errors. Improvement of the volumetry precision is a required assignment in multi-site imaging trials. In addition, it is ideal to improve accuracy.

The third limitation is that we cannot deny that variations for the fundamentally inaccurate atlas-based volumetry may be reduced by the N3 correction. However, the previous study showed the relatively high reproducibility in atlas-based volumetry (24), and reported that the N3 correction demonstrated a high degree of stability for the signal intensity non-uniformity correction (8). In addition, visually, the smaller Regularization values had a decreased change ratio in present study. Therefore, we believe that system dependency was reduced by N3 correction. In conclusion, to the best of our knowledge, the present study is the first atlas-based study to clarify the effect of bias-correction level on brain volumetry, and showed that system dependency was reduced by the N3 correction. This study could help investigators evaluate the impact of combining data from different MRI systems in a multisite study.

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Classification of First-Episode Schizophrenia Patients and Healthy Subjects by Automated MRI Measures of Regional Brain Volume and Cortical Thickness

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Abstract

Background: Although structural magnetic resonance imaging (MRI) studies have repeatedly demonstrated regional brain structural abnormalities in patients with schizophrenia, relatively few MRI-based studies have attempted to distinguish between patients with first-episode schizophrenia and healthy controls.

Method: Three-dimensional MR images were acquired from 52 (29 males, 23 females) first-episode schizophrenia patients and 40 (22 males, 18 females) healthy subjects. Multiple brain measures (regional brain volume and cortical thickness) were calculated by a fully automated procedure and were used for group comparison and classification by linear discriminant function analysis.

Results: Schizophrenia patients showed gray matter volume reductions and cortical thinning in various brain regions predominantly in prefrontal and temporal cortices compared with controls. The classifiers obtained from 66 subjects of the first group successfully assigned 26 subjects of the second group with accuracy above 80%.

Conclusion: Our results showed that combinations of automated brain measures successfully differentiated first-episode schizophrenia patients from healthy controls. Such neuroimaging approaches may provide objective biological information adjunct to clinical diagnosis of early schizophrenia.

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Introduction

Schizophrenia is a disabling psychiatric disorder which usually begins to affect individuals during their adolescence or early adulthood and most patients continue to suffer social, economic, and psychological difficulties from the first manifestation of the illness. Currently, diagnoses of psychiatric disorders are made on the basis of clinical manifestations and associated psycho-social disturbances [1,2]. However, there is an evidence for diagnostic instability in psychotic patients at an early stage of illness [3,4]. Although an accurate diagnosis is considered a prerequisite for appropriate physical/psychological treatment for each patient, no objective biomarker has been identified.

Previous structural magnetic resonance imaging (MRI) studies have demonstrated gray matter reductions of fronto-temporolimbic brain regions in schizophrenia patients compared with those of healthy subjects [5–11]. Several MRI-based studies have attempted to distinguish schizophrenia patients from healthy subjects using a variety of approaches such as manually traced regions of interest (ROI) [12,13], voxel-based morphometry (VBM) [14–16], cortical pattern matching [17], and cortical thickness obtained by a surface-based approach [18]. These studies have generally reported high classification accuracies (ranging from 75% to 92%), suggesting the potential clinical (i.e., diagnostic) utility of structural MRI. The majority of such classification studies employed chronic schizophrenia patients [12,14–16,18]. To date, only two studies [13,17] have attempted to distinguish between first-episode patients and healthy subjects by structural MRI.

Recently, an automated surface-based approach which can reliably measure local mean cortical thickness has been developed [19]. Several MRI studies applying this technique to schizophrenia have yielded robust findings such as cortical thinning especially in prefrontal and temporal regions [20–25]. This surface-based approach also enables to perform cortical parcellation and

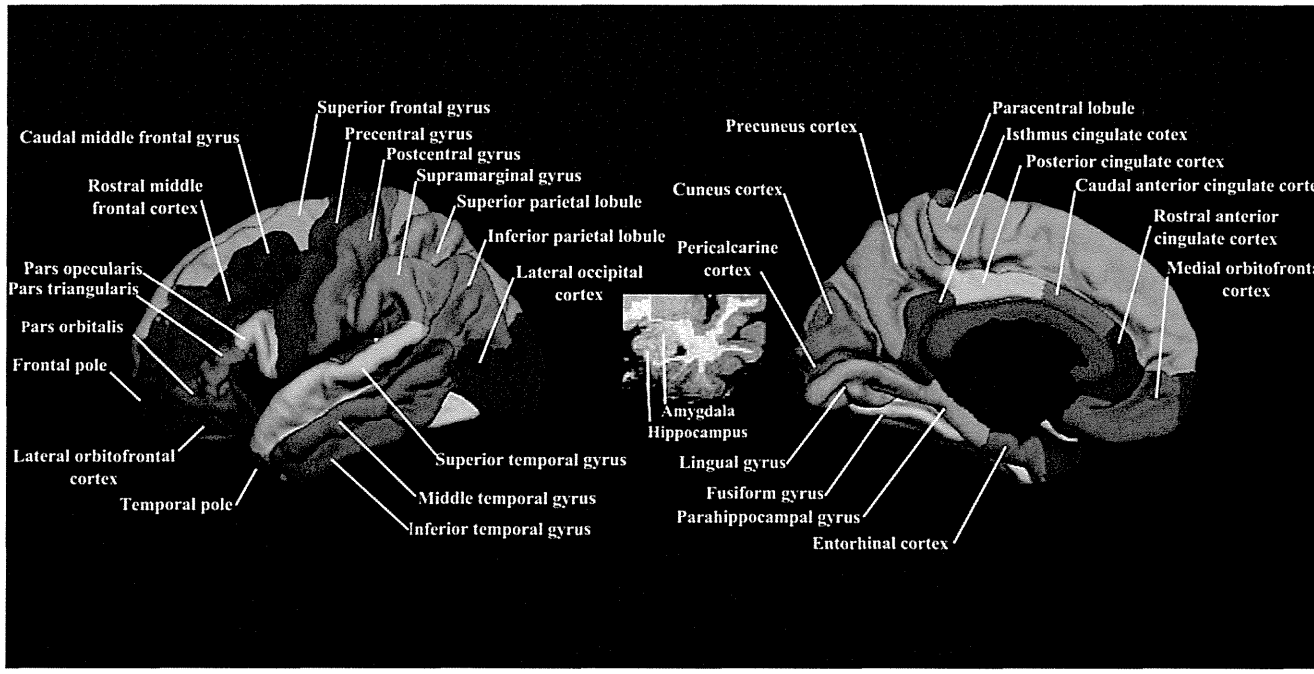

Figure 1. Representations of ROIs examined in this study on the left hemisphere. Cortical ROIs are shown in lateral view (left) and medial view (right). Two subcortical ROIs (i.e., amygdala and hippocampus) are visible in coronal view (middle).
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Table 1. Demographic and clinical characteristics of the male subjects.

A. First group	**Control subjects**		**Schizophrenia patients**		**Analysis of variance**	
	n = 16		**n = 20**			
	Mean	**SD**	**Mean**	**SD**	**F**	**p**
Age (years)	29.9	5.6	27.8	6.0	1.19	0.28
Handedness (number of right-handed)	16.0		19.0			
Socio-economic status	1.6	0.5	2.7	1.0	13.07	0.001
Parental socio-economic status	2.3	0.6	2.4	0.8	0.23	0.63
Estimated IQ	108.8	7.9	103.0	9.7	3.68	0.06
Duration of illness (months)			9.9	11.1		
Total BPRS score			40.2	11.5		
Antipsychotic medication (mg/day, chlorpromazine equiv.)			1074.5	487.9		
B. Second group	**Control subjects**		**Schizophrenia patients**		**Analysis of variance**	
	n = 6		**n = 9**			
	Mean	**SD**	**Mean**	**SD**	**F**	**p**
Age (years)	30.8	6.0	27.9	6.8	0.74	0.41
Handedness (number of right-handed)	6.0		7.0			
Socio-economic status	1.8	0.5	3.9	1.6	9.68	0.01
Parental socio-economic status	2.3	0.4	2.3	0.7	0.37	0.56
Estimated IQ	111.7	5.1	106.2	10.6	1.38	0.26
Duration of illness (months)			12.5	13.0		
Total BPRS score			42.5	9.9		
Antipsychotic medication (mg/day, chlorpromazine equiv.)			864.4	637.7		

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measurement of regional cortical volumes [25–27]. These approaches have been validated by several studies [21,26,28,29]. By using these newly developed automated methods to assess brain morphology (i.e., cortical thickness and regional brain volumes). Desikan et al. [30] demonstrated successful classification of subjects with mild cognitive impairment, patients with Alzheimer's disease, and controls. To our knowledge, however, no studies have attempted to classify patients with schizophrenia and healthy subjects with this fully automated MRI-based analysis.

