deficits in different cognitive domains. Dickinson et al. (2004) reported that generalized cognitive deficits might be a core feature of schizophrenia, in contrast to more specific, independent cognitive deficits. Researchers have attempted to determine whether or not there is a relationship between cognitive deficits and localized pathological changes in patients with schizophrenia. However, the biological basis of such generalized cognitive deficits remains unclear.

Proton magnetic resonance spectroscopy (^{1}H-MRS) provides a noninvasive means of examining endogenous brain metabolites, including *N*-acetylaspartate (NAA), choline (Cho), and Creatine (Cr). NAA is one of the most abundant free amino acids in the human brain, and is synthesized and stored primarily in neurons (Baslow, 2003). Since MRS can readily detect endogenous NAA in the human brain, NAA has been used as an intracellular marker of neuronal function (Jenkins et al., 2000); indeed, NAA appears to be an indirect measure of neuronal integrity and synaptic abundance in an expanding number of disorders (Tsai and Coyle, 1995). Cho is a precursor for the neurotransmitter acetylcholine and for the membrane constituent phosphatidylcholine. Levels of Cho have been reported to fluctuate when cellular membranes are degraded or rapidly synthesized (Miller, 1991). Cr reflects the total creatine plus phosphocreatine pool. The working memory function of patients with schizophrenia has been associated with NAA levels in the dorsolateral prefrontal cortex (Bertolino et al., 2000). Low levels of NAA/creatine (NAA/Cr) in the medial prefrontal cortex of schizophrenia patients subclassified as having a deficit syndrome have also been detected (Delamillieure et al., 2000). Although these and related studies have provided evidence of a relationship between prefrontal dysfunction and poor cognitive performance in patients with schizophrenia, it remains unclear whether pathological abnormalities in other brain regions are related to particular cognitive functions.

The *N*-methyl-D-aspartate (NMDA) receptor is a ligand-gated ion channel that participates in a number of neurophysiological processes, including learning and memory (Nakanishi, 1992; Shimizu et al., 2000; Tang et al., 1999). The NMDA receptor antagonists, phencyclidine (PCP) (Javitt and Zukin, 1991), MK-801, and ketamine (Newcomer et al., 1999; Umbricht et al., 2000), are known to induce psychotic behavior and cognitive deficits in both animals and humans. Therefore, hypofunction of the NMDA receptors has been implicated in the pathophysiology of schizophrenia (Hashimoto and Iyo, 2002; Hashimoto et al., 2003; Olney and Farber, 1995; Tsai and Coyle, 2002). Olney et al. (1989) reported that NMDA receptor antagonists induce transient neuropathological changes (neuronal vacuolization) in the posterior cingulate gyrus (PCG)/retrosplenial cortex of the rat brain (Fix et al., 1993). Such findings suggest, based on the putative pathogenetic role of NMDA in schizophrenia, that the effects of NMDA are local rather than generalized, and that MRS could be a candidate technique for the detection of NMDA-induced damage. Furthermore, pathology of the PCG can be suspected to be pathogenetically related to cognitive deficits observed in patients with schizophrenia.

Based on the results of these previous studies, we hypothesized that metabolic abnormalities in the PCG play a pivotal role in the cognitive dysfunctions in patients with schizophrenia. However, to the best of our knowledge, there have been no reports with a focus on the relationship between metabolic changes in the PCG and cognitive dysfunction in patients with schizophrenia. To test our hypothesis regarding the putative role played by PCG in the cognitive deficits observed in patients with schizophrenia, we measured metabolic ratios in three brain regions, namely, in the PCG, and in the left and right medial temporal lobes (MTL) by ^{1}H-MRS; and we also assessed neuropsychological cognitive function in patients with chronic schizophrenia.

2. Methods

2.1. Subjects

The ethics committee of the Chiba University Graduate School of Medicine approved the present study. The procedure was fully explained to all of the subjects, who then provided written informed consent prior to participation in the study. Handedness was determined using the Edinburgh Inventory (Oldfield, 1971), and right-handed subjects underwent the procedure (Table 1). Nineteen patients with schizophrenia (11 men and 8 women) were recruited from Chiba University Hospital and Kimura Hospital, Chiba, Japan. Eighteen age- and gender-matched healthy control subjects (12 men and 6 women) also participated in this study. We selected only healthy control subjects who had neither a medical nor a psychiatric history or diagnosis according to our clinical interview. All patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria (American Psychiatric Association, 1994). Patients with any other mental or physical illnesses were excluded. At the time of testing, the patients were taking antipsychotic medication. Sixteen patients were receiving treatment with atypical antipsychotic drugs including risperidone and olanzapine, while the remaining patients were being treated with conventional antipsychotic medication. The mean daily chlorpromazine equivalent dose was 650.5 mg (standard deviation = 536.3). Clinical symptoms were assessed using the 18-item Brief Psychiatric Rating Scale (BPRS) (Kolakowska, 1976; Overall and Gorham, 1962). The DSM-IV axis V Global Assess-

Table 1
Characteristics of the patients with chronic schizophrenia and the healthy controls

	Schizophrenia	Healthy controls	p value
Age, years	40.4 ± 13.1	34.9 ± 11.4	0.12[a]
Onset, years	24.1 ± 5.0		
Duration, years	16.3 ± 11.9		
Subtype	10 Residual, 9 paranoid		
BPRS	26.5 ± 6.1		
GAF	40.9 ± 5.1		

Normally distributed data are presented as mean ± standard deviation (SD).
Abbreviations: BPRS, Brief Psychiatric Rating Scale; GAF, Global Assessment of Functioning.
[a] Not significant. The comparison between two groups was performed using t-test (two-tailed).

ment of Functioning (GAF) scores (American Psychiatric Association, 1994) were also rated as part of the examination.

2.2. Cognitive functioning

The subjects performed a battery of tests designed to assess several domains of cognitive functioning. Verbal memory was evaluated with the Japanese version (Sugishita and Omura, 2001) of the Logical Memory I (immediate) and II (30-min delayed) subtests of the Wechsler Memory Scale-Revised (WMS-R) (Wechsler, 1987). Visuospatial memory was evaluated with the Visual Reproduction I (immediate) and II (30-min delayed) tests of the WMS-R.

Verbal fluency was assessed with the letter fluency test and the category fluency test. In the former test, the subjects were instructed to generate as many words as possible beginning with a given letter in Japanese, "Ka", in 60 s. For the latter test, the subjects were instructed to name as many animals as they could, according to the standard protocol (Spreen and Strauss, 1991).

The Trail Making Test (Reitan and Wolfson, 1985) examines psychomotor speed, attention, and set alternation. Part A requires the subjects to draw lines to connect 25 consecutively numbered circles on one worksheet as quickly as possible. In Part B, the subjects are asked to connect 25 consecutively numbered and lettered circles by alternating between the two sequences (e.g. 1–A–2–B–3 etc.). The time taken to complete Parts A and B of the Trail Making Test was recorded in seconds.

2.3. MRS protocol

The subjects and controls were scanned using a 1.5-T Signa MR scanner (General Electric Medical Systems, Waukesha, WI) with a standard quadrature head coil for MR imaging and ^{1}H-MRS. Both the anatomic and spectroscopic data were obtained within approximately 30 min. All MRI scans were reviewed in order to rule out any clinically significant abnormalities. We performed serial axial T1-weighted MR images with a slice thickness of 10 mm to establish a region of interest (ROI) for the proton MR spectroscopic studies. MRS was performed with the automated Proton Brain Examination (PROBE-p) sequence, which consists of a Point Resolved Spectroscopy (PRESS) sequence (TE = 144 ms, TR = 2000 ms) with Chemical-Shift Selective (CHESS) water suppression, to acquire localized spectra in the PCG, and in the left and right MTL (including hippocampal formation) (Fig. 1). The voxel size was 3.375 mL (15 × 15 × 15 mm). Voxel placements were carried out by a trained radiologist. All of the data were

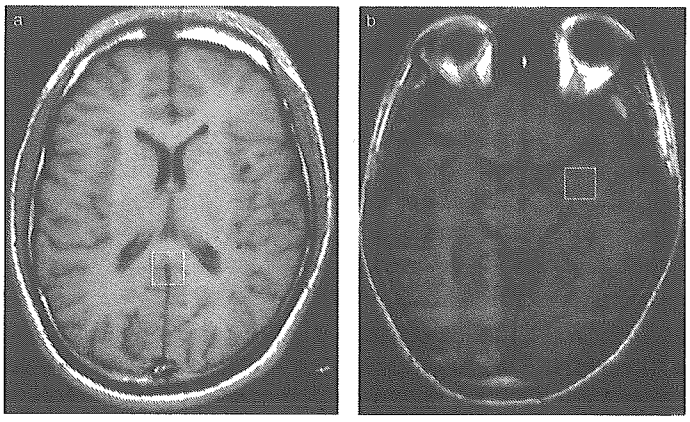

Fig. 1. Voxel size and position in the posterior cingulate gyrus (PCG) (a) and left medial temporal lobe (MTL) (b).

processed using the PROBE/single voxel quantification tool (Webb et al., 1994). Peaks of NAA (2.0 ppm), Cr (3.0 ppm), and Cho (3.2 ppm) were identified. Cr is present throughout various regions of the brain, and levels of Cr do not appear to be affected by neuronal fluctuations (Frahm et al., 1989). Therefore, the Cr peak was used as a reference for comparison with the peaks of the other substrates. The data reflecting the metabolite profiles were described as ratios, i.e., NAA/Cr and Cho/Cr.

2.4. Statistical analyses

Normally distributed data are presented as the mean ± standard deviation (SD); data that were not normally distributed are reported as medians, with interquartile ranges. Calculations were performed using the statistical software package SPSS 12.0 base and advanced systems for Windows (SPSS Inc., Chicago, IL). Fisher's exact test was used for categorical variables, and Student's *t*-test was employed for the continuous variables. As the scores on the cognitive tests were not found to have normal distributions, the differences between the two groups (patients and controls) were examined using the non-parametric Mann–Whitney *U*-test with Bonferroni's correction for multiple comparisons (Bland and Altman, 1995). A value of $p < 0.05$ was taken as the standard for statistical significance. Eight cognitive tests were conducted. Because of the multiple comparisons (the number of comparisons was 8), Bonferroni's correction was applied. With this adjustment, the critical level for significance, p, was reduced from the standard value of 0.05 to 0.00625 (i.e., 0.05/8).

Potential differences between patients and controls were tested separately for each metabolite ratio (NAA/Cr and Cho/Cr) by repeated-measures multivariate analysis of variances (MANOVAs), with region (the PCG, or the left or right MTL) as the within-group factor, and diagnosis (patients, controls) as the between-group factor. A post hoc analysis was performed by Tukey's honest significant difference test. Interactions were decomposed with a univariate analysis of variance (ANOVA).

Multiple stepwise regression analyses were used to determine the relative contribution of the metabolite ratios from the three brain regions as predictors of each cognitive score or age. On the other hand, the parameters (clinical and cognitive variables) that predicted the metabolite ratio were determined by multiple stepwise regression analysis. When association between highly significant and normally distributed measured variables was investigated, Pearson's correlation coefficient was obtained.

3. Results

3.1. Cognitive performance measures

Compared to the healthy controls, the patients with schizophrenia showed significant cognitive deficits on all neuropsychological tests, including the episodic memory tests (verbal memory, visuospatial memory) and executive function tests (verbal fluency and the Trail Making Test) (Fig. 2).

3.2. Metabolite ratios by ^{1}H-MRS

Analysis of the within-group effect revealed a significant diagnosis-by-region interaction for NAA/Cr ($F(2,35) = 3.588$, $p = 0.033$). A significant main effect of region was found ($F(2,35) = 42.837$, $p < 0.001$).

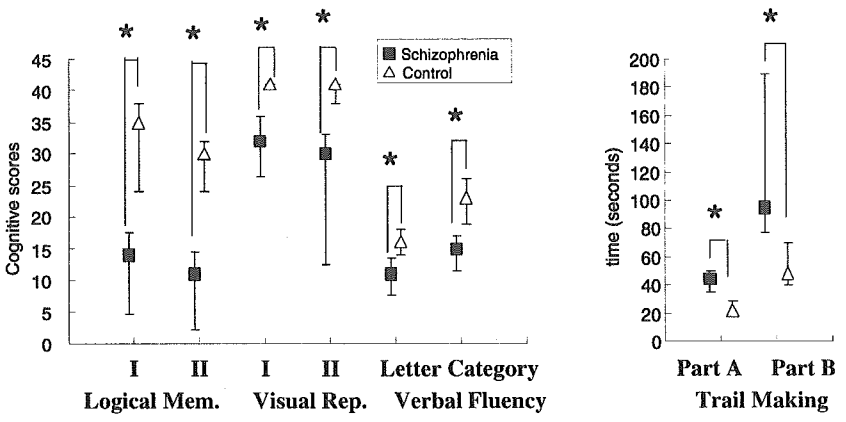

Fig. 2. Scores on cognitive tests of patients with chronic schizophrenia and healthy controls: Logical Memory and Visual Reproduction subtests from the Wechsler Memory Scale-Revised (WMS-R) (I, immediate recall; and II, 30-min delayed recall), verbal fluency (the letter fluency test and the category fluency test), and the Trail Making Test (Parts A and B). The data are represented as median ± interquartile range. The p values were determined by Mann–Whitney *U*-test with Bonferroni's correction on two groups. *Statistically significant difference for $p < 0.00625$ (corrected for multiple comparisons).

