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Behavioral/Systems/Cognitive

Effects of the Antipsychotic Risperidone on Dopamine Synthesis in Human Brain Measured by Positron Emission Tomography with L-[β-^{11}C]DOPA: A Stabilizing Effect for Dopaminergic Neurotransmission?

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Effects of antipsychotic drugs have widely been considered to be mediated by blockade of postsynaptic dopamine D_2 receptors. Effects of antipsychotics on presynaptic functions of dopaminergic neurotransmission might also be related to therapeutic effects of antipsychotics. To investigate the effects of antipsychotics on presynaptic functions of dopaminergic neurotransmission in relation with occupancy of dopamine D_2 receptors, changes in dopamine synthesis capacity by antipsychotics and occupancy of dopamine D_2 receptors were measured by positron emission tomography (PET) in healthy men. PET studies using [^{11}C]raclopride and L-[β-^{11}C]DOPA were performed under resting condition and oral administration of single dose of the antipsychotic drug risperidone on separate days. Although occupancy of dopamine D_2 receptors corresponding dose of risperidone was observed, the changes in dopamine synthesis capacity by the administration of risperidone were not significant, nor was the relation between the occupancy of dopamine D_2 receptors and these changes. A significant negative correlation was observed between the baseline dopamine synthesis capacity and the changes in dopamine synthesis capacity by risperidone, indicating that this antipsychotic can be assumed to stabilize the dopamine synthesis capacity. The therapeutic effects of risperidone in schizophrenia might be related to such stabilizing effects on dopaminergic neurotransmission responsivity.

Introduction

Effects of antipsychotic drugs have widely been considered to be mediated by blockade of postsynaptic dopamine D_2 receptors (Carlsson and Lindqvist, 1963; Creese et al., 1976; Seeman et al., 1976). This hypothesis has been supported by positron emission tomography (PET) studies to determine the occupancy of dopamine D_2 receptors in schizophrenia patients treated with first-generation antipsychotics, e.g., haloperidol (Farde et al., 1988; Baron et al., 1989) and second-generation antipsychotics, e.g., risperidone (Nyberg et al., 1993).

Effects of antipsychotics on presynaptic functions of dopaminergic neurotransmission might also be related to the therapeutic effects of antipsychotics. It has been reported that antipsychotic drugs, chlorpromazine and haloperidol, increased dopamine metabolites in mouse brain tissue (Carlsson and Lindqvist, 1963; O'Keeffe et al., 1970), and also that risperidone and clozapine increased dopamine release in rat brain (Hertel et al., 1996). Increases and decreases in the activity of aromatic L-amino acid decarboxylase (AADC) by antagonists and agonists of dopamine D_2 receptors were also observed in rat brain tissue, respectively (Zhu et al., 1992, 1993). The regional activity of AADC in brain indicating the dopamine synthesis capacity can be estimated using radiolabeled L-DOPA (Gjedde et al., 1991). Significant increases and decreases in dopamine synthesis capacities by antagonists and agonists of dopamine D_2 receptors were observed in animal studies using [^{3}H]DOPA, L-[β-^{11}C]DOPA, or 6-[^{18}F]fluoro-L-DOPA, respectively (Cumming et al., 1997; Torstenson et al., 1998; Danielsen et al., 2001). These findings indicate that pharmacological effects on dopaminergic autoreceptors might cause changes in the presynaptic dopamine synthesis capacity (Carlsson and Lindqvist, 1963).

Effects of antipsychotics on the dopamine synthesis capacity in brain have been investigated in human subjects. A significant increase in dopamine synthesis capacity after acute administration of antipsychotic drug haloperidol was observed using PET with 6-[^{18}F]fluoro-L-DOPA in healthy human subjects (Vernaleken et al., 2006). On the other hand, a significant decrease in dopamine synthesis capacity after chronic administration of haloperidol was observed using 6-[^{18}F]fluoro-L-DOPA in patients with schizophrenia (Gründer et al., 2003). A significant in-

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crease in the plasma concentration of homovanillic acid after acute administration of antipsychotics, haloperidol or fluphenazine, was observed in patients with schizophrenia, indicating an increase in dopamine turnover (Davila et al., 1988; Pickar et al., 1988). During chronic administration, a significant decrease in the plasma concentration of homovanillic acid was also observed (Davila et al., 1988; Pickar et al., 1988). However, the effects of antipsychotics on the dopamine synthesis capacity have not been investigated in relation to the occupancy of dopamine D_2 receptors in human subjects.

Recently, we have validated quantitative analyses in L-[β-^{11}C]DOPA PET studies (Ito et al., 2006, 2007). In the present study, to elucidate changes in dopamine synthesis capacity by antipsychotics in relation to the occupancy of dopamine D_2 receptors, dopamine D_2 receptor bindings and dopamine synthesis capacities at resting condition and after oral administration of a single dose of the antipsychotic drug risperidone were measured in the same human subjects by PET with [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively.

Materials and Methods

Subjects. The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. Twelve healthy men (21–29 years of age, 24.3 ± 2.9 years [mean ± SD]) were recruited and written informed consent was obtained. 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 according to interview.

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 10 min transmission scan using a ^{68}Ge-^{68}Ga line source was performed for correction of attenuation. A head fixation device with thermoplastic attachments for individual fit minimized head movement during the PET measurements.

PET studies were performed under resting condition (baseline study) and oral administration of risperidone (drug challenge study) on separate days. The interval between the 2 studies was 7 d in 10 subjects, and 14 d in 2 subjects. In each study, both PET scans with [^{11}C]raclopride and L-[β-^{11}C]DOPA were performed sequentially. After intravenous rapid bolus injection of [^{11}C]raclopride, dynamic PET scanning was performed for 60 min. After 1 h from the end of the [^{11}C]raclopride PET measurement, dynamic PET scanning was performed for 89 min after intravenous rapid bolus injection of L-[b-^{11}C]DOPA. The frame sequence consisted of twelve 20 s frames, sixteen 1 min frames, and ten 4 min frames for [^{11}C]raclopride, and seven 1 min frames, five 2 min frames, four 3 min frames, and twelve 5 min frames for L-[β-^{11}C]DOPA. The radioactivity injected was 220–230 MBq and 342–395 MBq in the baseline studies, and 205–274 MBq and 344–388 MBq in the drug challenge studies for [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively. The specific radioactivity was 168–517 GBq/μmol and 26–88 GBq/μmol in the baseline studies, and 162–535 GBq/μmol and 39–90 GBq/μmol in the drug challenge studies for [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively. A venous blood sample was taken at the beginning of L-[β-^{11}C]DOPA PET scanning for measurement of natural neutral amino acid (NAA) concentration in plasma. NAA concentration was measured by HPLC (L-8500 amino acid analyzer system, Hitachi Corp.). The amino acids are phenylalanine, tryptophan, leucine, methionine, isoleucine, tyrosine, histidine, valine and threonine, which are transported via the same carrier at the blood–brain barrier as L-DOPA (Sugaya et al., 2001). A weighted sum of the NAAs, which was the L-DOPA corresponding concentration of the nine NAAs for the carrier system, was calculated according to our previous work (Ito et al., 2006).

Table 1. Dose of risperidone and ranges of occupancy of dopamine D_2 receptors

Dose of risperidone (mg)	Occupancy (%) Putamen	Caudate
0.5	39–46%	33–44%
1.0	48–52%	48–60%
1.5	61–69%	63–71%
2.0	71–75%	75–79%

In the drug challenge studies, risperidone at 0.5–2.0 mg was orally administrated 2 h before the start of PET scanning with [^{11}C]raclopride. The dose of risperidone was 0.5 mg in 3 subjects, 1.0 mg in 3 subjects, 1.5 mg in 3 subjects, and 2.0 mg in 3 subjects. To estimate the plasma concentration of risperidone and its active metabolite (9-hydroxy-risperidone), venous blood sampling was performed at the start and end of each PET scan. The plasma concentrations of risperidone and 9-hydroxy-risperidone were determined by validated liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method. Since risperidone and 9-hydroxy-risperidone have similar binding profiles to neuroreceptors (Leysen et al., 1994), the sum of their plasma concentrations was used as the plasma concentration of the antipsychotic drug in the present study.

All MR imaging studies were performed with a 1.5-tesla 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 (echo time, 9.2 ms; repetition time, 21 ms; flip angle: 30°; field of view: 256 mm; acquisition matrix: 256 × 256; slice thickness: 1 mm).

Regions of interest. All MR images were coregistered to the PET images with the statistical parametric mapping (SPM2) system (Friston et al., 1990). Regions of interest (ROIs) were drawn on coregistered MR images and transferred to the PET images. ROIs were defined for the cerebellar cortex, putamen, caudate head, and occipital cortex. Each ROI was drawn in three adjacent sections and data were pooled to obtain the average radioactivity concentration for the whole volume of interest. To obtain regional time-activity curves, regional radioactivity was calculated for each frame, corrected for decay, and plotted versus time. In-house software was used to draw ROIs.