In this study, we intended to classify schizophrenia patients and healthy subjects using discriminant analysis with automated MRI-based measures of regional brain volume and cortical thickness. On the basis of findings of previous studies, we hypothesized that (1) cortical thinning and gray matter volume reductions in prefrontal and temporal regions would be seen in schizophrenia patients compared with controls, (2) and these MRI measures would differentiate schizophrenia patients from healthy subjects with good accuracy.

Materials and Methods

Subjects

Fifty-two patients (29 males, 23 females) with first-episode schizophrenia were recruited from the inpatient population at Tokyo Metropolitan Matsuzawa Hospital. Inclusion criteria for first-episode schizophrenia patients were (1) first psychiatric hospitalization, (2) younger than 45 years old, (3) currently psychotic as reflected by the presence of at least one "positive" symptom, and (4) fulfilling the ICD-10 research criteria for schizophrenia. Two experienced psychiatrists separately examined the patients within two weeks of admission and diagnostic consensus was confirmed. Furthermore, thorough medical record review was performed to confirm the diagnostic stability for all the patients during the follow-up periods (1 to 5 years) after first admission. All but three male patients with schizophrenia were right-handed. All patients had received antipsychotic medications at the time of scanning.

The control subjects consisted of 40 healthy volunteers (22 males, 18 females) who were recruited from the hospital staff and college students. All of the control subjects were right-handed. All control subjects were interviewed by psychiatrists using the questionnaire concerning their family and past histories, and present illness. Individuals who had a personal history of psychiatric illness or a family history of psychiatric disorders in their first degree relatives were excluded.

For the discriminant analysis described below, the subjects were randomly assigned to two independent groups. The first group consisted of 36 males (16 healthy subjects and 20 schizophrenia patients) and 30 females (13 healthy subjects and 17 schizophrenia patients). The second group for the prospective validation consisted of 15 males (6 healthy subjects and 9 schizophrenia patients) and 11 females (5 healthy subjects and 6 schizophrenia patients). Since the sample size of the present study is relatively modest, we assigned more subjects to the first group (i.e., about 70%) than to the second group to enhance the discriminating ability of the classifier.

In the schizophrenia patients, clinical symptoms were assessed using the Brief Psychiatric Rating Scale (BPRS) [31]. The premorbid IQ for schizophrenia patients and the present IQ for control subjects were estimated using the shortened version of the

Table 2. Demographic and clinical characteristics of the female subjects.

A. First group	**Control subjects**		**Schizophrenia patients**		**Analysis of variance**	
	n = 13		**n = 17**			
	Mean	**SD**	**Mean**	**SD**	**F**	**p**
Age (years)	27.5	4.8	28.1	5.8	0.16	0.69
Handedness (number of right-handed)	14.0		17.0			
Socio-economic status	1.6	0.5	3.1	1.1	17.14	<0.001
Parental socio-economic status	2.4	0.8	2.9	0.8	3.03	0.09
Estimated IQ	107.0	8.1	103.5	7.8	2.55	0.12
Duration of illness (months)			13.0	12.6		
Total BPRS score			37.4	9.7		
Antipsychotic medication (mg/day, chlorpromazine equiv.)			930.8	451.6		
B. Second group	**Control subjects**		**Schizophrenia patients**		**Analysis of variance**	
	n = 5		**n = 6**			
	Mean	**SD**	**Mean**	**SD**	**F**	**p**
Age (years)	28.4	3.8	28.3	8.6	0.05	0.83
Handedness (number of right-handed)	4.0		6.0			
Socio-economic status	1.6	0.5	2.5	1.0	4.65	0.06
Parental socio-economic status	2.0	0.0	2.6	0.9	2.25	0.17
Estimated IQ	108.1	10.8	103.0	8.1	0.18	0.68
Duration of illness (months)			14.5	19.8		
Total BPRS score			36.5	4.4		
Antipsychotic medication (mg/day, chlorpromazine equiv.)			483.3	263.9		

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Japanese version of the National Adult Reading Test (JART) [32]. The subjects' socio-economic status (SES) as well as parental SES was assessed using the Hollingshead's Index [33].

All subjects were physically healthy at the time of the study, and none had a lifetime history of serious head trauma, neurological illness, or serious medical or surgical illness. Individuals who met the ICD-10 research criteria for mental and behavioral disorders due to psychoactive substance use were excluded. All schizophrenia patients participated in this study after providing written informed consent. In addition, regal representatives of schizophrenia patients gave written informed consent. In case of unable to directly access to a patient's legal representative, oral informed consent was obtained using telephone, and this procedure was witnessed by at least two hospital staff and recorded in the medical chart. All control subjects also provided written informed consent. Since control group of this study consisted of only healthy adults, their legal representatives were not asked to give informed consents. This study was approved by the Committee on Medical Ethics of Tokyo Metropolitan Matsuzawa Hospital.

MRI data acquisition

MR images were obtained using a Philips Intera 1.5-T scanner (Philips Medical Systems, Best, Netherlands) with a three-dimensional sequence yielding 192 contiguous T1-weighted slices of 1.0-mm thickness in the axial plane. The imaging parameters were as follows: repetition time = 21 ms, echo time = 9.2 ms, flip angle = 30°, field of view = 256 mm, matrix size = 256×256 pixels, voxel size = 1.0×1.0×1.0 mm^3.

Automated MRI data processing

Cortical reconstruction and volumetric segmentation were performed with the Freesurfer image analysis suite (version 4.5), which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). This processing includes motion correction and averaging of multiple volumetric T1-weighted images, removal of non-brain tissue using a hybrid watershed/surface deformation procedure [34], automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures (including hippocampus and amygdala) [35,36], intensity normalization [37], tessellation of the gray matter/white matter boundary, automated topology correction [38,39], and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid (CSF) borders at the location where the greatest shift in intensity defines the transition to the other tissue class [19,40,41]. Once the cortical models are completed, a number of deformable procedures can be performed for further data processing and analysis.

Cortical thickness measurements were obtained by calculating the shortest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface [19]. The cerebral cortex of each MRI scan was automatically parcelled into regions of interest (ROIs) based on gyral and sulcal structure [26,42]. Both automated cortical thickness measurements and cortical parcellation have already been validated [21,26,28,29]. Figure 1 presents the neocortical ROIs and two limbic ROIs (hippocampus and amygdala) examined in this study. To control for head size in statistical analyses, the total intracranial volume (ICV) was calculated automatically [43].

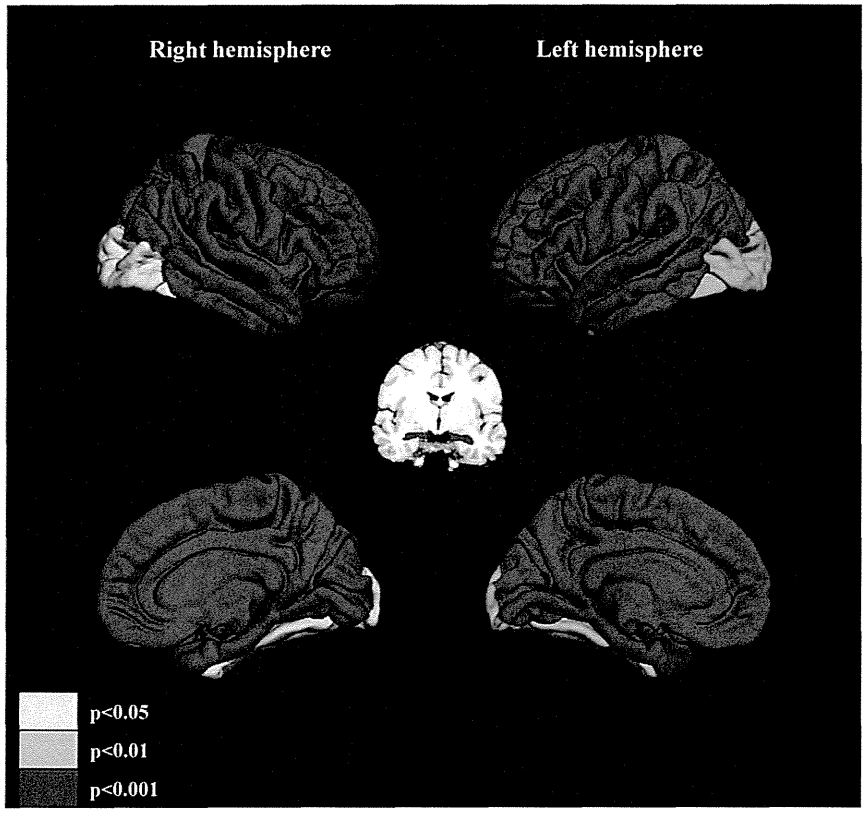

Figure 2. ROIs for which the volumes were significantly reduced in schizophrenia patients compared with those of healthy subjects. ROIs were differentially colored according to the p values of the post hoc tests.
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Statistical analysis-1 Group comparison

Demographic and clinical variables were compared by analysis of variance (ANOVA). The ROI volumes and the mean cortical thickness of ROIs were analyzed by repeated measures analysis of covariance (ANCOVA) with diagnosis and gender as between-subject factors, hemisphere (left, right) as a within-subject factor, and age and ICV as covariates. To prevent possible type 1 error, we used false positive discovery rate (FDR) correction. For variables of which p-values remained significant even after the FDR correction, post hoc Scheffe's tests were used to follow up significant main effects or interactions.