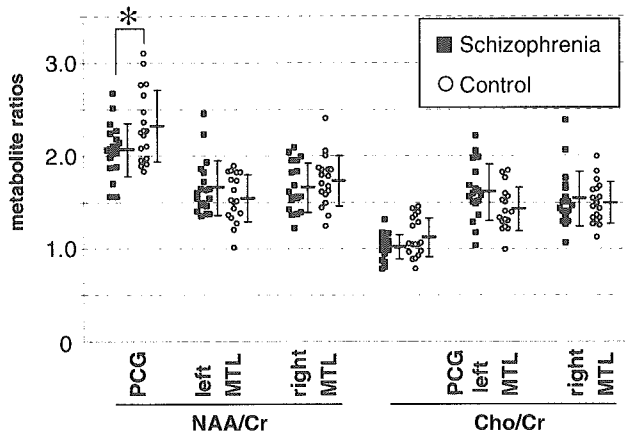

Fig. 3. ^{1}H-MRS metabolite ratios in the posterior cingulate gyrus (PCG) and the left and right medial temporal lobes (MTL) of patients with chronic schizophrenia and healthy controls. The horizontal bars represent mean ± standard deviation. *Statistically significant between-group difference ($F = 5.31$, df = 1,35, $p = 0.027$).

Comparison of the main effect showed that the NAA/Cr ratios of the patients with schizophrenia (2.07 ± 0.28) were significantly ($p = 0.027$, Bonferroni) lower than those (2.30 ± 0.38) of the healthy controls in the PCG (Fig. 3). MANOVA revealed a significantly simple main effect in each group for NAA/Cr, respectively. (patients: Wilks's lambda 0.373, $F(2,34) = 28.62$, $p < 0.001$; controls: Wilks's lambda 0.618, $F(2,34) = 10.509$, $p < 0.001$). Finally, follow-up ANOVA was carried out to analyze the interactions in terms of NAA/Cr; this analysis revealed a significantly simple main effect of diagnosis in the PCG ($F(1,35) = 5.313$, $p = 0.027$).

Comparison of the main effect showed no significant differences of the Cho/Cr ratios between the patients and controls in any of the three regions studied (Fig. 3). Moreover, follow-up ANOVA for the analysis of interactions in terms of Cho/Cr revealed that there was no significantly simple main effect of diagnosis in any of the regions studied.

3.3. Multivariate stepwise linear regression analyses

When the metabolite ratios were considered separately as the PCG and the left and right MTL values in a multivariate stepwise linear regression model, the NAA/Cr ratio in the PCG alone reflected 18.0% of Logical Memory I ($\beta = 0.425$, $p = 0.010$), 20.3% of Logical Memory II ($\beta = 0.451$, $P = 0.006$), and 16.3% of age ($\beta = -0.403$, $p = 0.013$) parameters in all subjects, respectively. A multiple stepwise linear regression model was designed with the NAA/Cr in the PCG as the independent variables with respect to the dependent clinical and cognitive valuables. Only Logical Memory II was accepted by the model, and it significantly accounted for 16.5% of the NAA/Cr ratio in the PCG ($\beta = 0.406$, $P = 0.015$) in all subjects.

Due to the lack of a significant correlation between the NAA/Cr in the PCC and Logical Memory II in the controls ($r = +0.195$, $p = 0.453$) and in the patients ($r = +0.347$, $p = 0.148$), a significant positive correlation ($r = +0.451$, $p = 0.006$) in all subjects was interpreted as a possible indicator of artificially stretched variance. Age-associated memory impairment appeared likely to be a phenomenon of normal aging. Therefore, the Logical Memory scores were excluded from the following selected correlation analysis. A significant negative correlation was found between the NAA/Cr in the PCG and age in all subjects ($r = -0.403$, $p = 0.013$), and also between the NAA/Cr in the PCG in the controls ($r = -0.470$, $p = 0.049$), whereas no correlation was detected among the patients only ($r = -0.217$, $p = 0.371$) (Fig. 4).

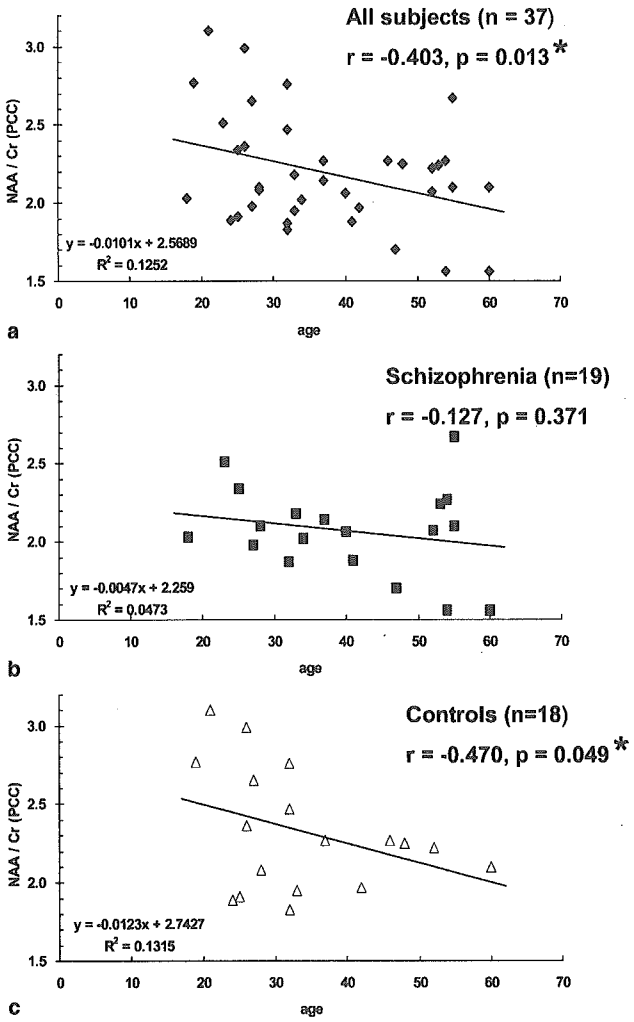

Fig. 4. Scatterplot and least regression line illustrating the relationship between the NAA/Cr ratio in the posterior cingulate gyrus (PCG) and age in all of the subjects (a), in patients with schizophrenia (b), and in healthy controls (c).

4. Discussion

Our results revealed decreased NAA/Cr ratios in the PCG of patients with chronic schizophrenia exhibiting generalized cognitive deficits, as compared to the healthy controls. Moreover, we found that the NAA/Cr of the PCG in healthy controls exhibited age-related decline, whereas corresponding value in patients with schizophrenia were consistently low, regardless of age. The lack of healthy, aging-related decline among the patients with schizophrenia suggested disease-associated neuronal pathology in the PCG. These observations suggested that a reduced NAA/Cr ratio in the PCG might be associated with the pathophysiology of chronic schizophrenia with generalized cognitive deficits.

Valenstein et al. (1987) reported a male case of retrosplenial amnesia following hemorrhage from an arteriovenous malformation. Using positron emission tomography (PET), several researchers reported that the use of the PCG was common to episodic memory (Andreasen et al., 1995; Desgranges et al., 1998; Nyberg et al., 1996; Shallice et al., 1994). Using PET, Minoshima et al. (1994, 1997) also demonstrated the functional importance of the PCG in impairments of learning and memory, which is a feature of very early Alzheimer's disease and mild cognitive impairment. In addition, the NAA/Cr ratios in the PCG have been shown to be decreased in cases of mild cognitive impairment, Alzheimer's disease (Kantarci et al., 2000), and non-demented Parkinson's disease (Camicioli et al., 2004). Taken together, these results suggest that cognitive decline is associated with hypofunction of the PCG. Our regression analysis data, which revealed a relationship between the NAA/Cr in the PCG and the verbal memory of all subjects, suggested that the NAA/Cr in the PCG might be related to episodic memory function.

NMDA receptor antagonists are known to induce neuronal damage in the rodent PCG; these antagonists are thought to be responsible for psychotomimetic activity in humans (Olney and Farber, 1995). Newcomer et al. (1999) reported dose-dependent increases in ketamine in healthy males exhibiting schizophrenia-like symptoms, as well as robust dose-dependent decreases in verbal declarative memory performance. Our data indicating PCG dysfunction in patients with chronic schizophrenia with generalized cognitive deficits provide support for the NMDA receptor hypofunction hypothesis that suggests a link between cognitive impairments and schizophrenia-like symptoms.

Several previous proton MRS studies have demonstrated decreased NAA/Cr ratios in the MTL of chronically medicated patients with schizophrenia (Bertolino et al., 1996, 1998; Deicken et al., 1998; Fukuzako et al., 1995; Maier et al., 1995; Nasrallah et al., 1994; Yurgelun-Todd et al., 1996), while other researchers have reported no such significant alterations (Buckley et al., 1994; Delamillieure et al., 2002; Heimberg et al., 1998). Our results regarding the NAA/Cr in the MTL were consistent with those of the latter group of studies. On the other hand, the results of the present study showing no significant changes in Cho/Cr in the MTL were consistent with those of other studies (Bertolino et al., 1996; Deicken et al., 1998; Delamillieure et al., 2002; Maier et al., 1995; Yurgelun-Todd et al., 1996). One possible explanation for the inconsistency regarding NAA alterations among medicated patients with chronic schizophrenia may be the differential effects associated with atypical vs. typical antipsychotic medications (Bertolino et al., 2001; Fannon et al., 2003; Heimberg et al., 1998). The longitudinal changes in brain chemistry after long-term treatment with antipsychotic agents remain undetermined at the present time.

A number of limitations merit further consideration in this context. As our sample size may be regarded as a weakness of the present study, these results will need to be confirmed using a much larger sample. In addition, because we focused on the relationship between cognitive function and the PCG or the MTL, MRS of the prefrontal cortex and anterior cingulate gyrus was not performed in the current study. Instead of the single-voxel approach used here, further studies using the MRS imaging technique should be conducted to clarify the involvement of other brain regions in schizophrenic patients with generalized cognitive deficits. An additional limitation of the present study was that we derived our conclusions based on metabolite ratios, instead of absolute concentrations. In our study, no significant correlation was found between the NAA/Cr in the PCG and the duration of illness. This finding, when taken together with the finding of a lack of a physiological age-related decline in patients with chronic schizophrenia, suggests the possibility that the NAA/Cr in the PCG might decrease during the initial years of illness, rather than reflect linear neurodegenerative processes. Whether or not the reduced NAA/Cr in the PCG reflects a basic underlying pathophysiological process that is present at the onset of illness, and then progresses during the course of the illness remains unclear. Follow-up MRS examinations for several years, not only in patients with chronic schizophrenia, but also in first-episode patients, will be necessary to clarify the role of the PCG during the disease process in schizophrenia.

In the present study, patients with schizophrenia exhibited not only memory deficits, but also executive dysfunction. Schizophrenia appears to be an amalgamation of many different disorders. For example, Wexler et al. (1998) found a selective deficit in the working memory of a subgroup of patients with schizophrenia who performed as well as healthy controls on a screening test of attention and auditory perception. Moreover, the existence of a subgroup of patients with schizophrenia

having a specific verbal memory deficit was reported (Bruder et al., 2004). Further MRS studies on a subgroup with only specific verbal memory deficits will be necessary in order to confirm the relationship between the NAA/Cr in the PCC and verbal memory in patients with schizophrenia.

In conclusion, a significant disease-specific decrease in the NAA/Cr ratio in the PCG, beyond a physiological age-related decline, was revealed in the present study. These findings are consistent with current speculation focusing on neuronal dysfunction in the PCG based on the NMDA hypofunction hypothesis regarding the pathophysiology of chronic schizophrenia.

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References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 4th ed. Washington (DC): American Psychiatric Press; 1994..

Andreasen NC, O'Leary DS, Cizadlo T, Arndt S, Rezai K, Watkins GL, et al.. Remembering the past: two facets of episodic memory explored with positron emission tomography. Am J Psychiatry 1995;152:1576–85.

Baslow MH. *N*-Acetylaspartate in the vertebrate brain: metabolism and function. Neurochem Res 2003;28:941–53.

Bertolino A, Nawroz S, Mattay VS, Barnett AS, Duyn JH, Moonen CT, et al.. Regionally specific pattern of neurochemical pathology in schizophrenia as assessed by multislice proton magnetic resonance spectroscopic imaging. Am J Psychiatry 1996;153:1554–63.

Bertolino A, Kumra S, Callicott JH, Mattay VS, Lestz RM, Jacobsen L, et al.. Common pattern of cortical pathology in childhood-onset and adult-onset schizophrenia as identified by proton magnetic resonance spectroscopic imaging. Am J Psychiatry 1998;155:1376–83.

Bertolino A, Esposito G, Callicott JH, Mattay VS, Van Horn JD, Frank JA, et al.. Specific relationship between prefrontal neuronal *N*-acetylaspartate and activation of the working memory cortical network in schizophrenia. Am J Psychiatry 2000;157:26–33.

Bertolino A, Callicott JH, Mattay VS, Weidenhammer KM, Rakow R, Egan MF, et al.. The effect of treatment with antipsychotic drugs on brain *N*-acetylaspartate measures in patients with schizophrenia. Biol Psychiatry 2001;49:39–46.

Bilder RM, Lipschutz-Broch L, Reiter G, Geisler SH, Mayerhoff DI, Lieberman JA. Intellectual deficits in first-episode schizophrenia: evidence for progressive deterioration. Schizophr Bull 1992;18:437–48.

Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170.

Braff DL, Heaton R, Kuck J, Cullum M, Moranville J, Grant I, et al.. The generalized pattern of neuropsychological deficits in outpatients with chronic schizophrenia with heterogeneous Wisconsin Card Sorting Test results. Arch Gen Psychiatry 1991;48:891–8.

