bodies, septal region, prefrontal cortex, and nucleus accumbens. Since all of these fornix-connected regions are deeply involved in the pathophysiology of schizophrenia (Gothelf et al., 2000; Kajimoto et al., 2003; Lauer et al., 2001; Weinberger et al., 2001), the fornix should have a key role in schizophrenia.

A previous diffusion tensor imaging (DTI) study (Kuroki et al., 2006) reported disrupted integrity of the fornix such as reduced fractional anisotropy (FA) and increased mean diffusivity (MD) in male patients with schizophrenia compared with male controls. However, the functional significance of the DTI abnormalities of the fornix in patients with schizophrenia has not been fully studied yet. Only one recent study (Nestor et al., 2007) reported a correlation between reduced FA of the fornix and general memory dysfunction in patients with schizophrenia.

Among memory functions, patients with schizophrenia have a particular deficit in 'memory organization', that is, an ability to utilize latent semantic organizational structure in their verbal recall (Koh et al., 1973; Matsui et al., 2006). Prefrontal cortex is indicated as an anatomical basis of memory organization deficits in schizophrenia (Nohara et al., 2000). Together with a central role of the hippocampus in memory functions, memory organization should require connectivity between the hippocampus and other regions such as prefrontal cortex via the fornix. Consequently we chose to assess an association between structural integrity of the fornix and memory organization in schizophrenia.

To assess memory organization, the current study employed two neuropsychological tests; stimulus category repetition (SCR) of the verbal learning task (Nohara et al., 2000) and the category fluency test (CFT). SCR reflects the degree to which the subject has utilized the implicitly provided conceptual categories to assist in organization (Koh et al., 1973), and has been used to evaluate memory organization (Araki et al., 2006). On the other hand, the CFT reflects the ability to recall categorized semantic words. While patients with schizophrenia have been shown to be impaired on both category and letter fluency measures when compared to healthy controls, patients are particularly impaired on the CFT (Bokat and Goldberg, 2003). Patients' disproportionate impairment of category fluency is presumably caused by impaired clustering, and disorganization of semantic memory storage (Bozikas et al., 2005). Thus the CFT assesses the organization of the long-term semantic memory system. Importantly, both prefrontal cortex (Audenaert et al., 2000) and hippocampus (Gleissner and Elger, 2001) are implicated in the retrieval of categorized words such as indexed by the CFT.

The present study hypothesized that disrupted integrity of the fornix correlates with impairment of memory organization, not with other cognitive function than memory organization, in patients with schizophrenia. Based on the hypothesis, it was predicted that DTI measures of the fornix would correlate with SCR scores of the verbal learning task and with performance of the CFT, not with verbal memory subscale of the Wechsler Memory Scale-Revised (WMS-R), the letter fluency test (LFT) or premorbid IQs estimated from Japanese version of National Adult Reading Test (JART) in patients with schizophrenia.

2. Methods

2.1. Instruments

The full list of instruments used in the current study was as follows:

2.1.1. Demographical and clinical assessments

Edinburgh handedness Inventory (Oldfield, 1971)
Structured Clinical Interview for DSM-IV Axis I Disorder (SCID-I) (First et al., 1997)
Positive and Negative Syndrome Scale (PANSS) and its five factor version (Kay et al., 1987; Bell et al., 1994)
Hollingshead socioeconomic status (SES) (Hollingshead, 1965)

2.1.2. Neuropsychological tests

Japanese version of National Adult Reading Test (JART) (Nelson, 1982; Matsuoka et al., 2006)
Stimulus category repetition (SCR) of the verbal learning task (Nohara et al., 2000)
Verbal fluency tests (category fluency test (CFT), and letter fluency test (LFT)) (Sumiyoshi et al., 2004)
The Wechsler Memory Scale-Revised (WMS-R) (Wechsler, 1987)

2.1.3. Magnetic resonance images

Diffusion tensor imaging (DTI)

2.2. Subjects

Thirty-one right-handed (determined using the Edinburgh Inventory) in- and outpatients with schizophrenia were recruited from the Department of Neuropsychiatry, Hospital of Tokyo University, Japan. Of these, twelve were male. Those patients who had already been clinically diagnosed as schizophrenia were reviewed according to DSM-IV criteria through the Structured

Clinical Interview for DSM-IV Axis I Disorder (SCID-I) by a trained psychiatrist (K.K. or H.Y.), and patients with diagnostic correspondence were recruited. The subtypes of schizophrenia were disorganized ($n=1$), catatonic ($n=1$), paranoid ($n=22$), residual ($n=1$), and undifferentiated ($n=6$). Psychiatric symptoms were assessed by a trained psychiatrist (H.Y.) according to PANSS (Kay et al., 1987) within three days before the MRI scanning. A five component model based on factor analysis of the PANSS (Bell et al., 1994) was used as an alternative to the rationally derived categories, since the five components, including Positive, Negative, Cognitive, Hostility, and Emotional Discomfort, have the advantage of separating cognitive from negative symptoms (Table 1). Sixty-five right-handed age- and gender-matched healthy subjects were recruited for comparison. Socioeconomic status (SES) and parental SES were assessed using the Hollingshead scale.

The exclusion criteria for both groups were neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, a history of electroconvulsive therapy, and substance abuse or addiction. An additional exclusion criterion for the control group was a history of psychiatric disease in themselves or a family history of axis I disorder in their first-degree relatives. The ethical committee of the University of Tokyo Hospital approved this study. All subjects gave written informed consent after a complete explanation of the study.

