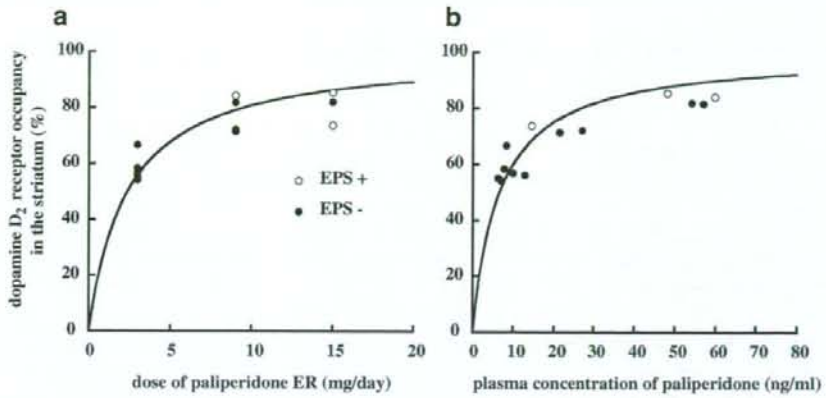

Fig. 1 Relationship between dopamine D_2 receptor occupancy in the striatum and dose (**a**) or plasma concentration (**b**) of paliperidone ER in the [^{11}C] raclopride study. ED_{50} in the striatum was 2.38 mg/day (r=0.86) and 6.65 ng/ml (r=0.82)

The dopamine D_2 receptor occupancy in the temporal cortex measured with [^{11}C]FLB 457 was 34.5 to 87.3%. Mean dopamine D_2 receptor occupancies were 53.1±14.5% at 3 mg/day, 76.2±9.5% at 9 mg/day, and 77.7±3.0% at 15 mg/day in the temporal cortex. ED_{50} in the temporal cortex was 2.84 mg/day (r=0.73) and 7.73 ng/ml (r=0.61; Fig. 2). There were no significant differences in plasma concentrations of paliperidone between the two scans (p= 0.24) and in dopamine D_2 receptor occupancy between the striatum and temporal cortex at any dose (p=0.30).

There were no correlations between striatal occupancy and age (p=0.07) or duration of illness (p=0.90).

Average PANSS scores of all patients were 62.9±16.5 before taking paliperidone ER and 58.5±16.8 after 6 weeks. Three patients, two taking 15 mg and one 9 mg (no. 10, 11, 12), showed EPS (Table 1).

Discussion

The present study demonstrated that the ED_{50} of striatal dopamine D_2 receptor occupancy of paliperidone ER was 2.38 mg/day and that of the temporal cortex was 2.84 mg/day. Previous studies reported that the striatal ED_{50} of risperidone was 1.2 mg/day (Nyberg et al. 1999) and that the limbic-cortical ED_{50} was 1.46 mg/day (Yasuno et al. 2001). These studies indicate that the equivalent ratio for a daily dose between risperidone and paliperidone ER seems to be about 1:2. The striatal and temporal ED_{50} values of plasma concentration of paliperidone were 6.65 and 7.73 ng/ml, respectively, almost matching the values previously reported for risperidone active moiety (6.87 ng/ml, Nyberg et al. 1999; 7.43 ng/ml, Yasuno et al. 2001) for striatal and limbic–cortical regions, respectively. The therapeutic dose ranges of paliperidone ER calculated from ED_{50} were 5.6–9.5 mg/day and 15.5–26.6 ng/ml. In two previous studies (Nyberg et al. 1999; Yasuno et al. 2001), the sum of risperidone and paliperidone was regarded as risperidone active moiety. Because paliperidone shows almost the same affinity for dopamine D_2 receptor as risperidone, the effect for dopamine D_2 receptor was about the same between risperidone active moiety and paliperidone. This suggests that similar dopamine D_2 receptor occupancy is achieved with comparable plasma concen-

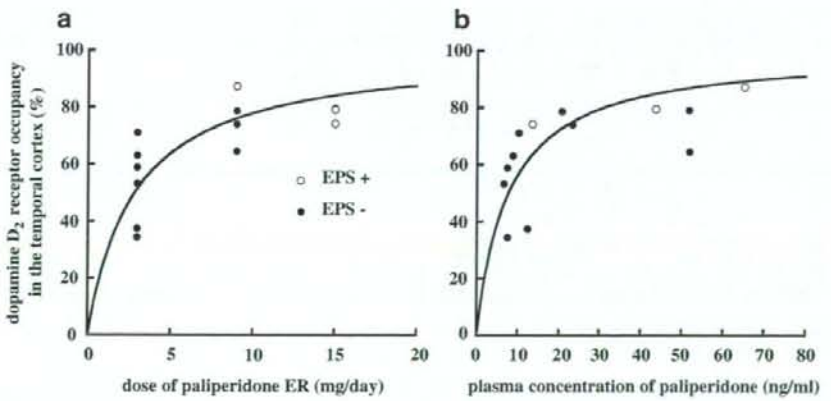

Fig. 2 Relationship between dopamine D_2 receptor occupancy in the temporal cortex and dose (**a**) or plasma concentration (**b**) of paliperidone ER in the [^{11}C]FLB 457 study. ED_{50} in the temporal cortex was 2.84 mg/day (r=0.73) and 7.73 ng/ml (r=0.61)

trations of paliperidone or risperidone active moiety. This finding confirms that paliperidone is as effective in crossing the blood–brain barrier as the active moiety of risperidone.

In the previous PET study that administered a single dose of paliperidone ER at 6 mg to four healthy Caucasian subjects, the striatal dopamine D_2 receptor occupancy fluctuation derived was 75–78%, and ED_{50} was 4.4 ng/ml (Karlsson et al., presented at WWS 2006). The differences between the two studies may be explained by the small number of observations and/or ethnicity. In the present study, occupancy was measured at steady-state drug levels (after multiple doses), whereas the previous study was carried out after a single dose.

There were no significant differences between striatal and extrastriatal dopamine D_2 receptor occupancy by paliperidone. Although the interval between the two scans was 2 h, the difference in plasma concentrations of paliperidone between them was about 7%, statistically not different as paliperidone ER tablets were made for flat plasma concentrations at a steady state. There have been discussions about the concept of 'limbic selectivity,' i.e., low dopamine D_2 receptor occupancy in the striatum and high occupancy in the extrastriatum (Pilowsky et al. 1997). It was reported in some second-generation antipsychotics such as clozapine (Grunder et al. 2006; Kessler et al. 2006; Pilowsky et al. 1997; Xiberas et al. 2001), olanzapine (Bigliani et al. 2000; Xiberas et al. 2001), amisulpiride (Bressan et al. 2003a; Xiberas et al. 2001), and quetiapine (Kessler et al. 2006; Stephenson et al. 2000) using [^{123}I] epidepride, [^{76}Br]FLB 457 or [^{18}F]fallypride. However, no significant difference between the striatum and extrastriatal regions have been reported using two different ligands, [^{11}C]raclopride and [^{11}C]FLB 457 (Agid et al. 2007; Talvik et al. 2001), or one ligand, [^{18}F]fallypride (Kessler et al. 2005). Human dopamine D_2 receptor occupancy by risperidone also showed inconsistent results. Two studies showed higher occupancy in the temporal cortex than in the striatum using [^{123}I]epidepride (75% in the temporal cortex and 50% in the striatum; Bressan et al. 2003b) and [^{76}Br] FLB 457 (91.6% in the temporal cortex and 63.3% in the striatum; Xiberas et al. 2001). On the other hand, similar occupancy values by risperidone were reported in the striatum (53–85%) using [^{11}C]raclopride (Nyberg et al. 1999) and extrastriatal regions (38–80%) using [^{11}C]FLB 457 (Yasuno et al. 2001). Because several factors such as scanning time, ligand selection, kinetic modeling, etc. need to be considered (Erlandsson et al. 2003; Olsson and Farde 2001), we used two different ligands to measure the different receptor density regions with appropriate scanning time and kinetic modeling for each ligand (Olsson and Farde 2001). Our results indicated no significant difference in regional occupancy (Agid et al. 2007; Kessler et al. 2005; Talvik et al. 2001; Yasuno et al. 2001). Although extrastriatal regions are suggested to be sites for antipsychotic action (Lidow et al. 1998), a recent study reported that extrastriatal dopamine D_2 receptor occupancy did not correlate with the antipsychotic effect (Agid et al. 2007).

In the present study, three patients complained of EPS. Average striatal occupancy of these three patients was 80.8%, a level in line with that known to increase the likelihood for EPS (Farde et al. 1992; Kapur et al. 2000; Nordstrom et al. 1993).

Previous studies indicated that over 70% of dopamine D_2 receptor occupancy is required for antipsychotic effects in patients with schizophrenia in the acute phase (Kapur et al. 2000; Nordstrom et al. 1993). In chronic treatment, haloperidol decanoate showed 73% occupancy at 1 week after injection and 52% occupancy at 4 weeks (Nyberg et al. 1995). Long-acting injectable risperidone showed 25–83 or 53–79% occupancy at a steady state (Gefvert et al. 2005; Remington et al. 2006). It is difficult to link the degree of dopamine D_2 receptor occupancy to a clinical effect, as almost all our patients (except nos. 5 and 11) had been undergoing long-term treatment when they entered the study. However, in all patients, these scores decreased with treatment or remained stable (Table 1) irrespective of dose. Furthermore, in all patients, striatal dopamine D_2 receptor occupancies above 50% were noted. This indicates that, for maintenance therapy of patients with schizophrenia, over 70% dopamine D_2 receptor occupancy might not necessarily be required. However, as this was an open-label study, further studies (such as randomized controlled trials) would be needed for an exact estimation of the threshold of dopamine D_2 receptor occupancy in the treatment of chronic patients with schizophrenia.