Bruder GE, Wexler BE, Sage MM, Gil RB, Gorman JM. Verbal memory in schizophrenia: additional evidence of subtypes having different cognitive deficits. Schizophr Res 2004;68:137–47.

Buckley PF, Moore C, Long H, Larkin C, Thompson P, Mulvany F, et al.. 1H-magnetic resonance spectroscopy of the left temporal and frontal lobes in schizophrenia: clinical, neurodevelopmental, and cognitive correlates. Biol Psychiatry 1994;36:792–800.

Camicioli RM, Korzan JR, Foster SL, Fisher NJ, Emery DJ, Bastos AC, et al.. Posterior cingulate metabolic changes occur in Parkinson's disease patients without dementia. Neurosci Lett 2004;354:177–80.

Desgranges B, Baron JC, Eustache F. The functional neuroanatomy of episodic memory: the role of the frontal lobes, the hippocampal formation, and other areas. Neuroimage 1998;8:198–213.

Deicken RF, Zhou L, Schuff N, Fein G, Weiner MW. Hippocampal neuronal dysfunction in schizophrenia as measured by proton magnetic resonance spectroscopy. Biol Psychiatry 1998;43:483–8.

Delamillieure P, Constans JM, Fernandez J, Brazo P, Benali K, Courtheoux P, et al.. Proton magnetic resonance spectroscopy (1H MRS) in schizophrenia: investigation of the right and left hippocampus, thalamus, and prefrontal cortex. Schizophr Bull 2002;28:329–39.

Delamillieure P, Fernandez J, Constans JM, Brazo P, Benali K, Abadie P, et al.. Proton magnetic resonance spectroscopy of the medial prefrontal cortex in patients with deficit schizophrenia: preliminary report. Am J Psychiatry 2000;157:641–3.

Dickinson D, Iannone VN, Wilk CM, Gold JM. General and specific cognitive deficits in schizophrenia. Biol Psychiatry 2004;55:826–33.

Egan MF, Goldberg TE, Gscheidle T, Weirich M, Rawlings R, Hyde TM, et al.. Relative risk for cognitive impairments in siblings of patients with schizophrenia. Biol Psychiatry 2001;50:98–107.

Elvevag B, Goldberg TE. Cognitive impairment in schizophrenia is the core of the disorder. Crit Rev Neurobiol 2000;14:1–21.

Fannon D, Simmons A, Tennakoon L, O'Ceallaigh S, Sumich A, Doku V, et al.. Selective deficit of hippocampal *N*-acetylaspartate in antipsychotic-naive patients with schizophrenia. Biol Psychiatry 2003;54:587–98.

Fix AS, Horn JW, Wightman KA, Johnson CA, Long GG, Storts RW, et al.. Neuronal vacuolization and necrosis induced by the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist MK(+)801 (dizocilpine maleate): a light and electron microscopic evaluation of the rat retrosplenial cortex. Exp Neurol 1993;123:204–15.

Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hanicke W, Sauter R. Localized proton NMR spectroscopy in different regions of the human brain in vivo. Relaxation times and concentrations of cerebral metabolites. Magn Reson Med 1989;11:47–63.

Fukuzako H, Takeuchi K, Hokazono Y, Fukuzako T, Yamada K, Hashiguchi T, et al.. Proton magnetic resonance spectroscopy of the left medial temporal and frontal lobes in chronic schizophrenia: preliminary report. Psychiatry Res 1995;61:193–200.

Gold JM, Weinberger DR. Cognitive deficits and the neurobiology of schizophrenia. Curr Opin Neurobiol 1995;5:225–30.

Hashimoto K, Iyo M. Glutamate hypothesis of schizophrenia and targets for new antipsychotic drugs. Nihon Shinkei Seishin Yakurigaku Zasshi 2002;22:3–13, Japanese.

Hashimoto K, Fukushima T, Shimizu E, Komatsu N, Watanabe H, Shinoda N, et al.. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the *N*-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. Arch Gen Psychiatry 2003;60:572–6.

Heimberg C, Komoroski RA, Lawson WB, Cardwell D, Karson CN. Regional proton magnetic resonance spectroscopy in schizophrenia and exploration of drug effect. Psychiatry Res 1998;83:105–15.

Heydebrand G, Weiser M, Rabinowitz J, Hoff AL, DeLisi LE, Csernansky JG. Correlates of cognitive deficits in first episode schizophrenia. Schizophr Res 2004;68:1–9.

Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991;148:1301–8.

Jenkins BG, Klivenyi P, Kustermann E, Andreassen OA, Ferrante RJ, Rosen BR, et al.. Nonlinear decrease over time in *N*-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington's disease mice. J Neurochem 2000;74:2108–19.

Kantarci K, Jack Jr CR, Xu YC, Campeau NG, O'Brien PC, Smith GE, et al.. Regional metabolic patterns in mild cognitive impairment and Alzheimer's disease: A ^{1}H MRS study. Neurology 2000;55:210–7.

Kolakowska T. Brief psychiatric rating scale. Oxford: Oxford University Press; 1976.

Maier M, Ron MA, Barker GJ, Tofts PS. Proton magnetic resonance spectroscopy: an in vivo method of estimating hippocampal neuronal depletion in schizophrenia. Psychol Med 1995;25:1201–9.

Miller BL. A review of chemical issues in 1H NMR spectroscopy: *N*-acetyl-L-aspartate, creatine and choline. NMR Biomed 1991;4:47–52.

Minoshima S, Foster NL, Kuhl DE. Posterior cingulate cortex in Alzheimer's disease. Lancet 1994;344:895.

Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann Neurol 1997;42:85–94.

Mohamed S, Paulsen JS, O'Leary D, Arndt S, Andreasen N. Generalized cognitive deficits in schizophrenia: a study of first-episode patients. Arch Gen Psychiatry 1999;56:749–54.

Nakanishi S. Molecular diversity of glutamate receptors and implications for brain function. Science 1992;258:597–603.

Nasrallah HA, Skinner TE, Schmalbrock P, Robitaille PM. Proton magnetic resonance spectroscopy (1H MRS) of the hippocampal formation in schizophrenia: a pilot study. Br J Psychiatry 1994;165:481–5.

Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, et al.. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 1999;20:106–18.

Nyberg L, McIntosh AR, Cabeza R, Nilsson LG, Houle S, Habib R, et al.. Network analysis of positron emission tomography regional cerebral blood flow data: ensemble inhibition during episodic memory retrieval. J Neurosci 1996;16:3753–9.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113.

Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 1989;244:1360–2.

Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 1995;52:998–1007.

Overall JE, Gorham DR. The Brief Psychiatric Rating Scale. Psychol Rep 1962;10:799–812.

Pantelis C, Barnes TR, Nelson HE, Tanner S, Weatherley L, Owen AM, et al.. Frontal-striatal cognitive deficits in patients with chronic schizophrenia. Brain 1997;120:1823–43.

Shallice T, Fletcher P, Frith CD, Grasby P, Frackowiak RS, Dolan RJ. Brain regions associated with acquisition and retrieval of verbal episodic memory. Nature 1994;368:633–5.

Sharma T, Antonova L. Cognitive function in schizophrenia. Deficits, functional consequences, and future treatment. Psychiatr Clin North Am 2003;26:25–40.

Shimizu E, Tang YP, Rampon C, Tsien JZ. NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. Science 2000;290:1170–4.

Spreen O, Strauss E. A compendium of neuropsychological tests. Administration, norms, and commentary. New York: Oxford University Press; 1991.

Sugishita M, Omura K. Learning Chinese characters may improve visual recall. Percept Mot Skills 2001;93:579–94.

Reitan RM, Wolfson D. The Halstead-Reitan Neuropsychological Test Battery. Tucson: Neuropsychology Press; 1985.

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, et al.. Genetic enhancement of learning and memory in mice. Nature 1999;401:63–9.

Tsai G, Coyle JT. *N*-Acetylaspartate in neuropsychiatric disorders. Prog Neurobiol 1995;46:531–40.

Tsai G, Coyle JT. Glutamatergic mechanisms in schizophrenia. Annu Rev Pharmacol Toxicol 2002;42:165–79.

Umbricht D, Schmid L, Koller R, Vollenweider FX, Hell D, Javitt DC. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. Arch Gen Psychiatry 2000;57:1139–47.

Valenstein E, Bowers D, Verfaellie M, Heilman KM, Day A, Watson RT. Retrosplenial amnesia. Brain 1987;110:1631–46.

Webb PG, Sailasuta N, Kohler SJ, Raidy T, Moats RA, Hurd RE. Automated single-voxel proton MRS: technical development and multisite verification. Magn Reson Med 1994;31:365–73.

Wechsler DA. Wechsler memory scale-revised, manual. New York: Psychological Corp.; 1987.

Weickert TW, Goldberg TE, Gold JM, Bigelow LB, Egan MF, Weinberger DR. Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect. Arch Gen Psychiatry 2000;57:907–13.

Wexler BE, Stevens AA, Bowers AA, Sernyak MJ, Goldman-Rakic PS. Word and tone working memory deficits in schizophrenia. Arch Gen Psychiatry 1998;55:1093–6.

Yurgelun-Todd DA, Renshaw PF, Gruber SA, Ed M, Waternaux C, Cohen BM. Proton magnetic resonance spectroscopy of the temporal lobes in schizophrenics and healthy controls. Schizophr Res 1996;19:55–9.

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Association between angiotensin I-converting enzyme insertion/deletion gene functional polymorphism and novelty seeking personality in healthy females

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Abstract

A certain type of personality is at risk for developing psychiatric diseases. Several lines of evidence support the interaction between brain angiotensins and central catecholamine systems, and suggest that angiotensin I-converting enzyme (ACE) may be a reasonable candidate gene for psychiatric disorders. The present study examined the possibility that ACE insertion (I)/deletion (D) functional polymorphism might be associated with particular personality traits. Healthy Japanese subjects ($N=184$) were administered the Temperament and Character Inventory (TCI) and the NEO Personality Inventory Revised version (NEO-PI-R), and their ACE I/D polymorphisms were determined. There was an ethnic difference in the genetic distribution of ACE I/D between Japanese (D=34.5%) and Caucasians (D=55.2%). We found that the scores of novelty seeking (NS) in the Low-ACE group (II genotype) of healthy female subjects were significantly lower than those in the High-ACE group (ID or DD genotype) ($p=0.018$). Our findings suggested that the ACE I/D polymorphism might be associated with the NS personality trait in females, but not males. Taking into account the effects of multiple comparisons, this result should be interpreted with caution, and needs confirmation in a larger sample.

Keywords: Angiotensin I-converting enzyme; Character; Dopamine; Temperament

1. Introduction

Evidence for genetic influence has been found for most behavioral disorders and personality traits (Cloninger et al., 1993). It has been demonstrated that common genetic polymorphisms, especially the dopamine D4 receptor and the serotonin transporter promoter region, are associated with the novelty seeking (NS) trait (Ebstein et al., 1996) and the anxiety-related trait (Neuroticism) (Lesch et al., 1996), respectively. A certain type of personality or temperament is at high risk for developing psychiatric diseases, including drug addiction (Spotts and Shontz, 1986), panic disorders (Martin et al., 1988), depression (Akiskal et al., 1983; Hirschfeld et al., 1989; Boyce et al., 1991), bipolar disorders (von Zerssen et al., 1994), and mood disorders (Lozano and Johnson, 2001).

It is now firmly established that a complete renin–angiotensin system (RAS) exists in the brain, and that the brain RAS is distinct from the peripheral RAS (Von Bohlen Und Halbach, 2003). The precursor angiotensinogen is cleaved by renin to form the inactive peptide angiotensin I. Angiotensin I-converting enzyme (ACE) catalyzes the conversion of angiotensin I to the physiologically active octapeptide angiotensin II, which controls the fluid–electrolyte balance and systemic blood pressure. The brain RAS has been implicated in mental function because of the psychotropic effects of angiotensin II and III (Braszko et al., 1987). Recent studies have suggested that an active (3–8) fragment of angiotensin II, named angiotensin IV, is involved in a broad range of brain functions, including the modulation of exploratory behavior and the processes attributed to learning and memory (Wright et al., 2002). Furthermore, there are reports supporting the pivotal roles of angiotensins in the central nervous system using

Abbreviations: ACE, Angiotensin I-converting enzyme; D, deletion; I, insertion; NEO-PI-R, NEO Personality Inventory Revised version; NS, novelty seeking; RAS, renin–angiotensin system; TCI, Temperament and Character Inventory.

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angiotensinogen-deficient mice (Kakinuma et al., 1997, 1998). The angiotensinogen-deficient mice have also displayed a reduction in depressive-like behavior in forced swim tests, and spontaneous locomotor activity has diminished (Okuyama et al., 1999a). Moreover, angiotensin II type 2 receptor-deficient mice display anxiety-like behavior in comparison with wild-type mice (Okuyama et al., 1999b; Ichiki et al., 1995). In addition, the angiotensin II antagonist losartan has been shown to possess antidepressant-like activity in the mouse forced swim test (Gard et al., 1999). Antidepressant effects have also been demonstrated for captopril, an ACE inhibitor (Vuckovic et al., 1991).