2.3. Neuropsychological assessment

All of the patients and 32 of 61 healthy controls performed the neuropsychological tests: the Japanese version of the verbal learning task and the CFT as neuropsychological indices of fornix integrity; the verbal memory subscale of the WMS-R, the LFT, and the JART as control indices.

The procedures followed for administration of the CFT and LFT were similar to those adopted by Sumiyoshi et al. (2004). Briefly, subjects were asked to utter as many words as possible in 60 seconds. For the CFT, subjects uttered words belonging to each category as follows: ANIMALS, VEGETABLES or ELECTRIC

Table 1
Demographic data, clinical information, and neuropsychological scores

		Control subjects ($n=65$)		Patients with schizophrenia ($n=31$)		t-tests		
		Mean	SD	Mean	SD	df	t value	P
Age (range)		34.7 (21–54)	9.7	33.8 (22–55)	9.0	94	0.46	0.64
Male/female		24/41		12/19				
Education, years				14.3	1.8			
SES[a]		1.9	0.7	3.5	1.2	92	−8.66	<0.001
Parental SES[a]		2.2	0.6	2.7	0.9	91	−2.70	0.008
Neuroleptic dose[b], mg/day				830	382			
Onset of illness, years				24.9	5.6			
Duration of illness, years (range)				9.7 (1–30)	8.1			
PANSS[c]	Positive			15.8	4.3			
	Negative			23.5	7.1			
	Cognitive			20.4	4.4			
	Hostility			7.3	2.7			
	Emotional discomfort			10.3	2.8			
JART		115.2	13.1	84.8	20.1	60	7.06	<0.001
WMS-R	Verbal memory	120.0	18.3	75.5	20.5	61	7.46	<0.001
Verbal learning task	Random	31.8	6.3	20.2	7.3	60	6.71	<0.001
	Blocked	43.1	4.0	29.1	10.5	60	6.91	<0.001
	Unblocked	38.0	5.9	24.2	9.5	60	6.90	<0.001
	SCR	22.6	8.2	9.0	7.9	60	6.66	<0.001
Verbal fluency test	Category fluency	50.7	12.0	37.0	13.0	59	4.28	<0.001
	Letter fluency	40.2	10.4	24.5	11.4	59	5.63	<0.001

PANSS, Positive and Negative Symptom Scale; JART, Japanese version of National Adult Reading Test; WMS-R, Wechsler Memory Scale-Revised; SCR, Stimulus Category Repetition.

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

[b] Based on chlorpromazine equivalents.

[c] Derived by a five component model based on factor analysis of the PANSS (Bell et al., 1994).

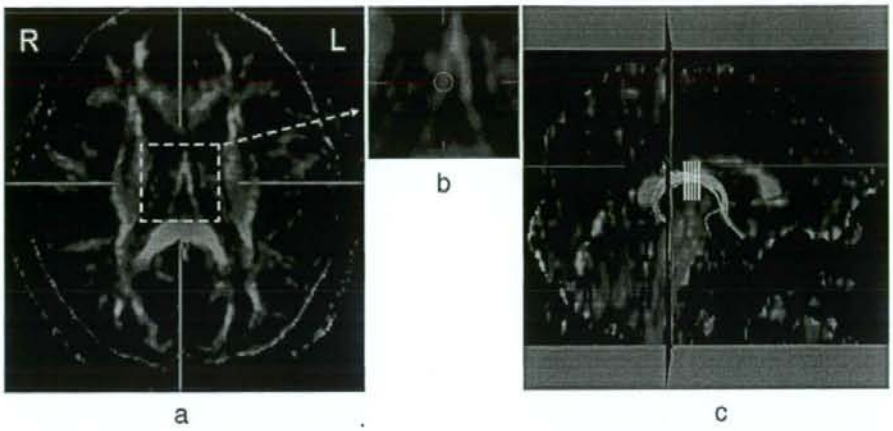

Fig. 1. Diffusion tensor tractography of the fornix on fractional anisotropy (FA) color maps. Red, green, and blue colors represent fibers running along the right–left, anterior–posterior, and superior–inferior orientations respectively. a) The axial slice showing the maximum size of the fornix (square dashed line). b) A magnified view of the square dashed line of (a). The circle on the right fornix shows the maximum cross-section of the seed-sphere from which tractography began. c) The bundle of the right fornix (pink) drawn by tractography method. Five equally spaced coronal cross-sections of the horizontal part of fornix tractography were measured. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

APPLIANCES. For the LFT, subjects uttered words beginning with each syllable as follows: /i/, /re/, or /si/. The number of words generated was defined as the measure of task performance.

The procedures of the verbal learning task are described in detail in a previous study (Nohara et al., 2000). Briefly, the verbal learning task was composed of three 20-word lists: a random list, a blocked list, and a semi blocked list. These three lists differed in degree of semantic organization. The random list consisted of 20 unrelated nouns. The blocked list contained subgroups of four taxonomic categories (stationery, vehicles, colors, and sports), each of which included five exemplars in a row. In the semi blocked list, five exemplars from each of four categories (animals, countries, musical instruments, and vegetables) were mixed so that related items never appeared consecutively. Thus, the words of the semi blocked list are categorized implicitly. The subjects were instructed to memorize the items they heard. For each list, three trials were repeated consecutively. Categorical clustering was evaluated as SCR (Bousfield and Bousfield, 1966) in recall of the semi blocked list. SCR is defined as the total number of occasions on which an item in a category is immediately

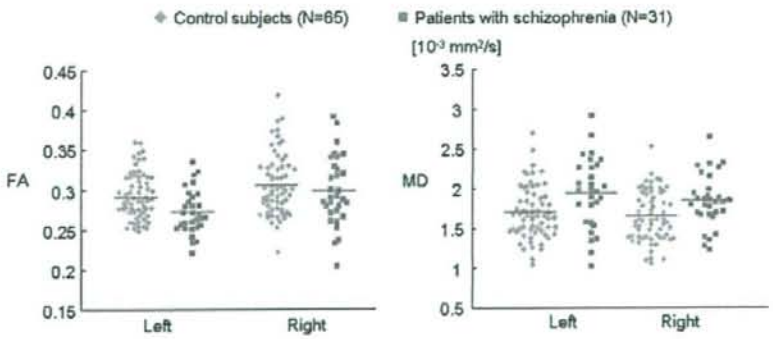

Fig. 2. Group difference in the diffusion tensor measures. Scatter plots of FA (left) and MD (right) of the fornix. Horizontal bars indicate mean values. FA, fractional anisotropy; MD, mean diffusivity.