The half-life of paliperidone is about 28 h (data on file). High receptor occupancy is sustained when the plasma half-life of the treatment is long (Takano et al. 2004). Sustained high dopamine D_2 receptor occupancy can be expected at dosages of 9 or 15 mg/day of paliperidone ER. As EPS are a frequent reason for interruption of drug treatment (Lieberman et al. 2005), although the therapeutic dose range of paliperidone ER calculated from ED_{50} was 5.6–9.5 mg/day, for chronic treatment, lower doses might be useful, avoiding dopamine D_2 receptor occupancy rates above 80%. The estimated dopamine D_2 receptor occupancy at 6 mg/day of paliperidone ER was about 72%, in a range associated with efficacy (dopamine D_2 receptor occupancy above 70%) but not above a level associated with increased risks of extrapyramidal side effects (dopamine D_2 receptor occupancy above 80%).

To calculate the dopamine D_2 receptor occupancy in this study, we used BP_{ND} of normal control subjects as a surrogate for BP_{ND} in the drug-free state. Although previous studies showed no difference in dopamine D_2 receptor density in the striatum (Farde et al. 1990) or in the

temporal cortex (Suhara et al. 2002; Talvik et al. 2003) between the normal subjects and the patients with schizophrenia, individual differences in dopamine D_2 receptor density might potentially lead to an error in the estimation of dopamine D_2 receptor occupancy (Farde et al. 1992). For example, if BP_{base} changes from −13% to +15%, the range of the present study, the calculated 50% occupancy could be changed from 43 to 57%. The effect of a small portion of displaceable binding in the cerebellum (Delforge et al. 2001; Hall et al. 1996) may lead to an underestimation from 50% of [^{11}C]FLB 457 occupancy to 46% (Olsson et al. 2004). These factors may explain the differences in dopamine D_2 receptor occupancy between the striatum and temporal cortex in some patients.

Conclusions

The data from this study suggest that paliperidone ER at 6–9 mg provides an estimated level of dopamine D_2 receptor occupancy between 70–80%. The magnitude of dopamine D_2 receptor occupancy is similar between the striatum and temporal cortex.

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$GABA_A$/Benzodiazepine receptor binding in patients with schizophrenia using [^{11}C]Ro15-4513, a radioligand with relatively high affinity for α5 subunit

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Abstract

Dysfunction of the GABA system is considered to play a role in the pathology of schizophrenia. Individual subunits of $GABA_A$/Benzodiazepine (BZ) receptor complex have been revealed to have different functional properties. α5 subunit was reported to be related to learning and memory. Changes of α5 subunit in schizophrenia were reported in postmortem studies, but the results were inconsistent. In this study, we examined $GABA_A$/BZ receptor using [^{11}C]Ro15-4513, which has relatively high affinity for α5 subunit, and its relation to clinical symptoms in patients with schizophrenia.

[^{11}C]Ro15-4513 bindings of 11 patients with schizophrenia (6 drug-naïve and 5 drug-free) were compared with those of 12 age-matched healthy control subjects using positron emission tomography. Symptoms were assessed using the Positive and Negative Syndrome Scale. [^{11}C]Ro15-4513 binding was quantified by binding potential (BP) obtained by the reference tissue model. [^{11}C]Ro15-4513 binding in the prefrontal cortex and hippocampus was negatively correlated with negative symptom scores in patients with schizophrenia, although there was no significant difference in BP between patients and controls. $GABA_A$/BZ receptor including α5 subunit in the prefrontal cortex and hippocampus might be involved in the pathophysiology of negative symptoms of schizophrenia.

Keywords: γ-Amino-butyric acid; Schizophrenia; Negative symptoms; Prefrontal cortex; Hippocampus; PET

1. Introduction

γ-Amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system.

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$GABA_A$/Benzodiazepine (BZ) receptors are heteropentamiric GABA-gated chloride channels, and mediate fast synaptic inhibition (Moss and Smart, 2001). Benzodiazepines enhance the action of the neurotransmitter GABA at $GABA_A$/BZ receptors by interaction with their modulatory benzodiazepine sites.

Dysfunction of GABA neurotransmission in the brain is thought to play a role in the pathology of schizophrenia (Simpson et al., 1989; Reynolds et al., 1990). Postmortem studies using [^{3}H]muscimol showed that binding was increased in the hippocampal formation (Benes et al., 1996a), anterior cingulate cortex (Benes et al., 1992) and prefrontal cortex (Benes et al., 1996b; Dean et al., 1999) in patients with schizophrenia. The axon terminals of chandelier GABA neurons are reported to be reduced substantially in the middle layers of the prefrontal cortex in schizophrenia (Lewis et al., 1999).

$GABA_A$/BZ receptor chloride channel complex consists of two α subunits, two β subunits and one γ subunit (Barnard et al., 1998; Lüddens et al., 1995; Mehta and Ticku, 1999). It has been reported that the diversity of α subunits is responsible for various functional properties and ligand selectivity to the $GABA_A$/BZ receptor (Barnard et al., 1998; Low et al., 2000; Mehta and Ticku, 1999; Tobler et al., 2001). α1 subunit has been suggested to be related to hypnotic and sedative amnesic actions, whereas α2, α3 and α5 subunits to anxiolytic, anticonvulsant, and antipsychotic actions, and to the function of learning and memory (Crestani et al., 2001; Mohler et al., 2001; Serwanski et al., 2006).

Alterations in individual subunits of $GABA_A$/BZ receptor in schizophrenia have been the focus of recent postmortem studies. Expression of α1 subunit was reported to increase in the prefrontal cortex of patients with schizophrenia (Ohnuma et al., 1999; Ishikawa et al., 2004), α2 subunit was reported to increase in the prefrontal cortex (Volk et al., 2002), and α5 subunit expression was reported to show no significant change (Akbarian et al., 1995) or increase (Impagnatiello et al., 1998).

Several ligands such as [^{11}C]flumazenil and [^{11}C]Ro15-4513 were developed to visualize $GABA_A$/BZ receptors by positron emission tomography (PET) (Inoue et al., 1992; Halldin et al., 1992; Pappata et al., 1988). Both [^{11}C]flumazenil and [^{11}C]Ro15-4513 have the imidazobenzodiazepine core structure. However, flumazenil is a $GABA_A$/BZ receptor antagonist while Ro15-4513 is known as a $GABA_A$/BZ receptor partial inverse agonist. A different distribution pattern has been reported for the binding of [^{11}C]Ro15-4513 compared to that of [^{11}C]flumazenil (Inoue et al., 1992; Halldin et al., 1992). Ro15-4513 was reported to have relatively higher affinity for the α5 subunit-containing $GABA_A$/BZ receptor *in vitro* (Lüddens et al., 1994; Wieland and Lüddens, 1994). [^{11}C]Ro15-4513 bindings in the cingulate and temporal cortical regions showed relatively higher binding to α5 subunit of $GABA_A$ receptor (Lingford-Hughes et al., 2002; Maeda et al., 2003).

A simplified method without arterial blood sampling for [^{11}C]Ro15-4513 in the living human brain has been evaluated recently, and it can be used in clinical studies (Asai et al., in press).

In this study, we measured [^{11}C]Ro15-4513 binding to examine $GABA_A$/BZ receptors with α5 subunit and their relation to clinical symptoms in patients with schizophrenia.

2. Methods and materials

2.1. Subjects

Eleven patients with schizophrenia (5 women, 6 men; 32.8±10.2 years old, mean±SD) meeting DSM-IV criteria for schizophrenia or schizophreniform disorder were enrolled in this study. Demographic and clinical data on subjects are shown in Table 1. Six of the patients (3 women, 3 men; 29.2±7.3 years old) were neuroleptic-naïve and five (2 women, 3 men; 37.2±12.2 years old) had been neuroleptic-free for at least one year before the PET measurement except one subject who took

Table 1
Demographic and clinical characteristics asstudy entry

	N	Age (years)	Male/female	Duration of illness (months)	Schizophrenia/schizophreniform	PANSS			
						Positive	Negative	General	Total
Patient	11	32.8±10.2	6/5	1–444	9/3	24.4±5.1	21.4±6.0	44.6±10.2	90.4±19.6
Drug-naïve	6	29.2±7.3	3/3	1–36	3/3	24.8±3.9	20.3±8.0	45.3±12.0	90.5±23.0
Drug-free	5	37.2±12.2	3/2	24–444	6/0	23.8±6.8	22.6±2.5	43.8±8.8	90.2±17.4
Normal controls	12	29.0±10.2	12/0	–	–	–	–	–	–

neuroleptics two weeks before the PET measurement. Three neuroleptic-naïve patients satisfying criteria for schizophreniform disorder (duration of illness 1 to 4 months at the time of PET measurement) met criteria for schizophrenia at 6-month follow-up. The patients were recruited from the outpatient units of university-affiliated psychiatric hospitals, psychiatric divisions of general hospitals, and a mental clinic in the urban environments of Tokyo and Chiba prefectures in Japan. Exclusion criteria were current or past substance or cannabis or alcohol abuse, mood disorders, organic brain disease, and medication of antipsychotics, antidepressants, or benzodiazepines or mood stabilizers within two weeks before PET measurement. Five out of 11 subjects were smokers.

Psychopathology was assessed by the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). PANSS was completed by three experienced psychiatrists on the same day as PET measurements was performed. They reviewed the ratings after the interviews, and disagreements were resolved by consensus; the consensus ratings were used in this study. The symptom scores were calculated as the total scores, positive symptom, negative symptom, and general symptom subscores of PANSS. The total PANSS score ranged from 60 to 124 (90.4±19.6, mean±SD), mean positive symptom scores were 24.4±5.1, negative symptom scores were 21.4±6.0, and general symptom scores were 44.6±10.2.