ACE, a key enzyme in RAS, is encoded by the gene DCP1 (dipeptidyl carboxypeptidase 1), and consists of 26 exons spaced over approximately 24 kb on chromosome 17q23 (Rieder et al., 1999). Cloning of the ACE gene has revealed the presence (insertion; I) or absence (deletion;.D) of a 291-bp Alu repeat element in the 16th intron (Rigat et al., 1990, 1992). The ACE I/D polymorphism determines functional variants of the ACE gene, which have a major impact on plasma ACE levels. The polymorphism accounts for 47% of the total phenotypic variance of plasma ACE (Rigat et al., 1990; Tiret et al., 1992). The gene encoding ACE shows three genotypes: II, ID, and DD. The plasma ACE activity in people with the DD genotype is approximately double the values in those with II (Rigat et al., 1990). The individuals homozygous for the II allele constitute the low-activity ACE group (Low-ACE) and those individuals with either the ID or DD genotype constitute the high-activity ACE group (High-ACE). Researchers have investigated the possible importance of this ACE polymorphism on skeletal muscle performance (Montgomery et al., 1998; Williams et al., 2000), left ventricular hypertrophy (Kuznetsova et al., 2000), carotid artery intima-media thickness (Sayed-Tabatabaei et al., 2003), and cerebrovascular disease (Sharma, 1998).

Moreover, researchers have focused on the potential influence of the ACE polymorphism on the psychopathology of schizophrenia (Arinami et al., 1996; Ouyang et al., 2001; Segman et al., 2002) or mood disorders (Arinami et al., 1996; Pauls et al., 2000; Furlong et al., 2000; Meira-Lima et al., 2000; Baghai et al., 2001, 2002, 2003; Segman et al., 2002; Bondy et al., 2002; Hong et al., 2002). However, they have shown inconsistent results. It therefore remains unclear whether the ACE gene influences the potential risk for these psychiatric diseases.

Considering the role of ACE and personality traits in psychiatric diseases, it may be of interest to examine the relationship between the ACE I/D gene polymorphism and the personality inventory score. In the present study, the authors examined the association between personality traits and the ACE genotype in 184 Japanese healthy subjects.

2. Methods

2.1. Subjects

One hundred and eighty-four Japanese healthy subjects were recruited. The procedure was fully explained to all of the subjects, who then provided written informed consent prior to participation in the study. We selected only healthy control subjects who were medical staffs and students, and had neither a medical nor a psychiatric history or diagnosis according to our clinical interview. There were 74 males (mean; 29.1 years (S.D. 8.2), range; 22–62 years) and 110 females (mean; 28.9 years (S.D. 11.4), range; 20–58 years). This study was approved by the ethics committee of the Chiba University Graduate School of Medicine.

2.2. Personality inventory

All subjects were instructed to complete self-report inventories using the Japanese version of the Revised NEO Personality Inventory (NEO-PI-R) and the Temperament and Character Inventory (TCI). The NEO-PI-R consists of 30 facet scales that define the broad domains of the five-factor (N: Neuroticism, E: Extraversion, O: Openness, A: Agreeableness, C: Consciousness) model of personality (Costa and McCrae, 1997). The TCI is the extended version of the Tridimensional Personality Questionnaire (TPQ). It evaluates four temperament dimensions (NS; Novelty Seeking, HA; Harm Avoidance, RD; Reward Dependence, PS; Persistence) and three character (SD: Self-Directedness, C: Cooperativeness, ST: Self-Transcendence) dimensions based on a seven-factor psychobiological model of personality proposed by Cloninger et al. (1993).

2.3. Genotyping

Genomic DNA was extracted from blood samples obtained after obtained written informed consent. The ACE I/D polymorphism was determined by polymerase chain reaction (PCR) using constructed primers (forward: 5′-CTGGAGAC-CACTCCCATCCTTTCT-3′ and reverse: 5′-GGA,TGG,CTC,-TCC,CCG,CCT,TGT,CTC3′-3′) that flank the polymorphic region of intron 16. This primer pair produced the 534-bp product (corresponding to the insertion, I) or a 243-bp fragment (corresponding to the deletion, D). The reactions were carried out according to the protocol described by Lindpaintner et al. (1995) with a minor modification (Shimizu et al., 2004). After initial denaturation at 94 °C for 5 min, thermocycling consisted of denaturation of 94 °C for 30 s, annealing at 56 °C for 45 s, and extension at 72 °C, 2 min for 35 cycles, followed by a final extension at 72 °C for 5 min. PCR products were separated on 2% agarose gel with ethidium bromide staining, visualized by ultraviolet transillumination, and stored in digital form. Possible mistyping of the ACE I/D genotype as DD was controlled by the inclusion of dimethyl sulfoxide in the PCR reaction (Shanmugam et al., 1993; Fogarty et al., 1994).

2.4. Statistical analysis

Statistical calculations were performed using the statistical software package SPSS for Windows (SPSS Inc., Chicago, IL, USA). The data are presented as the mean±standard deviation (S.D.). Homogeneity of variance was assessed by F test. The Chi-squared test was used for the categorical variables, and the

Student's t-test was employed for the continuous variables. Potential differences of temperament between the two groups were tested separately by repeated measures multivariate analysis of variances (MANOVAs), with temperament score (NS, HA, RD and PS) as the within-group factor, and genotype (low-activity ACE and high-activity ACE group) as the between-group factor. Interactions were decomposed with a univariate analysis of variance (ANOVA). The p values <0.05 were considered statistically significant.

3. Results

We analyzed DNA samples from 184 healthy Japanese subjects. An ethnic difference in the genetic distribution between Japanese (D allele 34.5%) and Caucasians (D allele 55.5%) (Lindpaintner et al., 1995) were reconfirmed (Ishigami et al., 1995). As there were remarkable differences in personality scores between males and females (Table 1), we divided the subjects into males and females for further analyses. According to the Hardy–Weinberg equilibrium, there was no significant deviation in the ACE genotype distribution for males (DD 9 (12.2%), ID 32 (43.2%), II 33 (44.6%)), for females (DD 15(13.6%), ID 47(42.7%), II 48(43.6%)).

We compared the personality scores between the following two groups; the individuals homozygous for the II allele as the low-activity ACE group (Low-ACE), and those individuals who had either the ID or DD genotype as the high-activity ACE group (High-ACE). In the repeated measures MANOVA, the sphericity assumption was not valid, so we used the Greenhouse–Geiser adjusted degrees of freedom for the sphericity violation. In females, analysis of the within-group effect revealed a significant genotype (low-activity ACE and high-activity ACE group) by temperament (NS, HA, RD and PS) interaction ($F(2.107,227.515)=4.3$, $p=0.013$). A significant main effect of temperament was found ($F(2.107,227.515)=275.821$, $p<0.001$), whereas no main effect of genotype was found ($F(1,108)=0.068$, $p=0.794$). Comparison of the main effect showed that the novelty seeking (NS) of the Low-ACE group (II genotype) were significantly ($p=0.018$, Bonferroni) lower than those of the High-ACE group (ID or DD genotype). MANOVA revealed a significantly simple main effect within group, respectively (the High-ACE group (ID or DD genotype): Wilks lambda 0.087, $F(3,106)=368.853$, $p<0.001$; the Low-ACE group (II genotype): Wilks lambda 0.116, $F(3,106)=269.4$, $p<0.001$). Finally, follow-up ANOVA for analysis of the interactions revealed that there was a significantly simple main effect of genotype in the novelty seeking (NS) ($F(1,108)=5.767$, $p=0.018$). No significant differences between the two groups in males were found in each temperament score of the TCI (Table 1). There were no significant differences between the two groups in males and females in each character score of the TCI (SD: Self-Directedness, C: Cooperativeness, ST: Self-Transcendence) and personality score of the NEO-PI-R (N: Neuroticism, E: Extraversion, O: Openness, A: Agreeableness, C: Conscientiousness) (data not shown).

4. Discussion

Our findings indicate that female, but not male, subjects with the Low-ACE genotype (II) have lower NS, as compared to those with High-ACE genotype (DD or ID). NS is a tendency to respond to novelty and cues for reward that lead to exploratory activity. According to Cloninger's hypothesis (Cloninger et al., 1993), NS primarily utilizes dopaminergic pathways; Harm Avoidance utilizes serotonin pathways; and Reward Dependence utilizes norepinephrine pathways. It is therefore of interest that the ACE genotype could be associated with NS regulated by dopaminergic pathways. Several lines of evidence support an interaction between brain angiotensins and central catecholamine systems. Because angiotensin II receptors have been identified on dopamine containing cells in the substantia nigra and striatum of the human brain (Jenkins et al., 1995), an influence of angiotensin II on cerebral dopamine content is probable. Moreover, locally administered angiotensin II stimulates dopamine release from the striatum in the rat (Mendelsohn et al., 1993). From those viewpoints, the High-ACE group (DD or ID genotype) might have had high angiotensin II levels, which stimulate the release of more dopamine, resulting in high NS. These observations seem consistent with our results, suggesting an association between the ACE gene and NS thorough dopaminergic pathways.

Table 1
TCI scores as a function of ACE genotype in female healthy subjects

Genotype [n]	Age	NS	HA	RD	PS
Males [74]					
Low-ACE II [33]	27.9 (6.93)	21.4 (5.20)	19.1 (5.50)	14.2 (3.70)	3.91 (2.10)
High-ACE DD/ID [41]	30.1 (9.10)	21.2 (5.66)	19.4 (7.38)	15.7 (4.14)	4.54 (1.78)
Genotype [n]	Age	NS*	HA	RD	PS
Females [110]					
Low-ACE II [48]	29.3 (12.1)	19.9 (5.23)	20.3 (5.63)	16.9 (3.38)	4.67 (2.10)
High-ACE DD/ID [62]	28.6 (10.8)	22.4 (5.38)	18.2 (6.26)	16.6 (3.28)	4.16 (2.02)

NS: Novelty Seeking, HA: Harm Avoidance, RD: Reward Dependence, PS: Persistence, Low-ACE: the low-activity ACE group who had the insertion/insertion (II) genotype, High-ACE: the high-activity ACE group who had insertion/deletion (ID) or deletion/deletion (DD) genotype.
Data is shown as mean (SD; standard deviation); [n], number.
* Statistically significant between-group difference ($F=5.767$, $df=1,108$, $p=0.018$).

NS is known to be a risk factor for substance abuse (Howard et al., 1997; Hale et al., 2003). Our data regarding ACE and NS suggest that the D allele (High-ACE) is a risk factor for drug abuse as a NS-related behavior. From this perspective, our hypothesis is consistent with the report regarding an association between the ACE I/D polymorphism and alcohol abuse (Garrib and Peters, 1998). In their study, higher frequencies of the D alleles were observed in patients with alcoholism. However, their results were never replicated and must be interpreted with caution.

To our knowledge, our study is the first to suggest an association between NS and the ACE I/D genotype in female subjects. It is unclear why only females, but not males, show an association between NS and the ACE I/D genotype. A possible explanation is that the ACE gene is composed of two homologous regions and codes for both a somatic and testis isoenzyme (Bernstein et al., 1989; Rieder et al., 1999). The testis-specific form of ACE has its own promoter within intron 12 (Howard et al., 1990), is encoded by the 3′ region of the gene, and is found only in postmeiotic spermatogenic cells and sperm. Krege et al. (1995) have reported that ACE-deficient mice show male–female differences in fertility and blood pressure. Researchers have found that gender differences in cardiac ACE expression (Freshour et al., 2002) and plasma ACE activity (Lim et al., 2002) are normalized in androgen-deprived male mice. Gandhi et al. (1998) have shown that the renal vasoconstrictor response to angiotensin I and angiotensin II infusion is significantly increased in women compared to that in men. These observations, indicating gonadal effects on ACE activity, suggest that females may be more influenced by the ACE gene than males. However, the mechanisms of the possible gender differences on the association between ACE I/D polymorphism and NS are still unclear. As our sample size may be regarded as weakness of this study, these data needed to be confirmed in a much larger sample.

5. Conclusions

In healthy Japanese females, the novelty seeking (NS) of the Low-ACE group (II genotype) were significantly lower than those of the High-ACE group (ID or DD genotype). Our findings suggest that the ACE I/D polymorphism might be associated with NS personality in females, but not males. Taking into account the effects of multiple comparisons, this result should be interpreted with caution, and needs confirmation in a larger sample.

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References

Akiskal, H.S., Hirschfeld, R.M., Yerevanian, B.I., 1983. The relationship of personality to affective disorders. Arch. Gen. Psychiatry 40, 801–810.

Arinami, T., Li, L., Mitsushio, H., Itokawa, M., Hamaguchi, H., Toru, M., 1996. An insertion/deletion polymorphism in the angiotensin converting enzyme gene is associated with both brain substance P contents and affective disorders. Biol. Psychiatry 40, 1122–1127.

Baghai, T.C., Schule, C., Zwanzger, P., Minov, C., Schwarz, M.J., de Jonge, S., Rupprecht, R., Bondy, B., 2001. Possible influence of the insertion/deletion polymorphism in the angiotensin I-converting enzyme gene on therapeutic outcome in affective disorders. Mol. Psychiatry 6, 258–259.

Baghai, T.C., Schule, C., Zwanzger, P., Minov, C., Zill, P., Ella, R., Eser, D., Oezer, S., Bondy, B., Rupprecht, R., 2002. Hypothalamic–pituitary–adrenocortical axis dysregulation in patients with major depression is influenced by the insertion/deletion polymorphism in the angiotensin I-converting enzyme gene. Neurosci. Lett. 328, 299–303.

Baghai, T.C., Schule, C., Zwanzger, P., Zill, P., Ella, R., Eser, D., Deiml, T., Minov, C., Rupprecht, R., Bondy, B., 2003. Influence of a functional polymorphism within the angiotensin I-converting enzyme gene on partial sleep deprivation in patients with major depression. Neurosci. Lett. 339, 223–226.

Bernstein, K.E., Martin, B.M., Edwards, A.S., Bernstein, E.A., 1989. Mouse angiotensin-converting enzyme is a protein composed of two homologous domains. J. Biol. Chem. 264, 11945–11951.

