METHOD

Subjects

Patients with schizophrenia who met ICD-10 diagnostic criteria for research (WHO, 1993) were recruited from the in-patient and out-patient clinics of the Department of Neuropsychiatry, Toyama Medical and Pharmaceutical University Hospital, Japan. Diagnoses were made following structured clinical interviews by psychiatrists with the Comprehensive Assessment of Symptoms and History (CASH; Andreasen *et al.* 1992*a*). Clinical symptoms were rated using the Scale for Assessment of Positive Symptoms (SAPS; Andreasen, 1984) and the Scale for Assessment of Negative Symptoms (SANS; Andreasen, 1983). A Schneiderian score was calculated by summing the seven items (described below) of the first-rank symptoms in the SAPS (Franck *et al.* 2002). Right-handed patients who underwent brain MRI scan were screened for study eligibility by an experienced psychiatrist (M.S.) on the basis of structured clinical interview data and exhaustive review of the clinical records. Inclusion criteria were:

(1) young patients aged less than 40 years who were in active psychotic episode at the time of symptom rating;

(2) psychotic symptoms were assessed, if possible, when the patients were maximally psychotic, or as soon as possible once they had become cooperative by the initial treatment for the psychotic episode;

(3) patients had definite Schneider's first-rank symptoms according to the criteria that a score was more than 3 (moderate) in any of the following seven items in the SAPS: item 2 (voices commenting); item 3 (voices conversing); item 15 (delusions of being controlled); item 16 (delusions of mind reading); item 17 (thought broadcasting); item 18 (thought insertion); and item 19 (thought withdrawal);

(4) brain MRI scan was performed within 1 month of the symptom ratings.

Among the initial total of 63 patients, 22 patients remained eligible. All patients were receiving neuroleptic medication. They were physically healthy, and none had a history of head trauma, neurological illness, serious medical or surgical illness, or substance abuse disorder. Demographic and clinical data are presented in Table 1.

Table 1. *Demographic and clinical characteristics of schizophrenia patients with Schneiderian symptoms and healthy comparison subjects*

	Schizophrenia patients ($n=22$)	Healthy comparison subjects ($n=44$)
Male/female	9/13	18/26
Age (years)	25·4±5·5 (range, 19–36)	25·4±5·1 (range, 19–38)
Height (cm)	162·5±8·1	165·1±7·5
Weight (kg)	56·9±10·8	57·0±9·2
Education (years)	13·1±1·7**	16·4±2·5
Parental education (years)	12·0±2·6	12·8±2·5
Age at onset (years)	21·8±4·5	—
Duration of illness (years)	3·9±3·9	—
Schneiderian score	13·8±7·3	—
Total SAPS score	42·6±20·0	—
Total SANS score	52·6±23·9	—
Drug dose (mg/day, haloperidol equivalent)	14·4±10·8	—
Duration of medication (years)	2·5±2·7	—

Values represent mean±S.D.

SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms.

** $p<0·01$, Student's t test.

Forty-four control subjects were healthy volunteers recruited from among the community, hospital staff, and university students. They matched the patients for age, gender, handedness, and parental education. They were interviewed by psychiatrists using a questionnaire concerning their family and past histories, and present illness. Subjects were excluded if they had a history of psychiatric illness, head trauma, neurological illness, serious medical or surgical illness, or substance abuse disorder. They were also screened for history of psychiatric disorders in their first-degree relatives. All control subjects were given the Minnesota Multiphasic Personality Inventory (MMPI), and were excluded if they had abnormal profiles with any T score exceeding 70.

Written informed consent was obtained from all subjects. This study was approved by the Committee on Medical Ethics of Toyama Medical and Pharmaceutical University, Japan.

MRI acquisition

MRI scans were acquired with a 1.5 T scanner (Vision, Siemens Medical System, Inc., Erlangen, Germany). A three-dimensional T_1-weighted gradient-echo sequence FLASH (fast

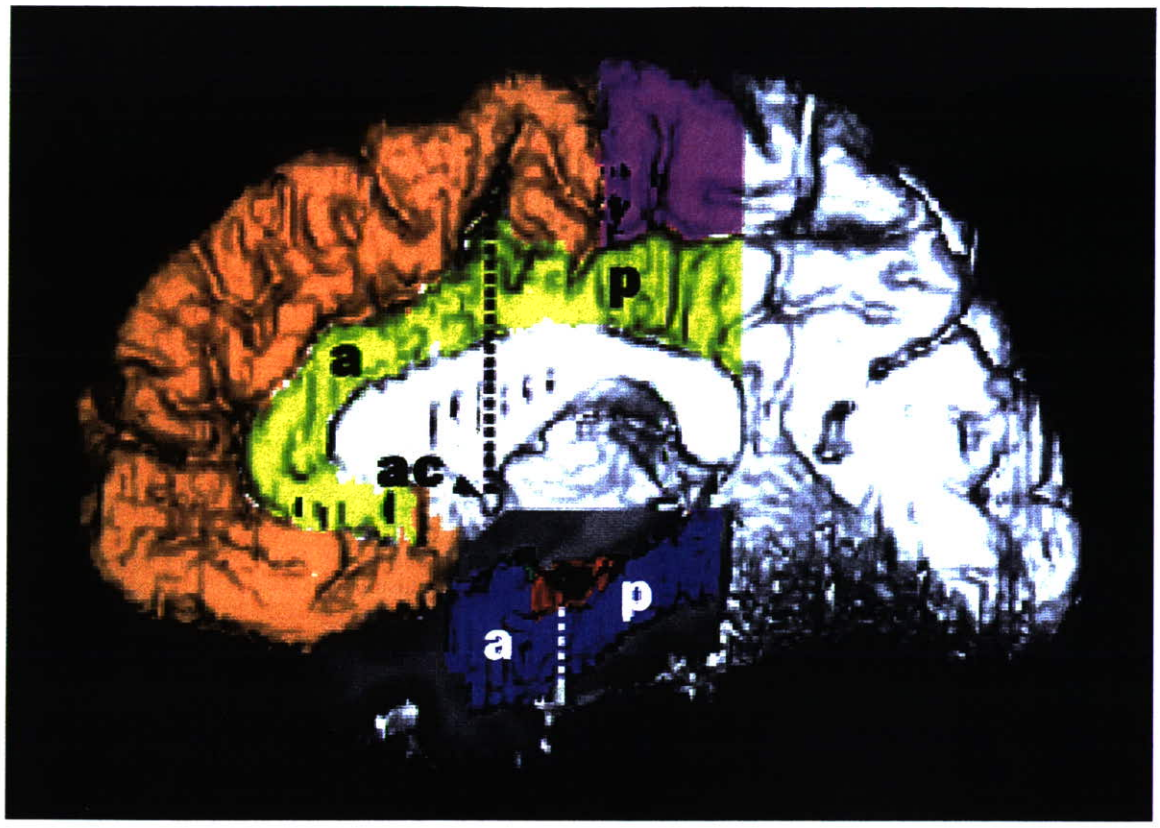

FIG. 1. Midsagittal view of a three-dimensional reconstructed image of the right hemisphere showing the regions of interest (ROIs), each of which is differentially colored: cingulate cortex (yellow), prefrontal cortex (orange), precentral cortex (purple), amygdala (green), hippocampus (red), and parahippocampal gyrus (blue). Part of the temporal lobe was removed to disclose the ROIs of the medial temporal lobe structures. The amygdala is only seen as a small piece in this aspect. The dotted lines (black, and white) indicate the level of the center of the anterior commissure and that of the posterior edge of mammillary body, respectively. a, anterior part; ac, anterior commissure; p, posterior part.

low-angle shots) with $1 \times 1 \times 1$ mm voxels was used. Imaging parameters were: TE = 5 ms; TR = 24 ms; flip angle = 40°; field of view = 256 mm; matrix size = 256×256.

Image processing

Image processing for volumetric region-of-interest (ROI) analysis has been described in detail previously (Takahashi *et al.* 2002). Briefly, on a Unix workstation (Silicon Graphics, Inc., Mountain View, CA, USA), the image data were processed with the software package Dr. View 5.0 (Asahi Kasei Joho System Co. Ltd, Tokyo, Japan). Brain images were realigned in three dimensions and reconstructed into entire contiguous coronal slices, with a 1-mm thickness, perpendicular to the anterior commissure–posterior commissure (AC–PC) line. The whole cerebrum was separated from the brainstem and cerebellum. The signal-intensity histogram distributions across the whole cerebrum were used to segment the voxels semi-automatically into gray matter, white matter, and cerebrospinal fluid. Intracranial volume (ICV) was measured as described previously (Zhou *et al.* 2003).