Statistical analysis-2 Classification by brain measures

The following statistical procedures were carried out separately for each gender, as was the case in our previous studies [12,13], on the basis of the gender differences in brain morphology found in this study (described below) as well as the evidence for gender differences in brain morphology among healthy subjects [44] and gender-specific brain structural changes in schizophrenia patients [45,46].

Transformation of brain measures into z scores. The volumes and mean cortical thickness of ROIs were expressed as standardized z scores corrected by regression analysis for the variations in head size and age of the control subjects, as described in our previous studies [12,13]. Briefly, the ROI volume and mean cortical thickness for the control group were regressed against ICV and age, yielding a residual value for each control subject. The ROI volume and mean cortical thickness for the patient groups were entered into the same equation as for the control group to calculate the residual value for each patient. The mean residual values and standard deviation (SD) derived from the control subjects were used to calculate z scores (z = [residual value - mean residual value for control subjects]/SD). For the control subjects, the expected mean z score was 0 with an SD of 1. The use of standardized z scores allows analysis of disease-related changes independent of head size and normal aging.

Linear discriminant function analysis. For the first group, discriminant function analysis was conducted using z scores as independent variables to assess the possibility of classifying diagnostic groups by a combination of brain measures. The variables were entered in a stepwise manner. Since we employed a stepwise variable selection, the number of variables which were entered into the discriminant analysis varied depending on the inclusion and exclusion criteria. In this study, relatively conservative inclusion criteria were used for the stepwise selection, which were set at $p<0.05$ to enter and $p>0.1$ to remove. If we used a more liberal criterion, more variables could be used for the discriminant function, vice versa. For each step, always a measure whose p-value is the smallest and smaller than 0.05 is entered to the discriminant function. Similar to a stepwise linear regression analysis, however, p-values of variables vary for each step. If a p-value of a measure that has already been entered to the model exceeds 0.1, this variable is removed at this step. If a p-value of the measure is 0.06 (i.e., <0.1), it remains in the model. However, if a measure with a p-value of 0.06 has yet to be entered in the model, it is still out of the model at this step. For each subject of the second group, the discriminant score was calculated using the discriminant function derived from the first group and his/her diagnosis was predicted based on the discriminant score. Since the p-value for the stepwise variable selection was computed

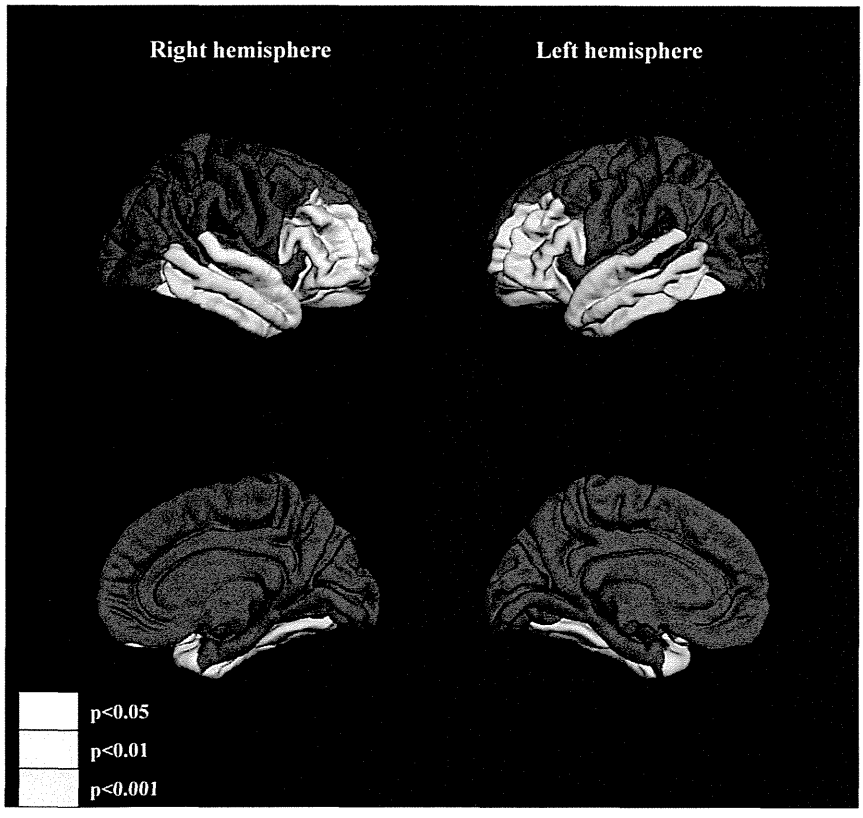

Figure 3. Significant cortical thinning of ROIs in schizophrenia patients compared with that of healthy subjects observed in this study. ROIs were differentially colored according to the p values of the post hoc tests.
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solely from the first group, the classification of the second group was achieved independently of subjects' diagnosis of the second group. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), and false positive rate (FPR) of the classifier were calculated. Detailed descriptions of discriminant function analysis and stepwise variable selection can be found at the Statsoft website (http://www.statsoft.com/textbook/).

All statistical analyses were performed using the STATISTICA 06J software package (Statsoft, Tulsa, OK).

Results

Demographic and clinical characteristics

Tables 1 and 2 present the results of group comparison of the demographic and clinical measures of male subjects and female subjects, respectively. When all subjects were combined, there were significant main effects of diagnosis on SES ($F = 41.77$, $df = 1,87$, $p<0.001$) and estimated IQ ($F = 6.90$, $df = 1,85$, $p = 0.01$). Post hoc tests showed that schizophrenia patients had lower SES ($p<0.001$) and lower estimated IQ ($p = 0.01$) than controls.

Comparison of the brain measures

Tables S1 and S2 show the comparisons of the volumes and the mean cortical thicknesses of ROIs among diagnostic groups, respectively. Below, we describe the significant results of post hoc tests.

Comparison of the ROI volumes. Post hoc tests demonstrated significant gray matter volume reductions of the bilateral hippocampus ($p<0.001$ for both hemispheres), the bilateral fusiform gyri ($p = 0.002$ for left, $p = 0.024$ for right), and the bilateral lateral occipital cortices ($p = 0.001$ for left, $p = 0.014$ for right) in schizophrenia patients compared with those of healthy subjects (Figure 2). Gender differences of ROI volumes were seen in the bilateral amygdala (male>female, $p<0.001$ for both hemispheres).

Comparison of the mean thickness of ROIs. Significant cortical thinning in schizophrenia patients compared with controls was observed in the bilateral rostral middle frontal gyri ($p = 0.007$ for left, $p = 0.007$ for right), the bilateral pars opecularis ($p = 0.002$ for left, $p<0.001$ for right), the bilateral pars triangularis ($p<0.001$ for left, $p = 0.009$ for right), the bilateral pars orbitalis ($p = 0.002$ for left, $p<0.001$ for right), the bilateral lateral orbitofrontal cortices ($p<0.001$ for both hemispheres), the bilateral superior temporal gyri ($p<0.001$ for left, $p = 0.001$ for right), the bilateral middle temporal gyri ($p<0.001$ for both hemispheres), the bilateral inferior temporal gyri ($p<0.001$ for both hemispheres), the bilateral fusiform gyri ($p = 0.005$ for left, $p<0.001$ for right), and the bilateral temporal pole ($p = 0.004$ for left, $p = 0.04$ for right) (Figure 3).