Calculation of occupancy of dopamine D_2 receptors. For both PET studies with [^{11}C]raclopride, the binding potential (BP_{ND}) was calculated by the reference tissue model method (Lammertsma et al., 1996; Lammertsma and Hume, 1996). With 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 non-displaceable radioligand binding:

$$C_i(t) = R_1 \cdot C_r(t) + \{k_2 - R_1 \cdot k_2/(1 + BP_{ND})\} \cdot C_r(t) \otimes \exp\{-k_2 \cdot t/(1 + BP_{ND})\},$$

where C_i is the radioactivity concentration in a brain region; C_r 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_{ND}, R_1, and k_2) were estimated by nonlinear least-squares curve fitting. The cerebellum was used as a reference region. The occupancy of dopamine D_2 receptors by risperidone was calculated as follows:

$$\text{Occupancy (\%)} = 100 \cdot BP_{ND(baseline)} - BP_{ND(drug)})/BP_{ND(baseline)},$$

where $BP_{ND(baseline)}$ is the BP_{ND} value in the baseline study, and $BP_{ND(drug)}$ is the BP_{ND} value in the drug challenge study.

Calculation of dopamine synthesis capacity. The uptake rate constant for L-[β-^{11}C]DOPA indicating the dopamine synthesis capacity was estimated by graphical analysis (Patlak and Blasberg, 1985; Gjedde, 1988; Hartvig et al.,

1991), which allows for the calculation of the uptake rate constant k_i using time-activity data in a reference brain region with no irreversible binding. The k_i values can be estimated by using simple linear least-squares fitting as follows:

$$\frac{C_i(t)}{C_i'(t)} = k_i \cdot \frac{\int_0^t C_i'(\tau)d\tau}{C_i'(t)} + F \qquad t < t^*,$$

where C_i and C_i' are the total radioactivity concentrations in a brain region with and without irreversible binding, respectively, and t^* is the equilibrium time of the compartment for unchanged radiotracer in brain tissue. Plotting $C_i(t)/C_i'(t)$ versus $\int_0^t C_i'(\tau)d\tau/C_i'(t)$, after time t^* yields a straight line with the slope k_i and intercept F. In the present study, the occipital cortex was used as a reference region with no irreversible binding, because 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). The range of equilibrium time t^* of 29–89 min was used (Ito et al., 2006, 2007). The percentage change in k_i by oral administration of risperidone was calculated as follows:

$$\% \text{ change} = 100 \cdot (k_{i(drug)} - k_{i(baseline)})/k_{i(baseline)},$$

where $k_{i(baseline)}$ is the k_i value in the baseline study, and $k_{i(drug)}$ is the k_i value in the drug challenge study.

Results

The ranges of occupancy of dopamine D_2 receptors in the striatum for each dose of risperidone measured by PET with [^{11}C]raclopride are given in Table 1. The occupancies of dopamine D_2 receptors ranged from 39% to 75% in the putamen and from 33% to 79% in the caudate. The sums of the plasma concentrations of risperidone and 9-hydroxy-risperidone during [^{11}C]raclopride and L-[β-^{11}C]DOPA PET studies, averaged between the start and end of each scanning, ranged from 3.8 to 23.1 ng/ml (12.2 ± 6.6 ng/ml, mean ± SD) and from 2.6 to 19.5 ng/ml (10.5 ± 5.8 ng/ml), respectively.

The uptake rate constant k_i of L-[β-^{11}C]DOPA in the striatum indicating the dopamine synthesis capacity for baseline and drug challenge study results are shown in Figure 1. The k_i values were 0.0136 ± 0.0017 min^{-1} and 0.0142 ± 0.0010 min^{-1} (mean ± SD) in the putamen and 0.0121 ± 0.0018 min^{-1} and 0.0125 ± 0.0015 min^{-1} in the caudate for baseline and drug challenge studies, respectively. No significant differences in k_i were observed between the two studies. Weighted sums of the natural neutral amino acids (NAAs) in plasma were 1251 ± 198 nmol/ml for the baseline studies and 1207 ± 199 nmol/ml (mean ± SD) for the drug challenge studies. No significant differences in values were observed between the two studies.

The relations between the occupancy of dopamine D_2 receptors and the percentage change in k_i by drug challenge are

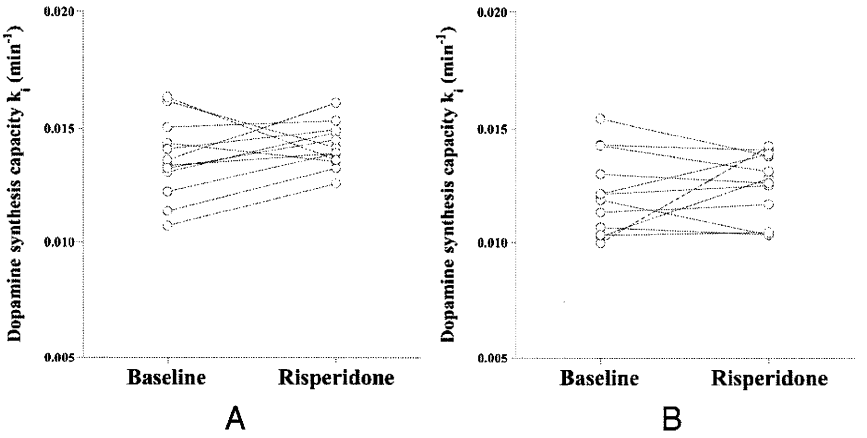

Figure 1. The uptake rate constant k_i indicating the dopamine synthesis capacity for the baseline study and drug challenge study with risperidone in the putamen (***A***) and caudate (***B***).

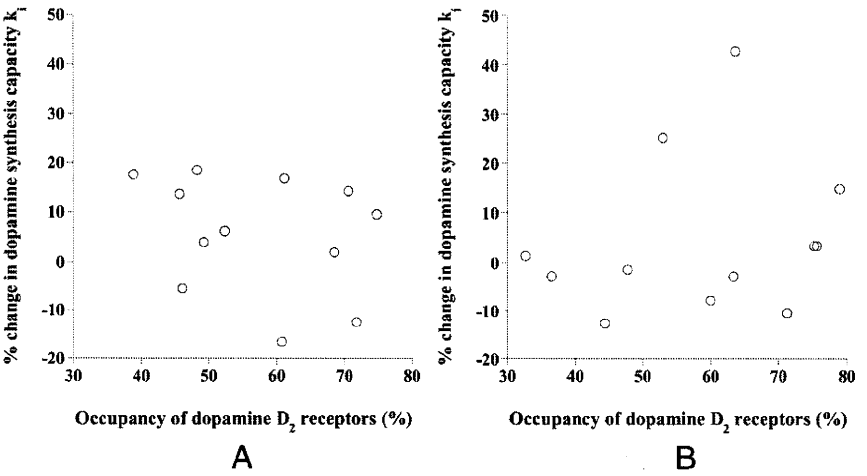

Figure 2. The relations between the occupancy of dopamine D_2 receptors and the percentage change in k_i by drug challenge with risperidone in the putamen (***A***) and caudate (***B***).

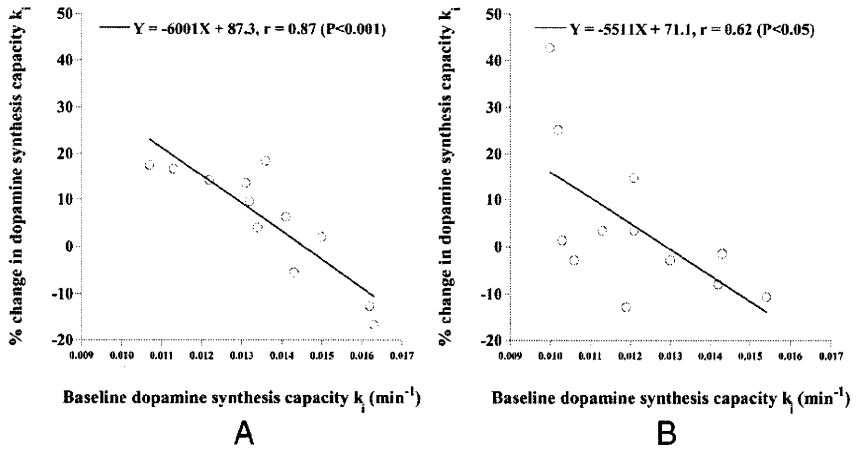

Figure 3. The relations between k_i in the baseline study and the percentage changes in k_i by drug challenge with risperidone in the putamen (***A***) and caudate (***B***).

shown in Figure 2. No significant correlations were observed. The relations between k_i in the baseline study and percentage change in k_i by the drug challenge are shown in Figure 3. Significant negative correlations were observed (putamen: $p < 0.001$, caudate: $p < 0.05$).

Discussion

Effects of antipsychotics on presynaptic dopamine synthesis might be caused by pharmacological activity on dopaminergic autoreceptors (Carlsson and Lindqvist, 1963). Although occupancy of dopamine D_2 receptors corresponding to the dose of risperidone was observed, no significant changes in the dopamine synthesis capacity k_i by administration of risperidone were observed in the present study. Furthermore, there were no significant correlations between the occupancy of dopamine D_2 receptors and changes in dopamine synthesis capacity k_i by risperidone. No significant changes in the dopamine synthesis capacity after acute administration of risperidone in healthy human subjects were also reported using 6-[^{18}F]-L-*m*-tyrosine (Mamo et al., 2004). On the other hand, a significant increase in the dopamine synthesis capacity measured using 6-[^{18}F]fluoro-L-DOPA and a significant increase in the plasma concentration of homovanillic acid have been observed after acute administration of antipsychotics, haloperidol or fluphenazine, in healthy human subjects (Vernaleken et al., 2006) and patients with schizophrenia (Davila et al., 1988; Pickar et al., 1988), respectively. The discrepancy between the present and previous results might have resulted from the use of different antipsychotics. However, in rat brain, it has been reported that risperidone and clozapine also increased dopamine release (Hertel et al., 1996). Another reason for this discrepancy might due to differences in the radiotracers used. However, in animal studies with [^{3}H]DOPA, L-[β-^{11}C]DOPA, or 6-[^{18}F]fluoro-L-DOPA, significant increases and decreases in dopamine synthesis capacities by antagonists and agonists of dopamine D_2 receptors, respectively, have been observed (Cumming et al., 1997; Torstenson et al., 1998; Danielsen et al., 2001).