Volumetric analysis of ROIs

ROIs for volumetric measurements were placed on the frontal and medial temporal structures as presented in Fig. 1.

Frontal lobe

Delineation of the frontal lobe was partially based on the works of Rademacher *et al.* (1992) and Crespo-Facorro *et al.* (1999). First, the whole frontal lobe was separated from the rest of the brain. Taking account of the intrinsic anatomical landmarks (sulci/gyri), the whole frontal lobe was subdivided into three large functional parts: the precentral gyrus, the cingulate gyrus, and the prefrontal area. The prefrontal area was defined as the part of the whole frontal lobe from which the precentral gyrus and cingulate gyrus were excluded. With the

availability of synchronous-orthogonal views in three dimensions in conjunction with the context of gyri/sulci on successive coronal slices, decisions of anatomical landmarks (e.g. central sulcus, precentral sulcus, and cingulate sulcus) to separate and subdivide the frontal lobe could be readily made. The cingulate gyrus was further subdivided into the anterior part and the posterior part at the level of the center of anterior commissure (Fig. 1). The anterior and posterior parts approximately corresponded to Brodmann's area (BA) 24 and BA 23/31, respectively (Talairach & Tournoux, 1988). The procedure of the present study did not allow us to include part of the posterior cingulate gyrus and the retrosplenial cortex (Fig. 1). All the volumetric measurements were performed in reformatted consecutive 1-mm coronal slices (voxel size = 1 × 1 × 1 mm) by manual outlining. Gray-matter volumes of the regional cortices were calculated by applying the segmentation procedure described previously.

Medial temporal lobe structures

The amygdala, the hippocampus and the parahippocampal gyrus were manually outlined on consecutive coronal 1-mm slices, from anterior to posterior, with the corresponding sagittal and axial planes simultaneously presented for reference. Volumes of gray and white matter in each of these structures were measured together. The detailed procedures for delineations of these structures have been described previously (Niu *et al.* in press; Suzuki *et al.* in press).

Amygdala: The most anterior slice of the amygdala was that on which the amygdala just appeared as oval-shaped gray matter. As the slices proceed posteriorly, the rounded cortical nucleus of the amygdala transitions to a thin strip of gray matter, known as the hippocampal-amygdala transitional area. This area was included in the amygdala ROI. Thus the most posterior slice of the amygdala was the plane where the transitional area disappeared. The inferior border of the amygdala in contact with the hippocampal head was determined by reference to the sagittal plane since the boundary between the hippocampus and the amygdala is more readily identified on the sagittal plane (Convit *et al.* 1999). The alveus was used to differentiate the hippocampal head from the amygdala. The amygdala was separated by thin strips of white matter from the entorhinal cortex medially, and from the claustrum and tail of the caudate nucleus superio-laterally. The inferio-lateral boundary of the amygdala was the temporal lobe white matter and the extension of the temporal horn.

Hippocampus and parahippocampal gyrus: The alveus served as the superior boundary of the whole hippocampus. The inferior boundary was the white matter of the parahippocampal gyrus. The lateral and medial boundaries were the inferior horn of the lateral ventricle and the mesial edge of the temporal lobe respectively. For the parahippocampal gyrus, the most anterior slice was defined by the appearance of the white-matter tract (temporal stem) linking the temporal lobe with the rest of the brain. The parahippocampal gyrus was separated laterally by using a line drawn from the most lateral border of the hippocampal flexure to the collateral sulcus, and superiorly by the inferior gray border of the hippocampal formation. The most posterior slice for both the hippocampus and the parahippocampal gyrus was at the level of the last appearance of the fiber of the fornix. The parahippocampal gyrus was further subdivided into the anterior part and the posterior part at the level of the posterior edge of mammillary body (Fig. 1).

Three trained raters (S.Z., L.N. and H.H.), who were blinded to the subjects' identity, measured the volumes of the frontal lobe regions, the amygdala, and the hippocampus and parahippocampal gyrus respectively. Inter- and intra-rater intra-class correlation coefficients in five randomly selected brains were over 0·92 for the frontal ROIs and over 0·93 for the medial temporal ROIs.

Statistical analysis

Statistical analyses were performed using repeated-measures multivariate analysis of variance with age and ICV as covariates (MANCOVA) for each region, with group (patients, control subjects) and gender (male, female) as between-subject factors, and hemisphere (right, left) as a within-subject factor. *Post-hoc* Tukey's honestly significant difference tests modified for unequal sample sizes were employed to follow up the significant main effects or interactions yielded by MANCOVA.

Table 2. *Volumes of the regions of interest in schizophrenia patients with Schneiderian symptoms and healthy comparison subjects*

Regions of interest	Schizophrenia patients ($n=22$)	Healthy comparison subjects ($n=44$)	Diagnosis effect		
			F	df	p
Intracranial volume	1480±178	1483±136	<0·01	1, 61	0·987
Prefrontal gray matter[a]			0·72	1, 60	0·396
Left	91·55±11·25	94·57±8·75			
Right	88·37±11·23	89·57±7·66			
Cingulate gyrus gray matter[b,c]			9·47	1, 60	0·003
Left	7·81±1·87	8·77±2·25			
Right	9·43±2·31	10·82±1·80			
Anterior part[c]			4·17	1, 60	0·045
Left	3·69±1·46	4·16±1·69			
Right	5·14±1·84	5·88±1·59			
Posterior part[b]			10·17	1, 60	0·002
Left	4·12±0·80	4·61±0·94			
Right	4·29±1·23	4·94±0·67			
Precentral gyrus gray matter[a]			2·05	1, 60	0·157
Left	18·60±2·90	19·34±2·02			
Right	17·44±2·51	18·21±2·09			
Amygdala[b,c]			13·75	1, 60	<0·001
Left	0·99±0·13	1·11±0·14			
Right	1·05±0·15	1·14±0·13			
Hippocampus[c]			1·50	1, 60	0·225
Left	3·02±0·44	2·89±0·40			
Right	3·20±0·52	3·15±0·36			
Parahippocampal gyrus			1·41	1, 60	0·239
Left	7·28±0·83	7·07±0·84			
Right	7·38±0·93	7·24±0·78			
Anterior part[c]			1·97	1, 60	0·164
Left	2·58±0·43	2·41±0·60			
Right	2·88±0·56	2·74±0·55			
Posterior part[a]			<0·01	1, 60	0·970
Left	4·70±0·61	4·69±0·49			
Right	4·50±0·60	4·54±0·48			

Values are mean±S.D. of measured volume (cm^3).

Post-hoc comparisons followed multivariate analysis of variance with age and intracranial volume as covariates (MANCOVA) revealed: [a] Volume is larger on left hemisphere than on right hemisphere ($p<0·001$ for prefrontal area and precentral gyrus; $p=0·003$ for posterior parahippocampal gyrus). [b] Volume is smaller in patients than in controls ($p=0·009$ for whole cingulate gyrus; $p=0·007$ for posterior cingulate gyrus; $p=0·002$ for amygdala). [c] Volume is larger on right hemisphere than on left hemisphere ($p<0·001$ for whole cingulate gyrus, anterior cingulate gyrus, hippocampus, and anterior parahippocampal gyrus; $p=0·003$ for amygdala).

In the patient group, Pearson's correlation coefficients were calculated to examine relationships between the ROI volumes relative to ICV (100 × ROI volume/ICV) and the Schneiderian score. The Schneiderian score was log-transformed in order to remove skewness. To prevent a possible Type I error due to multiple comparisons, we limited the correlation analysis to the relative ROI volumes and the Schneiderian score. When significant correlations were obtained, they were also examined by partial correlation coefficients controlling for potential confounders such as duration of illness, duration of medication, dose of neuroleptics, or age. Further, the specificity of such correlations to the Schneiderian symptoms was examined by partial correlation coefficients controlling for the overall severity of symptoms assessed by total scores of the SAPS and SANS. Statistical significance was defined as $p<0·05$ (two-tailed).

RESULTS

Comparisons of volume measures between patients and controls

Volumes of intracranial cavity, gray matter of the frontal lobe regions, amygdala, hippocampus, and parahippocampal gyrus are presented in Table 2.