Classification of schizophrenia patients and healthy subjects by brain measures

Among male subjects, the following 2 measures were entered in a stepwise manner: the left lateral occipital cortex volume and right lateral orbitofrontal cortex thickness (Figure 4). Accuracy, sensitivity, specificity, PPV, NPV and FPV of the obtained classifier were 86.1%, 80.0%, 93.8%, 94.1%, 78.9%, and 5.9%, respectively in the first male cohort. In the second cohort, the

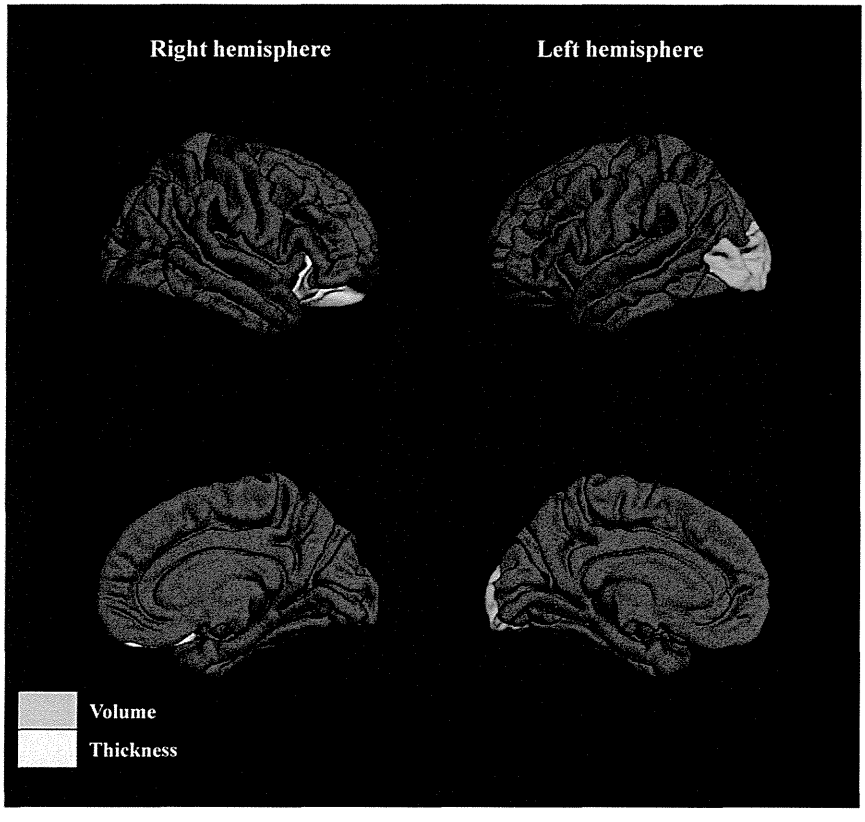

Figure 4. Discriminative pattern for male subjects. Selected regions were differentially colored when volume (blue) or thickness (yellow) of those regions were entered into the model.
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classifier correctly assigned 86.7% of the subjects. Accuracy, sensitivity, specificity, PPV, NPV and FPV for second cohort were 86.7%, 88.9%, 83.3%, 88.9%, 83.3%, and 11.1%, respectively (Table 3).

During the stepwise procedure, the following 5 measures were selected as variables in female subjects: the left temporal pole volume, the right medial orbitofrontal cortex volume, the right pars triangularis volume, the left pars orbitalis thickness, and the left superior temporal gyrus thickness (Figure 5). Accuracy, sensitivity, specificity, PPV, NPV and FPV of the classifier were 96.7%, 94.1%, 100%, 100%, 92.9%, and 0%, respectively in the first female cohort. Obtained classifier correctly classified 81.2% of the subjects of the second cohort. Accuracy, sensitivity, specificity, PPV, NPV and FPV for the second cohort were 81.2%, 66.7%, 100%, 100%, 71.4%, and 0%, respectively (Table 3).

Discussion

Classification performance

To the best of our knowledge, this is the first MRI study to reliably classify first-episode patients with schizophrenia and healthy subjects using fully automated MRI-based discriminant analysis based on both brain regional volumes and regional cortical thicknesses. Our results were comparable to those of previous MRI-based classification studies in chronic [14–16,18] and first-episode [13,17] schizophrenia patients. Our results suggest that the combination of automated brain measures is a candidate for an objective biological marker of early schizophrenia adjunct to clinical diagnosis.

In the present study, the fronto-temporolimbic regions as well as the occipital cortex exhibited the discriminative patterns among the diagnostic groups. These patterns appear to be somewhat different from those of previous classification studies between schizophrenia patients and healthy controls using whole brain analysis by VBM [16] or cortical pattern matching [17], which highlighted the fronto-temporal regions as contributing to between-group differentiation. Interestingly, we replicated recent findings by Rimol et al. [47] in showing robust cortical thinning of posterior cortices in first-episode schizophrenia. Our results might thus suggest that combination of cortical thickness (including occipital regions) and gray matter volume contributed to high classification accuracies reported in this study.

Several studies have attempted to distinguish between persons with psychiatric conditions and healthy controls using neuropsychological tests [48], a combination of structural brain measures and neuropsychological tests [49], and functional MRI [50]. Although these previous studies also reported high classification accuracy, neuropsychological and functional measures are considered more susceptible to the subjects' condition (i.e., state-dependent). In contrast, brain morphologic changes in schizophrenia are considered to be more static and already exist at the first episode of the illness [5] or even before/during the onset of overt psychosis [51–53] Our findings that MRI measures alone could reliably differentiate healthy controls and schizophrenia patients might thus suggest a role of brain structural measures in the earlier detection of psychosis. In fact, a recent VBM-based classification study demonstrated successful discrimination of individuals with at risk mental state (ARMS) who later developed psychosis from those without transition to psychosis [54].

Table 3. Classification performance.

A. First group	Male (n = 36)		Female (n = 30)	
	Predicted diagnosis		Predicted diagnosis	
	HC	SZ	HC	SZ
Clinical diagnosis				
HC	15	1	13	0
SZ	4	16	1	16
Accuracy (%)	86.1		96.7	
Sensitivity (%)	80.0		94.1	
Specificity (%)	93.8		100.0	
PPV (%)	94.1		100.0	
NPV (%)	78.9		92.9	
FPR (%)	5.9		0.0	
B. Second group	**Male (n = 15)**		**Female (n = 11)**	
	Predicted diagnosis		Predicted diagnosis	
	HC	SZ	HC	SZ
Clinical diagnosis				
HC	5	1	5	0
SZ	1	8	2	4
Accuracy (%)	86.7		81.2	
Sensitivity (%)	88.9		66.7	
Specificity (%)	83.3		100.0	
PPV (%)	88.9		100.0	
NPV (%)	83.3		71.4	
FPR (%)	11.1		0.0	

FPR, false positive rate; HC, healthy control; NPV, negative predictive value; PPV, Positive predictive value; SZ, schizophrenia.

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Volume reductions and cortical thinning of ROIs in patients

This study demonstrated significant gray matter volume reductions of temporal, limbic, and occipital regions in schizophrenia patients compared with those of controls. In schizophrenia patients, significant cortical thinning was more widely observed, relative to volume reductions, in prefrontal and temporal regions. These results are consistent with previous studies that reported fronto-temporolimbic gray matter volume reductions [5–11] and cortical thinning of prefrontal/temporal regions [20–25] in schizophrenia patients. Prefrontal and temporolimbic regions are considered to be involved in cognitive function, auditory/visual processing, speech, emotional processing, executive function, and decision-making, all of which are often impaired in schizophrenia patients [55–57]. Onitsuka et al. [58] demonstrated volume reductions of the bilateral occipital sub-region (the visual association areas), which largely includes the lateral occipital cortex where the schizophrenia patients had a decreased volume in this study. In general, the present study has replicated the brain structural abnormalities in schizophrenia patients demonstrated in previous MRI-based studies.

Gender difference was seen in the bilateral amygdala volume (male>female) in accordance with previous studies [44]. In order to exclude such gender effect which potentially confounds classification analyses, we divided the subjects into male and female cohorts in this study.