Bondy, B., Baghai, T.C., Zill, P., Bottlender, R., Jaeger, M., Minov, C., Schule, C., Zwanzger, P., Rupprecht, R., Engel, R.R., 2002. Combined action of the ACE D- and the G-protein beta3 T-allele in major depression: a possible link to cardiovascular disease? Mol. Psychiatry 7, 1120–1126.

Boyce, P., Parker, G., Barnett, B., Cooney, M., Smith, F., 1991. Personality as a vulnerability factor to depression. Br. J. Psychiatry 159, 106–114.

Braszko, J.J., Wisniewski, K., Kupryszewski, G., Witczuk, B., 1987. Psychotropic effects of angiotensin II and III in rats: locomotor and exploratory vs cognitive behaviour. Behav. Brain Res. 25, 195–203.

Cloninger, C.R., Svrakic, D.M., Przybeck, T.R., 1993. A psychobiological model of temperament and character. Arch. Gen. Psychiatry 50, 975–990.

Costa Jr., P.T., McCrae, R.R., 1997. Stability and change in personality assessment: the revised NEO Personality Inventory in the year 2000. J. Pers. Assess. 68, 86–94.

Ebstein, R.P., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., Bennett, E.R., Nemanov, L., Katz, M., Belmaker, R.H., 1996. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of NS. Nat. Genet. 12, 78–80.

Fogarty, D.G., Maxwell, A.P., Doherty, C.C., Hughes, A.E., Nevin, N.C., 1994. ACE gene typing. Lancet 343, 851.

Freshour, J.R., Chase, S.E., Vikstrom, K.L., 2002. Gender differences in cardiac ACE expression are normalized in androgen-deprived male mice. Am. J. Physiol., Heart Circ. Physiol. 283, H1997–H2003.

Furlong, R.A., Keramatipour, M., Ho, L.W., Rubinsztein, J.S., Michael, A., Walsh, C., Paykel, E.S., Rubinsztein, D.C., 2000. No association of an insertion/deletion polymorphism in the angiotensin I converting enzyme gene with bipolar or unipolar affective disorders. Am. J. Med. Genet. 96, 733–735.

Gandhi, S.K., Gainer, J., King, D., Brown, N.J., 1998. Gender affects renal vasoconstrictor response to Ang I and Ang II. Hypertension 31, 90–96.

Gard, P.R., Mandy, A., Sutcliffe, M.A., 1999. Evidence of a possible role of altered angiotensin function in the treatment, but not etiology, of depression. Biol. Psychiatry 45, 1030–1034.

Garrib, A., Peters, T., 1998. Angiotensin-converting enzyme (ACE) gene polymorphism and alcoholism. Biochem. Soc. Trans. 26, S136.

Hale, R.L., Whiteman, S., Muehl, K., Faynberg, E., 2003. Tridimensional personality traits of college student marijuana users. Psychol. Rep. 92, 661–666.

Hirschfeld, R.M., Klerman, G.L., Lavori, P., Keller, M.B., Griffith, P., Coryell, W., 1989. Premorbid personality assessments of first onset of major depression. Arch. Gen. Psychiatry 46, 345–350.

Hong, C.J., Wang, Y.C., Tsai, S.J., 2002. Association study of angiotensin I-converting enzyme polymorphism and symptomatology and antidepressant response in major depressive disorders. J. Neural. Transm. 109, 1209–1214.

Howard, T.E., Shai, S.Y., Langford, K.G., Martin, B.M., Bernstein, K.E., 1990. Transcription of testicular angiotensin-converting enzyme (ACE) is

initiated within the 12th intron of the somatic ACE gene. Mol. Cell. Biol. 10, 4294–4302.
Howard, M.O., Kivlahan, D., Walker, R.D., 1997. Cloninger's tridimensional theory of personality and psychopathology: applications to substance use disorders. J. Stud. Alcohol 58, 48–66.
Ichiki, T., Labosky, P.A., Shiota, C., Okuyama, S., Imagawa, Y., Fogo, A., Niimura, F., Ichikawa, I., Hogan, B.L., Inagami, T., 1995. Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. Nature 377, 748–750.
Ishigami, T., Iwamoto, T., Tamura, K., Yamaguchi, S., Iwasawa, K., Uchino, K., Umemura, S., Ishii, M., 1995. Angiotensin I converting enzyme (ACE) gene polymorphism and essential hypertension in Japan. Ethnic difference of ACE genotype. Am. J. Hypertens. 8, 95–97.
Jenkins, T.A., Allen, A.M., Chai, S.Y., Mendelsohn, F.A., 1995. Interactions of angiotensin II with central catecholamines. Clin. Exp. Hypertens. 17, 267–280.
Kakinuma, Y., Hama, H., Sugiyama, F., Goto, K., Murakami, K., Fukamizu, A., 1997. Anti-apoptotic action of angiotensin fragments to neuronal cells from angiotensinogen knock-out mice. Neurosci. Lett. 232, 167–170.
Kakinuma, Y., Hama, H., Sugiyama, F., Yagami, K., Goto, K., Murakami, K., Fukamizu, A., 1998. Impaired blood–brain barrier function in angiotensinogen-deficient mice. Nat. Med. 4, 1078–1080.
Krege, J.H., John, S.W., Langenbach, L.L., Hodgin, J.B., Hagaman, J.R., Bachman, E.S., Jennette, J.C., O'Brien, D.A., Smithies, O., 1995. Male–female differences in fertility and blood pressure in ACE-deficient mice. Nature 375, 146–148.
Kuznetsova, T., Staessen, J.A., Wang, J.G., Gasowski, J., Nikitin, Y., Ryabikov, A., Fagard, R., 2000. Antihypertensive treatment modulates the association between the D/I ACE gene polymorphism and left ventricular hypertrophy: a meta-analysis. J. Hum. Hypertens. 14, 447–454.
Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527–1531.
Lim, Y.K., Retnam, L., Bhagavath, B., Sethi, S.K., bin Ali, A., Lim, S.K., 2002. Gonadal effects on plasma ACE activity in mice. Atherosclerosis 160, 311–316.
Lindpaintner, K., Pfeffer, M.A., Kreutz, R., Stampfer, M.J., Grodstein, F., LaMotte, F., Buring, J., Hennekens, C.H., 1995. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N. Engl. J. Med. 332, 706–711.
Lozano, B.E., Johnson, S.L., 2001. Can personality traits predict increases in manic and depressive symptoms? J. Affect. Disord. 63 (1–3), 103–111.
Martin, N.G., Jardine, R., Andrews, G., Heath, A.C., 1988. Anxiety disorders and neuroticism: are there genetic factors specific to panic? Acta Psychiatr. Scand. 77, 698–706.
Meira-Lima, I.V., Pereira, A.C., Mota, G.F., Krieger, J.E., Vallada, H., 2000. Angiotensinogen and angiotensin converting enzyme gene polymorphisms and the risk of bipolar affective disorder in humans. Neurosci. Lett. 293, 103–106.
Mendelsohn, F.A., Jenkins, T.A., Berkovic, S.F., 1993. Effects of angiotensin II on dopamine and serotonin turnover in the striatum of conscious rats. Brain Res. 613, 221–229.
Montgomery, H.E., Marshall, R., Hemingway, H., Myerson, S., Clarkson, P., Dollery, C., Hayward, M., Holliman, D.E., Jubb, M., World, M., Thomas, E.L., Brynes, A.E., Saeed, N., Barnard, M., Bell, J.D., Prasad, K., Rayson, M., Talmud, P.J., Humphries, S.E., 1998. Human gene for physical performance. Nature 393, 221–222.
Okuyama, S., Sakagawa, T., Sugiyama, F., Fukamizu, A., Murakami, K., 1999a. Reduction of depressive-like behavior in mice lacking angiotensinogen. Neurosci. Lett. 261, 167–170.
Okuyama, S., Sakagawa, T., Chaki, S., Imagawa, Y., Ichiki, T., Inagami, T., 1999b. Anxiety-like behavior in mice lacking the angiotensin II type-2 receptor. Brain Res. 821, 150–159.
Ouyang, W.C., Wang, Y.C., Hong, C.J., Cheng, C.Y., Tsai, S.J., 2001. Association study of angiotensin-converting enzyme gene polymorphism with schizophrenia and polydipsia. Neuropsychobiology 44, 31–35.
Pauls, J., Bandelow, B., Ruther, E., Kornhuber, J., 2000. Polymorphism of the gene of angiotensin converting enzyme: lack of association with mood disorder. J. Neural. Transm. 107, 1361–1366.
Rieder, M.J., Taylor, S.L., Clark, A.G., Nickerson, D.A., 1999. Sequence variation in the human angiotensin converting enzyme. Nat. Genet. 22, 59–62.
Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., Soubrier, F., 1990. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J. Clin. Invest. 86, 1343–1346.
Rigat, B., Hubert, C., Corvol, P., Soubrier, F., 1992. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 20, 1433.
Sayed-Tabatabaei, F.A., Houwing-Duistermaat, J.J., van Duijn, C.M., Witteman, J.C., 2003. Angiotensin-converting enzyme gene polymorphism and carotid artery wall thickness: a meta-analysis. Stroke 34, 1634–1639.
Segman, R.H., Shapira, Y., Modai, I., Hamdan, A., Zislin, J., Heresco-Levy, U., Kanyas, K., Hirschmann, S., Karni, O., Finkel, B., Schlafman, M., Lerner, A., Shapira, B., Macciardi, F., Lerer, B., 2002. Angiotensin converting enzyme gene insertion/deletion polymorphism: case–control association studies in schizophrenia, major affective disorder, and tardive dyskinesia and a family-based association study in schizophrenia. Am. J. Med. Genet. 114, 310–314.
Shanmugam, V., Sell, K.W., Saha, B.K., 1993. Mistyping ACE heterozygotes. PCR Methods Appl. 3, 120–121.
Sharma, P., 1998. Meta-analysis of the ACE gene in ischaemic stroke. J. Neurol. Neurosurg. Psychiatry 64, 227–230.
Shimizu, E., Hashimoto, K., Kobayashi, K., Mitsumori, M., Ohgake, S., Koizumi, H., Okamura, N., Koike, K., Kumakiri, C., Nakazato, M., Komatsu, N., Iyo, M., 2004. Lack of association between angiotensin I-converting enzyme insertion/deletion gene functional polymorphism and panic disorder in humans. Neurosci. Lett. 363, 81–83.
Spotts, J.V., Shontz, F.C., 1986. Drugs and personality: dependence of findings on method. Am. J. Drug Alcohol Abuse 12, 355–382.
Tiret, L., Rigat, B., Visvikis, S., Breda, C., Corvol, P., Cambien, F., Soubrier, F., 1992. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. Am. J. Hum. Genet. 51, 197–205.
Von Bohlen Und Halbach, O., 2003. Angiotensin IV in the central nervous system. Cell Tissue Res. 311, 1–9.
von Zerssen, D., Tauscher, R., Possl, J., 1994. The relationship of premorbid personality to subtypes of an affective illness. A replication study by means of an operationalized procedure for the diagnosis of personality structures. J. Affect. Disord. 32, 61–72.
Vuckovic, A., Cohen, B.M., Zubenko, G.S., 1991. The use of captopril in treatment-resistant depression: an open trial. J. Clin. Psychopharmacol. 11, 395–396.
Williams, A.G., Rayson, M.P., Jubb, M., World, M., Woods, D.R., Hayward, M., Martin, J., Humphries, S.E., Montgomery, H.E., 2000. The ACE gene and muscle performance. Nature 403, 614.
Wright, J.W., Reichert, J.R., Davis, C.J., Harding, J.W., 2002. Neural plasticity and the brain renin–angiotensin system. Neurosci. Biobehav. Rev. 26, 529–552.

ORIGINAL ARTICLE

Brain Serotonin Transporter Density and Aggression in Abstinent Methamphetamine Abusers

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Context: In animals, methamphetamine is known to have a neurotoxic effect on serotonin neurons, which have been implicated in the regulation of mood, anxiety, and aggression. It remains unknown whether methamphetamine damages serotonin neurons in humans.

Objective: To investigate the status of brain serotonin neurons and their possible relationship with clinical characteristics in currently abstinent methamphetamine abusers.

Design: Case-control analysis.

Setting: A hospital research center.

Participants: Twelve currently abstinent former methamphetamine abusers (5 women and 7 men) and 12 age-, sex-, and education-matched control subjects recruited from the community.

Interventions: The brain regional density of the serotonin transporter, a structural component of serotonin neurons, was estimated using positron emission tomography and *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio) phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652). Estimates were derived from region-of-interest and statistical parametric mapping methods, followed by within-case analysis using the measures of clinical variables.

Main Outcome Measures: The duration of methamphetamine use, the magnitude of aggression and depressive symptoms, and changes in serotonin transporter density represented by the [^{11}C](+)McN-5652 distribution volume.

Results: Methamphetamine abusers showed increased levels of aggression compared with controls. Region-of-interest and statistical parametric mapping analyses revealed that the serotonin transporter density in global brain regions (eg, the midbrain, thalamus, caudate, putamen, cerebral cortex, and cerebellum) was significantly lower in methamphetamine abusers than in control subjects, and this reduction was significantly inversely correlated with the duration of methamphetamine use. Furthermore, statistical parametric mapping analyses indicated that the density in the orbitofrontal, temporal, and anterior cingulate areas was closely associated with the magnitude of aggression in methamphetamine abusers.

Conclusions: Protracted abuse of methamphetamine may reduce the density of the serotonin transporter in the brain, leading to elevated aggression, even in currently abstinent abusers.