Frontal lobe regions

MANCOVA revealed a significant main effect of diagnosis in gray matter of the cingulate gyrus ($F=9{\cdot}47$, df $=1, 60$, $p=0{\cdot}003$). When its anterior and posterior parts were compared separately, there were also significant main effects of diagnosis ($F=4{\cdot}17$, df $=1, 60$, $p=0{\cdot}045$ for the anterior part; $F=10{\cdot}17$, df $=1, 60$, $p=0{\cdot}002$ for the posterior part). *Post-hoc* analyses showed significant gray-matter reductions of the whole cingulate gyrus ($p=0{\cdot}009$) and posterior part of the cingulate gyrus ($p=0{\cdot}007$) in the patients. There was no significant main effect of diagnosis in MANCOVA for gray matter of the precentral gyrus or prefrontal area. Significant main effects of hemisphere were observed in gray matter of the whole cingulate gyrus ($F=37{\cdot}32$, df $=1, 62$, $p<0{\cdot}001$), anterior part of the cingulate gyrus ($F=35{\cdot}36$, df $=1, 62$, $p<0{\cdot}001$), precentral gyrus ($F=24{\cdot}29$, df $=1, 62$, $p<0{\cdot}001$), and prefrontal area ($F=68{\cdot}00$, df $=1, 62$, $p<0{\cdot}001$). *Post-hoc* analyses revealed that the whole cingulate gyrus and the anterior part of the cingulate gyrus were larger on the right than on the left, whereas the precentral gyrus and prefrontal area were larger on the left than on the right (all $p<0{\cdot}001$). A significant interaction of gender by hemisphere was observed in the prefrontal area ($F=8{\cdot}81$, df $=1, 62$, $p=0{\cdot}004$). *Post-hoc* analyses revealed that the bilateral prefrontal area volumes were larger in males than in females ($p<0{\cdot}001$ for both hemispheres).

Medial temporal lobe

MANCOVA revealed a significant main effect of diagnosis in the amygdala ($F=13{\cdot}75$, df $=1, 60$, $p<0{\cdot}001$). *Post-hoc* analysis showed significant volume reduction in the patients ($p=0{\cdot}002$). There was no significant main effect of diagnosis in the hippocampus or parahippocampal gyrus. Nor was a significant diagnosis effect observed when the parahippocampal gyrus was subdivided into the anterior and posterior parts. There were significant main effects of hemisphere for the amygdala ($F=8{\cdot}57$, df $=1, 62$, $p=0{\cdot}005$), hippocampus ($F=47{\cdot}64$, df $=1, 62$, $p<0{\cdot}001$), anterior parahippocampal gyrus ($F=28{\cdot}54$, df $=1, 62$, $p<0{\cdot}001$), and posterior parahippocampal gyrus ($F=8{\cdot}33$, df $=1, 62$, $p=0{\cdot}005$). *Post-hoc* tests revealed that the amygdala ($p=0{\cdot}003$), hippocampus ($p<0{\cdot}001$), and anterior parahippocampal gyrus ($p<0{\cdot}001$) in the right hemisphere were larger than those in the left, while the posterior parahippocampal gyrus ($p=0{\cdot}003$) showed an asymmetry of left larger than right.

Correlations between volume measures and Schneiderian symptom severity

The Schneiderian score showed a trend toward a significant inverse correlation with the relative volume of gray matter of the right cingulate gyrus ($r=-0{\cdot}45$, $p=0{\cdot}051$). When the cingulate gyrus was subdivided into anterior and posterior parts, the Schneiderian score was significantly correlated with the relative volume of the right posterior cingulate cortex ($r=-0{\cdot}59$, $p=0{\cdot}004$) (Fig. 2*a*), but not with that of the anterior cingulate cortex ($r=-0{\cdot}09$, $p=0{\cdot}693$). This correlation between the Schneiderian score and the right posterior cingulate volume remained significant even after excluding a single subject with the lowest posterior cingulate volume ($r=-0{\cdot}52$, $p=0{\cdot}017$). There was also a significant inverse correlation between the Schneiderian score and the relative volume of the left anterior parahippocampal gyrus ($r=-0{\cdot}46$, $p=0{\cdot}032$) (Fig. 2*b*).

In this study, the illness duration in the patients varied among individuals, even though only young patients with relatively short duration were included. It was possible that progressive morphological changes, which have been suggested to occur in schizophrenia (Shenton *et al.* 2001), may have confounded the correlation results. In order to eliminate such effects, we conducted a partial correlation analysis controlling for the illness duration. The correlations between the Schneiderian score and the right posterior cingulate or the left anterior parahippocampal gyrus remained significant. Similarly these correlations controlling for duration of medication, dose of neuroleptic drugs, age, or the total scores of the SAPS and SANS also remained significant.

DISCUSSION

In the present study, the schizophrenia patients with Schneiderian symptoms had smaller volumes in the cingulate cortex and amygdala compared to the comparison subjects. The severity of Schneiderian symptoms in the patients was negatively correlated with the volumetric

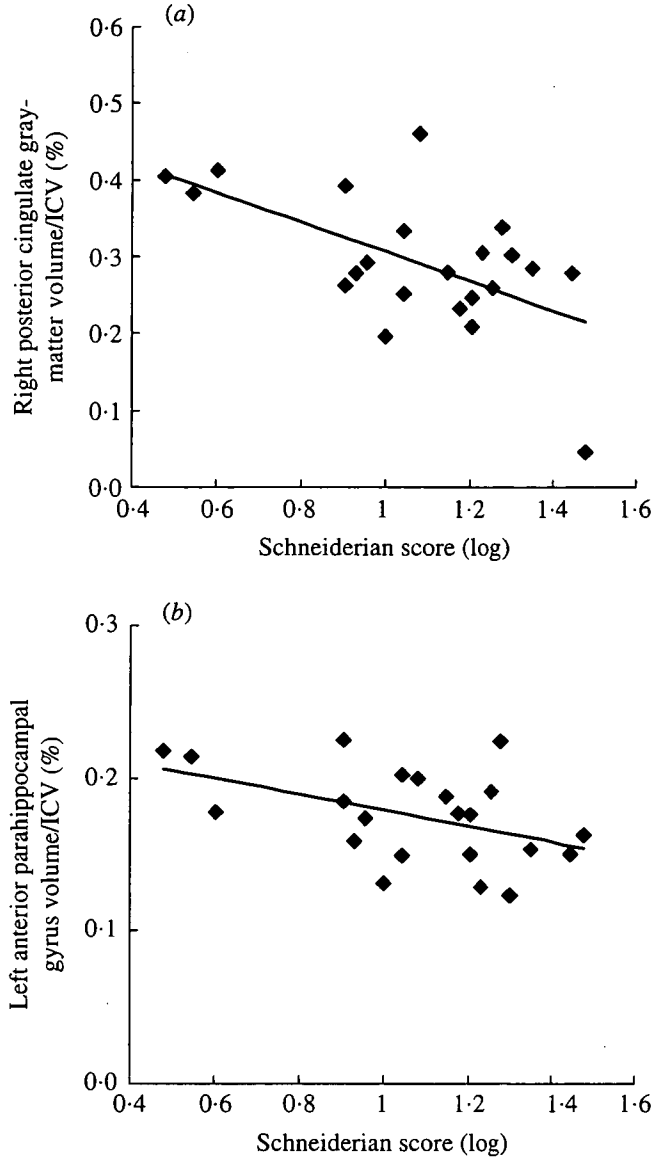

FIG. 2. (*a*) Correlations between Schneiderian score and relative right posterior cingulate gray-matter volume ($r = -0{\cdot}59$, $p = 0{\cdot}004$). (*b*) Correlations between Schneiderian score and relative left anterior parahippocampal gyrus volume ($r = -0{\cdot}46$, $p = 0{\cdot}032$).

measures of the right cingulate cortex, especially of the posterior part, and the left anterior parahippocampal gyrus. As these brain regions are intimately interconnected, our results suggest that structural abnormalities in the limbic-paralimbic neural circuit may underlie the schizophrenic syndrome with alienated features. To our knowledge, this is the first study to show the relation of overall severity of Schneiderian symptoms to the structural changes in specific brain regions in schizophrenia. In accordance with our hypothesis, these regions partly overlap with those which have been implicated in the self-monitoring function (Frith & Done, 1988; Gray *et al.* 1991).

