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ARTICLE

CINP

Peripheral benzodiazepine receptors in patients with chronic schizophrenia: a PET study with [^{11}C]DAA1106

Akihiro Takano[1], Ryosuke Arakawa[1], Hiroshi Ito[1], Amane Tateno[2], Hidehiko Takahashi[1], Ryohei Matsumoto[1], Yoshiro Okubo[2] and Tetsuya Suhara[1]

[1] *Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan*
[2] *Department of Neuropsychiatry, Nippon Medical School, Tokyo, Japan*

Abstract

Inflammatory/immunological process and glial contribution are suggested in the pathophysiology of schizophrenia. We investigated peripheral benzodiazepine receptors in brains of patients with chronic schizophrenia, which were reported to be located on mitochondria of glial cells, using [^{11}C]DAA1106 with positron emission tomography. Fourteen patients and 14 age- and sex-matched normal controls participated in this study. PET data were analysed by two-tissue compartment model with metabolite-corrected plasma input. Clinical symptoms were assessed using the Positive and Negative Syndrome Scale. There was no significant difference between [^{11}C]DAA1106 binding of the cortical regions of normal controls and patients with schizophrenia, whereas the patients showed a positive correlation between cortical [^{11}C]DAA1106 binding and positive symptom scores. There was also a positive correlation between [^{11}C]DAA1106 binding and duration of illness. Although the correlations need to be interpreted very cautiously, involvement of glial reaction process in the pathophysiology of positive symptoms or progressive change of schizophrenia might be suggested.

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Key words: Microglia, peripheral benzodiazepine receptor, positive symptoms, schizophrenia.

Introduction

An accumulating body of evidence has suggested that the pathophysiology of schizophrenia could be related to the dysregulation of the inflammatory response system, such as increased levels of *in vivo* IL-1RA, sIL-2R, and IL-6 (Lin *et al.* 1998; Nawa & Takei, 2006; Potvin *et al.* 2008; Zhang *et al.* 2004). Microglia has been regarded as a mediator of neuroinflammation via the release of pro-inflammatory cytokines, nitric oxide (NO) and reactive oxygen species (ROS) in the central nervous system (CNS). Peripheral benzodiazepine receptor (PBR) was reported to reflect neuronal injury and inflammatory lesions in the brain by increased expression of the number of binding sites in glial cells including activated microglia and reactive astrocytes as visualized *in vivo* using PET with [^{11}C]PK11195 (Shah *et al.* 1994). Recent reports demonstrated that [^{11}C]PK11195 binding was increased in patients with acute-onset schizophrenia (van Berckel *et al.* 2008) and in patients with schizophrenia during psychosis (Doorduin *et al.* 2009). However, the affinity (Chaki *et al.* 1999) and permeability of the blood–brain barrier was low for PK11195, reportedly a substrate of efflux transporter P-glycoprotein (Jakubikova *et al.* 2002; Vaalburg *et al.* 2005). Low uptake of [^{11}C]PK11195 in the brain could hamper stable quantitative analysis.

(*N*-5-fluoro-2-phenoxyphenyl)-*N*-(2,5-dimethoxylbenzyl) acetamide (DAA1106) is a potent and selective ligand for PBR with high affinity (Chaki *et al.* 1999; Okuyama *et al.* 1999). [^{11}C]DAA1106 is accumulated at high levels in the mouse brain (Zhang *et al.* 2003), and the radioactivity of [^{11}C]DAA1106 at 30 min after injection was reported to be four times higher than that of [^{11}C]PK11195 in the monkey brain (Maeda *et al.* 2004). A quantitative analysis method for [^{11}C]DAA1106 binding in the human brain has been well established with the two-tissue compartment model (Ikoma *et al.* 2007). [^{11}C]DAA1106 was

Address for correspondence: T. Suhara, M.D., Ph.D., Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba, 263-8555, Japan.
Tel.: +81-43-206-3251 *Fax*: +81-43-253-0396
Email: suhara@nirs.go.jp

Table 1. Demographic and clinical characteristics of the patients with schizophrenia