Normal control subjects (12 men, 29.0±10.2 years old) were recruited through notices on bulletin boards at the universities and among the staffs of the affiliated hospitals where the patients had been diagnosed. None of the controls had a history of psychiatric or neurological illness, brain injury, chronic somatic illness, or substance abuse. None had taken any drug including benzodiazepines within two weeks before PET measurements. Seven out of 12 subjects were smokers. All the subjects were examined by T1-weighted magnetic resonance image (MRI) using 1.5 T Philips Gyroscan NT to rule out organic brain diseases. This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from all subjects.

2.2. PET measurement

[^{11}C]Ro15-4513 was synthesized by *N*-methylation of a corresponding *N*-desmethyl precursor with [^{11}C] methyl iodide. The reaction mixtures were purified by liquid chromatography, eluted with CH_3CN/6mM-phosphoric acid=175/325. The radiochemical purities were more than 95%.

The PET system used was ECAT EXACT HR+(CTI-Siemens, Knoxville, TN, USA), which provides 63 planes and a 15.5-cm field of view and was used in 3-dimensional mode. After a 10-minute transmission scan, a bolus of 352.3±66.9 MBq (mean±SD) of [^{11}C] Ro15-4513 with high specific radioactivities (103.4±38.9 GBq/μmol; mean±SD) was injected into the antecubital vein with a 20-ml saline flush. Radioactivity in the brain was measured in a series of sequential frames up to 60 min (total 28 frames).

2.3. PET data analysis

All emission scans were reconstructed with a Hanning filter cut-off frequency of 0.4 (FWHM 7.5 mm). Regions-of-interest (ROIs) were delineated on PET/MRI coregistered images for ten target regions (anterior cingulate, hippocampus, amygdala, thalamus, temporal cortex, prefrontal cortex, insula, caudate, putamen, cerebellum) and the pons as a reference region. Regional binding potentials were calculated using a simplified reference tissue model (SRTM)(Lammertsma and Hume, 1996). In brief, based on the three-compartment model, regional radioactivities in a target region (C_T) can be described by the following equation:

$$C_T(t) = R_1 C_R(t) + (k_2 - R_1\theta_3) C_R(t) {}^{*} e^{-\theta_3 t},$$

where C_R represents the radioactivity in the reference region, R_1 is the ratio of K_1 in a target region to the reference region, $\theta_3 = k_2/(1+BP)$, K_1 and k_2 are rate constants corresponding to the influx and efflux rates from plasma to the tissue compartments, and * is the

Table 2
Binding potentials for regions of interest

	BP values		*T* test (df=21)	
	Controls (*N*=12)	Patients (*N*=11)	*T* score	*p*
Anterior cingulated	6.08±0.72	6.14±0.63	−0.213	0.833
Hippocampus	5.43±0.77	4.95±0.80	1.432	0.167
Amygdala	5.49±0.56	5.25±0.48	1.118	0.276
Thalamus	2.00±0.28	1.83±0.24	1.534	0.14
Temporal cortex	4.20±0.52	4.12±0.38	0.438	0.666
Prefrontal cortex	3.60±0.35	3.59±0.34	0.09	0.929
Insula	5.79±0.63	5.56±0.46	1.011	0.324
Caudate	2.99±0.43	3.32±0.81	−1.199	0.249
Putamen	2.86±0.36	3.10±0.45	−1.445	0.165
Cerebellum	1.32±0.25	1.34±0.23	0.148	0.883

Values are mean±SD.

convolution operator. In this study, the pons was chosen as the reference tissue because this region is almost devoid of $GABA_A$/BZ receptor complex (Abadie et al., 1992).

2.4. Statistical analysis

Statistical analysis of the difference of regional BP or R_1 for each ROI between patients and controls was performed by repeated measures analysis of variance (ANOVA). When any interaction was found, post hoc Bonferroni correction was used for multiple comparisons. $p<0.05$ was considered significant.

Correlations between regional BP and PANSS scores were analyzed with Pearson's correlation method. $p<0.05$ was considered significant.

3. Results

Regarding regional BP values of [^{11}C]Ro15-4513, two-way repeated ANOVA revealed significant group-region interaction [$F_{4.3,90.6}=2.6$, $p=0.037$]. However, post hoc Bonferroni correction showed no significant differences of BPs for 10 ROIs between patients and controls (Table 2). As for R_1 values, two-way repeated ANOVA revealed no significant main effect of the groups [$F_{4.9,103.9}=1.613$, $p=0.164$] nor group-region interaction [$F_{1,21}=1.532$, $p=0.229$].

For the reference tissue, time activity curves of the pons between patients with schizophrenia and controls were compared with repeated-measures ANOVA with Green–Geisser correction. There was no significant main effect of groups [$F_{1,21}=1.027$, $p=0.323$] or no significant group by time interaction [$F_{2.09,43.9}=0.203$, $p=0.826$].

Regarding the relation to clinical symptoms, there were significant negative correlations between [^{11}C]Ro15-4513 binding in the prefrontal cortex and negative symptom scores ($R=-0.733$, $p=0.010$)(Fig. 1A), general symptom scores ($R=-0.655$, $p=0.029$) (Fig. 1C), and total PANSS scores ($R=-0.690$, $p=0.019$) (Fig. 1D). There was also a negative correlation between [^{11}C]Ro15-4513 binding in the hippocampus and negative symptom scores ($R=-0.605$, $p=0.048$) (Fig. 1B). No other regions

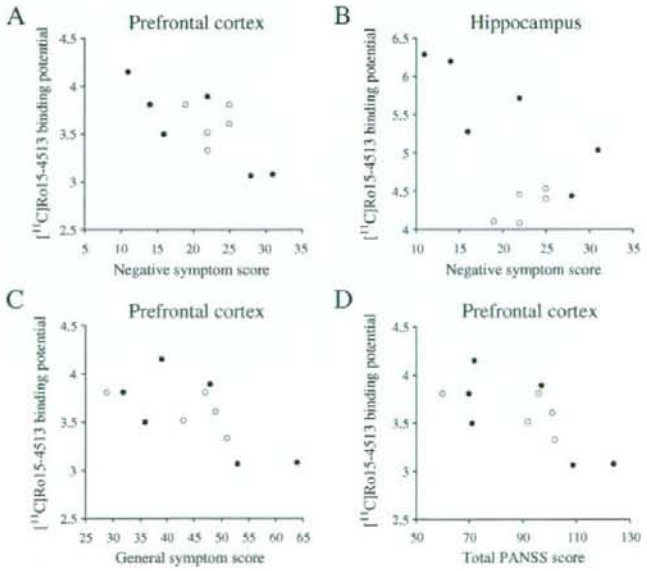

Fig. 1. Relationship between regional [^{11}C]Ro15-4513 binding potentials and PANSS scores in 11 patients with schizophrenia. Filled circles indicate neuroleptic-naïve patients ($N=6$). Open circles indicate drug-free patients ($N=5$). Total PANSS scores consist of positive symptom scores, negative symptom scores, and total symptom scores. There were significant negative correlations between [^{11}C]Ro15-4513 binding in the prefrontal cortex and negative symptom scores ($R=-0.733$, $p=0.010$)(A), general symptom scores ($R=-0.655$, $p=0.029$) (C), and total PANSS scores ($R=-0.690$, $p=0.019$) (D). There was also a negative correlation between [^{11}C]Ro15-4513 binding in the hippocampus and negative symptom scores ($R=-0.605$, $p=0.048$) (B).

Table 3
Correlation between regional [11C]Ro15-4513 binding potentials and PANSS scores

Region	Positive symptoms		Negative symptoms		General symptoms		Total scores	
	R	*p*	*R*	*p*	*R*	*p*	*R*	*p*
Anterior cingulate	−0.123	0.718	−0.312	0.350	−0.079	0.817	−0.169	0.620
Hippocampus	−0.008	0.982	−0.605	0.048*	−0.221	0.513	−0.302	0.367
Amygdale	−0.394	0.231	−0.307	0.359	−0.282	0.401	−0.343	0.302
Thalamus	−0.298	0.373	−0.163	0.633	0.005	0.987	−0.125	0.714
Temporal cortex	−0.415	0.205	−0.594	0.054	−0.564	0.070	−0.583	0.060
Prefrontal cortex	−0.485	0.131	−0.733	0.010*	−0.655	0.029*	−0.690	0.019*
Insula	−0.146	0.668	−0.541	0.085	−0.281	0.403	−0.349	0.292
Caudate	−0.164	0.630	−0.118	0.729	0.031	0.929	−0.063	0.854
Putamen	−0.383	0.245	−0.287	0.393	−0.184	0.587	−0.283	0.398
Cerebellum	0.057	0.868	−0.120	0.725	−0.010	0.976	−0.027	−0.937

showed significant correlation with clinical symptom scores (Table 3).

4. Discussion

In this study, significant negative correlation between clinical symptoms (especially negative symptoms) and $GABA_A$/BZ receptor binding in the prefrontal cortex (Fig. 1A, C, D) and the hippocampus (Fig. 1B) of the patients with schizophrenia was found. The significant relation between $GABA_A$/BZ receptor binding and clinical symptoms would suggest dysfunctions of the GABA system in schizophrenia.

Our results showed no significant difference of $GABA_A$/BZ receptor binding between patients with schizophrenia and controls (Table 2). This is consistent with some of the previous postmortem studies (Akbarian et al., 1995; Impagnatiello et al., 1998). However, inconsistent results have also been reported (Benes et al., 1996a, 1996b; Dean et al., 1999). Inconsistency can be attributed to methodological differences between PET study and postmortem study, as well as to the effects of prolonged antipsychotic and benzodiazepine administration. None of the patients in this study had taken any antipsychotics or benzodiazepines for at least two weeks before PET measurement. On the other hand, most of the subjects investigated in the postmortem studies had taken antipsychotics and/or benzodiazepines on a long-term basis. Recently, it was suggested from an animal experiment that antipsychotic drug administration would result in a "reshuffling" of $GABA_A$ receptor subtypes (Skilbeck et al., 2007).