Limitations

A few limitations in this study should be taken into account. First, this study was partly limited by the lack of inclusion of other

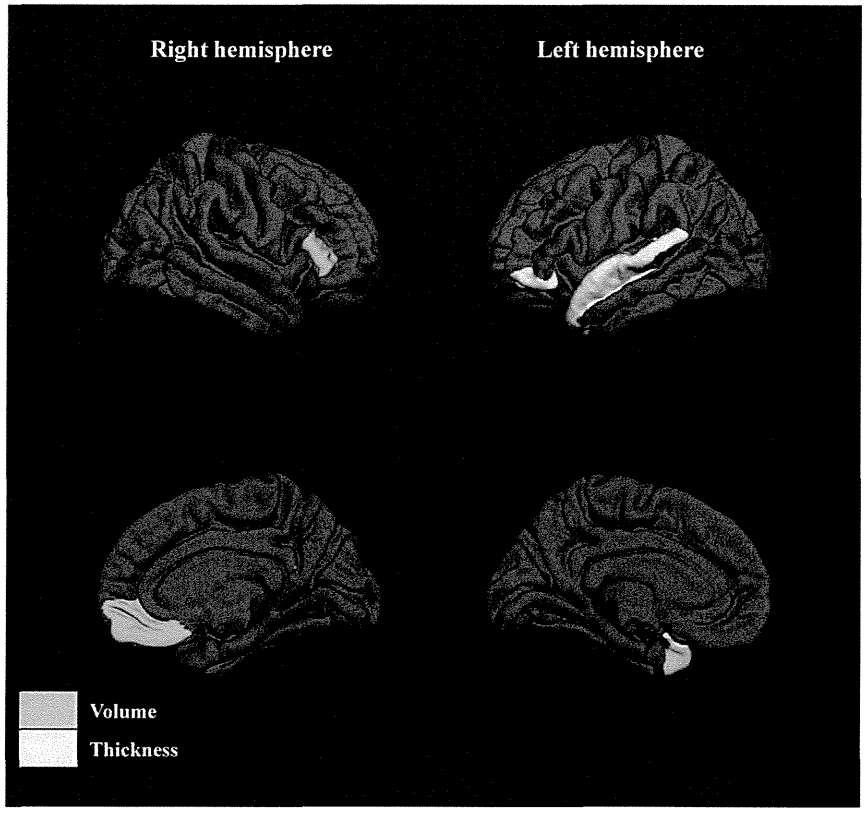

Figure 5. Discriminative pattern for female subjects. Selected regions were differentially colored when volume (blue) or thickness (yellow) of those regions were entered into the model.
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psychiatric disorders such as bipolar affective disorder (BD). Our preliminary classification analysis using the current sample as well as 15 BD patients [8 males (mean age, 33.5 years) and 7 females (mean age, 33.7 years)] correctly assigned 81.4% of male subjects and 87.5% of female subjects, respectively (unpublished data). However, larger number of BD patients will be needed to delineate the conclusion that such technique may possibly attribute to the clinical diagnosis of different psychiatric conditions. Second, the higher socio-economic status of control group compared to schizophrenia patients might have confounded the analyses, although parental socio-economic status was not different between groups. Third, the results may have been influenced by antipsychotic medication that all patients in this study had received prior to scanning [59–61]. Finally, as the sample size of this study is modest (51 males and 41 females), we needed to assign more subjects to the training cohort than to the validation cohort in order to obtain more reliable classifiers. A larger number of subjects should be tested for validation in future study.

Conclusion

In conclusion, our results showed that combinations of fully automated brain measures successfully classified diagnostic groups (i.e., schizophrenia patients and controls), and suggest that such neuroimaging approaches may provide objective biological information adjunct to clinical diagnosis of early schizophrenia.

Supporting Information

Table S1 The results of comparison of the ROI volumes between schizophrenia patients and healthy controls.
(XLSX)

Table S2 The results of comparison of the mean thickness of the ROIs between schizophrenia patients and healthy controls.
(XLSX)

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Author Contributions

Conceived and designed the experiments: YT MS MK. Performed the experiments: YT LO YM YS. Analyzed the data: YT MS TT YK KN. Wrote the paper: YT MS TT HY KK. Supervised the overall research project: MI YO.

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Reduced amygdala and hippocampal volumes in patients with methamphetamine psychosis

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ABSTRACT

The similarity between psychotic symptoms in patients with schizophrenia such as hallucinations and delusions and those caused by administration of methamphetamine has been accepted. While the etiology of schizophrenia remains unclear, methamphetamine induced psychosis, which is obviously occurred by methamphetamine administration, had been widely considered as a human pharmaceutical model of exogenous psychosis. Although volume reductions in medial temporal lobe structure in patients with schizophrenia have repeatedly been reported, those in patients with methamphetamine psychosis have not yet been clarified. Magnetic resonance images (MRI) were obtained from 20 patients with methamphetamine psychosis and 20 age, sex, parental socio-economic background, and IQ matched healthy controls. A reliable manual tracing methodology was employed to measure the gray matter volume of the amygdala and the hippocampus from MRIs. Significant gray matter volume reductions of both the amygdala and hippocampus were found bilaterally in the subjects with methamphetamine psychosis compared with the controls. The degree of volume reduction was significantly greater in the amygdala than in hippocampus. While the total gray, white matter and intracranial volumes were also significantly smaller-than-normal in the patients; the regional gray matter volume reductions in these medial temporal structures remained statistically significant even after these global brain volumes being controlled. The prominent volume reduction in amygdala rather than that in hippocampus could be relatively specific characteristics of methamphetamine psychosis, since previous studies have shown significant volume reductions less frequently in amygdala than in hippocampus of the other psychosis such as schizophrenia.

1. Introduction

Methamphetamine administration could cause psychotic symptoms including visual and auditory hallucinations and persecutory delusions in subjects with non-preexisting psychotic manifestations (Iwanami et al., 1991; Buffenstein et al., 1999; McKetin et al., 2006; Matsuzawa et al., 2007; Kishimoto et al., 2008). This obviously exogenous psychosis is induced by enhanced dopaminergic transmissions due to methamphetamine (Sato et al., 1992), and is usually well treated by dopamine antagonist. The similarities in clinical features between psychosis induced by methamphetamine and schizophrenia ranged from clinical symptomatology to pharmacotherapeutical characteristics, which implied that methamphetamine psychosis might be a human pharmaceutical model of schizophrenia (Snyder, 1973).

Methamphetamine users also shows affective symptoms including acute manic excitement comprises motor hyperactivity, elevated mood, excitement, hostility, grandiosity and subsequent depressive condition comprises depressive mood, apathy and suicidal idea (Tatetsu et al., 1956; Matsumoto et al., 2002). Further consumption of methamphetamine results in severe psychosis (McKetin et al., 2006). Previous literature has repeatedly reported a role of hippocampus and amygdala in the pathophysiology of psychostimulant addiction (Makris et al., 2004), affective psychosis (Usher et al., 2010),

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[1] The location where the work was done.

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and schizophrenia (Shenton et al., 2001). Therefore, these medial temporal lobe structures may be important in the pathophysiology of methamphetamine psychosis.

Previous structural MRI studies have consistently reported hippocampal volume reduction in patients with schizophrenia (Shenton et al., 2001). Smaller-than-normal hippocampal volume has been reported in subjects at early phase of their illness (Hirayasu et al., 1998; Kubicki et al., 2002) and even in individuals at high-risk for schizophrenia (Keshavan et al., 2002; Pantelis et al., 2003). Therefore, smaller-than-normal hippocampal volume has been thought to reflect vulnerability for predisposition to develop schizophrenia. The other line of evidence revealed a significant smaller-than-normal hippocampal volume in methamphetamine abusers (Thompson et al., 2004). A previous study using MRI (Harano et al., 2004) examined brain morphology in patients with methamphetamine psychosis, although the lengths between limited brain structures (e.g., ventricles and corpus callosum) were employed as quantitative brain measures instead of brain volume. To our knowledge, no previous study has examined whether patients with methamphetamine psychosis was associated with volume reduction in the hippocampus.

Although results of structural abnormality in amygdala, in contrast to hippocampus, have been divergent in previous literature in patients with schizophrenia (Shenton et al., 2001; Steen et al., 2006), smaller-than-normal amygdala volumes have been reported in subjects with cocaine addiction (Makris et al., 2004) and bipolar disorders (Usher et al., 2010). The elevated activation in amygdala during emotional processing has been repeatedly reported in patients with bipolar disorder who shows exacerbated emotional responsibility (Strakowski et al., 2005), which is also occasionally observed in subjects with methamphetamine use (Tatetsu et al., 1956). In addition, lower-than-normal dopamine transporter density was reported in amygdala of methamphetamine users, including patients having psychotic symptoms (Sekine et al., 2003). Although previous literature suggests that the structural and/or functional abnormalities of amygdala might play a role in the pathophysiology of methamphetamine psychosis, no study has examined brain structural abnormality in this region of such patients.