Subject	Age (yr), sex	PANSS				Duration of illness (yr)	Duration of drug treatment (yr)	Haloperidol equivalent (mg)	Main antipsychotics
		Positive	Negative	General	Total				
1	29, F	12	12	25	49	11	9	3	Olanzapine
2	34, F	17	12	33	62	7	5	6	Risperidone
3	37, F	14	23	27	64	0.5	0.5	3	Olanzapine
4	43, F	21	27	49	97	22	19	17	Risperidone
5	46, F	16	15	34	65	33	21	10	Nemonapride
6	49, F	24	20	33	77	23	16	19.4	Haloperidol
7	42, M	15	22	27	64	4	4	4	Olanzapine
8	43, M	15	26	33	74	26	23	9	Haloperidol
9	44, M	22	25	40	87	22	22	8.5	Olanzapine
10	44, M	16	26	37	79	4	4	14	Haloperidol
11	46, M	29	26	56	111	26	26	3.5	Olanzapine
12	46, M	16	16	25	57	24	24	4	Risperidone
13	52, M	24	35	58	117	18	17	16.5	Olanzapine
14	59, M	27	24	47	87	43	39	10.3	Mosapramine
		19.1±5.3	22.1±6.5	37.4±11.1	77.9±20.1	18.8±12.2	16.4±10.8	9.2±5.7	

PANSS, Positive and Negative Syndrome Scale; F, female; M, male.
Haloperidol (1 mg) was equivalent to chlorpromazine (50 mg).

demonstrated to be useful in the study of neurodegerative disorders such as Alzheimer's disease (Yasuno *et al.* 2008).

In this study, we investigated PBR binding in patients with chronic schizophrenia using [^{11}C]DAA1106 to evaluate whether glial reaction was involved in the pathophysiology of schizophrenia.

Materials and methods

Subjects

Fourteen patients with schizophrenia [six females, eight males; 43.9±7.4 yr (mean±S.D.)] and 14 normal control subjects (five females, nine males; 42.5±9.0 yr) were enrolled in this study. Patients were recruited from the outpatient and in-patient units of Nippon Medical School Hospital, Asai Hospital and Sobu Hospital, located in Tokyo and Chiba prefecture in Japan. The patients were diagnosed as having schizophrenia and treated by attending physicians at each hospital, and their diagnoses were re-evaluated with structured interviews at our PET centre. All 14 patients were diagnosed with schizophrenia according to DSM-IV criteria. Exclusion criteria were current or past substance, cannabis or alcohol abuse, mood disorders, and organic brain disease. The patients' demographic and clinical data are shown in Table 1. None of the patients had taken benzodiazepines within more than 1 month prior to PET measurements.

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 the PET measurements. 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 total scores, positive symptom, negative symptom, and general symptom subscores of PANSS. The total PANSS score ranged from 49 to 117 (78.6±20.7). The mean positive symptom score was 19.1±5.3, negative symptom score was 22.1±6.5, and general symptom score was 37.4±11.1.

The normal control subjects were recruited from the surrounding community. Based on psychiatric screening interviews, they were free of current and past psychiatric or major medical disease, and had no relatives with neuropsychiatric disorders.

This study complied with the current laws of Japan, and 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.

Radiochemistry

[^{11}C]DAA1106 was prepared as described in detail previously (Ikoma *et al.* 2007; Zhang *et al.* 2003). The precursor was supplied by Taisho Pharmaceutical Co. (Japan).

PET data acquisition

PET scans were performed with ECAT EXACT HR+ (CTI-Siemens, USA), which provides 63 planes and a 15.5-cm axial field of view (FOV). A 10-min transmission scan with a ^{68}Ge–^{68}Ga source was followed by a 90-min dynamic scan (20s × 9, 60s × 5, 120s × 4, 240s × 11, and 300s × 6) with a bolus injection of 261–411 (369 ± 27) MBq of [^{11}C]DAA1106. Specific radioactivity was 15.4–220.7 GBq/μmol at the time of the injection. There was no significant difference in injected radioactivity and specific radioactivity between patients and normal controls (373 ± 20 MBq and 60.3 ± 44.4 GBq/μmol for patients, and 366 ± 32 MBq and 98.4 ± 70.7 GBq/μmol for normal controls). Radioactivity was measured in three-dimensional mode, and the data were reconstructed with a Hanning filter with a cut-off frequency of 0.4 (full width half maximum = 7.5 mm).