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METHAMPHETAMINE IS A powerfully addictive drug, and the number of its abusers has been steadily increasing worldwide.[1-5] Long-term methamphetamine abuse can produce various psychiatric symptoms, including psychosis, depression, anxiety, and aggression, under conditions of intoxication and withdrawal.[6,7] These psychiatric states are sometimes prolonged, in the form of residual symptoms, and are easily exacerbated in some long-term abusers by methamphetamine reuse or by psychological stress.[3,8-10]

In animal studies, the biochemical effects of the neurotoxicity of methamphetamine on mature neurons, especially on the dopaminergic and serotonergic axon arbors, are well documented,[11,12] although neurotoxic methamphetamine may also cause cell death through apoptosis or necrosis.[12,13] However, methamphetamine-induced neuronal damage is thought to vary across species.[14,15] For example, in contrast to the findings in rats,[11] which indicate that serotonergic neurons are more sensitive to the methamphetamine-induced toxicity than are dopaminergic neurons, recent findings[16] have suggested that serotonergic neurons in nonhuman primates seem to be less affected by methamphetamine administration than are dopaminergic neurons.

In vivo studies using positron emission tomography (PET) are helpful for understanding the contribution of methamphetamine neurotoxicity and induced

Table 1. Demographic and Clinical Characteristics of the 24 Study Participants

	Control Subjects (n = 12)		Methamphetamine Abusers (n = 12)*	
	Mean ± SD	Range	Mean ± SD	Range
Age, y	31.8 ± 6.6	21-44	31.4 ± 6.8	21-44
Education, y	11.5 ± 1.2	9-12	11.1 ± 2.1	9-12
Duration of methamphetamine use, y	NA	NA	6.7 ± 3.2	1.5-11.0
Duration of methamphetamine abstinence, y	NA	NA	1.6 ± 1.3	0.5-5.0
BPRS positive symptoms subscale score	NA	NA	5.3 ± 3.9	0-14
BPRS negative symptoms subscale score	NA	NA	0.0 ± 0.0	0.0
17-Item HAM-A score	NA	NA	3.8 ± 6.3	0-16
17-Item HAM-D score	NA	NA	4.0 ± 6.3	0-19
Scale for methamphetamine craving score	NA	NA	4.9 ± 3.4	1-10
Aggression Questionnaire score†	30.2 ± 1.7	29-34	75.0 ± 13.9‡	46-97

Abbreviations: BPRS, Brief Psychiatric Rating Scale; HAM-A, Hamilton Rating Scale for Anxiety; HAM-D, Hamilton Rating Scale for Depression; NA, not applicable.
*All the abusers took methamphetamine intravenously.
†Higher scores represent greater aggression.
‡Significantly difference from control subjects using the *t* test ($P<.001$).

neural damage to the long-term withdrawal syndrome. Recent PET studies have shown that long-term use of methamphetamine decreases the density of DA transporters, which are located on dopaminergic terminals in the human brain[1-3,17,18]; moreover, long-term use of methamphetamine may cause severe positive symptoms (eg, delusions and hallucinations) and an increased reduction in DA transporter density.[3,18] However, to date, no studies have addressed the alteration of serotonergic neurons in methamphetamine abusers. In addition, it is not known whether such changes, if found, could be related to the psychiatric symptoms frequently observed in currently abstinent methamphetamine abusers.

We, therefore, examined the possibility of changes in the density of the serotonin transporter, an index of serotonin neuronal damage,[19-25] in methamphetamine abusers by means of PET. This information was then considered as part of an evaluation of the potential associations between serotonin transporter density and participant clinical characteristics.

METHODS

PARTICIPANTS

The ethics committees of the Hamamatsu University School of Medicine and Hamamatsu Medical Center approved this study. Written informed consent was obtained from each participant after they were provided an explanation of the study procedures. Twelve currently abstinent methamphetamine abusers who had previously abused only methamphetamine (ie, monodrug abusers) and 12 age-, sex-, and education-matched control subjects participated in this study (**Table 1**). Potential participants were recruited from the community by means of poster advertisements and word of mouth in and around Hamamatsu City, which is located in the middle of the mainland of Japan. The participants in the methamphetamine group were required to attend a weekly meeting at the Drug Detoxification and Rehabilitation Program Center of Hattori Mental Hospital (Iwata, Japan) to maintain and ensure abstinence until the PET study was conducted.

All the methamphetamine abusers had used the drug recreationally and had no history of toxic or high-dose methamphetamine use. None of the abusers had any history of hospitalization or treatment at psychiatric hospitals. We assessed the participants regarding the use of other illicit drugs, including (±)3,4-methylenedioxymethamphetamine, cocaine, cannabis, heroin, and toluene, because these substances are known to cause psychiatric symptoms and to affect neural transmission in the brain.[19,26,27] However, none of the methamphetamine abusers recruited for the present study were found to have a history of such illicit drug use. All the methamphetamine abusers were naive to neuropsychiatric medications, such as antipsychotics and antidepressants. None of the methamphetamine abusers had a history of psychiatric disorders, including antisocial or intermittent explosive disorder, or a history of increased aggression before the use of methamphetamine. The controls were healthy and had never used methamphetamine or any other illicit drugs, and none of them met any of the relevant criteria according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.[28] The control and methamphetamine groups showed similar habits of occasional drinking and smoking, but none of the participants fulfilled either the alcohol- or the nicotine-related *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria. These evaluations were determined using the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.[29] To increase the accuracy of the abusers' profiles, detailed information on the duration of methamphetamine use and the history of psychiatric symptoms was retrospectively obtained using Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*–based interviews with the abusers and their family members. The period of methamphetamine use was defined as the duration between the first and last use. When intervals of abstinence longer than 1 month occurred during the duration of methamphetamine use as defined, these intervals were subtracted from the total duration value. The methamphetamine abstinence period was arbitrarily defined as the duration between the day of the last use of methamphetamine and that of the PET examination.

DRUG SCREENING

During the weekly meeting at the Drug Detoxification and Rehabilitation Program Center, the absence of recent methamphetamine and other drug use was regularly confirmed using a rapid

immunoassay for the qualitative detection of the metabolites of the following 8 classes of drugs: amphetamines, including methamphetamine and (±)3,4-methylenedioxymethamphetamine; barbiturates; benzodiazepines; cocaine; methadone; opiates; tetrahydrocannabinol; and tricyclic antidepressants (Triage8; Biosite Diagnostics, San Diego, Calif). In addition, the participants were tested for urinary hippuric acid, a biomarker of toluene use, using high-performance liquid chromatography according to the standard diagnostic methods.[27] These assessments were also performed on the same day as the PET examination. When necessary, we assessed hair samples using high-performance liquid chromatography, which enabled us to verify long periods of methamphetamine abstinence.[30]

CLINICAL EVALUATION

The severity of psychiatric symptoms in methamphetamine abusers was evaluated using the Aggression Questionnaire (AQ)[31]; the scores can range from 29 to 145, with higher scores representing greater aggression. In addition, the 17-item Hamilton Rating Scale for Anxiety,[32] the 17-item Hamilton Rating Scale for Depression,[33] and positive and negative symptom subscores[34] on the Brief Psychiatric Rating Scale[35] were included in the evaluation. The Subjective Drug Effect Rating Scale for Cocaine[36] was modified and used for the assessment of cravings for methamphetamine. The scores on this assessment can range from 1 to 10, with higher scores representing more intense craving sensations (Table 1). These evaluations were performed on the day of the PET examination by a trained research psychiatrist masked to the PET results.

MAGNETIC RESONANCE IMAGING AND MAGNETIC RESONANCE IMAGING–TO-PET COORDINATE PROCEDURES

Three-dimensional magnetic resonance imaging (MRI) was performed just before the PET examination using a 0.3-T MRI unit (MRP7000AD; Hitachi Medical Corp, Tokyo, Japan) and the following acquisition parameters: repetition time, 200 milliseconds; echo time, 23 milliseconds; flip angle, 75°; slice thickness, 2 mm with no gap; and matrix, 256 × 256. In reference to the measurements of the tilt angle and spatial coordinates obtained in the procedure for determining the anterior-posterior intercommissural line on each participant's sagittal MRIs, a PET gantry was set parallel to the anterior-posterior intercommissural line by tilting and moving the gantry for each participant, which permitted reconstruction of the PET images parallel to the anterior-posterior intercommissural line without reslicing; using this approach, we allocated regions of interest (ROIs) on the target areas of the original PET images.[37]

PET PROCEDURES

We used a high-resolution brain PET scanner (model SHR12000; Hamamatsu Photonics KK, Hamamatsu, Japan), which was capable of yielding 47 PET images simultaneously.[38] Before dynamic scanning, a 20-minute transmission scan was performed for attenuation correction using a germanium Ge 68/gallium Ga 68 source with the participant's head fixed by means of a radiosurgery-purpose thermoplastic face mask. Then, after a bolus intravenous injection of a 370-MBq dose of *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652), a ligand with high specificity to serotonin transporter,[21,39] 38 serial PET scans (time frames: 4 × 60, 20 × 120, and 14 × 300 seconds) were performed for 92 minutes. A total of 23 arterial blood samples were collected at intervals of 10 seconds to 15 minutes after the tracer injection. The blood samples were analyzed using thin-layer chromatography (Whatman AL SIL G/UV 20 × 20 cm; Whatman Japan KK, Tokyo) and a storage phosphorscreen bioimaging analyzer (model BAS-1500; Fuji Photo Film Co, Tokyo) to determine the levels of unmetabolized tracer.

IMAGE ANALYSIS AND KINETIC MODELING

At the beginning of the study, the MRI voxel size was adjusted to the PET voxel size 3-dimensionally using image processing software (DrView; Asahi Kasei Co, Tokyo) on a Sun workstation (HyperSPARC ss-20; Sun Microsystems, Santa Clara, Calif). These reformatted MRIs with 3-dimensional scales and coordinates identical to those of the PET images were used as anatomic landmarks for the ROI setting, which allowed for minimization of the partial volume effects.[3,18,40,41] An investigator masked to the participant's condition placed 10 ROIs bilaterally over the midbrain, thalamus, caudate nucleus, putamen, amygdala, anterior cingulate cortex, dorsolateral prefrontal cortex, orbitofrontal cortex, temporal cortex, and cerebellar cortex on the MRIs, as previously described.[40,42,43] After delineation of the ROIs was completed on the reformatted MRIs, the PET images were displayed side-by-side with the MRIs. Then, the determined ROIs were placed on the same area on the MRIs and the corresponding PET images.

To assess the brain serotonin transporter density, we analyzed the [^{11}C](+)McN-5652 binding data on the basis of a model that described the radioligand kinetics using a single-tissue compartment and 3 parameters—uptake of radioligand in brain tissue (K_1), release of radioligand from brain tissue (k_2), and blood volume—because the regional brain [^{11}C](+)McN-5652 distribution volume (DV) (ie, the ratio of K_1/k_2) estimated by this model is known to correlate with the known regional brain serotonin transporter density[21,39,44] and has been reported to be suitable for evaluating amphetamine-induced serotonergic neurotoxicity.[21] Cerebral radioactivity was corrected for the contribution of plasma radioactivity, assuming a 5% blood volume in the ROIs. The K_1 and k_2 values were estimated by fitting the metabolite-corrected plasma time–radioactivity curves and the blood volume–corrected brain time–radioactivity curves using a nonlinear least squares algorithm.[3,18,40]

STATISTICAL ANALYSIS

In addition to the ROI method described in the "Methods" section, we also performed a voxel-based whole-brain analysis using statistical parametric mapping (SPM) software (SPM99; Wellcome Department of Cognitive Neurology, Institute of Neurology, London). Based on the same kinetic model as that used for the ROI method, absolute parametric [^{11}C](+)McN-5652 DV images were generated for each participant using biomedical image quantification and kinetic modeling software (PMOD version 2.5; PMOD Technologies Ltd, Zurich, Switzerland) (**Figure 1**).[45,46] To normalize the absolute DV image to the standard stereotaxic brain atlas,[47] we used transformation parameters for early integrated images of [^{11}C](+)McN-5652 (0-20 minutes after injection).[48,49] Subsequently, *t* statistics were performed on a voxel-by-voxel basis (voxel size: 2.0 × 2.0 × 2.0 mm), resulting in *t* statistic maps. Then, the results were transformed to the unit normal distribution. For the SPM analysis, we assessed both group differences in the regional [^{11}C](+)McN-5652 DVs and the possible relationship between the regional changes in [^{11}C](+)McN-5652 DVs and the severity of clinical symptoms in methamphetamine abusers. Age and sex were treated as covariates, and the scores on the clinical measures (AQ, Hamilton Rating Scale for Anxiety, Hamilton Rating Scale for Depression, positive and negative symptoms on

the Brief Psychiatric Rating Scale, and the scale for methamphetamine craving) were considered to be variables of interest. To test hypotheses about the regional specific effects of these variables, the estimates were compared using 2 linear contrasts (positive or negative correlation). According to recently published PET studies[6,50] of methamphetamine abusers, the level of significance was determined using a voxel height threshold of $P=.05$ (corrected). The cluster significance threshold was also set at $P=.05$ (corrected).

To compare the mean values of the demographic and clinical variables in control subjects and methamphetamine abusers, an unpaired t test was used. We tested the main effect of methamphetamine use on [^{11}C](+)McN-5652 DVs derived from 10 brain regions using multivariate analysis of variance. Statistical significance was set at $P<.05$. To investigate the correlation between the [^{11}C](+)McN-5652 DV and the clinical variables in methamphetamine abusers, including the duration of methamphetamine use and abstinence, the Pearson correlation coefficient was computed, with age and sex adjusted for; after applying the Bonferroni correction, the level of statistical significance was set at $P=.005$ (SPSS version 11.0J; SPSS Japan Inc, Tokyo).