Table 2
Diffusion tensor measures in the fornix and statistical results

		Control subjects ($n=65$)		Patients with schizophrenia ($n=31$)		Repeated measures analysis of variance					
						Diagnosis		Side		Diagnosis × Side	
		Mean	SD	Mean	SD	F	P	F	P	F	P
FA	Left	0.291	0.029	0.273	0.027	3.95	0.049	24.4	<0.001	1.60	0.21
	Right	0.306	0.039	0.299	0.043						
MD [10^{-3} mm^2/s]	Left	1.71	0.33	1.94	0.45	8.96	0.004	5.60	0.02	0.46	0.50
	Right	1.66	0.32	1.85	0.33						

FA, fractional anisotropy; MD, mean diffusivity.

followed by an item in the same category during recall, and it reflects the degree to which the subject utilized the conceptual categories provided implicitly to assist in organization (Koh et al., 1973).

Premorbid IQs were estimated using JART (Matsuoka et al., 2006) (Table 1).

2.4. Diffusion tensor imaging acquisition and processing

All subjects underwent DTI. The methods of DTI acquisition and data analysis were similar to those in our previous studies (Yamasue et al., 2007). Briefly, MRI data were obtained using a 1.5-T Signa Echo Speed MRI system (General Electric Medical Systems, Milwaukee, WI). The pulse sequence was single-shot, diffusion-weighted, $1\sqrt{2}$, 0, $1\sqrt{2}$), ($-1\sqrt{2}$, 0, $1\sqrt{2}$), (0, $1\sqrt{2}$, $1\sqrt{2}$), (0, $1\sqrt{2}$, $-1\sqrt{2}$), ($1\sqrt{2}$, $1\sqrt{2}$, 0), ($-1\sqrt{2}$, $1\sqrt{2}$, 0). The structural distortion of diffusion-weighted MRI images was corrected based on each T2-weighted echo-planar image ($b=0$ s/mm^2) (Haselgrove and Moore, 1996). The six elements of diffusion tensor were estimated in each voxel, and the eigenvectors and eigenvalues ($\lambda_1>\lambda_2>\lambda_3$) of the diffusion tensor were determined. FA and MD were generated on a voxel-by-voxel basis. FA was defined as the standard deviation of the eigenvalues divided by the root mean square of the eigenvalues. MD was defined as the average of the eigenvalues (Basser et al., 1994).

2.5. Diffusion tensor tractography

DTI measurements were performed in native space of each subject, because spatial normalization of echo-planar DTI may cause error in highly-localized structure such as the fornix. A software package for medical image analysis (*dTV*; software available at http://www.ut-radiology.umin.jp/people/masutani/dTV/dTV_frame-e.htm) was used to visualize fiber tracking (tractography). The methodologies of tractography and DTI measurement were based on those in our previous studies (Aoki et al., 2005; Masutani et al., 2003). Briefly, "seed-sphere" was defined as the location for the initiation of the tracking algorithm. The maximum cross-section of seed-sphere was put on one side of the fornix in axial slice of FA maps (Fig. 1b). The axial slice in which the fornix is displayed for its maximum size was selected from FA maps for placement of a circle of the seed-sphere. The tracking algorithm then moved a distance of 0.66 mm along the principal axis. The diffusion tensor at the next location was determined from the adjacent voxels by trilinear filtering, and its principal axis was subsequently estimated. The tracking

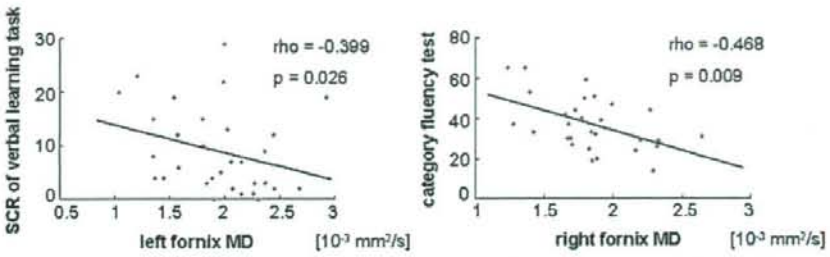

Fig. 3. Correlations between disrupted integrity of the fornix and impaired memory organization in the patients with schizophrenia. Scatter plots show correlations: left) between mean diffusivity in the left fornix and SCR of the verbal learning task, and right) between mean diffusivity in the right fornix and the category fluency test of the patients with schizophrenia. SCR, stimulus category repetition.

then traveled a further 0.66 mm along the same direction. Tracking lines were automatically traced in this way and were propagated in antegrade and retrograde directions until the FA fell below an assigned threshold (FA=0.2).

Five coronal cross-sections, which were equally spaced with 1.875 mm interval along the horizontal part of fornix tractography, were measured (Fig. 1c). Then, the mean and standard deviation (SD) of FA and MD for the five cross-sections were calculated.