The cingulate gyrus is a major component of the 'paralimbic belt' and has reciprocal connections with neocortical association areas as well as with the amygdala and hippocampus. The anterior and posterior parts of the cingulate cortex are differentially organized in cytoarchitecture as well as in function, although they are densely interconnected (Vogt *et al.* 1995; Devinsky *et al.* 1995). The anterior cingulate (BA 24/32) has been implicated not only in non-spatial attentional and executive functions (Pardo *et al.* 1990; Elliott & Dolan, 1998), but also in numerous other functions such as motor control, spatial working memory, selection for action, conflict monitoring, error monitoring, and performance evaluation (Paus *et al.* 1993; Carter *et al.* 1998; Botvinick *et al.* 1999; MacDonald *et al.* 2000). Posner & Rothbart (1998) proposed a broad role for the anterior cingulate in the conscious self-regulation of behavior via its reciprocal anatomical connectivity with the prefrontal and limbic regions. On the other hand, there is evidence that the posterior cingulate (BA 23/29/30) is involved in episodic memory, processing of emotionally salient stimuli, and spatial attention (Grasby *et al.* 1993; Maddock, 1999; Mesulam *et al.* 2001). Mesulam *et al.* (2001) have suggested that the posterior cingulate may enable the bias of anticipatory attention to be set in the direction of events that have intrinsic or experientially acquired significance.

In schizophrenia, functional and structural abnormalities of the anterior cingulate cortex have been reported in a large number of neuroimaging and post-mortem studies (Benes *et al.* 1991; Andreasen *et al.* 1992*b*; Carter *et al.* 1997; Takahashi *et al.* 2002, 2003), and its critical role in the pathophysiology of schizophrenia has been proposed (Benes, 1998; Tamminga *et al.* 2000). It has been suggested that impaired error monitoring in schizophrenia patients relates to dysfunction in the anterior cingulate (Carter *et al.* 2001; Alain *et al.* 2002). Laurens *et al.* (2003) recently reported decreased activity of the rostral anterior cingulate and associated limbic-paralimbic structures, including the posterior cingulate gyrus and hippocampus, during the commission of errors in schizophrenia patients. The posterior cingulate has been

examined less extensively in structural imaging studies of schizophrenia. However, a few voxel-based morphometric studies have shown gray-matter reduction in the posterior cingulate in patients with schizophrenia (Sowell *et al.* 2000; Hulshoff Pol *et al.* 2001), and a recent longitudinal MRI study demonstrated posterior cingulate gray-matter loss in parallel with psychosis development (Pantelis *et al.* 2003). Previous functional imaging studies revealed that patients with schizophrenia showed abnormal metabolism in the posterior cingulate (Andreasen *et al.* 1997; Haznedar *et al.* 1997), and cerebral blood flow in this region was negatively correlated with Schneiderian first-rank symptoms (Franck *et al.* 2002). In addition, the cingulate gyrus has been implicated in the etiology of 'alienation' in organic brain disorders (Feinberg *et al.* 1992). Thus, considering the anatomical and functional correlates of the cingulate cortex, its structural deficits might represent the liability that internally generated thoughts or actions are imbued with abnormal perceptual qualities and misattributed to external agencies.

The anterior parahippocampal gyrus was another region implicated in the Schneiderian symptoms in the current study. Cytoarchitectonic as well as volumetric abnormalities in the entorhinal cortex (anterior part of the parahippocampal gyrus) have been demonstrated in post-mortem and MRI studies in schizophrenia (Jakob & Beckmann, 1986; Falkai *et al.* 2000; Joyal *et al.* 2002). A positron emission tomography study demonstrated that abnormal cerebral blood flow in the left parahippocampal region correlated with overall symptom severity in schizophrenia (Friston *et al.* 1992). Our findings are consistent with the notion that subtle structural abnormality of the entorhinal cortex, especially of the left side, is associated with the expression of positive symptoms in schizophrenia (Bogerts, 1997; Kurachi, 2003*b*). The hippocampo-entorhinal complex is thought to play a critical role in memory and learning by acting as a neural gateway for encoding and retrieval, and thus, possibly orchestrate the coherent storage and reactivation of information distributed widely in the brain (Mesulam, 2000). A structurally deviant parahippocampal gyrus might conduct misguiding orchestration, which could contribute, to some extent, to adding an alienated nature to the experiences of patients.

Few attempts have been made to examine specific relationships between structural deficits of the amygdala and clinical symptoms of schizophrenia using MRI, since many of the previous studies measured the amygdala and hippocampus as a single ROI (Shenton *et al.* 2001). Kurachi (2003*b*) proposed the amygdala as a principal candidate for the substrate of Schneiderian symptoms based on theoretical considerations as well as on data from experimental studies. Among a limited number of studies which specifically measured the amygdala, Gur *et al.* (2000) observed smaller volume of the amygdala only in male patients with schizophrenia. Joyal *et al.* (2003) reported bilateral volume reductions of the amygdala in first-episode patients. Reduced volume of the amygdala found in the present study is consistent with these previous studies. However the amygdala volumes were not correlated with the severity of Schneiderian symptoms. The human amygdala, in general, plays crucial roles in modulating the impact of sensory stimuli according to the emotional valence and in emotional conditioning (Mesulam, 2000). So a possible contribution of the involvement of the amygdala may at least be to exert an abnormal modulation of emotional experiences elicited by the Schneiderian symptoms, and to add further distortion of psychological reality to the patients' experiences.

The findings of the current study should be interpreted with caution for several reasons. First, the relatively small sample size of patients with Schneiderian symptoms may have limited the ability to generalize our findings. Second, although we reduced the number of correlation analyses, the possibility of a Type I error due to the multiple comparisons must be taken into account, especially for the marginally significant correlation between the Schneiderian score and the left anterior parahippocampal gyrus volume. A further study with a larger sample is needed to confirm this finding. Third, the lack of implication of the prefrontal cortex may not exclude the possible differential contributions of the prefrontal subcomponents to the formation of Schneiderian symptoms. A future study subdividing the prefrontal cortex into functionally heterogeneous subcomponents will clarify this issue. Finally, the Schneiderian symptoms consist of certain types of auditory

hallucination and the so-called bizarre delusions (disturbance of the self), which are phenomenologically different, although all of them share an alienated feature. Thus, there should be some differences in the underlying neurobiology among these symptoms. Especially, the superior temporal gyrus has been suggested to be involved in the auditory hallucinations (Barta *et al.* 1990; Suzuki *et al.* 1993; Levitan *et al.* 1999), whereas the parietal cortex has been related to delusions of control (Spence *et al.* 1997; Blakemore *et al.* 2003). However, this point is somewhat out of the scope, since the specific aim of the current study was to find a common morphological basis (denominator) for the alienated features of schizophrenia syndrome. Involvement of additional structural deficits and/or functional changes elicited in more widespread interconnected regions may be responsible for the variety of the alienated symptoms.

In summary, the present study suggests that Schneider's first-rank symptoms are based on structural changes in the limbic-paralimbic circuit comprising the cingulate gyrus, the parahippocampal gyrus, and possibly the amygdala. Future studies should be worth being performed to confirm our findings with a larger sample and to clarify the relationships between the morphological changes in the brain and cognitive deficits possibly underlying Schneiderian symptoms, such as the self-monitoring dysfunction, in patients with a propensity for these symptoms.

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DECLARATION OF INTEREST

None.

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Volumetric MRI study of the short and long insular cortices in schizophrenia spectrum disorders

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Abstract

We have previously reported volume reductions of the insular cortex in schizophrenia, but it is still not clear whether insular cortex volume loss preferentially involves the anterior (short insular cortex) or posterior (long insular cortex) portion. On the other hand, no volumetric studies of the brain have examined changes in insular cortex volume in subjects with schizotypal features. In this study, we separately investigated the volumes of the short and long insular cortex portions using magnetic resonance imaging in 37 schizotypal disorder patients (24 males, 13 females), 62 schizophrenia patients (32 males, 30 females), and 69 healthy controls (35 males, 34 females). While the volumes of the short and long insular cortex were significantly reduced in schizophrenia patients compared with schizotypal disorder patients and control subjects, there was no difference between schizotypal disorder patients and control subjects. These results suggest that the volume reduction of the insular cortex may be specific to overt schizophrenia without topographically specific localization.

Keywords: Magnetic resonance imaging; Insula; Schizophrenia; Schizotypal disorder

1. Introduction

Post-mortem (Jakob and Beckmann, 1986, 1989) and functional neuroimaging (Curtis et al., 1998; Shergill et al., 2000; Crespo-Facorro et al., 2001a,b; Surguladze et al., 2001; Desco et al., 2003) studies have suggested that insular cortex abnormalities are involved in the pathophysiology of schizophrenia. With regard to the morphology of the insular cortex in schizophrenia, recent volumetric magnetic resonance imaging (MRI) studies have reported that schizophrenia patients have a significantly smaller insular cortex volume than do control subjects

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(Crespo-Facorro et al., 2000; Kasai et al., 2003; Kim et al., 2003; Takahashi et al., 2004a). Several voxel-based analyses of MRI have also revealed a gray matter reduction of the insular cortex in patients with schizophrenia (Wright et al., 1999; Hulshoff Pol et al., 2001; Paillère-Martinot et al., 2001; Kubicki et al., 2002; Shapleske et al., 2002; Kawasaki et al., 2004).