Applying automated techniques such as voxel-based morphometry (VBM) to assess structure of hippocampus and amygdala, however, might have methodological limitations. The VBM might not detect very small and localized gray-matter volume reductions, because false-positive or false-negative VBM findings might arise from changes in the shape or displacement of structures in the course of spatial normalization (Davatzikos, 2004). Accordingly, previous studies have shown the superiority of manual tracing methodology to advanced automated morphometries in detecting structural abnormality in hippocampus (Carmichael et al., 2005; Firbank et al., 2008; Bergouignan et al., 2009). Therefore, manual tracing volumetry could have advantage in measuring actual brain volume in native space, although the tracing is time-consuming.

The present study employing manual tracing volumetry was aimed to examine whether gray matter volume reductions in hippocampus and amygdala existed in patients with methamphetamine psychosis compared with healthy controls. Based on the previous literature, we hypothesized that patients with methamphetamine psychosis show smaller-than-normal hippocampus, which is similar to schizophrenia (Shenton et al., 2001), as well as smaller amygdala volume, which is similar to abusers of other psychostimulants (Makris et al., 2004).

2. Materials and methods

2.1. Subjects

Twenty right-handed in- and outpatients with methamphetamine psychosis were recruited from the Department of Psychiatry, Metropolitan Matsuzawa Hospital, Japan. Ten were male, and ten were female. Diagnosis was determined for each patient according to ICD-10 research criteria for methamphetamine psychosis (World Health Organization, 1993). At least two experienced psychiatrists separately examined the patients and diagnostic consensus was confirmed. Psychiatric symptoms were evaluated by one psychiatrist (Y.T.) using the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), Scale for the Assessment of Negative Symptoms (SANS) and Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen and Olsen, 1982), within three days prior to MRI scanning.

Similar to the previous studies examining subjects with methamphetamine psychosis (Matsuzawa et al., 2007; Kishimoto et al., 2008), methamphetamine abusers (Sekine et al., 2001, 2003) and cocaine abusers (Makris et al., 2004), detailed information on methamphetamine use and its relationship to onset and disappearance of psychotic symptoms was obtained carefully from the interviews with patient and medical record (Table 1). Seventeen of 20 patients received typical neuroleptics. Nine of the 17 patients received both typical and atypical neuroleptics. The other 3 patients received atypical neuroleptics only. Clinical assessment of baseline craving (a trait like measure) for methamphetamine focused on the 24 h interval prior to MR scan using the subjective craving scale (Mean = 1.00, S.D. = 2.52 [0–9 range; Higher scores representing stronger craving]) which was modified from the Quantitative Cocaine Inventory/Craving Scale (QCI/CS) (Elman et al., 2001).

Table 1
Subject characteristics and symptom scores.

Variable	Patients (n = 20)		Control subjects (n = 20)		t-tests		
	Mean	SD	Mean	SD	df	t value	p
Age (range)	33.8 (22–52)	7.8	33.7 (22–52)	7.5	38	0.021	0.98
Male/female	10/10		10/10				
SES[a]	4	1.1	1.6	0.5	37	−8.7	<0.001
Parental SES[a]	2.9	0.8	2.6	0.7	36	−1.4	0.18
JART (Japanese Adult Reading Test)	90.4	5.1	108.5	3.7	31	5.9	<0.001
Age at first use of methamphetamine	19.7	4.0					
First onset of psychotic symptom	26.5	6.2					
Transient phsychosis within 1 month/prolonged	6/12						
Neuroleptics dose (chlorpromazine equivalent)	625.7	687.5	.....	...	...	.....	...
ECT (elecroconvulsive tratment) treated/not	3/17		.....	...	...	.....	...
way of abuse: smoking/injection/both	8/10/2		.....	...	...	.....	...
Craving	1	2.5	.....	...	...	.....	...
Brief Psychiatric Rating Scale	39.3	14.7	.....		...	.....	...
Scale for the Assessment of Positive Symptoms	30.1	20.8	.....	...	...	.....	...
Scale for the Assessment of Negative Symptoms	24	14.4	.....	...	...	.....	...

[a] Socioeconomic status, assessed using the Hollingshead scale. Higher scores indicate lower status.

Twenty age-, gender-, and handedness-matched healthy subjects were employed as controls (Table 1). The socioeconomic status (SES) and parental SES were assessed using the Hollingshead scale (Hollingshead, 1957). The premorbid IQ for patients and the present IQ for control subjects were estimated using the shortened version of the Japanese version of the National Adult Reading Test (JART) (Nelson, 1982; Matsuoka et al., 2006). The patients with methamphetamine psychosis showed a significantly lower SES and estimated IQ than the control subjects, while the parental SES did not differ significantly between groups.

All subjects were physically healthy at the time of the study, and none had a lifetime history of serious head trauma, neurological illness, and serious medical or surgical illness. No control subjects had significant alcohol or substance abuse disorder. All subjects participated in this study after providing written informed consent. The ethical committee of Metropolitan Matsuzawa Hospital approved this study. After a complete explanation of the study to the subjects, written informed consent was obtained.

2.2. MRI acquisition

The methods of MRI acquisition were described in detail elsewhere (Takayanagi et al., 2010). Briefly, the MRI data were obtained using a 1.5-Tesla Gyroscan Intera MR scanner with SENSitivity Encoding (SENSE) (Philips Medical Systems, Best, Netherlands). The repetition time was 20 ms, the echo time 9.2 ms, the slice thickness 1 mm, the field of view 25.6×20.4×19.2 cm, and the acquisition matrix 256×256×192. The voxel (volume of pixel) dimensions were 0.9375×0.9375×1 mm. And we obtained two image data every one subject at one chance. The obtained images were realigned in the coronal and axial planes using the interhemispheric fissure. After this alignment in the two planes, the midsagittal plane was aligned to correct head tilt using the line between the anterior and posterior commissures. After realignment, to improve the signal-to-noise-ratio, the two sequential images obtained at one occasion were then averaged. The realignment and averaging were conducted using SPM 2 (Institute of Neurology, London, UK). A trained neuroradiologist (T.I.) evaluated the MRI scans and found no gross abnormalities in any of the subjects. Magnetic field inhomogeneity in our scanner was monitored with daily basic quality control, and has been stable over the MR acquisition time for this study.

2.2.1. Definition of region of interest (ROI)

The amygdala and hippocampus gray matter regions of interest (ROIs) were outlined manually by one rater (L.O.) who was blind to the group status or genotype. For the manual tracing, we used a software package for medical image analysis (*3D Slicer*; software available at http://www.slicer.org), which enables a simultaneous view of orthogonal planes. The landmarks to delineate the ROIs were the same as our previous studies (Inoue et al., 2010; Yamasue et al., 2008b) (Fig. 1) (see details of definition of ROIs in supplementary material).

For interrater reliability, two raters (L.O. and H.I.) blind to group membership, independently drew ROIs. Ten cases were selected at random, and the raters drew ROIs on every slice. The intraclass correlation coefficient was 0.81/0.80 for the left/right amygdala and 0.94/0.97 for the left/right hippocampus respectively. Intrarater reliability, computed by using all of the slices from one randomly selected brain and measured by one rater (H.I.) on two separate occasions (approximately 2 months apart), was >0.95 for all structures.

Global brain volumes including total gray matter, white matter, and cerebrospinal fluid volumes were calculated using SPM2 (Good et al.,