For interrater reliability, two raters (K.T. and S.S.) independently depicted tractographies and measured ten cases selected at random blind to diagnosis. The interclass correlation coefficient was 0.96/0.94 for left/right fornix FA and 0.99/0.98 for left/right fornix MD. Intrarater reliability, measured by one rater (K.T.) at two separate times (approximately 6 months apart), was 0.92/0.94 for left/right fornix FA and 0.98/0.97 for left/right fornix MD.

2.6. Statistical analyses

2.6.1. Group comparison

t-tests were employed in group comparisons of the demographic and neuropsychological data. For analysis of DTI measures, repeated measures ANOVAs were performed for between-group comparison of FA and MD in the fornix, adopting group (schizophrenia, control) as the between-subject factor, and side (left, right) as the within-subject factor. Then, in the case of a significant group-by-side interaction, post-hoc *t*-tests were conducted separately for each hemisphere. The significance level was set at $p<0.05$.

2.6.2. Correlational analysis

The association between DTI measures of the fornix in each hemisphere and neuropsychological scores was tested using Spearman's rank order correlation for each group separately. It was predicted that DTI measures in the fornix would show correlations with SCR of the verbal learning task and with CFT performance in the schizophrenia group. Thus, taking into account the hypothesis-driven nature of the correlational analysis, we set alpha at $p<0.05$.

Additionally, Spearman's rho was calculated for exploring the correlation between clinical measures (age, SES, parental SES, onset of illness, duration of illness, and neuroleptic dose) and DTI measures in each group separately. Alpha was set at $p<0.002$ (Bonferroni correction for 18 correlations [12 for schizophrenia group {2 DTI measures×6 clinical measures}; 6 for control group {2 DTI measures×3 clinical measures}]). Furthermore, Spearman's rho was calculated for exploring the correlation between factor analytic scores in the PANSS (Bell et al., 1994) and DTI measures in the schizophrenia group. Alpha was set at $p<0.0025$ (Bonferroni correction for 20 correlations (4 DTI measures×5 symptom measures).

3. Results

The patients with schizophrenia showed significantly lower self- and parental SES than the control subjects ($p<0.01$), whereas the age and sex ratio did not differ significantly between groups (Table 1).

3.1. Neuropsychological scores

The patients with schizophrenia had significantly lower scores than the controls on all of the neuropsychological tests (JART score, verbal memory of WMS-R, verbal learning task, CFT, and LFT) (each $p<0.001$). The variances did not differ significantly between the patients and the controls on all of these tests except for the 'blocked' subscale of the verbal learning task (Table 1).

3.2. Diffusion tensor imaging measures

For FA of the fornix, significant main effects of group ($F[1,94]=3.95$, $p=0.049$) and side ($F[1,94]=24.4$, $p<0.001$) were observed, while the group-by-side interaction was not significant ($F[1,94]=1.60$, $p=0.21$) (Fig. 2, Table 2). For MD of the fornix, significant main effects of group ($F[1,94]=8.96$, $p=0.004$) and side ($F[1,94]=5.60$, $p=0.02$) were observed with no significant group-by-side interaction ($F[1,94]=0.458$, $p=0.50$) (Table 2). These results suggested bilaterally reduced FA and elevated MD of the fornix in patients with schizophrenia compared with control subjects. Of note, no significant interaction between gender and diagnosis was observed (FA: $F[1,94]=0.718$, $p=0.40$; MD: $F[1,94]=0.122$, $p=0.73$), and separate statistical analysis for each gender did not change the statistical conclusions. Moreover, the main effects of group on FA and MD remained significant after a covariate analysis using age, which showed correlations with FA in the patient group.

The results of ANOVAs for right and left hemisphere separately are as follows: $t=0.82$, $p=0.41$ for FA of the right fornix; $t=2.91$, $p=0.005$ for FA of the left fornix; $t=-2.67$, $p=0.009$ for MD of the right fornix; $t=-2.81$, $p=0.006$ for MD of the left fornix. If the Bonferroni-type correction for multiple comparison was employed, the significantly reduced FA of fornix in left hemisphere and significantly increased MD of the fornix in both hemisphere were detected ($p<0.025$).

3.3. Correlations between DTI measures and neuropsychological scores and symptom severity

As for patients with schizophrenia, Spearman's rho showed significant relationships between elevated MD of the left fornix and lower SCR of the verbal learning task (rho$=-0.399$, $p=0.026$) and between elevated MD of the right fornix and poorer performance on the CFT (rho$=-0.468$, $p=0.009$). These correlations were specific to the patient group (Fisher's r to z transformation, $z>2.13$, $p<0.034$) (Fig. 3). Moreover, the correlation between MD of the fornix and SCR of the verbal learning task in the patients with schizophrenia was not specific to the left hemisphere, since the hemispheric difference in the correlations did not reach the statistically significant level (Fisher's r to z transformation, $z=0.42$, $p=0.67$). Similarly, the correlation between MD of the fornix and performance on the CFT in the patients with schizophrenia was not specific to the right hemisphere (Fisher's r to z transformation, $z=0.73$, $p=0.47$).

For the control subjects, none of the correlations between fornix measures and JART score ($-0.092<$rho<0.25, p's>0.19), verbal memory subscale of the WMS ($-0.18<$rho<-0.004, p's>0.34), or LFT ($-0.13<$rho<0.26, p's>0.16) reached the level for statistical significance. Similarly, for the patients with schizophrenia, none of the correlations between fornix measures and JART score ($-0.14<$rho<0.11, p's>0.47), verbal memory subscale of the WMS ($-0.30<$rho<0.03, p's>0.11), or LFT ($-0.30<$rho<0.06, p's>0.10) reached the level for statistical significance.