There are distinct differences in the connectivities and functions of the anterior (short insular cortex) versus posterior (long insular cortex) portions of the insular cortex; these two subregions are diagonally divided by the central insular sulcus (Augustine, 1996; Duvernoy, 1999; Türe et al., 1999). However, most previous volumetric MRI studies (Crespo-Facorro et al., 2000; Kim et al., 2003; Takahashi et al., 2004a) have not taken into account these differences. Kasai et al. (2003) separately examined the short and long insular cortex and reported that insular volume loss associated with schizophrenia is not localized to a particular subregion, although their study might also have been limited in part by having bounded the two subregions not by their own anatomical boundaries but other anatomical landmarks, i.e. mamillary bodies. Thus, it remains unresolved whether the insular cortex volume reduction in schizophrenia preferentially involves the anterior (short insular cortex) or posterior (long insular cortex) portion.

Subjects with schizotypal features such as schizotypal disorder in ICD-10 (World Health Organization, 1992) or schizotypal personality disorder (SPD) in DSM-IV (American Psychiatric Association, 1994) share genetic, biological, and psychological features with schizophrenia and are thought to be part of the schizophrenia spectrum (Siever et al., 1993; Siever and Davis, 2004). Several recent brain structural imaging studies have identified specific structural abnormalities in schizotypal subjects similar to those seen in schizophrenia, although generally to a lesser degree and with the sparing of some brain regions (reviewed by Dickey et al., 2002a; Siever et al., 2002; Siever and Davis, 2004). The abnormalities include increased lateral ventricular size (Siever et al., 1995; Buchsbaum et al., 1997; Silverman et al., 1998), greater cerebrospinal fluid volume (Dickey et al., 2000), volume reduction in temporal lobe structures (Dickey et al., 1999, 2002b; Seidman et al., 1999; Downhill et al., 2001), and volume reduction in the thalamus (Hazlett et al., 1999; Seidman et al., 1999; Byne et al., 2001), basal ganglia (Shihabuddin et al., 2001; Levitt et al., 2002), and internal capsule (Suzuki et al., 2004), along with shape and size differences in the corpus callosum (Downhill et al., 2000) and asymmetry anomalies in the parahippocampal gyrus (Dickey et al., 1999) and the anterior cingulate gyrus (Takahashi et al., 2002b). The shared brain abnormalities between schizotypal and schizophrenia patients might represent a common denominator in schizophrenia spectrum disorders, whereas the differences might account for the sparing of schizotypal patients from the development of overt psychotic symptoms. Therefore, assessing schizotypal patients on brain regions such as the insular cortex that have been identified previously as impaired in schizophrenia patients is one possible strategy for advancing our understanding of pathogenesis of schizophrenia spectrum disorders. In addition, it is of interest to know the morphologic characteristics of the insular cortex, a brain region interconnected with both temporal and frontal regions (Augustine, 1996; Türe et al., 1999), in schizotypal disorder patients since the differential involvement of the frontal regions has been suggested to underlie the differences in phenomenology between schizophrenia and schizotypal patients while the abnormalities in temporal regions have been considered to be common to both disorders (Kurachi, 2003a,b; Siever and Davis, 2004). To our knowledge, however, no volumetric MRI studies have examined the insular cortex volume in subjects with schizotypal features.

In the present study, we followed the course of the central insular sulcus and accurately distinguished between the short and long insular cortex using three-dimensional MRI. We separately measured the volumes of the short and long insular cortex in schizophrenia patients, schizotypal disorder patients, and normal control subjects. The aims of the present study were to determine if the short and long insular cortices exhibited different patterns in terms of structural abnormalities in schizophrenia and to test the hypothesis that schizotypal disorder patients would have structural abnormalities in the insular cortex that were partly similar to those seen in overt schizophrenia.

2. Methods

2.1. Subjects

Thirty-seven schizotypal disorder patients (24 males and 13 females; mean age=25.8 years, SD=5.4, range=18–37) who met the ICD-10 criteria for research (World Health Organization, 1993) were examined. They were recruited from patients who visited the clinics of the Department of Neuropsychiatry, Toyama Medical and Pharmaceutical University Hospital, with schizotypal features accompanied by distress or associated problems in their lives and who needed to receive consistent clinical follow-up. Candidates who had a previous history of overt psychotic episode or met the ICD-10 criteria for schizophrenia during the follow-up period were excluded. The mental condition of each subject was assessed by well-trained psychiatrists approximately every 2 weeks to check for the emergence of overt psychotic symptoms as part of an early intervention program for psychoses, and none of the 37 patients have developed overt schizophrenia to date (mean follow-up period after MRI scanning=2.0 years, SD=1.7). All available clinical information and data obtained from a detailed review of the clinical records and structured interviews using the Comprehensive Assessment of Symptoms and History (CASH) including the chapter on premorbid or intermorbid personality (Andreasen et al., 1992) were stored in a database. The subjects were diagnosed by consensus reached by at least two experienced psychiatrists based on these data. Twenty-nine patients were outpatients, and the other eight underwent closer clinical and medical examinations including MRI during short-term admission. At the time of MRI scanning, 32 of the 37 patients were treated with low-dose antipsychotics, of which 11 patients were treated with typical neuroleptics and 21 patients received atypical neuroleptics. The remaining five patients were neuroleptic-naïve. Clinical symptoms were rated within 1 month of scanning using the Scale for the Assessment of Negative Symptoms (SANS; Andreasen, 1984a) and the Scale for the Assessment of Positive Symptoms (SAPS; Andreasen, 1984b). Their mean scores for the SANS and SAPS were 42.4 (SD=23.0, range=5–84) and 16.0 (SD=8.9, range=0–31), respectively. Thirty-three of the 37 patients with schizotypal disorder were also assessed using the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1962). Their mean total BPRS score was 38.4 (SD=9.7, range=19–61).

The schizophrenic comparison group was composed of 62 patients with schizophrenia [32 males and 30 females, mean age=25.8±4.9 (SD) years, range=18–36], and this group contained 58 schizophrenia patients (31 males, 27 females) who were examined in our previous study of the whole insular cortex volume (Takahashi et al., 2004a). All patients fulfilled the ICD-10 diagnostic criteria for research on schizophrenia (World Health Organization, 1993). All but one of the schizophrenia patients were receiving neuroleptic medication; 30 patients were treated with typical neuroleptics and 31 patients with atypical neuroleptics. At the time of the MRI study, their mean scores on the SANS and SAPS were 46.8 (SD=23.4) and 25.2 (SD=20.4), respectively.

The control subjects consisted of 69 healthy volunteers [35 males and 34 females, mean age=24.0±6.5 (SD) years, range=18–38] recruited from among members of the community, hospital staff, and medical and pharmaceutical students, and included 61 subjects who participated in a previous study (Takahashi et al., 2004a). They were given a questionnaire consisting of 15 items concerning their family and past histories, as well as present illness. They did not have any personal or family history of psychiatric illness in their first-degree relatives. The control subjects were not screened with a standard measure such as the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II; First et al., 1997) and this may be a possible limitation of the study. However, all control candidates were interviewed and administered the Minnesota Multiphasic Personality Inventory (MMPI) by experienced clinical psychologists to obtain a rather homogenous control group without eccentric profiles on the MMPI. Although the MMPI has not proved very sensitive for the detection of schizotypy (Walters, 1983), approximately 17% of the candidates for normal controls were excluded for having an abnormal profile with a T-score for the validity scales or the clinical scales exceeding 70.

All subjects were physically healthy at the time of the study, and none had a lifetime history of serious head trauma, neurological illness or substance abuse. A handedness inventory developed by

Kameyama et al. (1981) consisting of 14 questions about hand preference was used to assess handedness; the subjects were right-handed except for one female patient with schizotypal disorder of unknown handedness.