Arterial blood sampling

To obtain the arterial input function, an automated blood sampling system was used for continuous (counts/s) blood radioactivity measurements during the first 12 min of PET measurement. At the same time, arterial blood samples were taken manually and their radioactivity concentration was measured 13 times during the initial 3 min after the injection, eight times during the next 17 min, and once every 10 min until the end of the scan. To analyse the metabolite fraction in the plasma, arterial blood samples were taken 10 times during PET measurements. The parent ligand, separated from the total radioactive compound, was measured as previously described (Ikoma *et al.* 2007). The mean time-course of the fraction of the parent ligand is shown in Fig. 1. There was a significant group × time interaction using repeated-measures ANOVA with Greenhouse–Geisser correction ($F_{3.4,81.1} = 4.92$, $p = 0.002$), although one subject from each group was excluded for the statistical analysis due to one missing data-point.

MR imaging

T1-weighted magnetic resonance imaging (MRI) of the brain was performed with Philips Intera 1.5 T (Philips Medical Systems, The Netherlands). T1-weighted images of the brain were obtained from all subjects. The scan parameters were 1-mm-thick 3D T1 images with a transverse plane [repetition time (TR)/echo time (TE) 22/9.2 ms, flip angle 30°, matrix 128 × 128, FOV 256 × 256). Voxel size of the magnetic resonance images was 1 mm × 1 mm × 1 mm.

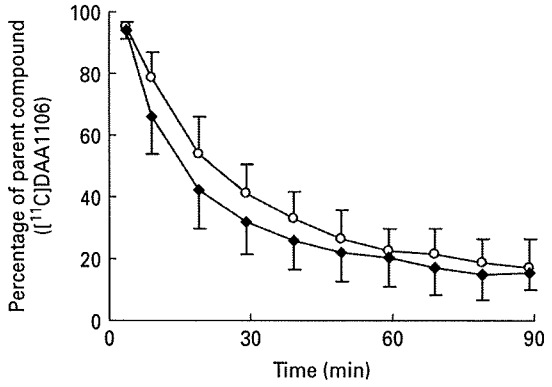

Fig. 1. Mean time-course of the percentage of parent compound ([^{11}C]DAA1106) after venous injection of [^{11}C]DAA1106 between normal controls (–○–) and patients (–◆–) with schizophrenia.

Data analysis

Eleven regions of interest (ROIs) (medial frontal cortex, dorsolateral frontal cortex, medial temporal cortex, lateral temporal cortex, parietal cortex, occipital cortex, thalamus, striatum, cerebellum, anterior cingulate cortex, and posterior cingulate cortex) were delineated on the co-registered PET/MRI images. In addition to each regional ROI, eight cortical ROIs (medial frontal cortex, dorsolateral frontal cortex, medial temporal cortex, lateral temporal cortex, parietal cortex, occipital cortex, anterior cingulate cortex, and posterior cingulate cortex) were also summed up as total cortical regions.

Regional time–activity data were analysed with two-tissue compartment model (2-TC) with the metabolite-corrected plasma input function, a model demonstrated to estimate binding potential (BP_{ND}) most reliably for [^{11}C]DAA1106 (Ikoma *et al.* 2007). Rate constants were estimated with weighted least squares and the Marquardt optimizer. For each region, k_1, k_2, k_3, k_4 and blood volume were estimated by 2-TC. BP_{ND} was calculated as k_3/k_4 in this analysis. Data analysis was performed with PMOD 2.65 (PMOD Technologies, Switzerland).

Statistical analysis

Regional ROIs

Statistical analysis of the difference of regional BP_{ND} for each ROI (for total 11 ROIs) between patients and normal controls was performed by repeated-measures ANOVA ($p < 0.05$ was considered significant). When any interaction was found, *post-hoc* Bonferroni correction was used for multiple comparisons.

Table 2. Significant correlation between PANSS scores and regional [^{11}C]DAA1106 binding

PANSS scores	Region	*p* value
Positive symptom	Medial frontal cortex	0.002*
	Dorsolateral frontal cortex	0.022
	Medial temporal cortex	0.003*
	Lateral temporal cortex	0.013
	Parietal cortex	0.005
	Occipital cortex	0.001*
	Cerebellum	0.022
	Striatum	0.010
Negative symptom	None	
General symptom	Medial frontal cortex	0.018
	Medial temporal cortex	0.027
	Occipital cortex	0.038
Total score	Medial frontal cortex	0.012
	Medial temporal cortex	0.029
	Parietal cortex	0.044
	Occipital cortex	0.017

PANSS, Positive and Negative Syndrome Scale.
* $p < 0.0045$ (0.05/11).