As for symptoms, none of the correlations between the five factors of the PANSS and DTI measures reached the level for statistical significance.

3.4. Correlations between DTI measures and demographic information

Lower FA of the left fornix in the schizophrenia group showed correlations with increased age (rho$=-0.415$, $p=0.020$), longer duration of illness (rho$=-0.399$, $p=0.036$), and lower class of parental SES (rho$=-0.370$, $p=0.048$), although the correlations did not reach statistical significance after Bonferroni correction. No significant correlation was observed between DTI measures and neuroleptic dosage. In addition, no significant correlation was observed between DTI measures and age, SES, or parental SES in the control subjects.

4. Discussion

The present study demonstrated reduced FA and increased MD in the fornix of patients with schizophrenia compared with matched healthy controls with no significant lateralization. In the patients, increased MD of the fornix further showed significant correlations with lower SCR scores of the verbal learning task and with poorer CFT performance. The correlations were specific to the patient group, as indicated by the significant group difference in the values of these correlations.

The current results from group comparison, both reduced FA and increased MD in the fornix of patients with schizophrenia compared with healthy controls, were consistent with those in a previous study employing ROI methodology in male patients with schizophrenia (Kuroki et al., 2006). In addition, FA reduction of the fornix was consistent with a previous voxel-based analysis study (Kubicki et al., 2005) from the overlapping samples with those in Kuroki et al. (2006). Thus, the present study replicated previous findings, and further extended the observation to both genders.

Various pathological changes can result in both MD increase and FA reduction. One of the putative pathological changes is impairment of axonal myelin sheath. Histological (Hof et al., 2003) and genetic (Hakak et al., 2001) studies indicate the possibility of myelin abnormalities in schizophrenia. However, contribution of myelin abnormalities to MD increase and FA reduction is tentative, because also other pathologies such as brain atrophy can cause both MD increase and FA reduction.

The present study identified worsened memory organization as a functional correlate of the disrupted integrity of the fornix in 31 patients with schizophrenia, while a recent study (Nestor et al., 2007) reported that reduced FA of the fornix correlated with lower scores for general memory in 14 male patients with schizophrenia. Implicit utilization of a strategy such as semantic clustering of encoded words is necessary for better performance on the memory organization task, which presumably recruits functional cooperation between prefrontal cortex and the hippocampus, as well as the hippocampus per se (Bussey et al., 2001; Nohara et al., 2000). Similarly, both prefrontal cortex (Audenaert et al., 2000) and hippocampus (Gleissner and Elger, 2001) are implicated in the performance of CFT, which requires organized word retrieval from the long-term semantic memory system. Thus, it is reasonable to expect that structural abnormality of the fornix, which connects hippocampus and other regions including prefrontal cortex, is associated with dysfunction of memory organization in patients with schizophrenia.

Regarding laterality, a previous study indicated a relationship between the CFT and the right hippocampus (Gleissner and Elger, 2001), and another study showed a relationship between SCR of the verbal learning task and the left prefrontal cortex (Nohara et al., 2000). These two studies are in line with the present correlational findings in terms of laterality. However, the present study could not further discuss the laterality of the findings, since Fisher's r to z transformation did not show significant hemispheric differences in the correlations.

The reason why only MD, not FA, of the fornix correlated with neuropsychological scores is difficult to be interpreted. One possibility is as follows. The white matter of the patients with schizophrenia may include fewer glia cells (Hof et al., 2003), followed by less water content in the white matter, causing MD increase without FA reduction. On the other hand, myelin abnormalities cause both MD increase and FA reduction. Given that patients with schizophrenia have both of these histological changes (fewer glia cells and myelin abnormalities), both MD increase and FA reduction are observed. Presumably, having fewer glia cells in the fornix is more strongly implicated in dysfunction of memory organization than having myelin abnormalities. However, it is not totally ruled out that the correlation with only MD reflects an increase of CSF space in schizophrenia through partial volume effect, although an increase of CSF is likely to be associated with FA reduction as well as MD increase.

The current study also showed correlations between reduced FA and increased age and between reduced FA and duration of illness in patients with schizophrenia although the significance levels were marginal. These correlations might indicate that illness progression affects deterioration of FA in the fornix of patients with schizophrenia. On the other hand, fornix MD correlated with cognitive function, which is comparatively unaltered throughout the duration of the disease (Rund, 1998), while MD did not correlate with either age or duration. Therefore, it is possible that fornix FA represents some aspect of progressive pathophysiology and fornix MD represents some aspect of stable pathophysiology of schizophrenia. This notion is supported by our previous study reporting that reduced FA was more strongly correlated with increased age in the frontotemporal white matter, compared with volume and MD (Abe et al., 2008). On the other hand, Kuroki et al. (2006) reported that age correlated positively with MD of the fornix for a healthy control group and age did not correlate with either FA or MD for a schizophrenia group. Inconsistency in the relationship between age and DTI measures of the fornix may partially derive from cross-sectional designs, thus, it will be important in future studies to employ longitudinal designs.

The present study has several limitations. First, although coronal sections are more suitable for seed-sphere placement in the fornix, we used axial sections for seed-sphere placement because DTI scans were acquired as axial slices. Second, the voxel size was not small enough relative to the size of the fornix. The comparatively large voxel size caused partial volume effect, resulting in relatively low FA values of the fornix. In addition, we had to adopt a low cut-off point ($FA > 0.18$) in tractography because tractography was otherwise difficult to be completed, which may be another cause of low FA values of the fornix. Third, the correlation with memory organization may not be specific to the fornix because we did not compare the fornix with any control region. Fourth, all the patients were taking antipsychotic medications. The medication might influence DTI measures, although neuroleptic dosage did not correlate with DTI measures in the present study. Thus, it will be important in future studies to examine integrity of the fornix in unmedicated patients with schizophrenia.