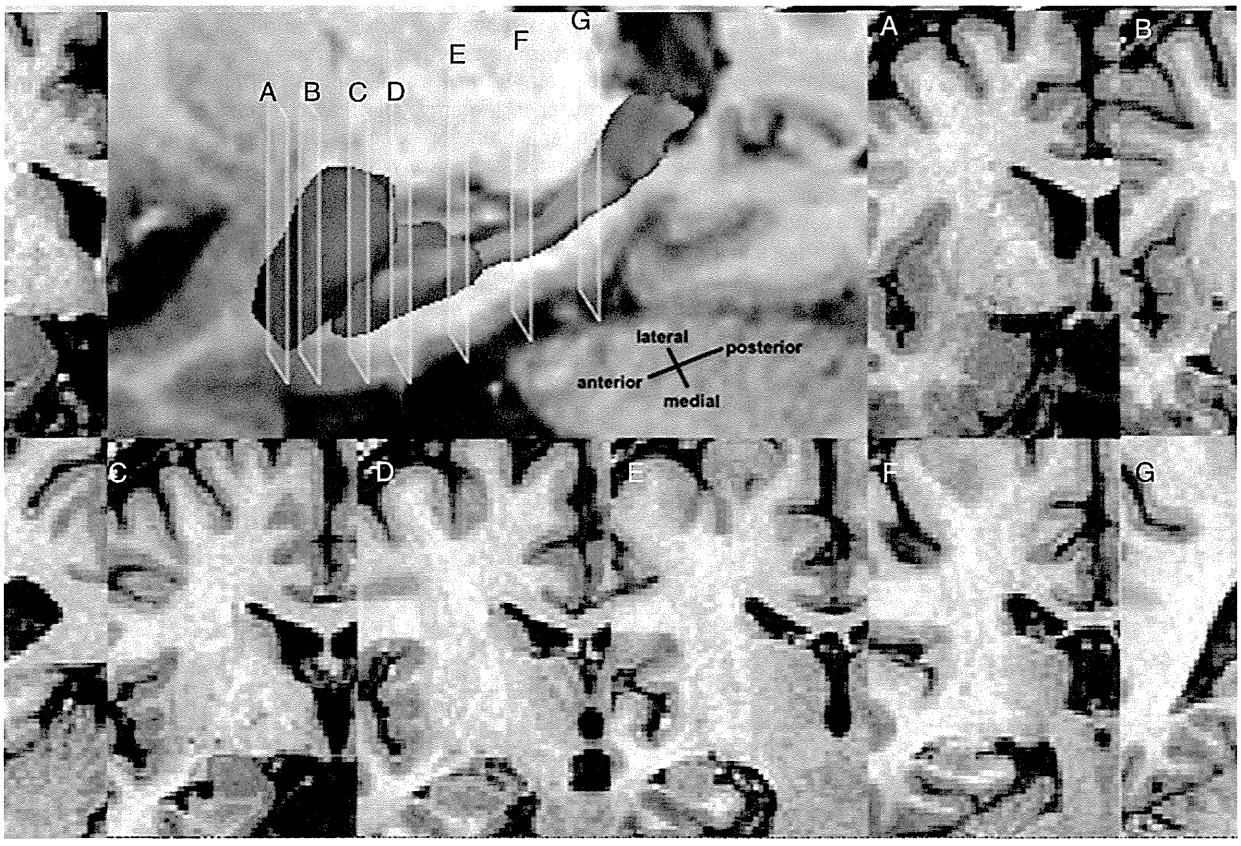

Fig. 1. Two- (panels A–G) and 3-dimensional images of regions of interest. The gray matter of the right amygdala is labeled light brown. The gray matter of right hippocampus is labeled light green. A–G: Delineation of the amygdala and hippocampus in coronal slices from rostral to caudal parts of regions of interest based on MRI data of a control subject. Three-dimensional reconstructions of the amygdala and hippocampus were superimposed on the sagittal plane of the right hemisphere. The coronal lines A–G correspond to the planes of panels A–G, respectively.

2001; Inoue et al., 2010). Then, whole brain volume and intracranial volume (ICV) were calculated by summing up the total gray matter and white matter volumes and the total gray matter, white matter, and cerebrospinal fluid (CSF) volumes respectively. To validate this method, these global brain volumes of an independent sample of MRI scans for 50 adult subjects were measured by both the current procedure and an intensity-based semi automated segmentation procedure using ANALYZE PC 3.0 (Yamasue et al., 2004). Then, we confirmed that the calculated intraclass correlation coefficients for the global brain volumes were satisfactory (more than 0.96).

2.3. Statistical analysis

Independent t-tests were used to examine the difference in total gray matter, white matter, whole brain volume, CSF, and ICVs between the patients and controls. For group comparison of ROI volumes, we employed a repeated measures ANCOVA with 1 between-subject factor (group: patients/controls) and 2 within-subject factors (hemisphere: left/right; region: hippocampus/amygdala). The absolute volumes of ROIs were used as the dependent variable with whole brain volume as a covariate, since the significant difference between the patients and controls was found in the whole brain volume. Once a significant group-by-region or group-by-region-by-hemisphere interaction was found, follow-up analyses using repeated measures ANOVA separately for each region were performed. Then, in the case of group-by-hemisphere interaction, post-hoc t-tests separately for each hemisphere were conducted. Statistical significance was set at $p<0.05$. Of note, the statistical conclusions reported below remained the same when ANOVA with relative volume [(absolute ROI volume)/(whole brain volume)×100] as the dependent variable was employed. In addition, the effect size between patients and controls was computed for each ROI using relative volumes, because the results might be biased by group difference in absolute whole brain volumes.

The associations between global brain volumes, including total gray matter, white matter, whole brain volume, CSF, and ICVs, and the relative volume of ROI and the clinical indices including symptom severities assessed by BPRS, SAPS and SANS and the ages at first use and at first onset of psychosis were tested with Spearman's rank correlation in the patients group. The association of way of methamphetamine usage with global brain volumes and relative volumes of ROIs was tested using multivariate ANCOVA with gender as covariate, since female ratio was significantly higher in smoking than in injection ($p=0.042$). Statistical significance was set at $p<0.05$ to exploratory detect potential associations between brain volumetric measures and severity of clinical symptoms or way of drug abuse.

Additionally, the associations between volumes of ROIs and potential confounds, including age, self SES, parental SES, dose of neuroleptics or IQ, were also tested using Spearman's rank correlation in each group separately. Statistical significance was set at $p<0.0014$ significance (Bonferroni correction for 36 correlations [20 for patient group {4 ROIs×5 clinical measures}; 16 for control group {4 ROIs×4 clinical measures}]).

3. Results

3.1. Volumes of global brain measures and ROIs

Total gray matter and whole brain volumes were significantly smaller in the patients with methamphetamine psychosis compared with the controls. The repeated measures ANCOVA showed a significant main effect of group ($F[1, 37]=42.87$, $p<0.001$) and group×region interaction ($F[1, 37]=9.658$, $p=0.004$), with no significant group×hemisphere ($F[1, 37]=0.853$, $p=0.362$), or group×hemisphere×region ($F[1, 37]=0.283$, $p=0.598$) interaction. Since the significant interaction between group×region was found, a follow up repeated measures ANOVA employing hemisphere as within subject factor was then employed for each region. The follow-up ANOVA showed that there was a significant main effect of group for amygdala ($F[1, 38]=27.16$, $p<.001$) and for hippocampus ($F[1,38]=16.99$, $p<.001$) with no significant interaction or other effects. These results indicated that the subjects with methamphetamine psychosis had significantly smaller volumes of amygdala and hippocampus with no significant laterality, while the significance of gray matter volume reduction was more pronounced for amygdala (Fig. 2). Although the patients showed significantly lower self-SES and premorbid IQ than the controls, the statistical conclusions were the same when ANCOVA with these variables as covariate. Moreover, exclusion of patients having history treated with electroconvulsive therapy ($n=3$) also did not alter the statistical conclusion. An evaluation of the effect size indicated that the effect size for the right amygdala (−1.27) was the largest among the ROIs (Table 2). The effect size for total (left+right) amygdala volume (−1.28) was larger than that for total hippocampus volume (−1.10).

3.2. Associations between clinical information and brain volumes

The total white matter ($F=4.93$, $p=0.024$) and whole brain volume ($T=3.86$, $p=0.046$) were significantly smaller in the patients with methamphetamine psychosis by intravenous injection than in those by smoking, while no significant difference between subjects with injection and smoking was found in the other volumes including amygdala or hippocampus volumes. No significant correlation was found between global and regional brain volumes and clinical information and symptom measures and potential confounds.

4. Discussion

To our knowledge, the present study is the first to demonstrate gray matter volume reduction in the amygdala and hippocampus of patients with methamphetamine psychosis compared with healthy controls. Of note, the extent of volume reduction was significantly greater in the amygdala than that in the hippocampus. These gray matter volume reductions in medial temporal lobe structures remained statistically significant when the significantly smaller-than-normal whole brain volume in the patients was adjusted.

No previous study has revealed volume reduction in amygdala in patients with methamphetamine abusers or psychosis compared with healthy controls. Previous reviews and meta-analyses have shown that patients with schizophrenia showed volume reduction less frequently in amygdala than in hippocampus (Shenton et al., 2001; Steen et al., 2006). However, the gray matter volume reduction in amygdala of current patients with methamphetamine psychosis seems to be similar to those in cocaine abusers in previous studies

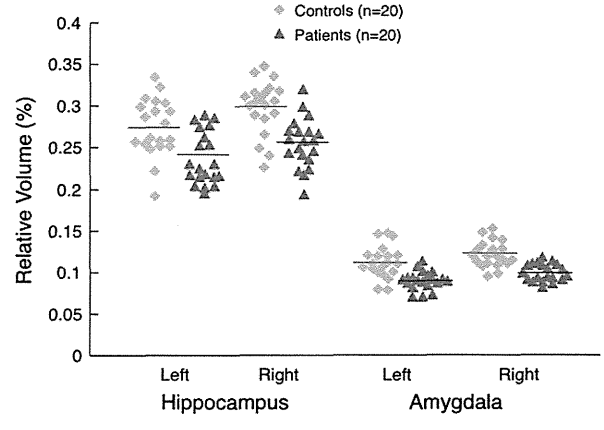

Fig. 2. Plots of gray matter volume of the regions-of-interest. Scatter plots show relative volumes of hippocampus and amygdala for patients with methamphetamine psychosis ($n=20$) and healthy controls ($n=20$). Horizontal lines indicate means of each group.