In the present study, significant negative correlations were observed between the baseline dopamine synthesis capacity k_i and the percentage changes in the dopamine synthesis capacity by risperidone. This indicates that the increase and decrease in dopamine synthesis capacity by administration of risperidone are observed in subjects with low and high baseline dopamine synthesis capacity, respectively, and the degrees of increase and decrease in dopamine synthesis capacity are greater as the baseline dopamine synthesis capacities are smaller and larger, respectively. Negative correlations between baseline cerebral 6-[^{18}F] fluoro-L-DOPA utilization and change in 6-[^{18}F]fluoro-L-DOPA storage capacity by haloperidol challenge have also been observed in healthy human subjects (Vernaleken et al., 2008), corresponding to our present results. In addition, the coefficients of variation of dopamine synthesis capacity k_i were smaller in studies with the administration of risperidone than in baseline studies. Thus, the antipsychotic drug risperidone can be assumed to stabilize the dopamine synthesis capacity. The concept of phasic and tonic dopamine release with relation to the modulation of dopaminergic neurotransmission has been proposed, and abnormal responsivity in both phasic and tonic dopamine release in schizophrenia has been considered (Grace, 1991). The therapeutic effects of risperidone might be related to stabilizing effects on such dopaminergic responsivity. In addition, it has been reported that an antipsychotic drug, clozapine, normalized dopamine turnover in the primate phencyclidine model, indicating that the effects of clozapine in schizophrenia might be related to the restoration of dopamine tone (Elsworth et al., 2008). In this study, only an acute intervention was performed on healthy subjects, and therefore, the chronic effects of antipsychotics on patients with schizophrenia should be investigated in future.

It has been reported that the working memory and learning functions were correlated with the baseline dopamine synthesis capacity (Cools et al., 2008, 2009). Further studies to investigate the effects of antipsychotics on such higher brain functions in relation with changes in dopamine synthesis capacity should be considered (Vernaleken et al., 2008).

Serotonin 5-HT_{2A} receptor antagonists have been reported to modulate endogenous dopamine release (Pehek et al., 2001), and to reduce extrapyramidal side effects (Balsara et al., 1979; Korsgaard et al., 1985; Hicks, 1990). Risperidone is an antagonist for dopamine D_2 receptors and serotonin 5-HT_{2A} receptors with high affinity (Leysen et al., 1994), and it has been reported to modulate endogenous dopamine release. These findings indicate that changes in the dopamine synthesis capacity by administration of risperidone might be due to not only pharmacological effects on dopaminergic autoreceptors, but also on serotonin 5-HT_{2A} receptors. Thus, the stabilizing effects of risperidone on the dopamine synthesis level might also be related to its antagonism toward serotonin 5-HT_{2A} receptors. To elucidate this, further studies based on the same design using a selective antagonist for dopamine D_2 receptors, such as sulpiride, should be considered. In addition, a new antipsychotic drug aripiprazole that is a partial agonist to dopamine D_2 receptors has recently been used for treatment of schizophrenia (Mamo et al., 2007). Further studies to investigate the effects of aripiprazole on dopamine synthesis capacity should also be considered.

In conclusion, dopamine D_2 receptor bindings and dopamine synthesis capacities at resting condition and after oral administration of a single dose of the antipsychotic drug risperidone were measured in the same human subjects. Although occupancy of dopamine D_2 receptors corresponding to the dose of risperidone was observed, no significant changes in dopamine synthesis capacity by administration of risperidone were observed. It was also noted that there was no significant correlation between occupancy of dopamine D_2 receptors and changes in dopamine synthesis capacity by risperidone. On the other hand, a significant negative correlation was observed between the baseline dopamine synthesis capacity and the changes in dopamine synthesis capacity by risperidone. This indicates that the antipsychotic drug risperidone can be considered to stabilize the dopamine synthesis capacity. This suggests that the therapeutic effects of risperidone in schizophrenia might be related to the stabilizing effects on dopaminergic neurotransmission responsivity.

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Increase in thalamic binding of [^{11}C]PE2I in patients with schizophrenia: A positron emission tomography study of dopamine transporter

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ABSTRACT

Previous *in vivo* imaging studies reported no difference in dopamine transporter (DAT) bindings in the striatum between control subjects and patients with schizophrenia. However, as the signals of radioligands with moderate affinity were insufficient for allowing the evaluation of small amounts of DAT, DAT binding in extrastriatal regions has not been determined. Positron emission tomography scanning using [^{11}C]PE2I was performed on eight patients with schizophrenia and twelve normal control subjects. Binding potential (BP_{ND}) for DAT in the caudate, putamen, thalamus and substantia nigra was calculated, using the cerebellum as reference region. In patients with schizophrenia, clinical symptoms were evaluated by Positive and Negative Syndrome Scale (PANSS). BP_{ND} in the thalamus of patients with schizophrenia was significantly higher than in control subjects ($P = 0.044$). In patients with schizophrenia, there were significantly positive correlations between BP_{ND} in the thalamus and total ($r = 0.75$), positive ($r = 0.78$) and negative PANSS scores ($r = 0.82$). Altered DAT in the thalamus might be related to the pathophysiology and clinical symptoms of schizophrenia.

1. Introduction

One of the most accepted hypotheses concerning the pathophysiology of schizophrenia are the hyperactivity of dopaminergic neurotransmission. This 'dopamine hypothesis' is supported by the facts that antipsychotic effects are mainly related to dopamine D_2 receptor antagonism and that dopamine stimulating agents can cause psychotic symptoms such as hallucination or delusion. Dopamine transporter (DAT) plays a role in the reuptake of dopamine into pre-synaptic nerves and regulates dopaminergic transmission in the synaptic cleft. DAT inhibitors such as cocaine increase dopamine concentration in the synaptic cleft (Schlaepfer et al., 1997) and worsen the clinical course of schizophrenia, e.g., exacerbating positive and negative symptoms, increasing the risk of relapse, or hospitalization (Green, 2005).

Previous *in vivo* imaging studies using positron emission tomography (PET) or single photon emission computed tomography (SPECT) reported no difference in DAT bindings between control subjects and patients with schizophrenia (Hsiao et al., 2003; Laakso et al., 2000; Laruelle et al., 2000; Lavalaye et al., 2001; Schmitt et al., 2005, 2006, 2008; Yang et al., 2004) except for one study reporting lower binding in patients with schizophrenia as compared with controls (Mateos et al., 2007). However, those studies evaluated DAT binding only in the striatum, as DAT density in extrastriatal regions is very low (in a postmortem human study, [^{125}I]PE2I binding in the thalamus was reported to be 15% of that in the striatum and negligible in the cortex) (Hall et al., 1999). The recent development of [^{11}C]PE2I, which has high affinity ($K_i = 17$ nM) and selectivity (more than 30-fold for other monoamine transporters) for DAT, allows the evaluation of extrastiatal DAT bindings (Halldin et al., 2003; Hirvonen et al., 2008; Jucaite et al., 2006). In this study, we evaluated DAT binding in the striatal and extrastriatal regions of patients with schizophrenia using [^{11}C]PE2I.

2. Materials and methods

2.1. Subjects

Eight patients (age range 25–52 yr, mean ± SD: 36.5 ± 9.5 yr) diagnosed with schizophrenia or schizophreniform disorder according to DSM-IV criteria participated in this study. Four patients with schizophreniform disorder met the criteria for schizophrenia at six month follow-up. Exclusion criteria were current

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Table 1
Demographic and clinical characteristics.

	Controls	Patients
N	12	8
Age (years)	33.2 ± 12.0	36.5 ± 9.5
Gender (M/F)	10/2	6/2
Naïve/free		6/2
Duration of illness (months)		32.1 ± 42.8
PANSS (total)		77.8 ± 18.8
Positive		17.8 ± 4.8
Negative		18.9 ± 6.5
General		41.1 ± 10.8

Values are mean ± SD.

or past substance abuse, organic brain disease, or epilepsy. Demographic and clinical data are shown in Table 1. Six of the patients were antipsychotic naïve and two had been antipsychotic-free for at least six months before the PET scan. Three patients had taken benzodiazepines the night before the PET scan.

Psychopathological symptoms were assessed by three experienced psychiatrists on the same day as the PET scans using the Positive and Negative Syndrome Scale (PANSS), and consensus ratings were used. PANSS scores used were total score and subscores for positive symptom, negative symptom and general symptom.

Twelve normal control subjects (age range 23–56 yr, mean ± SD: 33.2 ± 12.0 yr) also participated. None of them had a history of psychiatric or neurological disorders, brain injury, chronic somatic illness, or substance abuse. None had taken any drugs within two weeks before the PET scan.