Although there was no significant difference in [^{11}C] Ro15-4513 binding between patients and controls, [^{11}C] Ro15-4513 binding was found to be negatively correlated with clinical symptom scores. Although some previous SPECT studies using [^{123}I]iomazenil showed no significant difference of benzodiazepine binding between patients and controls (Abi-Dargham et al., 1999, Verhoeff et al., 1999), some reported that there were significant negative correlations between benzodiazepine binding and the severity of negative symptoms (Busatto et al., 1997), or cognitive impairment (Ball et al., 1998) in patients with schizophrenia. Our results were consistent with those studies, despite [^{11}C]Ro15-4513 having relatively high affinity for α5 subunit of $GABA_A$/BZ receptor while [^{123}I]iomazenil binds to $GABA_A$/BZ receptor non-selectively.

α5 subunit-containing $GABA_A$ receptors are reported to be concentrated in the apical dendrites of pyramidal neurons (Akbarian et al., 1995). In a postmortem study, α2 subunit of GABA in the axonal initial segment of pyramidal neurons was reported to be increased in patients with schizophrenia (Volk et al., 2002). The expression of subunits of $GABA_A$/BZ receptor was reported to be changed following chronic administration of phencyclidine, which induces schizophrenia-like symptoms in rats (Abe et al., 2000). Combining our results with these reports, the imbalance among α subunits in pyramidal neurons could be expected in patients with schizophrenia.

Dopamine receptors in the prefrontal cortex have been suggested to be involved in the pathophysiology of schizophrenia. Dopamine D1 receptor plays a key role in negative symptoms and cognitive dysfunctions of schizophrenia (Abi-Dargham et al., 2002; Okubo et al., 1997). Reduced prefrontal pyramidal neuron output could change the activity of dopamine neurons in the prefrontal cortex in schizophrenia (Lewis and Gonzalez-Burgos, 2006). The possible change of α5 subunit in the prefrontal cortex might cause the change of pyramidal neuron output, which might interact with dopamine D1 receptor.

Not only the prefrontal cortex but also the hippocampus was found to be correlated negatively with negative symptoms of patients with schizophrenia in this study (Fig. 1B). Hippocampal-dependent spatial learning was improved in α5 subunit of $GABA_A$ receptor-knockout mice (Collinson et al., 2002), or by systemic treatment of an inverse agonist selective for α5 $GABA_A$ receptors (Chambers et al., 2003). The change of α5 subunit of $GABA_A$ receptors in the prefrontal cortex in patients with schizophrenia might affect hippocampal function because of the plastic neuronal connections between the hippocampus and prefrontal cortex (Goldman-Rakic et al., 1984; Laroche et al., 2000; Maccotta et al., 2007; Tierney et al., 2004; Takahashi et al., 2007).

There has been some interest in treating negative symptoms and cognitive dysfunctions in schizophrenia with GABA-modulating drugs (Guidotti et al., 2005; Lewis et al., 2004; Menzies et al., 2007). Imidazenil, which selectively allosterically modulates cortical $GABA_A$ receptors containing α5 subunit, was reported to contribute to amelioration of the behavioral deficits without producing sedation or tolerance liability in mice (Guidotti et al., 2005), and it increased locomotor activity in a social isolation mouse model (Pinna et al., 2006).

There were several limitations to this preliminary study. The number of subjects was small, and five of the eleven patients were previously treated. Further study would be needed with a larger population of drug-naïve patients. Although age correction was not performed, we previously reported no significant age effect of [^{11}C] Ro15-4513 binding (Suhara et al., 1993). We also compared with age-matched subgroup of drug naïve paitents (N=6) with controls (N=12) and two-way repeated ANOVA revealed no significant group-region interaction of [^{11}C]Ro15-4513 binding.

Sex was not matched between patients and controls, but sex differences of [^{11}C]Ro15-4513 binding have not been reported.

In conclusion, the present study showed that [^{11}C] Ro15-4513 binding was negatively correlated with negative symptom scores in schizophrenia. $GABA_A$/BZ receptor including α5 subunit might be involved in the pathophysiology of schizophrenia with negative symptoms.

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Contributors

Y Asai, T Takano and T Suhara designed the study and wrote the protocol. Y Okubo, M Matsuura, A Otsuka, H Takahashi, T Ando, and S Ito recruited the subjects and made psychiatric evaluations. Y Asai, T Takano, and R Arakawa performed the data analysis. Y Asai wrote the first draft of the manuscript. H Ito gave fruitful comments to finalize the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All the authors have no conflict of interest.

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Normal database of dopaminergic neurotransmission system in human brain measured by positron emission tomography

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The central dopaminergic system is of interest in the pathophysiology of schizophrenia and other neuropsychiatric disorders. Both pre- and postsynaptic dopaminergic functions can be estimated by positron emission tomography (PET) with different radiotracers. However, an integrated database of both pre- and postsynaptic dopaminergic neurotransmission components including receptors, transporter, and endogenous neurotransmitter synthesis has not yet been reported. In the present study, we constructed a normal database for the pre- and postsynaptic dopaminergic functions in the living human brain using PET. To measure striatal and extrastriatal dopamine D_1 and D_2 receptor bindings, dopamine transporter binding, and endogenous dopamine synthesis rate, PET scans were performed on healthy men after intravenous injection of [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, or L-[β-^{11}C]DOPA. All PET images were anatomically standardized using SPM2, and a database was built for each radiotracer. Gray matter images were segmented and extracted from all anatomically standardized magnetic resonance images using SPM2, and they were used for partial volume correction. These databases allow the comparison of regional distributions of striatal and extrastriatal dopamine D_1 and D_2 receptors, dopamine transporter, and endogenous dopamine synthesis capability. These distributions were in good agreement with those from human postmortem studies. This database can be used in various researches to understand the physiology of dopaminergic functions in the living human brain. This database could also be used to investigate regional abnormalities of dopaminergic neurotransmission in neuropsychiatric disorders.

Introduction

The central dopaminergic system is of major interest in the pathophysiology of schizophrenia and other neuropsychiatric disorders. Both pre- and postsynaptic dopaminergic functions can be estimated by positron emission tomography (PET) and single-photon emission computed tomography (SPECT) with the use of several radiotracers. The binding of dopamine receptors representing postsynaptic functions can be measured for each of D_1 and D_2 subtypes. For measurement of dopamine D_1 receptor binding, [^{11}C]SCH23390 (Farde et al., 1987a; Halldin et al., 1986) and [^{11}C]NNC112 (Halldin et al., 1998) are widely used. To measure the binding of striatal and extrastriatal dopamine D_2 receptors, which are quite different in densities, [^{11}C]raclopride (Farde et al., 1985; Ito et al., 1998; Kohler et al., 1985) and [^{11}C]FLB457 (Halldin et al., 1995; Ito et al., 2001; Suhara et al., 1999), respectively, are widely used. The bindings of striatal (Farde et al., 1987b, 1990; Nordstrom et al., 1995) and extrastriatal (Suhara et al., 2002; Yasuno et al., 2004) dopamine D_2 receptors in schizophrenia have been investigated. For estimation of the presynaptic dopaminergic function, dopamine transporter binding is measured by [^{11}C]β-CIT (Farde et al., 1994; Muller et al., 1993), [^{11}C]PE2I (Emond et al., 1997; Hall et al., 1999), and other radioligands. The endogenous dopamine synthesis rate measured by 6-[^{18}F]fluoro-L-DOPA (Gjedde, 1988; Gjedde et al., 1991; Huang et al., 1991) and L-[β-^{11}C]DOPA (Hartvig et al., 1991; Tedroff et al., 1992) can also indicate the presynaptic dopaminergic function. Dopamine transporter binding (Laakso et al., 2000; Laruelle et al., 2000) and the endogenous dopamine synthesis rate (Hietala et al., 1995; Laruelle, 1998; Lindstrom et al., 1999; Reith et al., 1994) in schizophrenia have been investigated.

An anatomic standardization technique, consisting of the transformation of brain images of individual subjects into a standard brain shape and size in three dimensions, allows inter-subject averaging of PET images (Fox et al., 1988; Friston et al., 1990). Using this technique with calculation of PET parametric images, a database of the regional distribution of neurotransmission functions can be constructed, and from this, group comparisons between normal control subjects and patients on a voxel-by-voxel basis can be performed. Previously, we built a normal database for the striatal and extrastriatal dopamine D_2 receptor bindings representing the postsynaptic dopaminergic neurotransmission components in the living human brain (Ito et al., 1999; Okubo et al., 1999). The in vivo regional distribution of dopamine D_2 receptor binding was in good agreement with that known from in vitro studies. For presynaptic dopaminergic function, a normal database for the endogenous dopamine synthesis rate in the living human brain has also been constructed with the use of 6-[^{18}F]fluoro-L-DOPA (Nagano et al.,

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2000). However, an integrated database for both pre- and postsynaptic dopaminergic neurotransmission components including receptors, transporter, and endogenous neurotransmitter synthesis, which allows to compare regional distributions between neurotransmission components on a same coordinate, has not been reported. In the present study, we constructed a normal database for pre- and postsynaptic dopaminergic neurotransmission components in the living human brain using PET and the anatomic standardization technique. Striatal and extrastriatal dopamine D_1 and D_2 receptor bindings, dopamine transporter binding, and endogenous dopamine synthesis rate were measured in healthy volunteers using the radiotracers [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA.

Materials and methods

Subjects

The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. A total of 37 healthy men were recruited, and they gave their written informed consent for participation in the study (Table 1). The subjects were free of somatic, neurological or psychiatric disorders on the basis of their medical history and magnetic resonance (MR) imaging of the brain. They had no history of current or previous drug abuse and had not taken dopaminergic drugs in the past two weeks. Both PET studies with [^{11}C]raclopride and [^{11}C]FLB457 were performed in the same subjects on the same day. For [^{11}C]SCH23390, [^{11}C]PE2I, and L-[β-^{11}C]DOPA studies, each subject underwent only one PET study except three subjects: two underwent L-[β-^{11}C]DOPA, [^{11}C]raclopride, and [^{11}C]FLB457 studies; one underwent L-[β-^{11}C]DOPA and [^{11}C]SCH23390 studies. L-[β-^{11}C]DOPA studies were conducted without L-DOPA decarboxylase inhibitor premedication.