Table 2
Volumetric measures and statistical results.

	Patients with MAP (n=20)		Controls (n=20)		Effect sizes*	Z-scores	Repeated measures analysis of variance			
							Group	Group×hemisphere	Group×region	Group×region×hemisphere
Variables	Mean	SD	Mean	SD			F (p)	F (p)	F (p)	F (p)
Global brain volumes										
Absolute volume (ml)								T	(p)	
Intracranial space	1499.5	143.9	1576.2	143.8	−0.52	−0.53		1.7	(0.100)	
Whole brain volume	1107.1	115.3	1189.5	99.0	−1.36	−0.72		2.4	(0.020)	
Gray matter	690.4	63.5	742.6	45.8	−0.86	−0.82		3.0	(0.005)	
White matter	416.7	56.1	447.0	58.0	−0.52	−0.54		1.7	(0.102)	
Cerebrospinal fluids	392.5	48.7	386.7	58.2	0.11	0.12		0.4	(0.736)	
Regions-of-interest										
Absolute volume (ml)										
Left hippocampus	2.614	0.343	3.244	0.377	−1.32	−1.84				
Left amygdala	1.003	0.172	1.329	0.214	−1.29	−1.90	42.87	0.853	9.658	0.283
Right hippocampus	2.819	0.344	3.533	0.323	−1.46	−2.07	(<0.001)	(0.362)	(0.004)	(0.598)
Right amygdala	1.103	0.143	1.439	0.158	−1.49	−2.35				
Relative volume (%: absolute volume×100/whole brain volume)										
Left hippocampus	0.237	0.032	0.274	0.035	−0.97	−1.16				
Left amygdala	0.090	0.011	0.112	0.019	−1.15	−1.95	26.09	0.656	4.255	0.73
Right Hippocampus	0.256	0.030	0.299	0.032	−1.14	−1.45	(<0.001)	(0.423)	(0.046)	(0.398)
Right amygdala	0.100	0.010	0.122	0.016	−1.27	−2.08				

Effect sizes are calculated as: (MEANnc-MEANmap)/SDnc, MEANnc (map): Group mean of relative volumes of normal controls (patients with methamphetamine psychosis).

(Makris et al., 2004). Cocaine, which is also a psychostimulant drug, is pharmacologically similar to methamphetamine (Hyman, 1996; Haile et al., 2009). Previous literature suggested that small amygdala plays a role as a vulnerability marker in developing trait that increases the risk for drug dependence (Makris et al., 2004). Similar to the current results, the small amygdala volume has also been reported in patients with bipolar disorder (Usher et al., 2010). In accordance, methamphetamine abusers tend to show manic symptoms in the acute phase of administration (Tatetsu et al., 1956). Although there has been some inconsistency among previous findings (e.g., Brambilla et al., 2003), a recent meta-analysis reported that amygdala volume was bilaterally reduced in an overall sample of patients with bipolar disorder and a pediatric subsample (Usher et al., 2010). The results of the adult studies were less homogeneous, and on average, no significant difference was found between adult patients and healthy controls. Their meta-regression analysis further revealed a positive correlation between mean age and amygdala volume in patients with bipolar disorder. The authors suggested that amygdala volume is reduced at the onset of bipolar disorder and increases with age after onset. This increase after onset in amygdala volume might be caused by the neurotrophic effects of lithium (e.g. Moore et al., 2000; Nakamura et al., 2007). It is thus plausible that the current results in lithium-naïve patients exhibiting methamphetamine-induced psychosis, whose traits might partially overlap with those of bipolar disorder patients, is similar to previous results reported in patients at the onset of bipolar disorder. The common findings to patients with methamphetamine psychosis, other psychostimulant abuse and bipolar disorder might indicate that preexisting small amygdala plays a role in seeking novelty and excitement that increases the risk for developing psychostimulant dependence in patients with methamphetamine psychosis.

The present study revealed a significant volume reduction in bilateral hippocampus in patients with methamphetamine psychosis similar to those in patients with schizophrenia in previous literature (Shenton et al., 2001; Yamasue et al., 2004; Steen et al., 2006). Since smaller-than-normal hippocampus has been observed in the individuals at early phase of their illness (Hirayasu et al., 1998; Kubicki et al., 2002) and even in individuals at genetically (Keshavan et al., 2002) and clinically (Pantelis et al., 2003) high-risk for schizophrenia, the current volume reduction in hippocampus might also be associated with a vulnerability to developing psychosis. In line with the notion, vulnerabilities to methamphetamine abuse and subsequent psychosis have at least partially been indicated as heritable (Matsuzawa et al., 2007; Kishimoto et al., 2008). A previous study reported that the methamphetamine users, at least a part of them could be thought as high-risk subjects to develop later psychosis, also showed small hippocampus (Thompson et al., 2004). The lack of association between duration of methamphetamine use or ways of methamphetamine and hippocampal volume further suggests that the hippocampal volume reduction represents pre-existing vulnerability for developing psychosis. In contrast, previous human (Chang et al., 2004) and animal studies (Williams et al., 2004) have reported morphological changes in hippocampus associated with methamphetamine exposure. Therefore, further studies using longitudinal follow-up of recent-onset patients are needed to test whether smaller-than-normal hippocampus reflects vulnerability or stimulant induced acquired changes.

Smaller whole brain and gray matter volumes were found in the current patients with methamphetamine psychosis. Moreover, the way of methamphetamine usage showed a significant effect on the total white matter and whole brain volumes but not on the amygdala or hippocampal volumes. While smoking methamphetamine is pharmacokinetically similar to intravenous injection (Cook et al., 1993), another line of evidence showed the differences in epidemiological background of abusers between these two methods (Matsumoto et al., 2002). Therefore, the brain structural difference associated with ways of abuse could be explained by the potential differences in patient populations and/or dose of methamphetamine use. Methamphetamine administration has been reported to be associated with white matter myelin alterations in humans (Alicata et al., 2009) and experimental animals (Melo et al., 2008). Thus, continuous psychostimulant use may arrest normal white matter maturation in the frontal and temporal lobes of addicts who continue using psychostimulant (Bartzokis et al., 2002).

Here we discuss the methodological considerations and limitations of the present study. First, although the sample size was sufficient to reveal the volumetric difference between the patients and controls, the number of patients was insufficient to detect potential differences within patient subtypes such as patients with transient v.s. prolonged psychosis, users with intravenous injection v.s. smoking, and male v.s. female patients. Second, the most patients were treated with neuroleptics. Since previous studies have shown

that neuroleptics could affect brain morphology (Selemon et al., 1999), future studies examining neuroleptic naïve patients with methamphetamine psychosis is needed to exclude the potential confounding effect of neuroleptics. Third, smaller hippocampal volumes have been reported in patients with various psychiatric disorders including depression (Sheline et al., 1996; Abe et al., 2010), post-traumatic stress disorder (Gilbertson et al., 2002) and also in individuals with higher trait anxiety (Yamasue et al., 2008a), the specificity of small hippocampus to methamphetamine psychosis should limitedly be interpreted. Finally, since the gray matter volume reductions have been reported in various brain regions in patients with schizophrenia (Shenton et al., 2001; Onitsuka et al., 2003; Yamasue et al., 2004) or the other psychostimulant abusers (Sim et al., 2007; Tanabe et al., 2009), future studies should examine the regional specificity of gray matter volume reduction in patients with methamphetamine psychosis.

In conclusion, we demonstrated significant gray matter volume reduction in both the amygdala and hippocampus in patients with methamphetamine psychosis compared with healthy controls, although the extent of reduction was significantly greater in amygdala than in hippocampus. The amygdala-dominant gray matter volume reduction may be relatively specific to exogenous methamphetamine psychosis.

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Contributors

L.O., H.Y. and K.K. designed the study. L.O., Y.T., M.Y., Y.S., I.T., I.M., M.S, K.M., and Y.O. were engaged in the data collection and/or clinical assessment and/or recruitment of participants. L.O., H.Y., and I.H. analyzed the data. L.O., H.Y. and K.K. discussed the results and wrote the paper. All authors contributed to and have approved the final manuscript.

Conflict of interest

All the authors have no biomedical financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.schres.2011.07.006.

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