After complete description of this study, written informed consent was obtained from all subjects. The study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. Data were collected from 4/2003 to 8/2006.

2.2. PET procedure

A PET scanner system, ECAT EXACT HR+(CTI-Siemens, Knoxville, TN, USA), was used for all measurements. A head fixation device was used to minimize head movement. A transmission scan for attenuation correction was performed using a ^{68}Ge–^{68}Ga source before each scan. A dynamic PET scan was performed for 90 min (20 s × 9, 1 min × 5, 2 min × 4, 4 min × 11, 5 min × 6) after intravenous bolus injection of 214.7 ± 13.7 MBq (mean ± SD) of [^{11}C]PE2I. The specific radioactivity of [^{11}C]PE2I was 344.5 ± 355.3 MBq/nmol. Injected dose and specific radioactivity between the control and patient groups were not significantly different (two-tailed *t*-test; $P = 0.15$ and $P = 0.16$, respectively). Since two previous quantitative studies of [^{11}C]PE2I had reported good reliability with scan times of 63 and 69 min, the scan time of 90 min was considered sufficient for estimation of DAT bindings especially in extrastriatal regions (Hirvonen et al., 2008; Jucaite et al., 2006). Magnetic resonance (MR) images of the brain were acquired with a 1.5 Tesla MR imaging system, Gyroscan NT (Philips Medical Systems, Best, Netherlands). T1-weighted images were obtained at 1 mm slices. All subjects were free of organic brain lesions.

2.3. Data analysis

All MR images were coregistered to the PET images using the statistical parametric mapping (SPM2) system. MR images were transformed into the standard brain size and shape by SPM2 (anatomic standardization). All PET images were also transformed into the standard brain size and shape using the same parameters as the MR image standardization. Thus, brain images of all subjects had the same anatomic format (Ito et al., 2008). Motion corrections were not made.

Regions of interest (ROIs) were drawn on all anatomically standardized PET images with reference to the T1-weighted MR images. ROIs were defined for the cerebellar cortex, caudate head, putamen, substantia nigra and thalamus (Fig. 1).

Binding potential (BP_{ND}) was calculated by the simplified reference tissue model (SRTM) method. The cerebellum was used as reference region because of its negligible density of DAT (Hall et al., 1999). In this study, the software package PMOD (PMOD Technologies, Zurich, Switzerland) was used to calculate BP_{ND}.

2.4. Statistics

Statistical analysis concerning the difference of BP_{ND} for each ROI between patients and controls was performed by two-tailed *t*-test. Correlations between BP_{ND} of patients with schizophrenia and age, duration of illness, and PANSS scores were evaluated using Pearson's correlation coefficient. In all analyses, $P < 0.05$ was considered significant.

3. Results

The BP_{ND} values of control subjects and patients with schizophrenia are shown in Table 2. The BP_{ND} value in the thalamus was significant higher in patients with schizophrenia than in con-

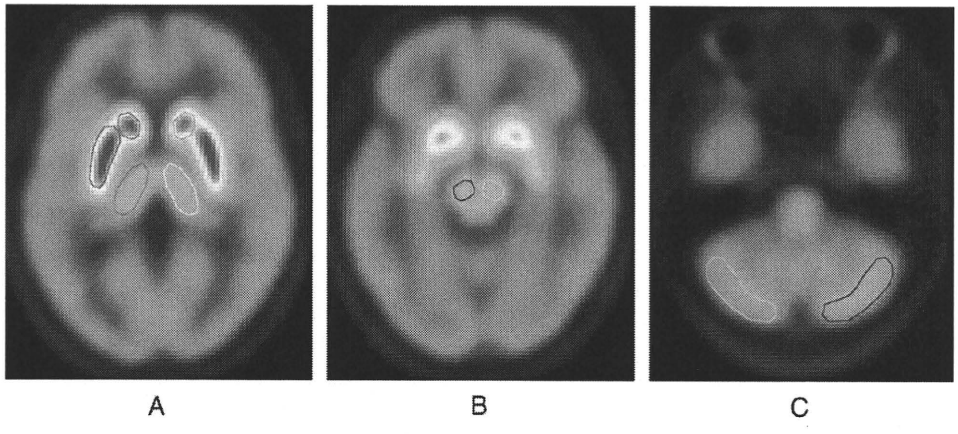

Fig. 1. Summated images of [^{11}C]PE2I with regions of interest. Average normalized images of twelve control subjects are shown at the level of caudate, putamen and thalamus (A), substantia nigra (B) and cerebellum (C).

Table 2
BP_{ND} in all regions.

	BP_{ND}[a]				t-test	
Region	Controls	Patients	% Change[b]	Effect size	t	P
Caudate	7.54 ± 1.22	8.21 ± 1.38	8.9 ± 18.4 (−6.5–24.2)%	0.55	1.14	0.27
Putamen	7.54 ± 1.25	8.23 ± 0.71	9.2 ± 9.4 (1.3–17.0)%	0.55	1.41	0.18
Thalamus	0.28 ± 0.08	0.36 ± 0.07	27.9 ± 25.8 (6.3–49.5)%	1.0	2.16	0.044*
Substantia nigra	1.09 ± 0.16	1.13 ± 0.12	4.1 ± 11.3 (−5.3–13.6)%	0.25	0.66	0.52

[a] Values are mean ± SD.
[b] Values are mean ± SD and 95% confidence interval.
* $P < 0.05$.

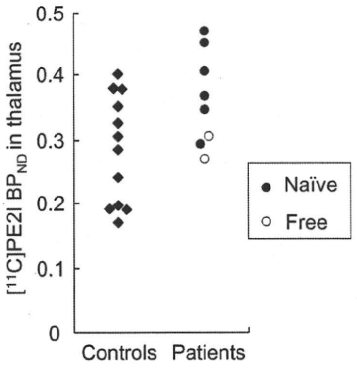

Fig. 2. BP_{ND} in the thalamus of normal controls and patients with schizophrenia. BP_{ND} of patients with schizophrenia was significantly higher than that of the control group (df = 18, $t = 2.16$, $P = 0.044$).

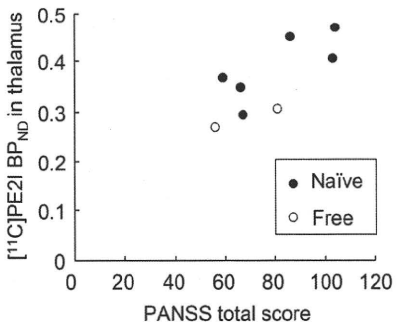

Fig. 3. Relationship between BP_{ND} in the thalamus of patients with schizophrenia and total PANSS score. There were significantly positive correlations between BP_{ND} and total PANSS score ($r = 0.75$, $P = 0.032$).

trols (df = 18, $t = 2.16$, $P = 0.044$) (Table 2, Fig. 2). There were no significant differences in BP_{ND} between the two groups in the caudate, putamen or substantia nigra. In patients with schizophrenia, there were significant positive correlations between BP_{ND} in the thalamus and total PANSS score ($r = 0.75$, $P = 0.032$), positive ($r = 0.78$, $P = 0.023$) and negative PANSS scores ($r = 0.82$, $P = 0.014$), but no correlation was observed with the general PANSS score (Table 3, Fig. 3). There was no significant correlation between BP_{ND} in other regions and clinical symptoms. There was also no significant correlation between BP_{ND} in each region and age or duration of illness.

4. Discussion

The *in vivo* evaluation of thalamic DAT had not been previously performed in detail due to its very low density as compared to that in the striatum (Hall et al., 1999). [^{11}C]PE2I allows the estimation of specific binding in low density regions because of its high affinity and selectivity for DAT (Halldin et al., 2003; Hirvonen et al., 2008; Jucaite et al., 2006). In this study, BP_{ND} in the thalamus of patients with schizophrenia was significantly higher than that of control subjects and was positively correlated with clinical symptoms. There was no significant difference in the area under the time activity curves of the cerebellum between controls and the patient group (two-tailed *t*-test; $P = 0.37$), suggesting that the higher DAT bindings were not due to cerebellar difference. An effect of endogenous dopamine on [^{11}C]PE2I binding has not been reported. However, as [^{11}C]PE2I is a high-affinity radioligand ($K_i = 17$ nM), it is reasonable to expect such an effect based on the result from a high-affinity ralioligand for serotonin transporter, [^{11}C]DASB ($K_i = 1.1$ nM) (Wilson et al., 2000). [^{11}C]DASB binding did not change by manipulation of endogenous serotonin in human brain (Praschak-Rieder et al., 2005; Talbot et al., 2005). Although these results may not apply directly to [^{11}C]PE2I binding, high [^{11}C]PE2I binding can nevertheless be interpreted as high DAT density.

The thalamus has been considered as the key brain structure of processing or integrating sensory information related to emotional or cognitive functions (Clinton and Meador-Woodruff, 2004). Several studies have reported morphological abnormalities of the thalamus in patients with schizophrenia using MR imaging or postmortem studies (Clinton and Meador-Woodruff, 2004). Regarding dopaminergic transmission, increased dopamine concentrations in the thalamus of patients with schizophrenia were reported in a postmortem study (Oke and Adams, 1987). The distribution of dopaminergic innervation in the thalamus was reported recently using immunohistochemistry in monkey (Melchitzky and Lewis, 2001) and human brain (Garcia-Cabezas et al., 2007). These studies reported that thalamic dopamine or DAT was relatively higher in the midline and mediodorsal nuclei. In patients

Table 3
Correlation between regional BP_{ND} and PANSS scores.