PET procedures

All PET studies were performed with a Siemens ECAT Exact HR+ system, which provides 63 sections with an axial field of view of 15.5 cm (Brix et al., 1997). The intrinsic spatial resolution was 4.3 mm in-plane and 4.2 mm full-width at half maximum (FWHM) axially. With a Hanning filter (cutoff frequency: 0.4 cycle/pixel), the reconstructed in-plane resolution was 7.5 mm FWHM. Data were acquired in three-dimensional mode. Scatter was corrected (Watson et al., 1996). A head fixation device with thermoplastic attachments for individual fit minimized head movement during PET measurements. A 10-min transmission scan using a ^{68}Ge-^{68}Ga line source was performed for correction of attenuation. After intravenous rapid bolus injection of [^{11}C]raclopride, [^{11}C]FLB457, or [^{11}C]PE2I, data were acquired for 90 min in a consecutive series of time frames. For [^{11}C]SCH23390 and L-[β-^{11}C]DOPA studies, data were acquired for 60 and 89 min after intravenous rapid bolus injection, respectively. The frame sequence consisted of twelve 20-s frames, sixteen 1-min frames, ten 4-min frames, and five 6-min frames for [^{11}C]raclopride, and nine 20-s frames, five 1-min frames, four 2-min frames, eleven 4-min frames, and six 5-min frames for [^{11}C]FLB457 and [^{11}C]PE2I. For [^{11}C]SCH23390, the frame sequence consisted of thirty 2-min frames. The frame sequence for L-[β-^{11}C]DOPA studies consisted of seven 1-min frames, five 2-min frames, four 3-min frames, and twelve 5-min. Injected radioactivity was 197–235 MBq, 213–239 MBq, 212–242 MBq, 197–230 MBq, and 320–402 MBq for [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA, respectively. Specific radioactivity was 23–81 GBq/μmol, 133–285 GBq/μmol, 72–371 GBq/μmol, 55–1103 GBq/μmol, and 29–82 GBq/μmol at the time of injection for [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA, respectively.

Table 1
Number of subjects per study and average age

Study	Number of subjects	Age (years, mean±SD)
[^{11}C]SCH23390	10	27.3±4.6
[^{11}C]raclopride	10	26.9±3.9
[^{11}C]FLB457	10	26.9±3.9
[^{11}C]PE2I	10	24.2±3.1
L-[β-^{11}C]DOPA	10	23.4±3.3

All subjects are male.
[^{11}C]raclopride and [^{11}C]FLB457 PET were conducted on same subjects. Two subjects underwent L-[β-^{11}C]DOPA, [^{11}C]raclopride, and [^{11}C]FLB457 studies; one underwent L-[β-^{11}C]DOPA and [^{11}C]SCH23390 studies.

MR imaging procedures

All MR imaging studies were performed with a 1.5-T MR scanner (Philips Medical Systems, Best, The Netherlands). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin transverse sections (TE: 9.2 ms; TR: 21 ms; flip angle: 30°; field of view: 256 mm; acquisition matrix: 256×256; slice thickness: 1 mm).

Calculation of parametric images

For PET studies with [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, and [^{11}C]PE2I, binding potential (BP) was calculated by the reference tissue model method on a voxel-by-voxel basis (Lammertsma et al., 1996; Lammertsma and Hume, 1996). By this method, the time–activity curve in the brain region is described by that in the reference region with no specific binding, assuming that both regions have the same level of nondisplaceable radioligand binding:

$$C_t(t) = R_1 \cdot C_r(t) + \{k_2 - R_1 \cdot k_2/(1+\mathrm{BP})\} \cdot C_r(t) \otimes \exp\{-k_2 \cdot t/(1+\mathrm{BP})\}.$$

where C_t is the radioactivity concentration in a brain region; $C_r(t)$ is the radioactivity concentration in the reference region. R_1 is the ratio of K_1/K_1' (K_1, influx rate constant for the brain region, K_1', influx rate constant for the reference region). k_2 is the efflux rate constant for the brain region, and ⊗ denotes the convolution integral. In this analysis, three parameters (BP, R_1, and k_2) were estimated by the basis function method (Cselenyi et al., 2006; Gunn et al., 1997). The cerebellum was used as a reference region. We used in-house written software to calculate parametric images.

For the L-[β-^{11}C]DOPA study, the dopamine synthesis index (I) was calculated on a voxel-by-voxel basis as follows (Dhawan et al., 2002; Hoshi et al., 1993; Ito et al., 2007):

$$I = \frac{\int_{t_1}^{t_2} C_t(t)\mathrm{d}t}{\int_{t_1}^{t_2} C_t'(t)\mathrm{d}t} - 1$$

where C_t is the radioactivity concentration in a brain region, and C_t' is the radioactivity concentration in a brain region with no

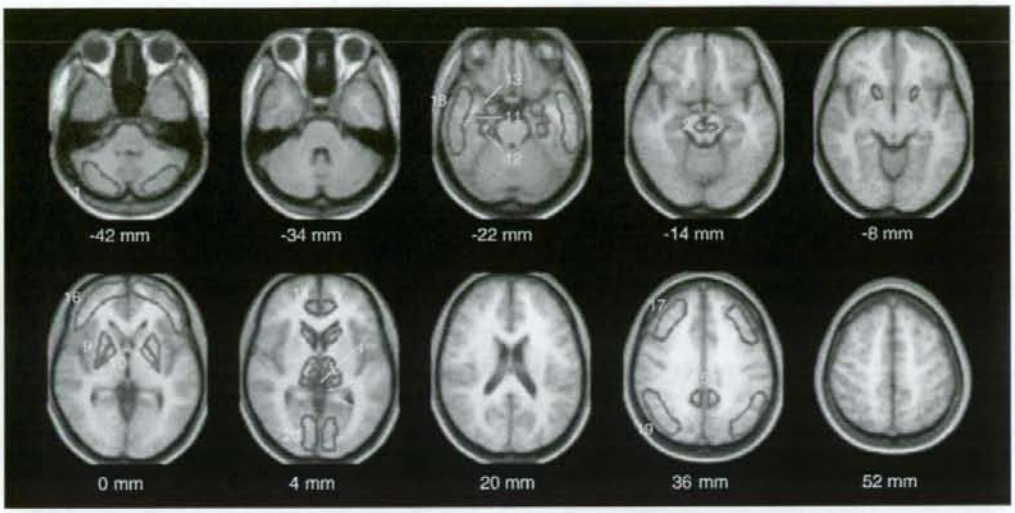

Fig. 1. Regions of interest drawn on all anatomically standardized images (1: cerebellum, 2: substantia nigra, 3: thalamus, 4: anterior nuclei, 5: dorsomedial nucleus, 6: pulvinar, 7: caudate head, 8: nucleus accumbens, 9: putamen, 10: globus pallidus, 11: hippocampus, 12: parahippocampal gyrus, 13: uncus, 14: anterior part of the cingulate gyrus, 15: posterior part of the cingulate gyrus, 16: base side of frontal cortex, 17: convexity side of frontal cortex, 18: lateral side of temporal cortex, 19: parietal cortex, 20: cuneus of occipital cortex). All images are transaxial sections parallel to the anterior-posterior commissure (AC–PC) line. The slice positions are −42, −34, −22, −14, −8, 0, 4, 20, 36 and 52 mm from the AC–PC line.

irreversible binding. The occipital cortex was used as a region with no irreversible binding, as this region is known to have the lowest dopamine concentration (Brown et al., 1979) and lowest aromatic L-amino acid decarboxylase activity (Lloyd and Hornykiewicz, 1972). Integration intervals (t_1 to t_2) of 29 to 89 min, representing late portions of the time–activity curves, were used (Ito et al., 2006b).

Data analysis

All MR images were coregistered to the PET images with the statistical parametric mapping (SPM2) system (Friston et al., 1990). MR images were transformed into the standard brain size and shape by linear and nonlinear parameters by SPM2 (anatomic standardization). The brain templates used in SPM2 for anatomic standardization were T1 templates for MR images, i.e., Montreal Neurological Institute (MNI)/International Consortium for Brain Mapping (ICBM) 152 T1 templates as supplied with SPM2. All PET images were also transformed into the standard brain size and shape by using the same parameters as the MR images. Thus, brain images of all subjects had the same anatomic format. Gray matter, white matter, and cerebrospinal fluid images were segmented and extracted from all anatomically standardized MR images by applying voxel-based morphometry methods with the SPM2 system (Ashburner and Friston, 2000). These segmented MR images indicate the tissue fraction of gray or white matter per voxel (mL/mL). All anatomically standardized PET, gray matter and white matter images were smoothed with an 8-mm FWHM isotropic Gaussian kernel, because final spatial resolution of PET camera was approximately 8 mm FWHM.