The demographic and clinical characteristics of the control subjects, patients with schizotypal disorder and patients with schizophrenia are summarized in Table 1. The three groups were matched on age, height or parental education. Although there were more male than female schizotypal patients, the difference in the gender ratios among the three diagnostic groups was not statistically significant (chi-square analysis, $\chi^2=2.20$, $P=0.333$). The control subjects had attained a higher mean level of education than had the patients with either disorder (control subjects, 15.7 ± 2.5 years; schizophrenia patients, 13.4 ± 1.9 years; schizotypal patients, 13.5 ± 1.9 years; ANOVA, $F=23.28$, $df=2,165$, $P<0.001$). The total SAPS score for the schizophrenia patients was significantly higher than that for the schizotypal patients (ANOVA, $F=6.60$, $df=1,96$, $P=0.012$), although there were no significant differences between patients with schizophrenia and schizotypal disorder for the total score for SANS. There were significant differences in medication dosage (ANOVA, $F=17.95$, $df=1,97$, $P<0.001$); the patients with schizotypal disorder took significantly smaller amounts of neuroleptics than did the patients with schizophrenia. This study was approved by the Committee on Medical Ethics of Toyama Medical and Pharmaceutical University. After a complete description of the study was given to the subjects, their written informed consent was obtained.

2.2. Magnetic resonance imaging procedures

Magnetic resonance images were obtained utilizing a 1.5-T Magnetom Vision (Siemens Medical System, Inc., Erlangen, Germany) with a three-dimensional gradient-echo sequence FLASH (fast low-angle shots) yielding 160–180 contiguous T1-weighted slices of 1.0-mm thickness in the sagittal plane. Imaging parameters were repetition time=24 ms, echo time=5 ms, flip angle=40°, field of view=256 mm, and matrix size=256×256 pixels. The voxel size was $1.0\times1.0\times1.0$ mm^3. Magnetic field inhomogeneities in our scanner were monitored with weekly phantom scanning and daily basic quality control, and had been stable over the MR acquisition time for this study.

Table 1
Clinical and demographic characteristics of normal control subjects, patients with schizotypal disorder, and patients with schizophrenia

Variable	Control subjects		Schizotypal patients		Schizophrenia patients	
	Male ($N=35$)	Female ($N=34$)	Male ($N=24$)	Female ($N=13$)	Male ($N=32$)	Female ($N=30$)
Age (years)	24.1 ± 5.1	23.8 ± 5.8	25.7 ± 5.8	25.9 ± 4.6	25.6 ± 4.8	26.0 ± 5.1
Height (cm)	171.9[a] ± 4.3	159.2 ± 4.5	170.8[a] ± 5.9	156.2 ± 4.6	170.7[a] ± 5.1	158.5 ± 4.0
Education (years)	16.6[b] ± 2.8	14.8 ± 1.6	13.4 ± 1.9	13.5 ± 2.0	13.5 ± 1.9	13.3 ± 1.9
Parental education (years)	13.0 ± 2.3	12.5 ± 2.3	12.1 ± 1.5	12.1 ± 2.3	12.2 ± 1.9	11.9 ± 2.4
Age at onset (years)	–	–	–	–	22.1 ± 4.5	21.9 ± 4.2
Duration of illness (years)	–	–	–	–	3.5 ± 3.9	4.4 ± 4.3
Duration of medication (years)	–	–	2.1 ± 3.8	0.9 ± 1.5	2.4 ± 2.9	3.3 ± 3.7
Drug (mg/day, haloperidol equiv.)[c]	–	–	5.1 ± 5.5	2.5 ± 1.6	11.7[d] ± 8.6	10.8[d] ± 10.4
Total SAPS score	–	–	15.8 ± 8.7	16.3 ± 10.1	23.0[e] ± 21.1	27.7[e] ± 19.8
Total SANS score	–	–	40.4 ± 23.4	46.5 ± 23.4	50.0 ± 22.4	43.4 ± 24.4

This values represent means ± SDs. SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms.
ANOVA followed by Scheffé's test was used.
[a] $P<0.01$: compared with females.
[b] $P<0.01$: compared with female schizophrenia patients, male schizotypal patients, and female schizotypal patients; $P<0.05$: compared with female controls and male schizophrenia patients.
[c] The different typical and atypical neuroleptic dosages were converted into haloperidol equivalents according to Toru (2001).
[d] $P<0.01$: compared with schizotypal patients.
[e] $P<0.05$: compared with schizotypal patients.

The images were transferred to a Unix workstation (Silicon Graphics, Inc, Mountain View, CA., USA), and the data were randomly coded and analyzed using the software package Dr View 5.3 (Asahi Kasei Joho System Co, Ltd, Tokyo, Japan) without knowledge of the subjects' gender and diagnosis. Details of the data analyses have been previously described (Takahashi et al., 2002a). Briefly, the scans were realigned in three dimensions to standardize for differences in head tilt during image acquisition and were then reconstructed into entire contiguous coronal images, with a 1-mm thickness, perpendicular to the anterior commissure–posterior commissure (AC-PC) line on the workstation. The signal-intensity histogram distributions from the T1-weighted images across the whole brain for each subject were used to segment the voxels semi-automatically into gray matter, white matter, and cerebrospinal fluid (CSF) according to the Alpert algorithm (Alpert et al., 1996). Although the images were not corrected for the magnetic field inhomogeneities, no visible effect on the quality of the segmentation was detected for any case. Before the volumetric analysis of the insular cortex, masks were semi-automatically created to demarcate the outer extent of the intracranial contents with the skull, scalp, and neck tissues removed, and therefore minimal manual editing of the masks was required.

2.3. Intracranial volume (ICV) measurements

Intracranial volume (ICV) was measured to correct for differences in head size. Before creation of the mask images, the 1-mm-thick coronal slices which had been corrected for head tilt were reformatted into consecutive 5-mm-thick sagittal slices with each voxel as $1 \times 1 \times 5$ mm^3. The intracranial cavity was manually traced for each slice using anatomical landmarks according to a study by Eritaia et al. (2000), and the ICV was calculated by summing the measured volumes of all slices.

2.4. Insular cortex measurements

First, based on the segmented gray matter images, the whole (short and long) insular cortex was traced on 1-mm consecutive coronal slices as described elsewhere (Takahashi et al., 2004a). Specifically, the most rostral coronal plane containing the insular cortex and the coronal plane containing the fusion of the superior and inferior circular insular sulci were chosen as anterior and posterior boundaries, respectively. On each coronal slice, the insular cortex was bounded superiorly by the superior circular insular sulcus and inferiorly by the inferior circular insular sulcus or the orbitoinsular sulcus.

Next, we followed the course of the central insular sulcus in three dimensions from the limen insulae and distinguished between the short and long insular cortex on coronal 1-mm slices (Fig. 1). The insular cortex rostral to the slice showing the limen insulae was regarded as the short insular cortex. On more caudal coronal slices, the short and long insular cortices were divided in a superior–inferior direction by the central insular sulcus, which was readily identified on the coronal slices in most cases. As previously noted by Naidich et al. (2004), the central insular sulcus provided a prominent landmark on conventional sagittal images, even when it was not clearly seen on coronal slices.

All volumetric data reported in this study were measured by one rater (TT) who was unaware of the subjects' identity, gender, and diagnosis. To determine the reliability of the measurements, five subjects were randomly selected for a total of approximately 275 slices (approximately 55 slices per brain). The short and long insular cortices in a subset of these five subjects were measured independently by two raters (TT and RT), and intraclass correlation coefficients (ICCs) were calculated. The inter-rater ICCs of the short and long insular cortex measurements were greater than 0.93. Each volume was then remeasured after at least 4 weeks by the first rater; the intra-rater ICCs of the short and long insular cortex measurements were greater than 0.98.

2.5. Statistical analysis

The absolute insular cortex volume was analyzed using repeated measures multivariate analysis of variance (MANCOVA) with age, ICV, and dosage of neuroleptic medication as covariates, diagnosis and gender as between-subject factors, and hemisphere (left, right) and subregion (short, long) as within-subject variables. Since a significant main effect for the subregion was observed (F=2469.84; df=1,162;