	Total		Positive		Negative		General	
Region	r	P	r	P	r	P	r	P
Caudate	−0.04	0.93	0.03	0.95	0.10	0.81	−0.14	0.74
Putamen	−0.44	0.28	−0.42	0.31	−0.03	0.93	−0.55	0.15
Thalamus	0.75	0.032*	0.78	0.023*	0.82	0.014*	0.47	0.24
Substantia nigra	0.04	0.93	0.26	0.53	0.03	0.94	−0.07	0.86

* $P < 0.05$.

with schizophrenia, lower dopamine D_2 receptor binding was observed in the thalamus using PET with [^{11}C]FLB457 (Buchsbaum et al., 2006; Talvik et al., 2003; Yasuno et al., 2004) and [^{11}C]raclopride (Talvik et al., 2006). Significant differences in calcyon and spinophilin, dopamine receptor-associated intracellular proteins, and no difference in vesicular monoamine transporter (VMAT) binding of the thalamus were reported in a postmortem study of patients with schizophrenia and controls (Clinton et al., 2005). Assuming that low dopamine D_2 receptor binding is related to the disruption of the feedback system of dopamine release mediated by GABA interneuron (Takahashi et al., 2006), a high turnover of dopamine at the synapse would exist as a hyper-dopaminergic state. Although the function of DAT in the thalamus has remained unclear, high DAT bindings may suggest a hyper-dopaminergic state of pre-synaptic dopamine function in patients with schizophrenia.

Most of the previous PET and SPECT studies reported that DAT binding in the striatum did not differ between subjects and patients with schizophrenia (Hsiao et al., 2003; Laakso et al., 2000; Laruelle et al., 2000; Lavalaye et al., 2001; Schmitt et al., 2005, 2006, 2008; Yang et al., 2004), and our present results were in line with these reports. DAT binding in the substantia nigra also showed no difference between control subjects and patients with schizophrenia. However, BP_{ND} in the striatum using SRTM can be underestimated as compared to the values by kinetic model analyses with arterial blood sampling (Hirvonen et al., 2008; Jucaite et al., 2006). This might affect the results in the striatum.

In this study, the number of subjects was small, and in the statistical analysis we did not perform multiple comparisons regarding group differences of BP_{ND} between patients and controls to avoid type II error. Moreover, two of the eight patients were in a drug-free state, not drug-naïve state. Nonetheless, even when the two drug-free patients were excluded, the group difference of BP_{ND} in the thalamus was still observed (two-tailed t-test; $P = 0.018$). Further study with larger numbers of subjects in a drug-naïve state will be needed.

In conclusion, [^{11}C]PE2I binding in the thalamus of patients with schizophrenia was significantly higher than in control subjects and was correlated with clinical symptoms. Altered DAT in the thalamus might be related to the pathophysiology and clinical symptoms of schizophrenia.

Conflict of interest

All authors have no conflicts of interest.

Contributors

R. Arakawa, T. Ichimiya, A. Takano, F. Yasuno, and T. Suhara designed the study and wrote the protocol. R. Arakawa, T. Ichimiya, A. Takano, M. Okumura, H. Takahashi, H. Takano, F. Yasuno, M. Kato, and Y. Okubo recruited the patients and made psychiatric evaluations. R. Arakawa, H. Ito, M. Okumura, H. Takahashi, and H. Takano participated in the data analysis. R. Arakawa wrote the first draft of the manuscript. R. Arakawa, H. Ito, H. Takahashi, H. Takano, M. Kato, Y. Okubo, and T. Suhara had discussions and corrected the manuscript. All authors contributed to and have approved the final manuscript.

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Time estimation during sleep relates to the amount of slow wave sleep in humans

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ABSTRACT

Humans have the ability to estimate the amount of time that has elapsed during sleep (time estimation ability; TEA) that enables a subset of individuals to wake up at a predetermined time without referring to a watch or alarm clock. Although previous studies have indicated sleep structure as a key factor that might influence TEA during sleep, which sleep parameters could affect the TEA has not been clarified. We carried out an experimental study in which 20 healthy volunteers participated in six time estimation trials during the 9-h nighttime sleep (NS) experiment or daytime sleep (DS) experiment. The time estimation ratio (TER, ratio of the subjective estimated time interval to actual time interval) decreased significantly from the first to the sixth trial in both the NS and DS experiments. TER correlated positively with slow wave sleep (SWS) in both experiments, suggesting that SWS was a determining factor in accurate time estimation, irrespective of circadian phase they slept. No other sleep parameters showed steady influence on TEA. The present findings demonstrate that longer period of SWS is associated with the longer sleep time they subjectively experienced during sleep.

1. Introduction

Growing evidence suggests that humans have the ability to estimate the amount of time that has elapsed on the order of milliseconds to several hours (time estimation ability, TEA) even under circumstances in which external time information is not available (Morell, 1996; Harrington et al., 1998; Lalonde and Hannequin, 1999; Rao et al., 2001; Ivry and Spencer, 2004). A series of studies has supported the notion that the TEA pervades sleep period; humans perceive the amount of time that has passed during sleep (Lewis, 1969; Tart, 1970; Zung and Wilson, 1971; Bell, 1972; Moiseeva, 1975; Lavie et al., 1979; Hartocollis, 1980; Campbell, 1986; Zepelin, 1986; Hawkins, 1989; Moorcroft et al., 1997; Born et al., 1999; Kaida et al., 2003; Aritake et al., 2004; Fichten et al., 2005). This ability enables a subset of individuals to wake up at a predetermined time without referring to a watch or alarm clock. Moorcroft et al. (1997) referred to this phenomenon as "self-awakening", and Born et al. (1999) referred to it as "anticipated sleep termination". Actually, several studies have reported that more than half of individuals surveyed were able to achieve "self-awakening" with a margin of error of plus or minus 10-odd min (Lavie et al., 1979; Moorcroft et al., 1997).

A large part of the physiological mechanism of TEA remains unclear, but previous studies have shown that several physiological and psychological factors influence TEA during sleep. These include psychological status prior to bedtime (Hawkins, 1989) altered neuroendocrine tonus (Born et al., 1999), and sleep structure (Kleitman, 1963; Tart, 1970; Zung and Wilson, 1971; Lavie et al., 1979; Zepelin, 1986; Aritake et al., 2004) preceding the predetermined wake time. For instance, strong motivation and the confidence that are will wake up at the predetermined time are associated with successful self-awakening (Hawkins, 1989; Moorcroft et al., 1997). Born et al. (1999) showed clearly that anticipated awakening at a predetermined time was preceded by an elevation in ACTH secretion (a particularly early, morning ACTH surge), a phenomenon that did not occur in relation to an unexpected ("surprise") awakening at the same clock time.

Several studies have focused on sleep structure as a key factor that might influence TEA during sleep; however, it remains controversial whether the preceding sleep stage or partial

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awakening prior to the predetermined wake time modifies TEA in humans (Kleitman, 1963; Zung and Wilson, 1971; Lavie et al., 1979; Zepelin, 1986; Aritake et al., 2004). We previously conducted a study to test whether the preceding sleep structure influenced the estimated passage of time during nighttime sleep which was divided into six time periods (90 min each) in healthy young subjects (Aritake et al., 2004). We found that, as sleep progressed, the subjects underestimated the amount of time that had passed in each time period. The estimated elapsed time correlated positively with the amount of slow wave sleep (SWS) and negatively with the amount of REM sleep. These findings support the notion that TEA pervades sleep and that it is affected by the preceding sleep status.

The aim of the present study was to clarify which sleep parameters could essentially influence on TEA by comparing the properties of estimated time interval during the usual nighttime sleep (NS) period with those during an arbitrary daytime sleep (DS) period in circadian antiphase. We expected REM sleep and SWS to show different time distributions between the two experimental conditions, and that this would enable us to more precisely detect functional interaction between the sleep structure and TEA during the sleep period.

2. Materials and methods

2.1. Participants

Twenty healthy men aged 18–23 years (mean, 21.1 ± 1.7 years), who had regular sleep habits, participated in the study. They were randomly allocated to on NS experiment or DS experiment. Three participants allocated to the DS experiment withdrew from the study (one due to infection during the pre-study period, one for an undisclosed reason, and one due to discomfort during the acute shift schedule). Thus, 10 participants completed the NS experiment (mean age, 20.2 ± 1.6 years) and 7 completed the DS experiment (mean age, 22.4 ± 0.7 years). They provided written informed consent after the possible risks and details of the study were explained to them. A physician and a psychiatrist examined all participants and found that none suffered from a neurological or psychiatric disorder, and none had a history of psychoactive drug use. Participants were instructed to keep to a regular sleep–wake schedule; record their sleep patterns in a sleep log; and abstain from caffeine, nicotine, and alcohol for 1 week prior to the experiment. All participants wore a wrist activity recorder (Actiwatch-L, Mini-Mitter Co., Inc., Bend, OR, USA) for 1 week prior to the experiment. Sleep onset and offset times were determined with Actiware Sleep software (V3.2 Mini-Mitter Co., Inc.). The details recorded in participants' sleep logs, together with their sleep onset and offset times, were used to confirm that they had regular sleep--wake schedules. Because participants' attention to time could potentially affect the experimental results, we told them that the aim of the study was to investigate correlation between sleep parameters and subjective feeling; we did not disclose the study objectives until the end of the study. We confirmed that none of the participants had sensed the real purpose of the investigation until the end of this study. The study protocol was approved by the Institutional Review Board of the National Center of Neurology and Psychiatry.