Regions of interest (ROIs) were drawn on all anatomically standardized PET, gray matter and white matter images with reference to the T1-weighted MR image (Fig. 1). ROIs were defined for the cerebellar cortex, substantia nigra with ventral tegmental area, thalamus and its subregions (anterior nuclei, dorsomedial nucleus, and pulvinar) (Okubo et al., 1999; Yasuno et al., 2004), caudate head, nucleus accumbens, putamen, globus pallidus, hippocampus, posterior part of parahippocampal gyrus, uncus including amygdala, anterior and posterior parts of the cingulate gyrus, base and convexity sides of frontal cortex, lateral side of temporal cortex, parietal cortex, and cuneus of occipital cortex,

Table 2
Representative MNI coordinates in ROIs drawn on anatomically standardized images

Region	Right			Left		
	X	*Y*	*Z*	*X*	*Y*	*Z*
Cerebellum	30	−74	−42	−30	−74	−42
Substantia nigra	6	−20	−14	−6	−20	−42
Thalamus	11	−18	4	−11	−18	4
AN	5	−12	4	−5	−12	4
DMN	8	−22	4	−8	−22	4
PUL	14	−29	4	−14	−29	4
Caudate head	12	16	4	−12	16	4
Nucleus accumbens	17	13	−8	−18	12	−8
Putamen	24	9	0	−24	7	0
Globus pallidus	19	0	0	−18	−2	0
Hippocampus	31	−12	−22	−30	−13	−22
Parahippocampal gyrus	28	−21	−22	−28	−23	−22
Uncus	21	−3	−22	−22	−2	−22
Anterior cingulate	7	44	4	−7	45	4
Posterior cingulate	8	−47	36	−8	−47	36
Frontal base	35	56	0	−34	56	0
Frontal convexity	35	31	36	−36	27	36
Lateral temporal cortex	59	−11	−22	−58	−13	−22
Parietal cortex	45	−62	36	−45	−65	36
Occipital cuneus	10	−81	4	−8	−81	4

AN: anterior nuclei, DMN: dorsomedial nucleus, PUL: pulvinar in the thalamus.

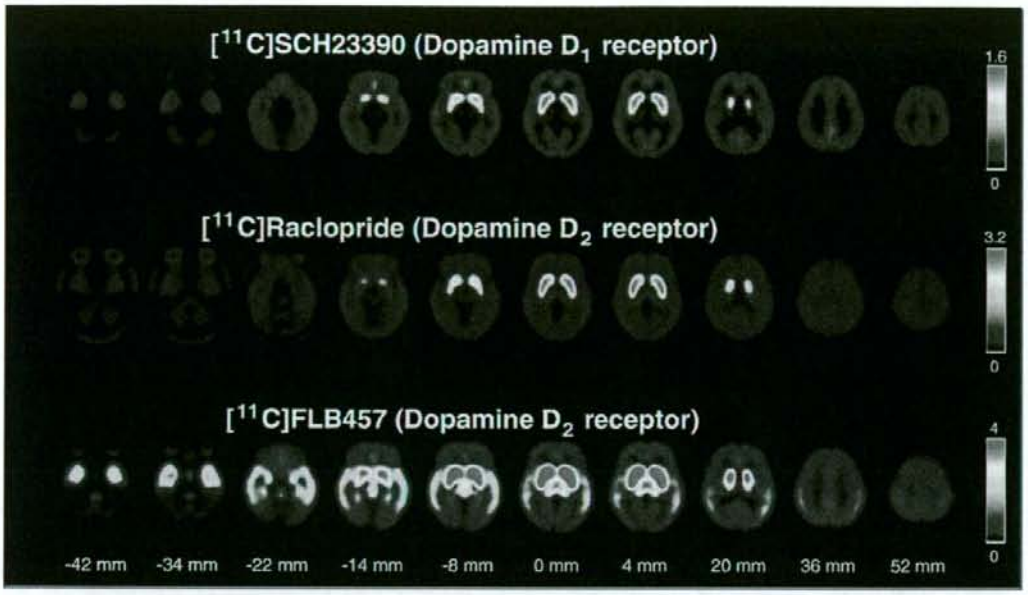

Fig. 2. Anatomically standardized averaged PET images obtained with [^{11}C]SCH23390, [^{11}C]raclopride, and [^{11}C]FLB457. All images are transaxial sections parallel to the AC–PC line. The slice positions are −42, −34, −22, −14, −8, 0, 4, 20, 36, and 52 mm from the AC–PC line. The anterior is at the top of the image and the subjects' right is at the left. Scale maximum and minimum values are 1.6 and 0 of BP for [^{11}C]SCH23390, 3.2 and 0 of BP for [^{11}C]raclopride, and 4 and 0 of BP for [^{11}C]FLB457, respectively.

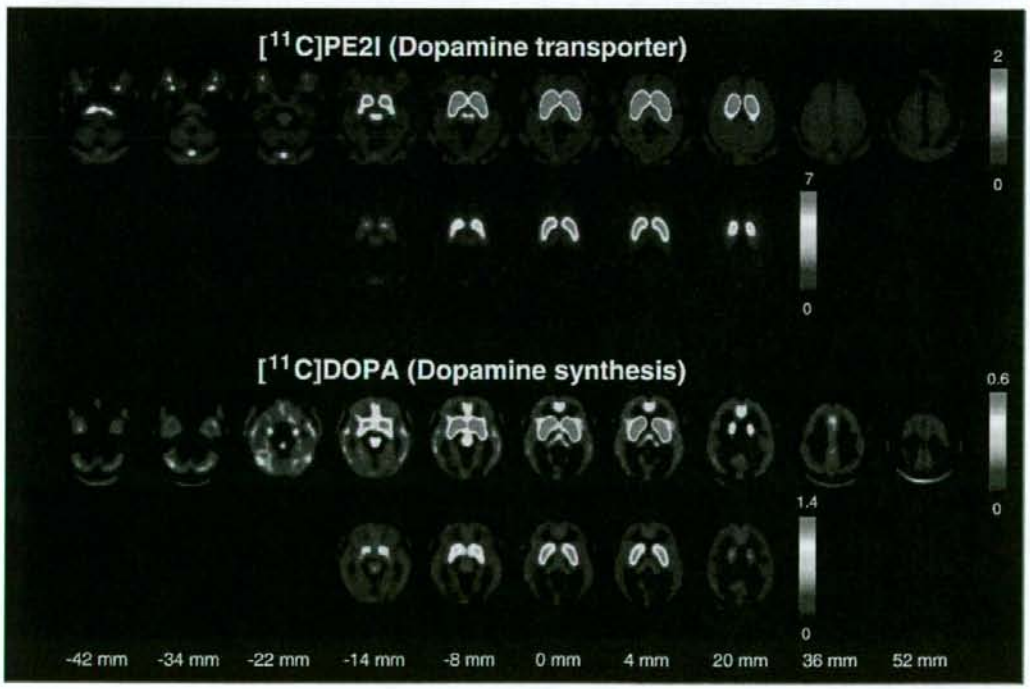

Fig. 3. Anatomically standardized averaged PET images obtained with [^{11}C]PE2I and L-[β-^{11}C]DOPA. All images are transaxial sections parallel to the AC-PC line. The slice positions are −42, −34, −22, −14, −8, 0, 4, 20, 36, and 52 mm from the AC–PC line. The anterior is at the top of the image and the subjects' right is at the left. Scale maximum and minimum values are 7 or 2 and 0 of BP for [^{11}C]PE2I, and 1.4 or 0.6 and 0 of *I* for L-[β-^{11}C]DOPA, respectively.

considering regional distribution of dopaminergic neurotransmission system. Representative MNI coordinates in ROIs (approximations of the arithmetic center of ROIs) are given in Table 2.

To compare BP and *I* values between tracers for each ROI, percentages of the putamen of BP or *I* were calculated for [^{11}C]SCH23390, [^{11}C]PE2I, and L-[β-^{11}C]DOPA, because the putamen showed highest BP and *I* values among all brain regions for all tracers. Since [^{11}C]FLB457 is not favorable for estimating striatal BP values, the percentage of BP of the putamen in the extrastriatal regions was calculated as the percentage of the putaminal BP values for [^{11}C]raclopride mediated with thalamic BP values:

$$\%\ \text{of putamen in extrastriatal regions} = \frac{\text{Extrastriatum(FLB)}}{\text{Putamen(Racl.)}} * \frac{\text{Thalamus(Racl.)}}{\text{Thalamus(FLB)}}$$

where Extrastriatum(FLB) is BP in the extrastriatal regions for [^{11}C]FLB457, Putamen(Racl.) is BP in the putamen for [^{11}C]raclopride, and Thalamus(Racl.) and Thalamus(FLB) are BP in the thalamus for [^{11}C]raclopride and [^{11}C]FLB457 studies, respectively. Although [^{11}C]raclopride binding in the extrastriatal regions is low (Farde et al., 1988), it has been reported that the specific binding of [^{11}C]raclopride in the thalamus was low but detectable (Ito et al., 1999). In these calculation, values without the partial volume correction (see below) were used.

Partial volume correction

BP and *I* values are affected by the regional gray matter fraction because of the limited spatial resolution of the PET scanner. The BP and *I* values per gray matter fraction in an ROI for cerebellar and cerebral cortical regions can be calculated as BP or *I* divided by the gray matter fraction obtained from segmented MR images for each ROI (Ito et al., 2006a).

Results

Anatomically standardized averaged images of BP and *I* are shown in Figs. 2 and 3, respectively. The BP and *I* values of each ROI for [^{11}C]SCH23390, [^{11}C]raclopride, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA are given in Table 3. Percentages of the putamen for BP or *I* values of [^{11}C]SCH23390, [^{11}C]FLB457, [^{11}C]PE2I, and L-[β-^{11}C]DOPA are shown in Fig. 4. In the substantia nigra with ventral tegmental area, binding to dopamine D_2 receptors, but very low binding to D_1 receptors, was observed. In the striatum, greatest bindings to dopamine D_1, D_2 receptors and transporters as well as the highest dopamine synthesis were observed. For the limbic regions, relatively high bindings of dopamine D_2 receptors were observed in the uncus. Relatively high dopamine synthesis was observed in the uncus and anterior part of the cingulate gyrus. For the other neocortical regions, the highest binding to dopamine D_2 receptors was observed in the temporal cortex. D_1 receptor binding among the neocortical regions was uniformly observed. Binding to dopamine transporter was very low in the neocortical regions. In the thalamus, relatively high binding to dopamine D_2 receptors was observed, but binding to D_1 receptors was very low.

