sclerosis, neoplastic disorders, vascular conditions, chronic alcoholism (Marchiafava–Bignami disease), fetal alcohol syndrome and HIV encephalopathy also involve structural abnormalities of the CC. The topography of corpus callosal atrophy has been suggested to provide an index of region-specific neuronal loss in the neocortex (Pantel et al., 1999; Hampel et al., 1998) in Alzheimer's disease. An integration of CC abnormalities in the various neuropsychiatric conditions and the corresponding behavioural features could pave the way for a better understanding of the connectivity disturbances underlying these disorders.

Females had significantly larger area of the whole CC as well as the anterior and middle callosal sub-regions in both the NRS and HS groups (Table 2). Reports of gender effects on CC morphology in schizophrenia have been inconsistent (Bishop and Wahlsten, 1997). Many previous studies have found a larger CC area relative to brain size in women, especially in the splenium or isthmus (Witelson, 1989; de Lacoste et al., 1990;). We did not find a significant gender * diagnosis interaction (Table 1) in agreement with two previous studies in first-episode schizophrenia patients (Bachmann et al., 2003; Keshavan et al., 2007). Our finding of increased area in females of whole CC as well as anterior and middle sub-regions in both NRS and HS groups provides strong support to the previous evidence for sexual dimorphism in CC areas, considering that we have attempted to control for most of the confounders, including handedness and TBV. The sexual dimorphism of CC area is commonly interpreted as reflecting increased inter-hemispheric connectivity underlying increased ambilaterality in females (McGlone, 1978; Jancke et al., 1997). It is well known that women with schizophrenia have better premorbid functioning, a more benign illness course, lower levels of disability and better integration into the community than men (Morgan et al., 2008). The callosal hyperconnection, along with the faster development of the CC noted in females may protect them against the 'misconnectivity phenomena' in the frontal lobes that males may encounter at a younger age, contributing to negative symptoms (Crow et al., 2007).

The major methodological advantage of the present study is that the schizophrenia subjects were neuroleptic-naïve. The schizophrenia sample was carefully selected to avoid possible confounding effects of gender, handedness, age, chronicity of illness, as well as clinical heterogeneity. Stringent rules were adopted to identify the mid-sagittal slice, resulting in very high inter-rater reliability in identifying the slice. Areas of CC sub-regions were computed using highly reliable geometric partitioning methods. We adjusted for total brain size by including TBV as a co-variate in group comparisons, thereby ruling out its effect on CC area. Nevertheless, the findings of the study should be interpreted in the light of certain methodological limitations which include modest sample sizes and non-equitable distribution of males and females in both NRS and HS samples (10:13). In this study on neuroleptic-naïve, recent-onset schizophrenia patients, limited power afforded by the modest sample sizes is indeed a limitation, as has been the case with similar studies in the past. It also needs to be pointed out that there were no inclusion/exclusion criteria stated *a priori* to limit the patients to only positive symptom schizophrenia subjects. However, post-hoc analysis of the NRS sample revealed that all the subjects had a rating of ≥ 3 on delusions and/or hallucinatory behavior on PANSS. Even though the patient sample comprised of recent-onset (≤ 5 years duration) schizophrenia subjects, there was substantial variance in the duration of illness (69.96 ± 63.35 weeks; range = 6–260 weeks), with four patients having a duration of illness of less than 6 months (schizophreniform disorder) (Table 1). However, all the major findings persisted even on reanalysis of the data excluding these 4 subjects. Another limitation of CC partition studies is the lack of a one-to-one correlation between the various partitioning methods and the texture of the CC at the cellular level.

In summary, right-handed, NRS subjects all of whom had scores of ≥ 3 on delusions and/or hallucinatory behavior were found to have increased area of the anterior truncus sub-region of CC, in comparison to age-, sex-, and education-matched right-handed HS. This finding of increased area in a specific region of the CC that could contain fibres connecting the temporal association cortices is in consonance with information processing models of schizophrenia which suggest "abnormal functional hyperconnection" as underlying the positive symptoms. Additionally, female subjects were found to have higher areas of the whole CC as well as anterior and middle sub-regions when compared to males, in both HS and NRS samples. This finding could be reflective of a "normal hyperconnection" underlying increased ambilaterality in females. The findings of the study also highlight the importance of controlling for the various confounders affecting CC morphometry to obtain internally consistent results.

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Contributors

J.P.J. and S.J. designed the study and wrote the protocol. J.P.J. and M.S.K. carried out morphometric analysis. J.P.J. carried out the statistical analysis. J.P.J. and M.S.K. wrote the manuscript. All authors have contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2008.04.035.

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