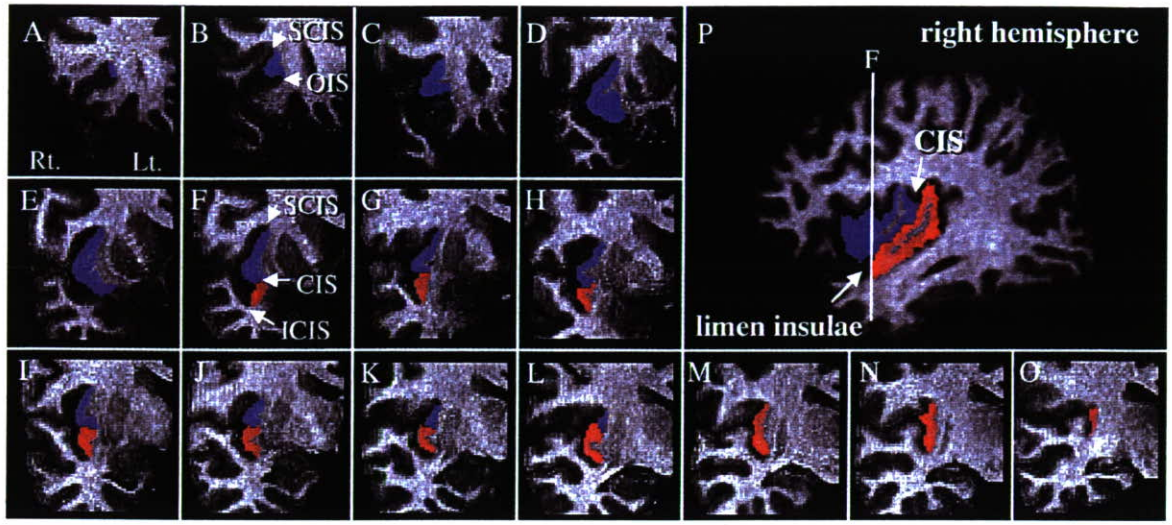

Fig. 1. Regions of interest manually traced in this study. The sample coronal slices (panels A-O) show delineations of the right short insular cortex (blue) and right long insular cortex (red), and panel P shows a sagittal view of the insular cortex in the right hemisphere. The coronal line F corresponds to panel F, a coronal slice showing the limen insulae. Abbreviations: CIS = central insular sulcus; ICIS = inferior circular insular sulcus; OIS = orbitoinsular sulcus; SCIS = superior circular insular sulcus.

$P<0.001$), the absolute volumes for the short and long insular cortex were then separately analyzed using the same model but with only hemisphere as a within-subject variable. As the schizotypal disorder patients took significantly smaller amounts of neuroleptics than the schizophrenia patients, the dosage of neuroleptic medication was used as the covariate for these analyses. For the comparison of the ICV, height was treated as covariate; groups did not significantly differ in ICV volume (Table 2). Post hoc Spjotvoll and Stoline tests, modified Tukey's tests for unequal sample size, were carried out to follow up the significant main effects or interactions yielded by these analyses (Fig. 2).

To analyze volume changes in relation to clinical symptoms, Spearman's rank correlation was calculated between the relative volumes for the long and short insular cortex and scores for the subscales of SAPS and SANS. The relative insular cortex volume, used to control for differences in head size, was obtained by dividing the absolute volume of the insular cortex by ICV and multiplying the result by 100. To examine the

Table 2
Intracranial volume (ICV) and absolute insular cortex volume in control subjects, patients with schizotypal disorder, and patients with schizophrenia

Brain region	Control subjects				Schizotypal patients				Schizophrenia patients				Analysis of covariance		
	Male ($N=35$)		Female ($N=34$)		Male ($N=24$)		Female ($N=13$)		Male ($N=32$)		Female ($N=30$)		Diagnosis effect[a]		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	df	P
ICV (cm^3)	1579	99	1384	108	1584	108	1420	154	1567	136	1391	101	1.13	2, 161	0.325[b]
Short insular cortex (mm^3)													3.19	2, 159	0.044[c]
Left	5588	601	4808	686	5347	652	4981	542	5095	661	4689	695			
Right	5349	637	4695	588	5261	699	5112	807	4855	556	4458	639			
Long insular cortex (mm^3)													5.26	2, 159	0.006[c]
Left	2795	493	2649	449	2911	628	2899	481	2763	500	2357	550			
Right	2678	429	2627	336	2906	377	2650	405	2676	448	2372	474			

[a] For the other main effects and interactions, and the results of post hoc tests, see the text.
[b] Height was used as covariate.
[c] Age, ICV, and dosage of neuroleptic medication were used as covariates.

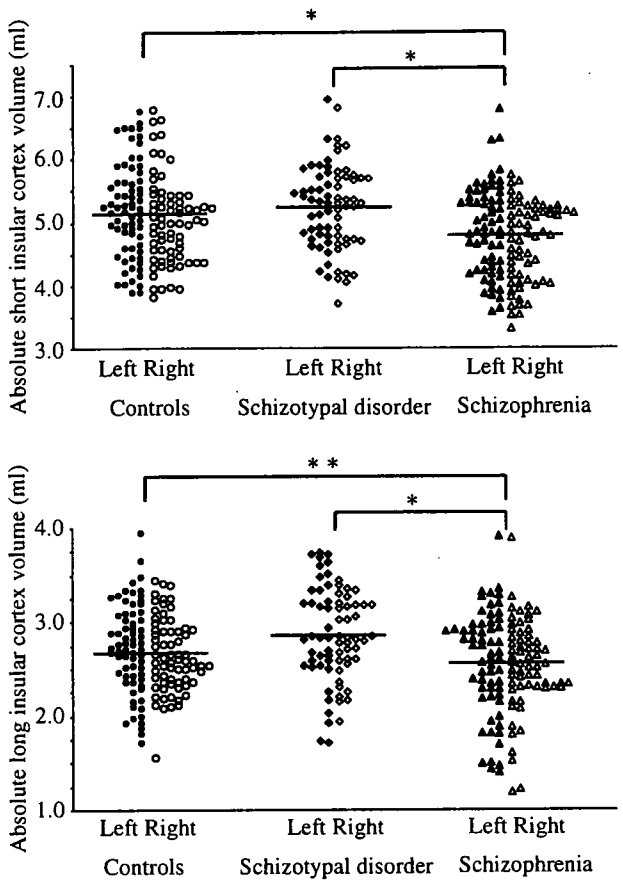

Fig. 2. Absolute volumes of the short and long insular cortex in control subjects (35 males, 34 females), schizotypal disorder patients (24 males, 13 females), and schizophrenia patients (32 males, 30 females). Horizontal lines indicate means. Post hoc Spjotvoll and Stoline tests: $^{*}P<0.01$, $^{**}P<0.05$.

effects of neuroleptic medication, correlations between the relative volumes for the long and short insular cortices and daily medication dosage and duration of neuroleptic medication were analyzed using Spearman's rank correlation coefficients. For the patients with schizophrenia, the correlation between the relative insular cortex volume and illness duration or age of onset was also analyzed. For these analyses, statistical significance was defined as $P<0.05$.

3. Results

3.1. Insular cortex measurements

Table 2 summarizes the short and long insular cortex measurements in schizophrenia patients, schizotypal disorder patients, and control subjects. Repeated measures MANCOVA revealed significant main effects for diagnosis ($F=6.06$; $df=2,159$; $P=0.003$), hemisphere ($F=23.55$; $df=1,162$; $P<0.001$), and subregion ($F=2469.84$; $df=1,162$; $P<0.001$). However, there was no significant diagnosis × subregion interaction ($F=1.80$; $df=2,162$; $P=0.169$). This indicates that the between-group difference in insular cortex volume was not specific for one subregion.

Lower order MANCOVA of the short insular cortex revealed significant main effects for diagnosis ($F=3.19$; $df=2,159$; $P=0.044$) and hemisphere ($F=8.91$; $df=1,162$; $P=0.003$), where patients with schizophrenia had a significantly smaller short insular cortex than schizotypal patients (post hoc test, $P=0.001$) and control subjects (post hoc test, $P=0.001$) bilaterally, and the short insular cortex volume was larger for the left than the right hemisphere for all diagnostic groups (post hoc test, $P=0.002$). There was no significant difference in short insular cortex volume between schizotypal disorder patients and control subjects (post hoc test, $P=0.827$), and no main effect for gender ($F=2.35$; $df=1,159$; $P=0.127$) or interaction among the factors was observed.

Lower order MANCOVA of the long insular cortex revealed significant main effects for diagnosis ($F=5.26$; $df=2,159$; $P=0.006$). Post hoc analyses showed the long insular cortex to be significantly reduced in the schizophrenia patients compared with the schizotypal disorder patients ($P<0.001$) and with the controls ($P=0.044$). No significant difference in the long insular cortex volume emerged between the schizotypal disorder patients and control subjects ($P=0.074$). There was no significant main effect for gender ($F=1.73$; $df=1,159$; $P=0.190$) or hemisphere ($F=3.79$; $df=1,162$; $P=0.053$), and no interaction among the factors was found.

3.2. Clinical correlations

For both patient groups, there were no significant correlations between the volumes for the short and long insular cortex and the scores for the subscales of the SAPS or SANS. For schizotypal disorder patients, the short and long insular cortex volumes were not correlated with the medication dosage or duration of

neuroleptic medication. For schizophrenia patients, insular cortex volume was negatively correlated with illness duration (right short insular cortex, Spearman's rho=0.39, $P=0.002$; left long insular cortex, Spearman's rho=0.47, $P<0.001$) and duration of neuroleptic medication (right short insular cortex, Spearman's rho=0.38, $P=0.002$) even after Bonferroni correction for multiple comparisons was made [i.e. $P<0.003$ (0.05/16)]. However, insular cortex volume was not correlated with age at onset of illness or dosage of neuroleptic medication.