2.2. Experimental procedures

Time estimation protocol is illustrated in Fig. 1.

2.2.1. NS experiment

The NS experiment was begun as follows: on day 1, the participant arrived at the laboratory at 19:00 h and slept in the laboratory bedroom from 0:00 h to 08:00 h for adaptation. After being woken at 08:00 h on day 2, the participant was kept awake until 00:00 h on day 3 under dim light conditions (150 lx). During waking hours, the participant was kept from knowing the clock time until the beginning of the time estimation protocol (TEP). His only awareness of the time of day would have been by the scheduled provision of an isocaloric meal (450 kcal) and mineral water every 4 h. At 00:00 h on day 3, the participant was instructed to go to bed and that the TEP would begin.

2.2.2. DS experiment

The DS experiment was begun as follows: on day 1, the participant arrived at the laboratory at 19:00 h and slept in the laboratory bedroom from 0:00 h to 08:00 h for adaptation. After being woken at 08:00 h on day 2, the participant was kept awake for 28 h until 12:00 h on day 3 under the same isolated condition as in the NS experiment. An isocaloric meal (450 kcal) and mineral water were provided every 4 h. After 28 h of enforced wakefulness,

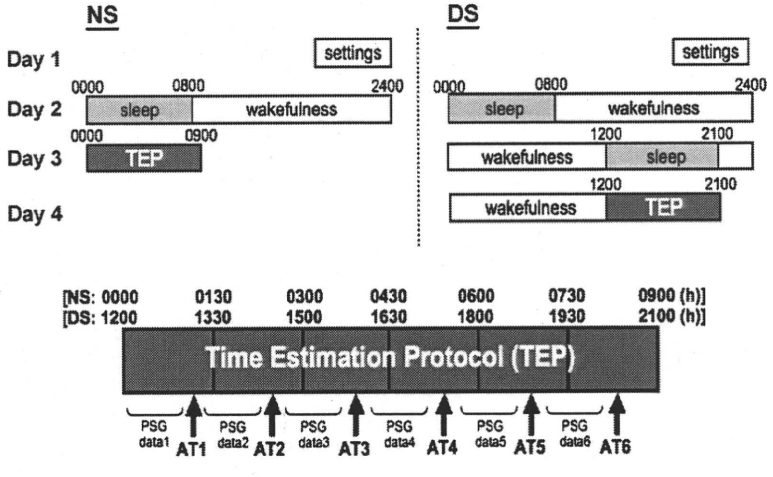

Fig. 1. Time estimation protocol (TEP). TEP was conducted between 00:00 h and 09:00 h (nighttime sleep: NS) or 12:00 h and 21:00 h (daytime sleep: DS). The 9-h polysomnography (PSG) recording periods were divided into six 90-min periods. We woke the participants and conducted a structured interview once during each 90-min period (awakening trial: AT). Participants were awakened for an AT when (1) they had slept for longer than 45 min after lights out or since the end of the prior AT; and (2) stage 2 sleep had continued for more than 3 min. PSG data between successive ATs were obtained. If these criteria were not satisfied until 75 min after the beginning of 90-min period, the participants were awakened at the end of each 90-min period. In the structured interview, we asked the several questions including, "What time do you think it is now? (subjective time of day)" to determine participants' spontaneous estimation of time, without encouraging them to focus their attention on time.

the participant was allowed recovery sleep from 12:00 h to 21:00 h on day 3. After being woken at 21:00 h on day 3, the participant was kept awake for 15 h. At 12:00 h on day 4, the participant was instructed to go to bed and that the TEP would begin.

2.3. Measures and condition

All experiments were performed in the time isolation laboratory of the National Center of Neurology and Psychiatry in Japan. Polysomnography (PSG) comprised electroencephalogram (EEG; C3–A2, C4–A1 and O1–A2, O2–A1) in conformity with the 10–20 electrode system, electrooculogram (EOG; left-A2 and right-A1), chin surface electromyogram (chin-EMG), and electrocardiogram (ECG) recordings. PSG data were obtained continuously during each experiment and stored in a digital EEG system (Neurofax, Nihon Kohden, Tokyo, Japan). Core body temperature (cBT) was measured every 2 min from 21:00 h on day 1 until the end of the experiment, the data were stored in a soft ware (V3.2 Mini-Mitter Co., Inc.). The PSG and cBT monitoring were set up between 19:00 h and 21:00 h on day 1. The participant' behavioral status and sleep–wake status were continuously monitored by two well-trained research attendants using a digital EEG system and by visual observation. Room temperature and humidity were controlled at 24 °C and 60%, respectively.

2.4. Time estimation protocol

The 9-h PSG recording period was divided into six 90-min periods (Fig. 1). During each 90-min period, the participant was awakened and given a brief structured interview with supine (lasting 2 min or less, <8 lx) about the perceived clock time. This procedure was termed the awakening trial (AT). The time of each AT was determined when (1) the participant had slept for more than 45 min after lights-out or since the end of the prior AT; and (2) stage 2 sleep had continued for more than 3 min. If these criteria were not satisfied before 75 min of each 90-min period has passed, the participant was awakened at the end of the 90-min period. During the structured interview, we asked several questions including, "What time do you think it is now?" to determine the participant's spontaneous estimation of time, without encouraging him to focus his attention on the amount of time that had passed since previous arousal. The interviewer was instructed not to give disclose the real purpose of the study, and the participant was given no information on the exact number or timing of the ATs.

2.5. Data analysis

2.5.1. TEA variables

The subjective time interval, defined as the difference between the estimated time of day during the AT and that during the previous AT (or 00:00 h) was determined. Time estimation ratio (TER), defined as the estimated time interval (subjective time interval: s_1 or s_2) divided by the actual clock time interval (actual time interval: a_1 or a_2) (Aritake et al., 2004) (Fig. 2), was also determined.

2.5.2. Sleep parameters

PSG data obtained between successive ATs were scored in epochs of 30 s according to the standard criteria (Rechtschaffen and Kales, 1968). Time percentages of stage W (%stage W), stage 1 (%stage 1), stage 2 (%stage 2), stage 3 + 4 (%stage 3 + 4) and stage REM (%stage REM) sleep for the entire sleep period and for each AT period were calculated for all PSG recordings.

2.5.3. cBT

To ensure comparability of the circadian phase between the NS and DS experiments, we determined the times of nadir and peak time of cBT in both experiments. The cBT data from 18:00 h on day 2 to 24:00 h on day 3 was smoothed by using a 24-h double cosine curve fit procedure (Kaleida Graph ver.3.6, Hulinks Inc., Tokyo, Japan) for both the NS and DS experiments, and the times of the fitted minimum (nadir) and maximum (peak time) of cBTs were determined.

2.6. Statistical analyses

Differences in variables between the NS and DS experiments were analyzed by *t*-test. Differences in TEA variables for each AT between the NS and DS experiments were analyzed by two-way repeated measures ANOVA (ATs × NS vs. DS) or two-way factorial ANOVA (sleep stages just before ATs × NS vs. DS). Correlations

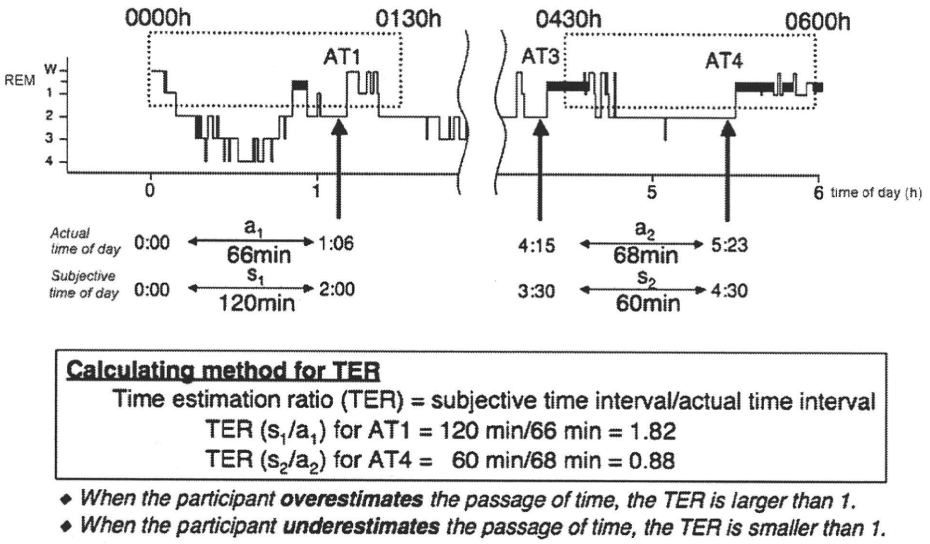

Fig. 2. Time estimation ratio (TER). Subjective time interval in both experiments was defined as the time difference between subjective times of the day, which were obtained at successive awakening trials (ATs). The actual time interval was defined as the actual time difference between successive ATs. The TER, as an indicator of subjective time estimation, was calculated by the dividing a subjective time interval (s_1 or s_2) by the actual time interval (a_1 or a_2).