RESULTS

PSYCHIATRIC STATES OF ABSTINENT METHAMPHETAMINE ABUSERS

Methamphetamine abusers showed no apparent negative symptoms as demonstrated by Brief Psychiatric Rating Scale assessment (Table 1). All methamphetamine abusers had previously experienced psychosis during methamphetamine use. Two methamphetamine abusers had persistent psychotic symptoms, such as persecutory delusions and auditory hallucinations; 5 had a depressed mood; 4 had anxiety; 4 showed severe aggression; and 4 had no psychiatric symptoms except for aggressive behavior. The mean AQ score was significantly higher in methamphetamine abusers than in controls ($t=-11.1$; $P<.001$).

ROI ANALYSIS

The traditional ROI-based analysis showed that methamphetamine abusers had significantly decreased [^{11}C](+)McN-5652 DVs in their global brain regions compared with control subjects (Wilks $\Lambda=0.001$; $P=.003$) (**Figure 2**). Subsequent univariate analysis of variance revealed that methamphetamine abusers had significantly lower [^{11}C](+)McN-5652 DVs than control subjects in all 10 ROIs studied ($P<.001$ for all). There was no group × sex interaction effect in the [^{11}C](+)McN-5652 DV, indicating no sex-specific effect in [^{11}C](+)McN-5652 DVs (Wilks $\Lambda=0.47$; $P=.37$).

Figure 3 shows the correlations between [^{11}C](+)McN-5652 DVs and clinical variables in methamphetamine abusers. The [^{11}C](+)McN-5652 DVs in 5 of the 10 ROIs (ie, the midbrain, thalamus, caudate nucleus, putamen, and orbitofrontal cortex) significantly correlated negatively with the duration of methamphetamine use ($P<.005$ for all by Pearson correlation coefficient) (Figure 3A). There was no correlation in any of the 10 ROIs between [^{11}C](+)McN-5652 DVs and the duration of methamphetamine abstinence, which lasted 6 months to 5 years in our participants (Figure 3B). The magnitude of aggression, as assessed using the AQ, increased significantly with decreasing [^{11}C](+)McN-5652 DVs in 8 of the 10 ROIs (ie, the thalamus, caudate nucleus, putamen, anterior cingulate cortex, temporal cortex, orbitofrontal cortex, dorsolateral prefrontal cortex, and cerebellar cortex) ($P<.005$ for all by Pearson correlation coefficient) (Figure 3C). Other clinical variables, including craving, were not statistically significantly correlated with changes in [^{11}C](+)McN-5652 DVs (data not shown).

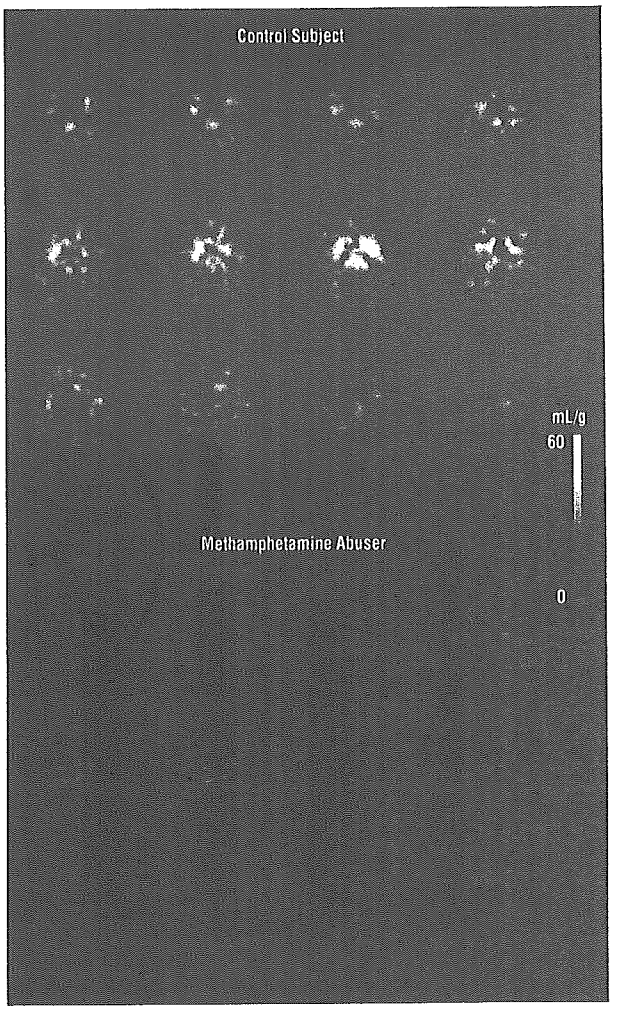

Figure 1. Voxel-based *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio) phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652) distribution volume images from a control subject and a methamphetamine abuser. These absolute parametric images were normalized to the standard stereotaxic brain atlas using transformation parameters for early integrated images of [^{11}C](+)McN-5652 (0-20 minutes after injection). The [^{11}C](+)McN-5652 distribution volumes in broad areas of the brain of the methamphetamine abuser were lower than those of the control subject.

SPM ANALYSIS

Figure 4 illustrates the results of the whole-brain voxel-based SPM analysis of [^{11}C](+)McN-5652 DVs. Figure 4A shows that the methamphetamine group had widely distributed reductions in [^{11}C](+)McN-5652 DVs compared with the control group ($P<.05$, corrected) (**Table 2**). In accord with the findings derived from the ROI analysis, the SPM analysis revealed an extensive clus-

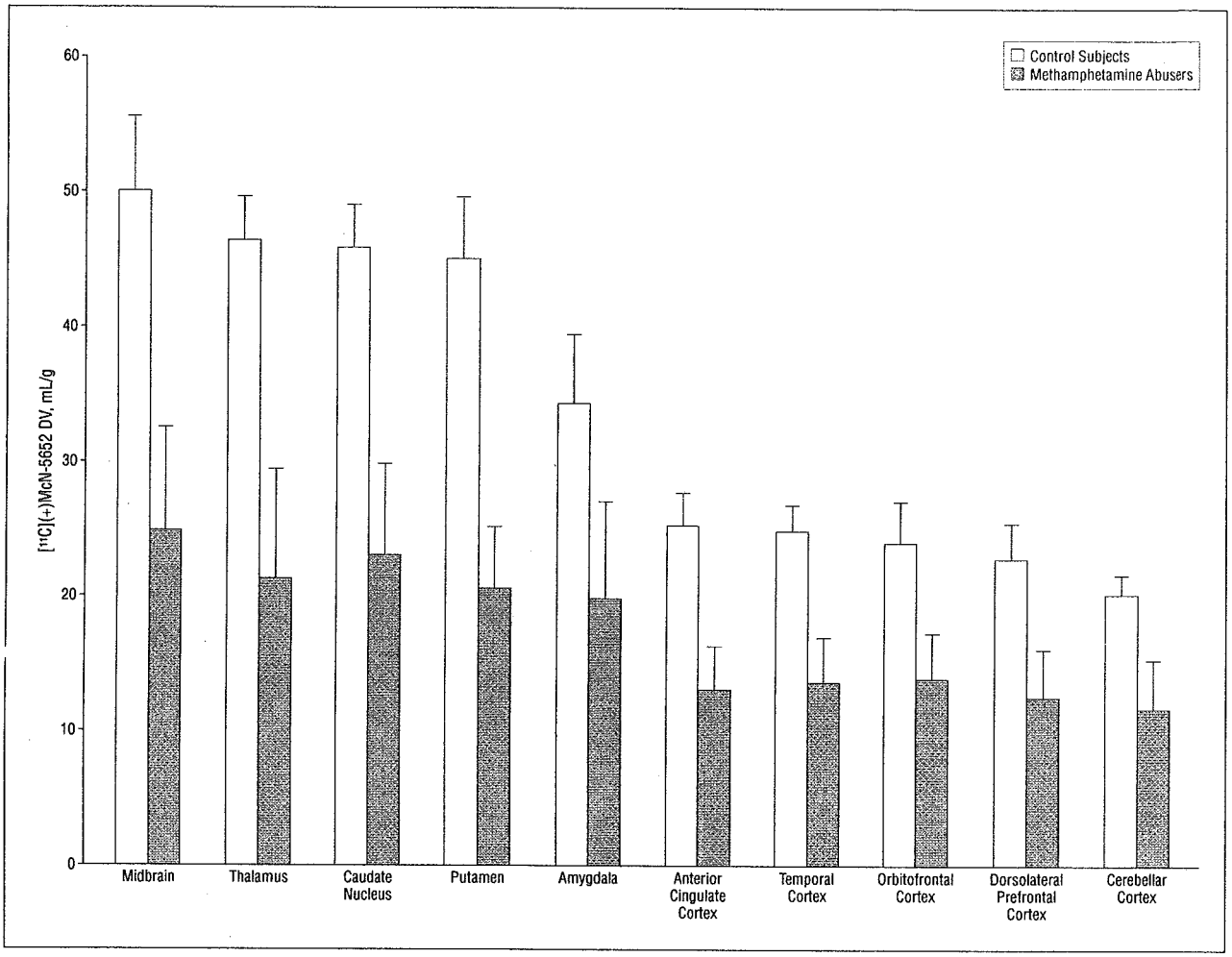

Figure 2. Mean regional brain *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652) distribution volumes (DVs) in control subjects and methamphetamine abusers. Methamphetamine abusers had significantly decreased [^{11}C](+)McN-5652 DVs in the global regions compared with controls (Wilks Λ=0.001; P=.003, by multivariate analysis of variance). Univariate analysis of variance revealed that methamphetamine users had significantly lower [^{11}C](+)McN-5652 DVs than controls in all regions studied (P<.001 for all). Error bars represent SE.

ter of voxels with reduced [^{11}C](+)McN-5652 DVs occupying the right insular area and extending out into the bilateral putamen, caudate, thalamus, hypothalamus, midbrain, temporal, parietal, frontal, occipital, cerebellar, anterior cingulate, and posterior cingulate areas. This cluster consisted of 45 315 voxels (363 mL). Figure 4B shows clusters in which the magnitude of aggression increased significantly with decreasing [^{11}C](+)McN-5652 DVs. These clusters were located on the bilateral orbitofrontal areas (P≤.001), left inferior temporal area (P<.001), and right anterior cingulate gyrus area (P<.001) (**Table 3**). The other clinical variables did not reach statistical significance (data not shown).

COMMENT

In the present study, methamphetamine abusers had statistically significantly decreased [^{11}C](+)McN-5652 DVs, a representative measure of serotonin transporter density,[21,39,44] in their global brain regions compared with control subjects. The finding of significantly reduced [^{11}C](+)McN-5652 DVs in a several brain regions in methamphetamine abusers, as revealed using the ROI approach, was in accord with the results of voxel-based SPM analysis. In addition, there was no group × sex interaction effect in terms of the [^{11}C](+)McN-5652 DV, indicating that abnormal [^{11}C](+)McN-5652 DVs in the brains of methamphetamine abusers are observed in both sexes. These findings suggest that the ingestion of methamphetamine leads to a global and severe reduction in the density of human brain serotonin transporters.

The values of the density of serotonin transporters in widely distributed brain regions, including the midbrain, hypothalamus, thalamus, caudate, putamen, amygdala, temporal cortex, and occipital cortex, were found to negatively correlate with the duration of methamphetamine use. This result implies that the longer methamphetamine is used, the more severe the decrease in serotonin transporter density will be. Although the duration of methamphetamine use is viewed as a proxy measure for the actual amount of intake of the drug, such a relationship in a dose-response manner strongly suggests a link between the use of methamphetamine and damage to serotonin neurons. This is compatible with the results of animal experiments[51] demonstrating dose-dependent methamphetamine-induced serotonin transporter reduction.

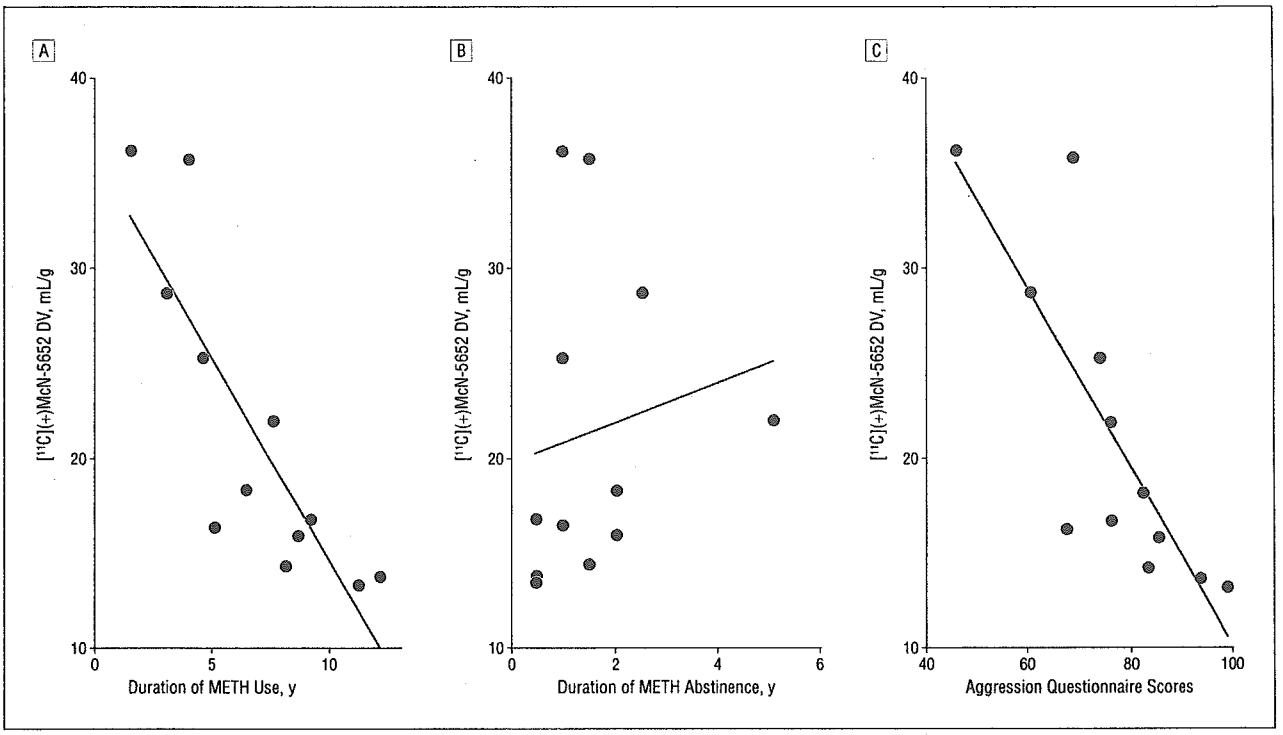

Figure 3. Correlations between *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652) distribution volumes (DVs) in a representative brain region (the thalamus) and clinical variables in methamphetamine (METH) abusers. A, Significant negative correlation between [^{11}C](+)McN-5652 DVs and the duration of METH use (r=−0.84; P=.001 by Pearson correlation coefficient). B, Correlation between [^{11}C](+)McN-5652 DVs and the duration of METH abstinence (r=0.16; P=.61). C, Correlation between Aggression Questionnaire scores and [^{11}C](+)McN-5652 DVs (r=−0.82; P=.001).