Anatomically standardized averaged T1-weighted MR images and averaged images of gray and white matter fractions are shown in Fig. 5. The tissue fraction values of gray and white matter per voxel in cerebellar and cerebral cortices are given in Table 4, and the BP and *I* values with correction for the gray matter fraction in an ROI are shown in Table 5. Almost the same order of BP and *I* values among cerebral cortical regions was observed between before and after the correction for the gray matter fraction.

Discussion

A normal database for pre and postsynaptic dopaminergic neurotransmission components, including the striatal and extra-

Table 3
Average binding potential (BP) and dopamine synthesis index (*I*) values

Region	BP				*I*
	[^{11}C]SCH23390	[^{11}C]raclopride	[^{11}C]FLB457	[^{11}C]PE2I	L-[β-^{11}C]DOPA
Cerebellum	–	–	–	–	0.09±0.05
Substantia nigra	0.01±0.06	0.20±0.07	1.72±0.26	0.82±0.12	0.33±0.08
Thalamus	0.08±0.06	0.38±0.05	2.80±0.40	0.29±0.08	0.12±0.07
AN	0.07±0.08	0.42±0.10	3.84±0.51	0.30±0.08	0.20±0.13
DMN	0.04±0.06	0.32±0.07	3.04±0.52	0.20±0.09	0.11±0.08
PUL	0.09±0.07	0.38±0.06	2.24±0.41	0.21±0.09	0.12±0.09
Caudate head	1.13±0.24	2.21±0.22	6.69±0.94	5.84±1.00	0.79±0.17
Nucleus accumbens	1.20±0.23	2.19±0.38	6.73±1.11	4.75±0.70	0.94±0.14
Putamen	1.39±0.24	2.84±0.30	7.97±1.15	6.22±0.90	1.20±0.16
Globus pallidus	0.83±0.23	1.80±0.25	5.57±0.91	3.38±0.72	0.74±0.13
Hippocampus	0.20±0.09	0.27±0.06	1.42±0.24	0.10±0.05	0.16±0.09
Parahippocampal gyrus	0.18±0.10	0.25±0.05	1.30±0.22	−0.01±0.03	0.14±0.09
Uncus	0.17±0.07	0.26±0.05	1.84±0.26	0.09±0.06	0.19±0.07
Anterior cingulate	0.34±0.11	0.31±0.07	0.93±0.16	0.16±0.05	0.22±0.05
Posterior cingulate	0.41±0.11	0.36±0.05	1.01±0.36	0.14±0.07	0.12±0.04
Frontal base	0.25±0.08	0.25±0.04	0.75±0.20	0.09±0.03	0.07±0.06
Frontal convexity	0.30±0.08	0.27±0.04	0.70±0.18	0.13±0.05	0.07±0.05
Lateral temporal cortex	0.31±0.07	0.32±0.04	1.61±0.30	0.03±0.02	0.12±0.05
Parietal cortex	0.29±0.10	0.29±0.02	1.02±0.35	0.11±0.04	0.04±0.04
Occipital cuneus	0.37±0.10	0.29±0.05	0.50±0.27	0.12±0.04	–

Values are shown as mean±SD.
AN: anterior nuclei, DMN: dorsomedial nucleus, PUL: pulvinar in the thalamus.

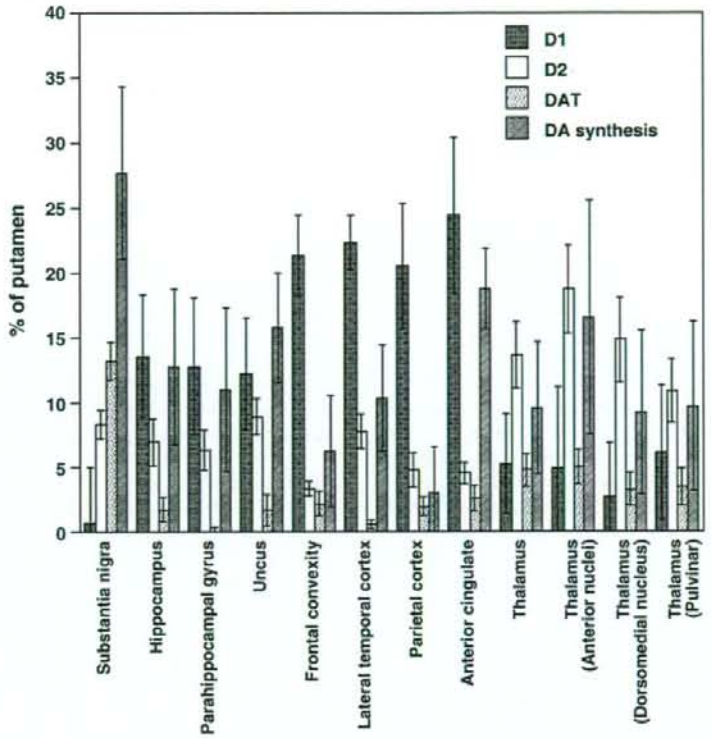

Fig. 4. Percentage of the putamen for BP or *I* values of [^{11}C]SCH23390 (dopamine D_1 receptor), [^{11}C]FLB457 (dopamine D_2 receptor), [^{11}C]PE2I (dopamine transporter (DAT)), and L-[β-^{11}C]DOPA (dopamine (DA) synthesis) for the substantia nigra, hippocampus, parahippocampal gyrus, uncus, frontal convexity, lateral temporal cortex, parietal cortex, anterior cingulate, thalamus and its subregions (anterior nuclei, dorsomedial nucleus, and pulvinar). Values are mean ± SD.

striatal dopamine D_1 and D_2 receptor bindings, dopamine transporter binding, and endogenous dopamine synthesis in the living human brain could be constructed by making use of the anatomic standardization technique. Although the subjects all differed in terms of the database (dopamine D_1, D_2 receptor, transporter, and synthesis), the anatomic standardization technique allowed us to compare between regional distributions of each dopaminergic neurotransmission component in vivo. While partial volume effects cause systemic underestimations of BP and *I* values, the partial volume correction did not change the order of BP and *I* values among cerebral cortical regions. This database is expected to be useful for various researches to understand the physiology of dopaminergic functions in the living human brain; however, regional differences in test-retest reliability in PET measurements should be considered (Hirvonen et al., 2001; Sudo et al., 2001). In addition, it has been reported that the reference tissue model method with the basis function method might cause overestimation and underestimation of BP in regions with low and high BP, respectively (Cselenyi et al., 2006; Gunn et al., 1997). Since BP values calculated from measured data may show some bias depending on kinetic models and calculation methods, it is not obvious if calculated BP values linearly reflect the biological pre- and postsynaptic functions. Thus, there might be some limitations in comparison of regional distributions between tracers using percentages of the putamen. This database can also be used in the investigation of regional abnormalities of dopaminergic neurotransmission in neuropsychiatric disorders, if database will be constructed from a large number of subjects. It might be difficult to use our database in other PET center due to between-center differences in data acquisition protocols, image reconstruction process, quantification methods, etc. (Ito et al., 2004). To solve these differences, further studies were required.

Nigrostriatal dopaminergic system

Midbrain

The ascending projections from the dopaminergic neurons in the substantia nigra to the striatum compose the nigrostriatal dopaminergic system (Bentivoglio and Morelli, 2005). Binding to dopamine D_2 receptors in the midbrain including the substantia nigra and ventral tegmental area was observed to be the same in the living human brain as in human postmortem studies (Hall et al., 1996; Joyce et al., 1991), suggesting the existence of receptors in dopaminergic neurons. On the other hand, no binding to dopamine D_1 receptors was observed in this region. These observations support the finding that dopaminergic autoreceptors in the midbrain are mainly of D_2 type (Meador-Woodruff et al., 1994; Morelli et al., 1987). In the living human brain, dopamine synthesis in the midbrain was greater than in the cerebral neocortical regions, although the aromatic L-amino acid decarboxylase activity in the substantia nigra was almost the same as in the parietal and occipital cortices in the human postmortem brain (Lloyd and Hornykiewicz, 1972). A medium amount of dopamine transporter was also found in the

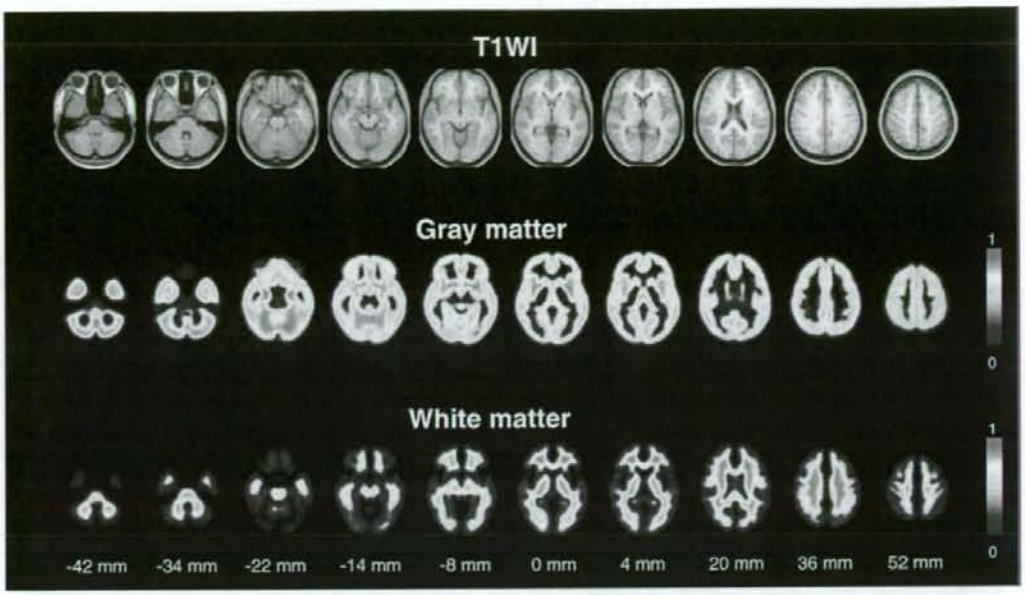

Fig. 5. Anatomically standardized averaged T1-weighted MR images and averaged images of gray and white matter fractions. All images are transaxial sections parallel to the AC–PC line. The slice positions are −42, −34, −22, −14, −8, 0, 4, 20, 36, and 52 mm from the AC–PC line. The anterior is at the top of the image and the subjects' right is at the left. Scale maximum and minimum values for gray and white matter images are 1 and 0 of gray and white matter fractions (mL/mL), respectively. T1WI, T1-weighted image.

midbrain, similar to a human postmortem study (Hall et al., 1999) and animal studies (Boja et al., 1994; Ciliax et al., 1995).