In conclusion, the current DTI study suggests that disrupted integrity of the fornix contributes to core cognitive deficits, specifically impairment of memory organization and the semantic memory system in patients with schizophrenia.

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Contributors

Authors Yamasue and Kasai designed the study and wrote the protocol. Author Takei managed the DTI measurement and the statistical analyses. Authors Abe, Yamada, Inoue, Suga, Sekita, Sasaki, and Aoki recruited the subjects, took MRI, and performed clinical evaluation. Authors Takei, Yamasue, Kasai and Rogers wrote the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

Kunio Takei, Hidenori Yamasue, Osamu Abe, Haruyasu Yamada, Hideyuki Inoue, Motomu Suga, Kayoko Sekita, Hiroki Sasaki, Mark Rogers, Shigeki Aoki, and Kiyoto Kasai declare that they have no conflicts of interest.

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BRIEF REPORT

Co-Administration of a D-Amino Acid Oxidase Inhibitor Potentiates the Efficacy of D-Serine in Attenuating Prepulse Inhibition Deficits After Administration of Dizocilpine

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Background: D-Serine, an endogenous agonist of the *N*-methyl-D-aspartate (NMDA) receptors, is effective in the treatment of schizophrenia. However, orally administered D-serine is metabolized substantially by D-amino acid oxidase (DAAO), diminishing its oral bioavailability. In this study, we examined the effects of oral D-serine administration with or without a DAAO inhibitor, 5-chloro-benzo[*d*]isoxazol-3-ol (CBIO), on the prepulse inhibition (PPI) deficits after administration of the NMDA receptor antagonist dizocilpine.

Methods: Vehicle or D-serine (30, 300, or 900 mg/kg) with or without CBIO (30 mg/kg) was orally administered to mice 1 hour before administration of dizocilpine (.1 mg/kg), and then the PPI of the acoustic startle response was measured. We measured the extracellular levels of D-serine in the frontal cortex after oral administration of D-serine with or without CBIO.

Results: Coadministration of CBIO with D-serine (30 mg/kg), but not D-serine (30 mg/kg) alone, significantly attenuated dizocilpine-induced PPI deficits. Furthermore, coadministration of CBIO significantly increased the extracellular levels of D-serine in the frontal cortex after administration of D-serine.

Conclusions: These findings suggest that coadministration of CBIO significantly enhanced the efficacy of D-serine in attenuating PPI deficits by administration of dizocilpine. Therefore, coadministration of D-serine and a DAAO inhibitor has therapeutic potential for the treatment of schizophrenia.

Key Words: Bioavailability, D-Amino acid oxidase, D-Serine, NMDA receptors, prepulse inhibition

Accumulating evidence suggests that a dysfunction in glutamatergic neurotransmission via the *N*-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of schizophrenia and that D-serine, an endogenous agonist at the NMDA receptors, plays a role in the pathophysiology of schizophrenia (1–3). First, it has been reported that levels of D-serine in the serum or cerebrospinal fluid of patients with schizophrenia are lower than those of normal control subjects (4–7). Second, Tsai *et al.* (8) reported that D-serine significantly improved schizophrenic symptoms when used as adjuvant to conventional antipsychotic drugs. A subsequent study demonstrated that the addition of D-serine to ongoing treatment with risperidone or olanzapine was beneficial for reducing positive, negative, and cognitive symptoms of patients with treatment-refractory schizophrenia (9). Third, the mRNA expression and activity of D-amino acid oxidase (DAAO) (10), which can metabolize D-serine, is increased in the postmortem brain of schizophrenic patients (11,12). Fourth, the G72 gene on chromosome 13q has been significantly associated with schizophrenia (13,14). The G72 gene has been designated a DAAO activator, because the G72 protein interacts physically with DAAO (13). A recent meta-analysis provided highly significant evidence of an association between nucleotide variations in the G72/G30 region and schizophrenia (15). In consideration of these findings, it is likely that reductions in brain D-serine levels play a role in the pathophysiology of schizophrenia (1–3).

In animals, D-serine is believed to be metabolized substantially by DAAO, diminishing its oral bioavailability (16). These findings prompted us to identify small molecule DAAO inhibitors that can be coadministered with D-serine to minimize its metabolism by DAAO. Recently, we reported that oral administration of a potent DAAO inhibitor, 5-chloro-benzo[d]isoxazol-3-ol (CBIO), in conjunction with D-serine could enhance the plasma and brain levels of D-serine in rats compared with the oral administration of D-serine alone (17).

In this study, we examined the effects of oral D-serine administration with or without CBIO on the prepulse inhibition (PPI) deficits in mice after administration of the NMDA receptor antagonist dizocilpine. We also measured the levels of D-serine in the brain and plasma after oral administration of D-serine with or without CBIO.

Methods and Materials

Animals

Male Slc:ddy mice (6 weeks old) weighing 25–30 g were purchased from SLC Japan (Hamamatsu, Shizuoka, Japan). The mice were housed in clear polycarbonate cages (22.5 × 33.8 × 14.0 cm) in groups of five or six per cage under a controlled 12-hour light-dark cycle (lights on from 7:00 AM to 7:00 PM), with room temperature at 23 ± 1°C and humidity at 55% ± 5%. The mice were given free access to water and food pellets. The experimental procedure was approved by the Animal Care and Use Committee of Chiba University.