Although the present study was not designed to directly assess recovery from brain damage induced by methamphetamine use, there was no correlation between the [^{11}C](+)McN-5652 DVs and the duration of methamphetamine abstinence. Along with this finding, the result showing that even individuals who had been abstinent for more than 1 year (n=9) had a substantial decrease in serotonin transporter density (approximately a 30% decrease compared with controls) (Figure 3B) suggests that reductions in the density of the serotonin transporter in the brain associated with habitual methamphetamine abuse could persist long after methamphetamine use ceases.

The magnitude of aggression in methamphetamine abusers increased significantly with decreasing serotonin transporter densities in some brain regions. Detoxification from methamphetamine in all the abusers in this study was confirmed by regular urine drug screening as described in the "Drug Screening" subsection, including a test on the day of PET examination; these tests were conducted to establish that the psychiatric symptoms, such as aggression, were residual rather than acute symptoms induced by methamphetamine use. As a result, the relationship between the degree of aggressiveness and the density of serotonin transporter found in this study was not ascribed to the process of detoxification from methamphetamine use. Thus, the present findings indicate that methamphetamine-induced serotonergic disturbances are responsible for the elevated aggressiveness that is frequently observed, as a residual symptom, in abstinent methamphetamine abusers. This contention is consistent with a variety of studies[52,53] that have documented associations between decreased serotonergic function and increased aggression. For example, cerebrospinal fluid 5-hydroxyindoleacetic acid, which is known to reflect presynaptic serotonergic activity in the brain, has been found to be reduced in aggressive psychiatric patients,[54,55] impulsive violent men,[56,57] and impulsive violent offenders.[58]

In the correlational region analysis using SPM in the methamphetamine group, the magnitude of aggression was substantially associated with a decrease in serotonin transporter density in the clusters located in the orbitofrontal cortex, anterior cingulate, and temporal cortex, although the clusters were localized to small areas and did not fully occupy the anatomic brain regions. This result suggests that the potential methamphetamine-induced decrease in serotonergic function around these 3 areas may play an important role in the pathogenesis of elevated aggression in methamphetamine abusers. This is supported by several lines of evidence. For example, studies of brain injuries suggest that damage to the orbitofrontal and anterior cingulate areas produces syndromes characterized by aggression and impulsivity.[52,59] Furthermore, recent PET and postmortem clinicopathologic correlation studies have indicated that low levels of serotonin$_{1A}$ receptors in the orbitofrontal, anterior cingulate gyrus, and temporal areas are related to aggressive behavior.[60,61]

However, we cannot rule out the possibility that the increased aggression observed in methamphetamine abusers could reflect a preexisting condition, for example, an "addictive personality," which might often involve a tendency toward aggression.[62] Nevertheless, in the present study, we selected methamphetamine abusers who had no history of abnormal aggression before the use of meth-

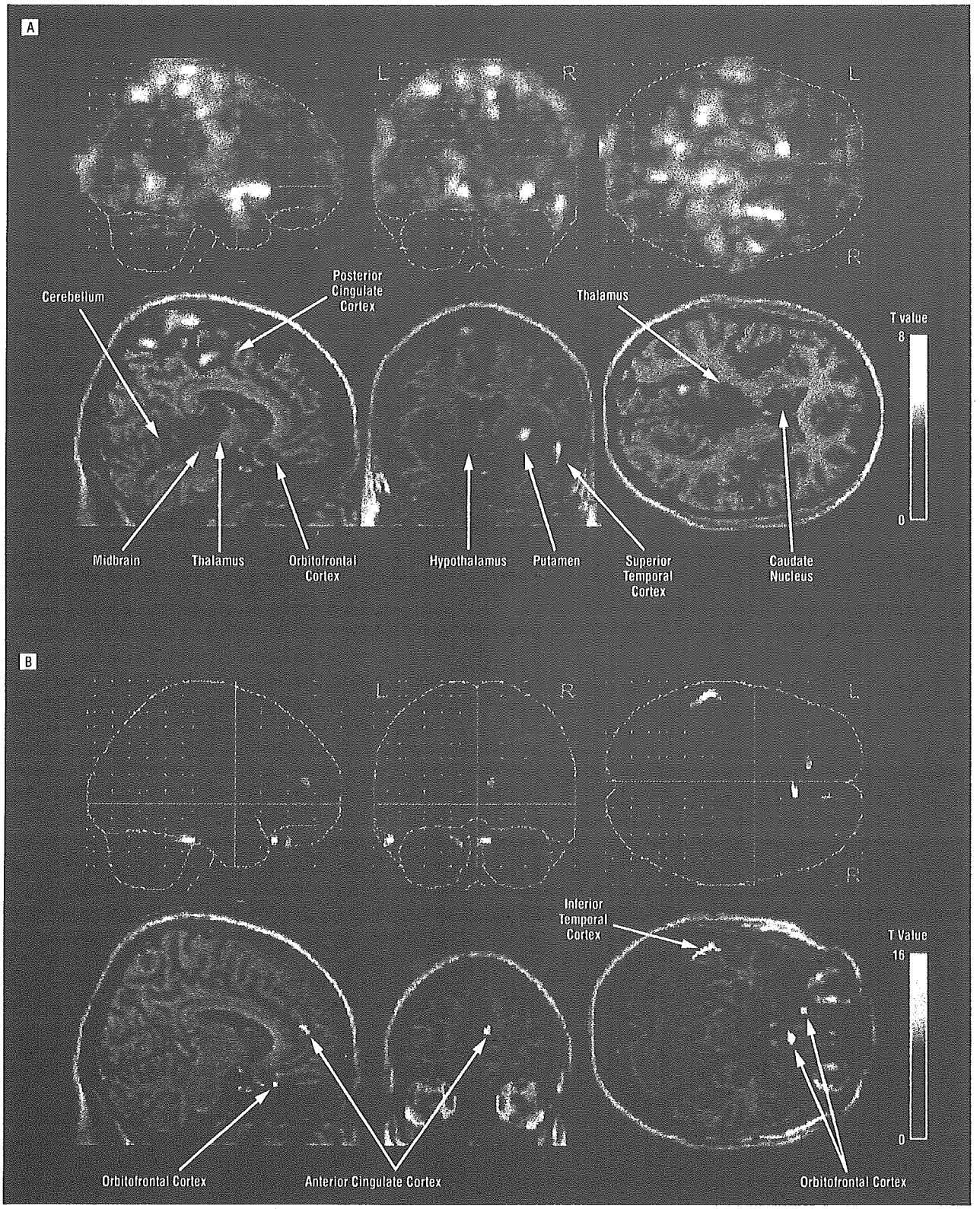

Figure 4. Results of the whole-brain voxel-based statistical parametric mapping analysis of the *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio) phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652) distribution volumes (DVs). A, Locations of methamphetamine abuser and control differences in [^{11}C](+)McN-5652 DVs. Areas with significantly reduced [^{11}C](+)McN-5652 DVs in methamphetamine abusers compared with those in controls ($P<.001$, corrected for cluster level) are given in Table 2. B, Locations of clusters with significant negative correlations between Aggression Questionnaire scores and [^{11}C](+)McN-5652 DVs in methamphetamine abusers ($P<.05$, corrected for voxel level) (Table 3). Each top row shows 3-dimensional glass brain views; each bottom row, detected area superimposed onto normal template magnetic resonance images.

amphetamine, and their histories were retrospectively confirmed by the abusers and their family members through detailed Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition–*

Table 2. Voxel-Based Analysis of Regional Brain [^{11}C](+)McN-5652 Distribution Volume Reductions in 12 Methamphetamine Abusers Compared With 12 Control Subjects*

	Cluster-Level Analysis		Voxel-Level Analysis		Talairach Coordinates		
Location	Corrected *P* Value	Voxels, No.	Corrected *P* Value	*z* Score	x	y	z
Right insular cortex	<.001	45 315	.009	5.33	34	13	−4
Left caudate nucleus	NA	NA	.02	5.12	−10	19	−4
Right claustrum	NA	NA	.03	5.02	32	0	−3

Abbreviations: [^{11}C](+)McN-5652, *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline; NA, not available.
*The significance threshold was $P<.05$ at the corrected voxel level and $P<.05$ at the corrected cluster level. Coordinates are given in millimeters from the origin at the midpoint of the anterior commissure for voxels of peak significance.

Table 3. Voxel-Based Analysis of Regional Brain [^{11}C](+)McN-5652 Distribution Volumes Negatively Associated With Aggression Questionnaire Scores in 12 Methamphetamine Abusers*

	Cluster-Level Analysis		Voxel-Level Analysis		Talairach Coordinates		
Location	Corrected *P* Value	Voxels, No.	Corrected *P* Value	*z* Score	x	y	z
Right orbitofrontal cortex	<.001	20	.007	5.49	6	26	−21
Left inferior temporal cortex	<.001	38	.007	5.48	−57	−30	−19
Left orbitofrontal cortex	.001	10	.02	5.17	−10	34	−24
Right anterior cingulate cortex	<.001	12	.03	5.13	10	49	10

Abbreviation: [^{11}C](+)McN-5652, *trans*-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline.
*The significance threshold was $P<.05$ at the corrected voxel level and $P<.05$ at the corrected cluster level. Coordinates are given in millimeters from the origin at the midpoint of the anterior commissure for voxels of peak significance.

based interviews. Furthermore, in this study, the severity of aggression clearly paralleled the decreases in serotonin transporter density in the brain, which in turn were found to be associated with the duration of methamphetamine use. Therefore, it seems unlikely that the increased aggression observed in these methamphetamine abusers reflected a preexisting disposition or personality trait.

Except for the scores on the AQ, none of the scores on the clinical rating scales for psychiatric symptoms were correlated with the decrease in serotonin transporter density. Methamphetamine has been reported to affect not only serotonergic neurons but also several other types of neurons, such as the dopaminergic, glutamatergic, and γ-aminobutyric acid (GABA)–ergic neurons, all of which have been implicated in the presence of a variety of psychiatric symptoms (eg, delusions, hallucinations, and anxiety).[63] It is possible that changes in various types of neurons might have affected or modified the clinical symptoms evaluated herein. Another plausible interpretation for the negative results is that, as seen in Table 1, the severity of most of the residual symptoms assessed in this study ranged from mild to moderate, and the variances of their distributions were relatively small; together, these factors may have biased the results toward the null hypothesis.

Herein, we recruited methamphetamine abusers from the community; they were recreational abusers of methamphetamine only, and none of them had used other illicit drugs or had taken toxic or high doses of methamphetamine. Although our strategy allowed us to evaluate the pure effects of methamphetamine on the human brain, the findings may not be generalized to the broad population of methamphetamine abusers. However, the combined use of methamphetamine with other illicit drugs is infrequent in Japan, as indicated by Japanese National Police Agency records in 2002.[64] One reason for this is that cannabis, cocaine, and major illicit drugs other than methamphetamine are not widely distributed in Japan.[64] Furthermore, a national survey of 233 methamphetamine abusers reported that only 2.6% of the abusers had undergone methamphetamine intoxication,[65] suggesting that abusers of an overdose of methamphetamine are rare in Japan. Consequently, our findings are considered to be fairly generalizable to the population of methamphetamine abusers, at least in Japan.

In this study, all the methamphetamine abusers exhibited some psychopathologic symptoms, even in an abstinent state. To our knowledge, no previous studies have examined the incidence of psychopathologic abnormalities in abstinent methamphetamine abusers recruited from the general community. In a study by Wada and Fukui,[65] who investigated the psychopathologic characteristics of 233 abstinent methamphetamine abusers recruited from hospitals in Japan (the period of abstinence exceeded 1 month; the mean ± SD duration of methamphetamine use was 11.1 ± 7.9 years), almost all the abusers exhibited some psychopathologic symptoms, such as auditory hallucinations, delusions of reference/persecution, mood disturbances, anxiety, insomnia, irritability, impulsivity, and personality changes, including the antisocial personality type. Such observations cannot be applied to absti-