4. Discussion

To our knowledge, this is the first volumetric MRI study to separately investigate sulcally defined short and long insular cortex volumes in schizophrenia spectrum disorders. The primary positive finding of this study was a significant volume reduction in the short and long insular cortices without a pattern of topographically specific localization for schizophrenia patients compared with schizotypal disorder patients and control subjects. In contrast, we found no volume differences in the short or long insular cortices between schizotypal disorder patients and normal controls.

The anterior and posterior portions of the insular cortex have been reported to have cytoarchitectural, connectional, and functional differences (Augustine, 1996; Duvernoy, 1999; Türe et al., 1999). The anterior portion, which is divided into three short insular gyri, has extensive connections with the frontal lobe. In contrast, the posterior portion of the insular cortex is formed by one or two long insular gyri and is seen to connect with both the parietal and temporal lobes. Functional neuroimaging studies have suggested that the short insular cortex is more involved in emotional and language-related functions, whereas the long insular cortex includes somatosensory and auditory processing areas [as reviewed by Augustine (1996), Nagai et al. (2001), Bamiou et al. (2003), and Naidich et al. (2004)]. Our findings are consistent with a recent MRI study by Kasai et al. (2003), who reported that both anterior and posterior insulae were significantly reduced in schizophrenia patients compared with control subjects and that group differences were not localized to a particular subregion. For the present study, we used the central insular sulcus as an anatomical boundary between the short and long insular cortex, whereas Kasai et al. (2003) used an alternative extrinsic landmark (mamillary body). Disruption of the paralimbic neural network including the insula has been proposed to contribute to the pathophysiology of schizophrenia by previous structural MRI studies (Goldstein et al., 1999; Shapleske et al., 2002). More specifically, functional neuroimaging studies have reported that various cognitive dysfunctions in schizophrenia such as emotional deficit (Crespo-Facorro et al., 2001a), recognition memory impairment (Crespo-Facorro et al., 2001b) or abnormal audiovisual speech perception (Surguladze et al., 2001) are mediated at least in part by the insular cortex. The insular cortex is engaged in a variety of cognitive functions, but its topographical localization has not been fully established. From the present and previous studies, it appears that the involvement of the insular cortex in schizophrenia is widespread and diffusely distributed rather than being specifically located in the anterior or posterior portion.

The sample characteristics of the clinic-based schizotypal disorder patients in our laboratory have been described in previous publications (Takahashi et al., 2002b, 2004b; Yoneyama et al., 2003; Kawasaki et al., 2004; Suzuki et al., 2004). The present study may not have been completely framed for direct comparisons with several previous studies in subjects with SPD since there are subtle but distinct differences between the diagnostic categories of schizotypal disorder (ICD-10) and SPD (DSM-IV). SPD is a stable personality, but schizotypal disorder in contrast requires a period of at least 2 years and the criteria include occasional transient quasi-psychotic episodes. Although all of the schizotypal subjects in this study also fulfilled DSM-IV criteria for SPD on Axis II, an additional diagnosis of brief psychotic disorder on Axis I was considered in eight subjects who experienced occasional transient quasi-psychotic episodes. In addition, schizotypal disorder "occasionally evolves into overt schizophrenia." Thus, schizotypal disorder in ICD-10 includes prodromal schizophrenia in addition to SPD as defined in DSM-IV. However, prior to the onset of psychosis, the clinical manifestations of two groups of patients who later develop schizophrenia or not are indistinguishable. The follow-up periods for the schizotypal patients in this

study were relatively short and some of them may have been at risk for developing psychosis later; they could be diagnosed as being in the prodromal phase of schizophrenia but not as SPD according to the concept of DSM-IV. We therefore adopted the ICD-10 criteria for schizotypal disorder in the present study. With regard to the symptom severity, the total BPRS score of our schizotypal subjects (mean=38.4, SD=9.7) was comparable to those (mean=37.5, SD=6.2) of previous MRI studies on mostly neuroleptic-free clinic-based subjects with SPD (Hazlett et al., 1999; Byne et al., 2001). However, our cohort may have included subjects with more serious symptoms than the SPD subjects in previous studies since most of the schizotypal disorder patients in the present study were taking neuroleptic medications.

Our results suggest that the volumes for the short and long insular cortices were reduced in overt schizophrenia but were preserved in schizotypal disorder. This may explain the decreased magnitude in cognitive/social deficits and symptomatology for schizotypal disorder relative to schizophrenia. Interestingly, it has been suggested that the abnormalities associated with the insular cortex are relevant to hallucinations (Crespo-Facorro et al., 2000; Shergill et al., 2000; Shapleske et al., 2002), which are a cardinal feature of schizophrenia but not prominently seen in schizotypal subjects. In a recent review of neurobiological abnormalities found in SPD, Siever and Davis (2004) hypothesized that while temporal volume reductions appear to be common to both SPD and schizophrenia, there may be preservation of frontal lobe volume in SPD compared with schizophrenia. Despite the above-mentioned differences in the sample characteristics between laboratories, Kurachi (2003a,b) suggested a similar hypothesis based on studies concerning cognitive characteristics and brain morphologic changes in schizotypal disorder and schizophrenia patients, i.e., the temporal lobe changes may underlie a vulnerability to schizophrenia and latent dysfunction in these lesions may become clinically apparent due to additional frontal lobe changes in schizophrenia. Based on these hypotheses, it may be reasonable to suppose that the long insular cortex, connecting with the temporal regions, is reduced in schizotypal patients as well as schizophrenia patients, while the short insular cortex, which has close connections with frontal cortex, is preserved in schizotypal patients. Such parallel reductions in associated regions in SPD were found in the volume of the thalamus; Byne et al. (2001) reported that size of the pulvinar, which projects to temporal lobe structures, was reduced in SPD as well as schizophrenia patients, while the size of the dorsomedial nucleus of the thalamus, associated with the prefrontal regions, was decreased only in the schizophrenia patients. Contrary to predictions, however, the present findings suggest that the insular cortex in schizotypal disorder patients shows no topographically specific volume changes. Although not supported directly by the present findings, the validity of these hypotheses seems worthy of further testing. Additional comprehensive assessment of multiple brain regions in the same group would be essential for the understanding of the brain morphologic characteristics of the schizotypal patients.

Some limitations of the present study should be mentioned. First, our results were not in agreement with those of a previous voxel-based MRI study carried out by our group (Kawasaki et al., 2004), in which reduced gray matter of the left insular cortex was found in schizotypal disorder patients. Although the validity of VBM has been tested in comparison with conventional region-of-interest (ROI) measurements (Wright et al., 1999; Suzuki et al., 2002), as discussed by Kasai et al. (2003), the results of the voxel-based methods could remain at odds with manual ROI methods, which are the current gold standard. Although we cannot clearly explain the reason for the differences in the results between the VBM and manual ROI analyses, the morphologic changes of the adjacent structures such as the superior temporal gyrus or the inferior frontal gyrus might have influenced the results for the insular cortex. A second limitation is that most of the patients were receiving neuroleptic medication. A relationship between brain morphologic features and neuroleptic medication has been reported in schizophrenia (Keshavan et al., 1994, 1998; Chakos et al., 1995; Gur et al., 1998), and insular cortex volume in schizophrenia was negatively correlated with duration of neuroleptic medication in the present study. This correlation was not found for the schizotypal patients, and the dosage of neuroleptic medication taken at the time of the scan in this study was not related to insular cortex volume. However, the

effects of cumulative years of medication treatment on the schizophrenia patients cannot be ruled out. A third limitation is that the control subjects in the present study were not selected to be educationally equivalent to the patients with both disorders. However, we optimally matched the parental education among the three groups according to the notion that matching on the basis of the educational level of the parents may reduce confounding factors in selection of control groups when brain measures are studied (Andreasen et al., 1990). In addition to these limitations, the relatively small sample size of female schizotypal disorder patients also limited our ability to generalize the findings of the present study. The morphologic characteristics of this disorder should be extensively examined with a larger female sample in future studies to confirm and extend the present findings.

In conclusion, the volume reduction of the insular cortex may be specific to overt schizophrenia, although there is no evidence for a topographically specific pattern of volume loss between the short and long insular cortices. The findings of the present study suggest that insular involvement may be implicated in the manifestation of overt psychosis.

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