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Measurement of Acoustic Startle Reactivity and Prepulse Inhibition of Startle

The mice were tested for their acoustic startle reactivity (ASR) in a startle chamber (SR-LAB; San Diego Instruments, San Diego, California) using the standard methods described previously (18). The test sessions were begun after an initial 10-min acclimation period in the chamber. The mice were subjected to one of six trials: 1) pulse alone, as a 40-msec broadband burst; a pulse (40-msec broadband burst) preceded by 100 msec with a 20-msec prepulse that was 2) 4 dB, 3) 8 dB, 4) 12 dB, or 5) 16 dB over background (65 dB); and 6) background only (no stimulus). The amount of prepulse inhibition (PPI) was expressed as the percentage decrease in the amplitude of the startle reactivity caused by presentation of the prepulse (% PPI).

D-Serine (30, 300, or 900 mg/kg; Sigma-Aldrich, St. Louis, Missouri) with or without CBIO (30 mg/kg) (16) or vehicle (.5% carbomethoxycellulose [CMC]; Wako Pure Chemical, Tokyo, Japan; 10 mL/kg) was administered 60 min (including the 10-min acclimation period) before the machine records, and dizocilpine (.1 mg/kg as a hydrogen maleate salt; Sigma-Aldrich) or saline (10 mL/kg) was administered subcutaneously 10 min (including the 10-min acclimation period) before the records. The PPI test lasted 20 min in total.

Measurement of D-Serine levels

Vehicle (10 mL/kg), D-Serine (30 or 900 mg/kg), D-serine (30 mg/kg) with CBIO (30 mg/kg), or CBIO (30 mg/kg) was administered 90 min before sacrifice. Levels of D-serine and L-serine in the frontal cortex and plasma were measured by high performance liquid chromatography (HPLC), as reported previously (19).

Mice were anesthetized with sodium pentobarbital before the stereotaxic implantation of a probe into the left frontal cortex (+2.1 mm anteroposterior, +1.0 mm mediolateral from the bregma, and −1.2 mm dorsoventral from the dura). Probes were secured onto the skull using stainless steel screws and dental acrylic. Twenty-four hours after surgery, in vivo microdialysis was performed on conscious mice. Probes were perfused continuously with artificial cerebrospinal fluid (147 mmol/L NaCl, 4 mmol/L KCl, and 2.3 mmol/L CaCl2) at a rate of 2 μL/min. D-Serine (30 mg/kg) with or without CBIO (30 mg/kg) was orally administered. The dialysate was collected in 30-min fractions, and then levels of D-serine and L-serine were measured as described earlier.

Statistical Analysis

The data are presented as the mean ± standard error of the mean (SEM). The PPI data were analyzed by multivariate analysis of variance (MANOVA). When appropriate, group means at individual dB levels were compared by one-way analysis of variance (ANOVA), followed by Bonferroni/Dunn test. The results of D-serine levels were also analyzed by one-way ANOVA, followed by Bonferroni/Dunn test. Significance for the results was set at $p < .05$.

Results

Effects of D-Serine With or Without CBIO on PPI Deficits After a Single Administration of Dizocilpine

There was no significant effect on the acoustic startle response at 120 dB in the all group. Figure 1 shows the effects of D-serine (30, 300, or 900 mg/kg) with or without CBIO (30 mg/kg) on dizocilpine (.1 mg/kg)-induced PPI deficits in mice. The MANOVA analysis of all PPI data revealed that there was a significant effect (Wilks lambda = .429, $p < .001$). Subsequent ANOVA analysis revealed the significant differences at all dB groups (69, 73, 77, and 81 dB). A posteriori analysis indicated a significant ($p < .001$) difference in PPI deficits between the vehicle + vehicle group and vehicle + dizocilpine (.1 mg/kg) group (Figure 1). Higher doses (300 or 900 mg/kg) of D-serine alone significantly attenuated PPI deficits induced by dizocilpine (.1 mg/kg; Figure 1). However, the low dose (30 mg/kg) of D-serine alone did not alter PPI deficits induced by dizocilpine. Interestingly, coadministration of CBIO (30 mg/kg) with D-serine (30 mg/kg) significantly attenuated dizocilpine-induced PPI deficits at 73 dB ($p = .010$), 77 dB ($p = .009$), and 81 dB ($p < .001$; Figure 1). In contrast, CBIO (30 mg/kg) alone did not alter PPI in normal and dizocilpine-treated mice (Figure 1).