Striatum

The highest bindings to dopamine D_1 and D_2 receptors were observed in the striatum among the all brain regions, indicating the highest density of receptors, the same as reported in human postmortem studies (Hall et al., 1994). The highest binding to dopamine transporter in the striatum was also observed, indicating the highest transporter density (Boja et al., 1994; Ciliax et al., 1995; Hall et al., 1999). These findings indicate that this region is rich in dopaminergic synapses. Although the microdistribution of dopamine D_2 receptors in the striatum is differential with D_2 predominating in the striosomes and D_1 in the matrix (Joyce et al., 1986), the distributions of dopamine D_1 and D_2 receptor bindings appeared almost uniform in the striatum on account of the limited spatial resolution of the PET scanner. The highest dopamine synthesis was also observed in the striatum. This finding is in good agreement with previous studies showing the highest aromatic L-amino acid decarboxylase activity (Lloyd and Hornykiewicz, 1972) and the highest dopamine concentration in the striatum (Brown et al., 1979). These findings, in total, reflect the dense dopamine innervation of this region from the substantia nigra (Moore et al., 2003).

Globus pallidus

In the globus pallidus, higher bindings to dopamine D_1 and D_2 receptors as compared with other extrastriatal regions were observed, much the same as in human postmortem studies (Hall et al., 1996, 1994). These findings support the concept of the dopaminergic pallidal projections from neurons in the substantia nigra and ventral tegmental area (Bentivoglio and Morelli, 2005; Parent et al., 1990). In addition, it has been reported that binding to dopamine D_2 receptors in the external segment of the pallidum was higher than that in the internal segment in the human postmortem brain (Hall et al., 1996; Joyce et al., 1991). However, spillover from striatal radioactivity owing to the limited spatial resolution of the PET scanner might hamper an accurate estimation of pallidal binding. BP values of [^{11}C]raclopride in the globus pallidus and nucleus accumbens were different from previous study of the living human brain (Ito et al., 1999). These differences might be caused by differences in anatomic standardization techniques, methods of calculation of BP, image reconstruction process, PET cameras, etc.

Mesocorticolimbic dopaminergic system

Limbic system

The ascending projections from dopaminergic neurons in the ventral tegmental area to the cerebral cortices and limbic system compose the mesocorticolimbic dopaminergic system (Bentivoglio and Morelli, 2005). The ventral striatum, the so-called limbic striatum, including the nucleus accumbens is also innervated by the ventral tegmental area (Bentivoglio and Morelli, 2005; Joel and Weiner, 2000).

Relatively high bindings of dopamine D_2 receptors were observed in the uncus including the amygdala, parahippocampal gyrus, and hippocampus as compared with cerebral neocortical regions. These distributions are in good agreement with those found in human postmortem studies (Hall et al., 1996, 1994; Joyce et al., 1991). It has been reported that densities of dopamine D_2 receptors in the hippocampus are lower than in the amygdala and parahippocampal gyrus (Hall et al., 1996, 1994). The present study also

Table 4
Average tissue fractions of gray and white matter per voxel in cerebellar and cerebral cortices (mL/mL)

Region	Tissue fraction	
	Gray matter	White matter
Cerebellum	0.75±0.04	0.16±0.03
Hippocampus	0.80±0.04	0.14±0.03
Parahippocampal gyrus	0.74±0.03	0.19±0.03
Uncus	0.83±0.03	0.03±0.01
Anterior cingulate	0.65±0.04	0.20±0.03
Posterior cingulate	0.72±0.04	0.18±0.05
Frontal base	0.55±0.02	0.29±0.02
Frontal convexity	0.57±0.04	0.29±0.04
Lateral temporal cortex	0.66±0.02	0.25±0.02
Parietal cortex	0.55±0.03	0.35±0.03
Occipital cuneus	0.64±0.04	0.27±0.04

Values are shown as mean±SD.

showed lower binding of dopamine D_2 receptors in the hippocampus than in the uncus. A human postmortem study showed aromatic L-amino acid decarboxylase activity in the amygdala but not in the hippocampus (Lloyd and Hornykiewicz, 1972). In the present study, dopamine synthesis was observed in the hippocampus in addition to the uncus including amygdala and parahippocampal gyrus although the spatial resolution of the PET scanner was limited. The present results might indicate the dopaminergic innervation to the hippocampus in addition to the amygdala and the parahippocampal gyrus (Joyce and Murray, 1994). As for the other extrastriatal regions, bindings to dopamine transporter were very low (Hall et al., 1999).

The anterior part of the cingulate gyrus representing the limbic system has projections from the dopaminergic neurons in the ventral tegmental area, and connections with hippocampal regions. In this region, bindings to dopamine D_1 and D_2 receptors similar to those in neocortical regions were observed. Binding to dopamine transporter was very low in this region, as in neocortical regions. It should be noted that dopamine synthesis was relatively higher in the anterior cingulate than in neocortical regions.

Neocortex

In the cerebral neocortical regions, binding to dopamine D_2 receptors was found to be low comparing with the other brain regions as previous studies of the living human brain (Okubo et al., 1999) and postmortem brain (Hall et al., 1996, 1994; Joyce et al., 1991; Lidow et al., 1989) have also reported. Regional differences in D_2 receptor binding among the neocortical regions were observed in the living human brain as in the human postmortem brain (Hall et al., 1996, 1994; Joyce et al., 1991), i.e., highest binding in the temporal cortex and lowest binding in the occipital cortex. Binding to dopamine D_2 receptors was higher in the parietal cortex than in the frontal and occipital cortices. Binding to dopamine D_1 receptors in the cerebral neocortical regions as compared with the striatum was higher than that to D_2 receptors as shown in a previous human postmortem study (Hall et al., 1994). Using the present database of dopamine D_1 and D_2 receptors, it could be observed that regional distribution of D_1 receptor binding among the neocortical regions was more uniform as compared with that to D_2 receptors. Percentages of the putamen for BP of dopamine D_2 receptors in the cerebral neocortical regions were around 5–10% in the present study, whereas those were reported to be about 1% in a previous human postmortem study with [^{125}I]epidepride (Hall et al., 1996). This discrepancy might be caused by the difference in used radioligands. The difference between in vivo and in vitro conditions might also cause such discrepancy.

In the present study, the binding to dopamine transporter was almost negligible level in the cerebral neocortical regions, as also reported in a human postmortem study (Hall et al., 1999), although both dopamine D_1 and D_2 receptors exist in these regions. These data indicate that released dopamine in the dopaminergic synapse might be inactivated by enzymatic degradation rather than by reuptake to the transporter (Hall et al., 1999). In addition, dopamine reuptake through the norepinephrine transporter in cerebral cortical regions with low levels of the dopamine transporter was observed in mice (Moron et al., 2002). Dopamine synthesis was observed in the cerebral neocortical regions except the occipital cortex. Among these regions, highest and lowest dopamine synthesis was observed in the temporal and frontal cortex, respectively. This regional difference in dopamine synthesis was in good agreement with that in aromatic L-amino acid decarboxylase activity (Lloyd and Hornykiewicz, 1972). In the temporal cortex, highest dopamine synthesis and highest dopamine D_2 receptor binding were observed among the neocortical regions. On the other hand, it should be noted that the parietal cortex showed relatively low dopamine synthesis and relatively high dopamine D_2 receptor binding as compared with the frontal cortex.

In the present study, database were constructed from male subjects. However, it has been reported that a gender difference in

Table 5
Average binding potential (BP) and dopamine synthesis index (*I*) values with correction for gray matter fraction in an ROI

Region	BP				*I*
	[^{11}C]SCH23390	[^{11}C]raclopride	[^{11}C]FLB457	[^{11}C]PE2I	L-[β-^{11}C]DOPA
Cerebellum	–	–	–	–	0.12±0.06
Hippocampus	0.24±0.11	0.34±0.07	1.77±0.33	0.12±0.06	0.20±0.12
Parahippocampal gyrus	0.25±0.13	0.33±0.07	1.75±0.30	−0.01±0.04	0.18±0.12
Uncus	0.21±0.08	0.31±0.06	2.22±0.31	0.11±0.07	0.23±0.08
Anterior cingulate	0.53±0.16	0.47±0.10	1.41±0.24	0.24±0.08	0.34±0.06
Posterior cingulate	0.57±0.11	0.50±0.06	1.38±0.47	0.20±0.10	0.17±0.05
Frontal base	0.45±0.13	0.46±0.07	1.35±0.37	0.16±0.05	0.13±0.11
Frontal convexity	0.52±0.13	0.46±0.07	1.18±0.29	0.24±0.10	0.12±0.08
Lateral temporal cortex	0.47±0.11	0.47±0.06	2.39±0.40	0.05±0.03	0.18±0.08
Parietal cortex	0.53±0.16	0.52±0.05	1.80±0.60	0.21±0.06	0.06±0.08
Occipital cuneus	0.58±0.15	0.45±0.07	0.78±0.40	0.18±0.06	–

Values are shown as mean±SD.