Table 1
Sleep and core body temperature parameters in normal NS and DS.

	NS (n = 10) (mean ± S.D.)	DS (n = 7) (mean ± S.D.)	t-Test (p-value)
Total recording time (min)	484.5 ± 25.7	502.1 ± 20.0	n.s.
Total sleep time (min)	436.9 ± 46.8	348.3 ± 56.9	0.003
Sleep efficiency (%)	90.5 ± 10.6	69.6 ± 12.9	0.002
Wake (min)	47.5 ± 55.9	153.8 ± 68.1	0.003
Stage 1 (min)	40.2 ± 19.0	48.8 ± 19.6	n.s.
Stage 2 (min)	240.1 ± 40.6	187.9 ± 42.1	0.021
Stage 3 + 4 (min)	58.8 ± 21.9	45.4 ± 9.5	n.s.
REM (min)	65.5 ± 31.9	59.00 ± 10.1	n.s.
Wake (%)	9.5 ± 10.6	30.4 ± 12.8	0.002
Stage 1 (%)	8.3 ± 3.9	9.8 ± 4.0	n.s.
Stage 2 (%)	49.7 ± 9.1	37.6 ± 9.3	0.017
Stage 3 + 4 (%)	12.1 ± 4.5	9.0 ± 1.7	n.s.
REM (%)	13.5 ± 6.6	11.8 ± 2.1	n.s.
Core body temperature parameters			
Nadir time (h)	5.5 ± 1.3	6.3 ± 2.3	n.s.
Peak time (h)	18.9 ± 2.9	20.36 ± 4.1	n.s.

p = probability, n.s. = not significant.

between variables were assessed by Pearson's correlation coefficient. Stepwise multiple regression analysis was used to evaluate relationship between TEA variables (dependent variables) and sleep structures or circadian phase (predictor variables). StatView ver.5.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses. Data were expressed as mean ± standard deviation. The level of significance was set at $p < 0.05$.

3. Results

3.1. PSG variables

PSG variables for the entire sleep period in the NS and DS experiments are shown in Table 1. There was no significant difference in total recording time between the two experiments. Total sleep time and sleep efficiency in the DS experiment were significantly deceased in comparison to corresponding values in the NS experiment. There were no significant differences in total duration and percentages of stage 1, stage 3 + 4, or stage REM sleep between the two experiments. However, sleep total duration and percentage of stage W sleep were significantly increased and those for stage 2 sleep were significantly decreased in the DS experiment in comparison to corresponding values in the NS experiment.

3.2. Circadian phase

There was no significant difference in the time of nadir or peak time of cBT between the NS and DS experiments (Table 1).

3.3. AT variables

PSG stages during which ATs were carried out differed between the NS and DS experiments; 91.67% and 64.29% ATs, respectively, were carried out in stage 2, 6.67% and 11.1% ATs were carried out in stage 1, and 1.67% and 44.44% ATs were carried out in stage W. However, two-way factorial ANOVA (sleep stage just before ATs × NS vs. DS) revealed that there was no significant main effect of sleep stages just before ATs on TER ($F(2, 96) = 1.615$, $p = 0.204$); neither was there a significant main effect of experimental condition ($F(1, 96) = 0.908$, $p = 0.343$) nor a significant interaction ($F(2, 96) = 0.076$, $p = 0.927$) between sleep stages just before ATs and experimental condition. Therefore, the TER data obtained in the three different PSG stages (stages 1, 2, and W) were combined in further analyses.

3.4. TER

There was no significant difference in the TER for the entire sleep period between the NS and DS experiments (NS experiment, 0.966 ± 0.717; DS experiment, 1.006 ± 0.747). Time course of the TER and the percentages of sleep stages are shown in Fig. 3. Two-way repeated measures ANOVA (ATs × NS vs. DS) revealed a significant main effect of the time course on TER ($F(5, 75) = 13.254$, $p < 0.0001$), whereas there was neither a significant main effect of experimental condition ($F(1, 75) = 0.110$, $p = 0.745$) nor a significant interaction ($F(5, 75) = 0.326$, $p = 0.896$) between time course and experimental condition. The TER value was at nearly two during AT1 and gradually decreased toward 0.5 as sleep progressed. The pattern was similar in the NS and DS experiments (Fig. 3a).

3.5. Sleep structures

Two-way repeated measures ANOVA (ATs × NS vs. DS) revealed a significant main effect of time course on stage 3 + 4 ($F(5, 75) = 12.285$, $p < 0.001$), whereas there was neither a significant main effect of experimental condition ($F(1, 75) = 2.266$, $p = 0.153$) nor a significant interaction ($F(5, 75) = 0.144$, $p = 0.981$) between time course and experimental condition (Fig. 3d). Two-way repeated measures ANOVA (ATs × NS vs. DS) also revealed a significant main effect of time course on %stage 3 + 4 ($F(5, 75) = 18.333$, $p < 0.001$), whereas there was neither a significant main effect of experimental condition ($F(1, 75) = 2.436$, p = 0.139) nor a significant interaction ($F(5, 75) = 0.184$, $p = 0.968$) between time course and experimental condition. The stage 3 + 4 decreased as sleep progressed in both the NS and DS experiments (Fig. 3i).

There was a significant interaction between time course and conditions in stage REM, stage W, and stage 2. Stage REM in NS increased toward morning, whereas stage REM in DS decreased toward nighttime. Stage W in NS did not change toward morning, whereas stage W in DS increased from AT5 to AT6. Stage 2 in NS did not change toward morning, whereas stage 2 in DS decreased from AT5 to AT6 (Fig. 3b, c and e). No significant effect of time course was found in stage 1 in either two conditions. We also found comparable results in corresponding percentage values for all sleep stages (Fig. 3g, h and j).

3.6. Correlation between TER and sleep structures

We averaged the TER and stage 3 + 4 sleep per AT data across all participants to reduce inter-individual variation in sleep

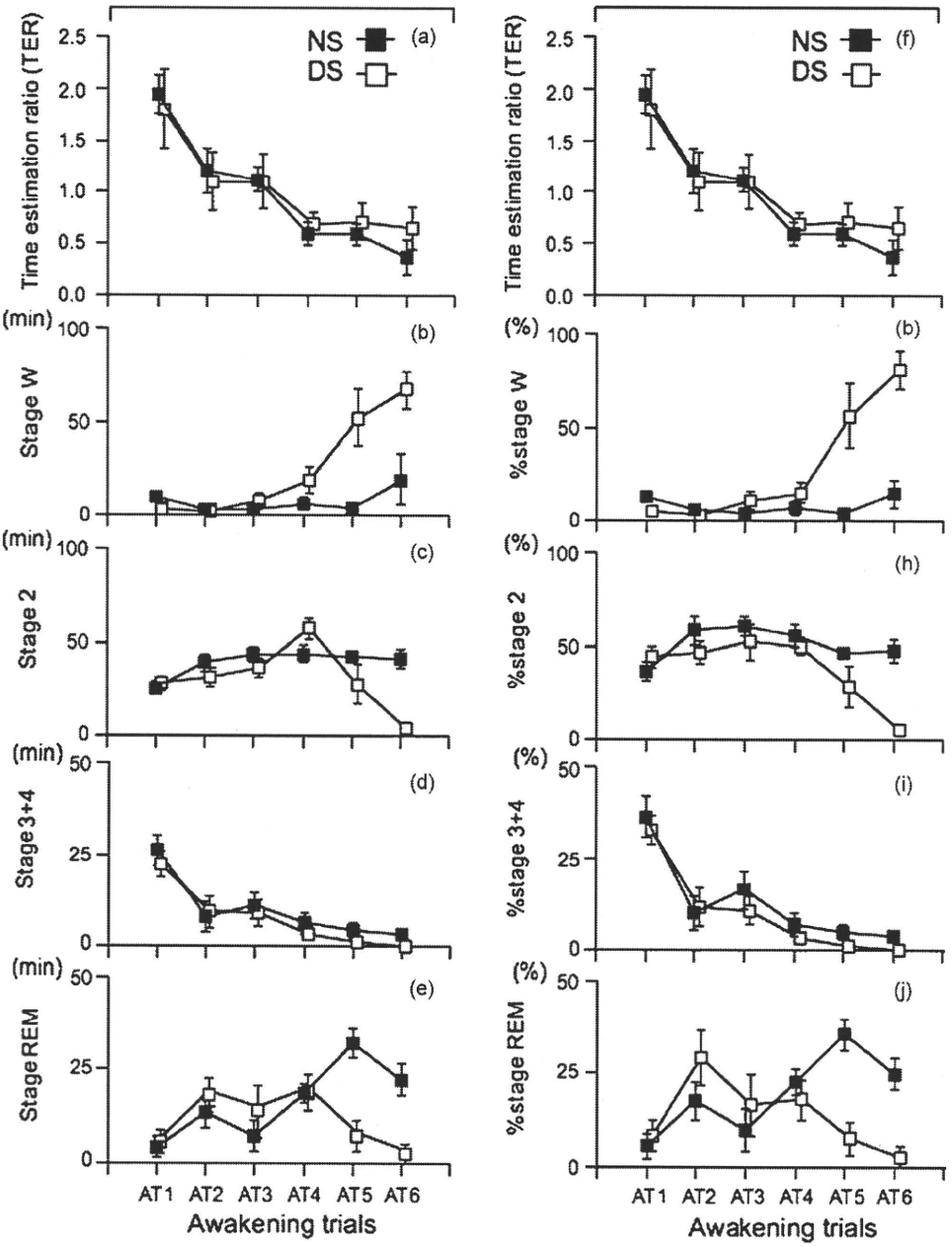

Fig. 3. (a–j) Time course of the mean time estimation ratio (TER) and the amounts (left panel) and the percentages (right panel) of sleep stages. Filled and open circles represent the data in nighttime sleep (NS) and daytime sleep (DS) experiments, respectively. The horizontal axes indicate the AT number. Two-way repeated measures ANOVA revealed a significant main effect of time course on TER and stage 3 + 4 sleep in both experiments. The value of TER was nearly 2.0 at AT1, and it decreased toward 0.5 as sleep progressed.