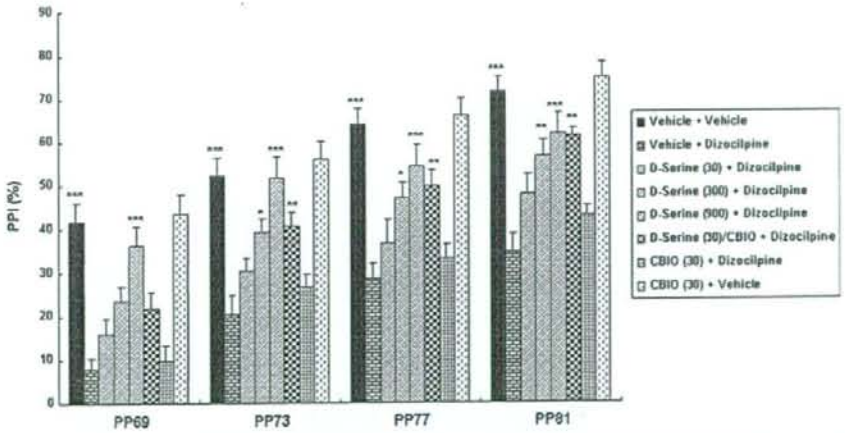

Figure 1. The effect of D-serine with or without 5-chloro-benzo[*d*]isoxazol-3-ol (CBIO) on dizocilpine-induced prepulse inhibition (PPI) deficits in mice. Sixty minutes after oral administration of vehicle (10 mL/kg), D-serine (30, 300, or 900 mg/kg), D-serine (30 mg/kg) plus CBIO (30 mg/kg), or CBIO (30 mg/kg) alone, with dizocilpine (.1 mg/kg), or with saline (10 mL/kg) was administered subcutaneously to the mice. Each value is mean ± SEM (n = 13–17 per group). The number in parentheses in the legend is the dose (mg/kg) of drugs. *$p < .05$, **$p < .01$, ***$p < .001$ compared with the vehicle + dizocilpine–treated group.

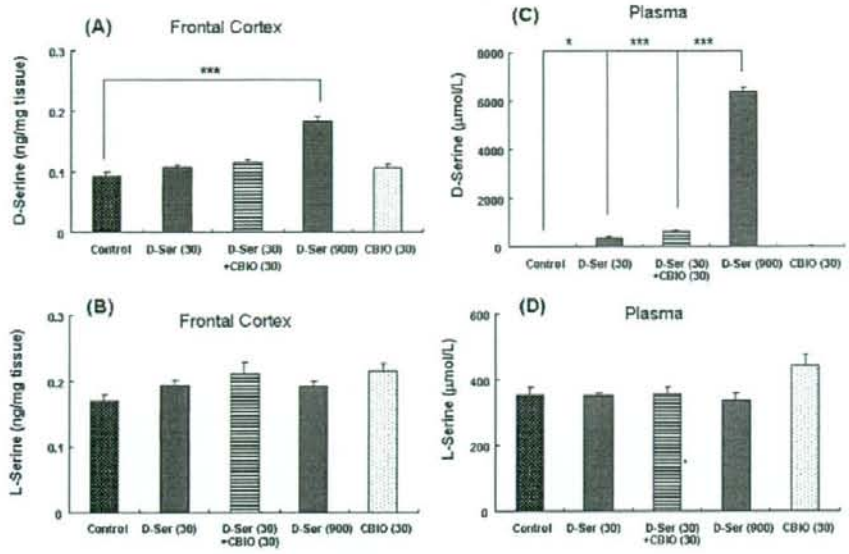

Figure 2. The effects of D-serine with or without 5-chloro-benzo[*d*]isoxazol-3-ol (CBIO) on levels of D-serine and L-serine in the frontal cortex and plasma. **(A–D)** Ninety minutes after oral administration of vehicle (10 mL/kg), D-serine (30, 300, or 900 mg/kg), D-serine (30 mg/kg) plus CBIO (30 mg/kg), or CBIO (30 mg/kg) alone, mice were sacrificed. Levels of D-serine and L-serine were measured by high-pressure liquid chromatography. The values are the mean ± SEM of six or seven mice. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ compared with the control group.

Effects of CBIO on D-Serine Levels in the Frontal Cortex and Plasma After Oral Administration of D-Serine

We measured the levels of D-serine in the frontal cortex and plasma 90 min after oral administration of D-serine (30 or 900 mg/kg) with or without CBIO (30 mg/kg). Levels of D-serine in the frontal cortex were significantly increased after a single administration of D-serine (900 mg/kg), but not D-serine (30 mg/kg) with or without CBIO (Figure 2A). Plasma levels of D-serine were significantly increased after a single administration of D-serine (30 or 900 mg/kg) or D-serine (30 mg/kg) with CBIO (Figure 2C). In contrast, CBIO (30 mg/kg) alone slightly increased the plasma levels of D-serine, although the difference was not significant (Figure 2A). Levels of L-serine were not altered in any of the groups (Figure 2B and 2D).

To explore the effects of CBIO on the extracellular levels of D-serine in the brain, we used an in vivo microdialysis technique to examine extracellular D-serine levels in the frontal cortex of conscious mice. Coadministration of D-serine (30 mg/kg) and CBIO (30 mg/kg) significantly increased the extracellular D-serine levels in the mouse frontal cortex compared with the group receiving D-serine (30 mg/kg) alone (Supplement 1). This data from the mouse brain is consistent with the previous results of rat brain (17), but the effects of CBIO in the mouse brain were higher than those in the rat brain (17).

Discussion

In this study, we found that coadministration of the DAAO inhibitor CBIO potentiated the bioavailability of D-serine in mice. Interestingly, we found that coadministration of CBIO (30 mg/kg) significantly enhanced the efficacy of D-serine (30 mg/kg) in attenuating dizocilpine-induced PPI deficits, although D-serine (30 mg/kg) alone was not effective in this model.

Plasma levels of D-serine after a single oral administration of D-serine with CBIO were significantly higher than those after administration of D-serine alone, indicating the increased oral bioavailability by CBIO. Furthermore, an in vivo microdialysis study revealed that extracellular D-serine levels in the frontal cortex after administration of D-serine with CBIO were significantly higher than those after treatment with D-serine alone. Taken together, these results suggest that the ability of CBIO to enhance the effects of D-serine on PPI deficits may be due to increased D-serine levels in the brain. In addition, combination therapy with D-serine and a DAAO inhibitor could be used to reduce the dose of D-serine in humans, because the dose of D-serine used for the treatment of schizophrenic patients is high (30–60 mg/kg) (8,9).

In conclusion, coadministration of D-serine and a DAAO inhibitor would be a new approach for the treatment of schizophrenia.

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Supplementary material cited in this article is available online.

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