structure. Significant positive correlation was found between averaged TER and averaged stage 3 + 4 in both the NS ($r = 0.943$, $p = 0.002$) and DS ($r = 0.993$, $p < 0.001$). We also found a significant positive correlation between the averaged TER and averaged %stage 3 + 4 in both the NS ($r = 0.944$, $p = 0.002$) and DS ($r = 0.993$, $p < 0.001$).

3.7. Stepwise multiple regression analysis for TER

The following variables were analyzed by stepwise multiple regression for prediction of TER (dependent variable): stage W, stage 1, stage 2, stage 3 + 4, stage REM, and acrophase of each AT (interval between the time of cBT nadir and the time of each AT). Only stage 3 + 4 was identified as a predictive variable that explained the variance of TER ($r = 0.251$, $p = 0.011$). We also found comparable results in percentage values for sleep stages; only %stage 3 + 4 was identified as a more prominent predictive variable that explained the variance of TER ($r = 0.327$, $p = 0.001$).

4. Discussion

In the present study, we investigated influences of the sleep architecture on TEA during NS and DS periods. We found that TER, as an indicator of a subjectively estimated time interval, was higher at the beginning of the sleep period (i.e., sleep time was overestimated than the actual time elapsed), and that it successively decreased toward the end of the sleep. Positive correlation between the amount of SWS and the TER was confirmed in both the NS and DS periods, despite the fact that the two sleep periods were located around the circadian antiphase

represented by the cBT. This suggests that the greater the amount of SWS the study subjects obtained, the longer the sleep time they subjectively experienced. We could not confirm a steady influence of REM sleep on TEA in our study participants. We observed negative correlation between the amount of REM sleep and the TER only in the NS period, as was reported previously (Aritake et al., 2004). This relation disappeared in the DS period during which the normal REM sleep pattern was distorted (Weitzman et al., 1980; Dijk and Czeisler, 1995; Borbely and Achermann, 1999). Comparison of sleep structures and TER properties between the NS and DS periods clearly highlighted the significant influence of SWS on TEA in humans.

The study subjects experienced poorer sleep continuity (shorter total sleep time, decreased sleep efficiency, and longer awake time) in the DS period than in the NS period, possibly due to the circadian antiphase, although the amounts of stage 1, stage 3 + 4, and stage REM sleep did not differ significantly between the two experimental conditions. However, it is not likely that the differences in sleep structure during the 9-h PSG recording period substantially influenced the relation between the sleep architecture and TEA because similar TER values close to 1 were obtained (0.966 ± 0.72 for the NS period, 1.006 ± 0.75 for the DS period), suggesting that participants could accurately estimate the length of sleep time (on average) through the entire sleep period.

While the underlying regulatory mechanism of TEA during sleep remains to be clarified, various brain sites have been revealed to be responsible for human TEA of different temporal range (Ivry, 1996; Lalonde, 1999; Lewis and Miall, 2003; Ivry, 2004). For instance, the cerebellum is reported to be involved in the short time estimation of less than 1 s (Jueptner et al., 1995; Rao et al., 1997; Spencer et al., 2003; Ivry and Spencer, 2004). Contrastingly, the prefrontal cortex is involved in the time estimation of more than 1 s (Mangels et al., 1998; Lalonde and Hannequin, 1999; Lewis and Miall, 2003). Concerning the TEA during sleep, greater cortical deactivation during a longer period of SWS might contribute to overestimation of the actual sleep time. Kajimura et al. (1999) studied cerebral blood flow during sleep by means of positron emission topography. Sleep-induced cortical deactivation started during light stages of nocturnal sleep and progressed in a sleep stage-dependent manner; cerebral blood flow during deep non-REM sleep was reduced in the midbrain, basal forebrain, and basal ganglia (caudate nucleus) and bilaterally in neocortical regions including the medial and inferior frontal gyrus. During wakefulness, the cerebellum, the prefrontal cortex and basal ganglia perform higher-order processing of sensory information, integrating cognitive information. Several neuroimaging studies in humans have shown that the cerebellum, the prefrontal cortex and a corticostriatal network in the basal ganglia are responsible for the ability to perceive time intervals during wakefulness (Jueptner et al., 1995; Maquet et al., 1996; Rao et al., 1997, 2001; Harrington et al., 1998; Pouthas et al., 1999; Gruber et al., 2000; Schubotz et al., 2000; Spencer et al., 2003; Coull et al., 2004). These neuronal systems might also contribute to the regulation of TEA during sleep. Thus, preceding deep sleep and associated cortical deactivation could substantially influence perceived passage of time during sleep.

During wakefulness, TEA has been reported to show diurnal fluctuation (Aschoff, 1998; Campbell et al., 2001; Kuriyama et al., 2005). A study involving a time production strategy (producing a predetermined time interval by pressing a button) during wake time has shown that TEA might be influenced by the circadian system in humans (Kuriyama et al., 2005). The produced time interval tended to be shorter than the actual time interval during the nighttime, and it became longer toward the morning time. This is analogous to individuals overestimating the perceived time interval in the first half of rather than the latter half of the sleep period, as was observed in our present study. However, in our study subjects, changes in TER for the NS and DS periods in reciprocally circadian antiphase showed remarkably similar time profiles and multiple stepwise regression analysis revealed no relation between acrophases of time estimation and the corresponding TER values. Although we examined the change in TEA for only 8–9 h of each sleep periods, our findings do not support the notion that the TEA during sleep time was primarily under the regulation of circadian system.

These findings were obtained using a time estimation protocol consisted of six 90-min period interval trials, which might interfere in the naturalistic sleep cycle including REM–NREM sleep cycles and TEA properties in the study subjects. Despite of the limitations, the present study support the notion that humans possess the TEA that pervades sleep period and that SWS can prolong the subjectively estimated time interval during sleep, irrespective of the circadian phase they slept. Future studies should focus on the physiological mechanism of TEA during sleep and reveal the pathophysiological features of TEA in several sleep disorders such as paradoxical insomnia in which subjective sleep disturbances appear without objective evidence of deteriorated sleep quality (Salin-Pascual et al., 1992; Edinger and Fins, 1995; Perlis et al., 1997; Vanable et al., 2000; ICSD, 2005; Edinger and Krystal, 2003). Time estimation protocol we applied in this study would be an useful option in the human sleep studies.

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Regular Article

Hyperfrontality in patients with schizophrenia during saccade and antisaccade tasks: A study with fMRI

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Aims: Antisaccadic eye movements, requiring inhibition of a saccade toward a briefly appearing peripheral target, are known to be impaired in schizophrenia. Previous neuroimaging studies have indicated that patients with schizophrenia show diminished activations in the frontal cortex and basal ganglia. These studies used target fixation as a baseline condition. However, if the levels of brain activities at baseline are not compatible between patients and healthy subjects, between-group comparison on antisaccade-related activations is consequently invalidated. One possibility is that patients with schizophrenia may present with greater activation during fixation than healthy subjects. In order to examine this possibility, here we investigated brain activities associated with antisaccade in the two groups without using target fixation at baseline.

Methods: Functional brain images were acquired during prosaccades and antisaccades in 18 healthy subjects and 18 schizophrenia patients using a boxcar functional magnetic resonance imaging design. Eye movements were measured during scanning.

Results: In the patient group, the elevated activities in the dorsolateral prefrontal cortex (DLPFC) and thalamus, normally seen in antisaccade tasks relative to saccade tasks, were no longer observed. Moreover, in normal subjects, activities in the DLPFC and thalamus were greater during the antisaccade task than during the saccade task. In patients, no such difference was observed between the two tasks, suggesting that these brain regions are likely to be highly activated even by a simple task such as fixation. In particular, the DLPFC and thalamus in patients were not activated at a level commensurate with the difficulty of the tasks presented.

Conclusions: From these results, it is suggested that schizophrenia entails dysfunctions in the fronto-striato-thalamo-cortical network associated with motor function control.

Key words: antisaccade, fMRI, hyperfrontality, saccade, schizophrenia.

SACCADIC EYE MOVEMENTS are the primary mechanism used by primates to visually explore their environments. A visually guided reflexive saccade can be defined as an automatic orienting response to a novel visual target in the peripheral field. Patients with schizophrenia perform prosaccades normally, making rapid and accurate eye movements to targets.[1–3] In contrast, the inhibition of

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