Correlation between regional BP_{ND} values and PANSS scores were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

Correlation between regional BP_{ND} values and duration of illness, duration of drug treatment, and chlorpromazine equivalent doses (Inagaki *et al.* 1999) were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

Changes in regional BP_{ND} values with age were analysed with Pearson's correlation method for patients and normal controls, respectively ($p < 0.05$ was considered significant).

Total cortical regions

For analysing differences in total cortical regions between patients and normal controls, Student's *t* test was used ($p < 0.05$ was considered significant).

Correlations between BP_{ND} values in total cortical regions and PANSS scores were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

Correlation between BP_{ND} values in total cortical regions and duration of illness, duration of drug treatment, and chlorpromazine-equivalent doses (Inagaki *et al.* 1999) were analysed with Pearson's correlation method ($p < 0.05$ was considered significant).

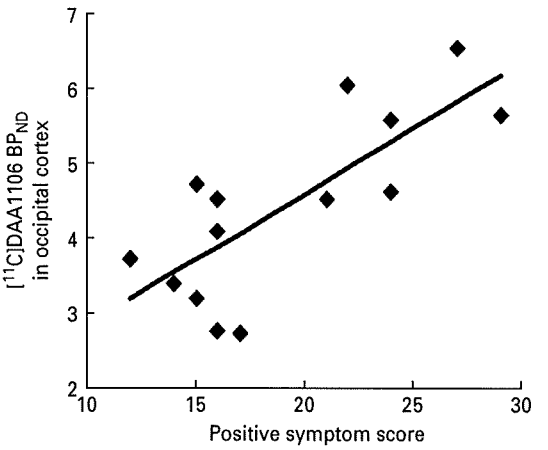

Fig. 2. Positive correlation between [^{11}C]DAA1106 BP_{ND} in the occipital cortex and positive symptom scores in the Positive and Negative Syndrome Scale.

Changes in BP_{ND} values in total cortical regions with age were analysed with Pearson's correlation method for patients and normal controls, respectively ($p < 0.05$ was considered significant).

Results

Regional ROIs

Comparison of regional BP_{ND} values for [^{11}C]DAA1106 between the patients with schizophrenia and normal controls by two-way repeated ANOVA with Greenhouse–Geisser correction showed no significant group × region interaction ($F_{1.7,44.4} = 0.542$, $p = 0.558$).

For the correlation analysis between BP_{ND} values in regional ROIs and positive symptom scores in the patient group, significant correlations were found in regions such as the medial frontal cortex, medial temporal cortex and occipital cortex (Table 2) (Fig. 2). No correlation was found between BP_{ND} values of each region and negative symptoms. Those three regions showed trends of positive correlation with general symptoms and total score (Table 2). There was no significant correlation between regional BP_{ND} and the duration of illness.

There was no significant change of regional BP_{ND} values with age in normal controls, whereas significant changes in BP_{ND} values with age in the patients with schizophrenia were observed in the occipital cortex ($p = 0.014$), lateral temporal cortex ($p = 0.023$), parietal cortex ($p = 0.023$), medial temporal cortex ($p = 0.031$), and medial frontal cortex ($p = 0.036$).

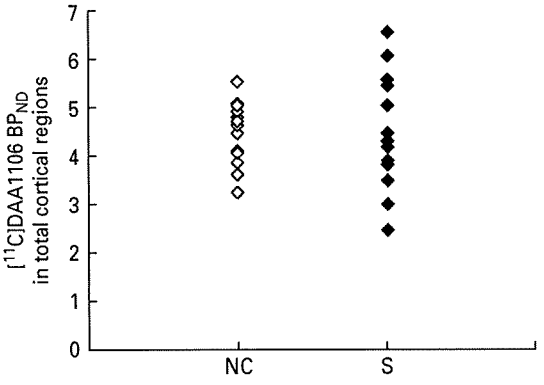

Fig. 3. Comparison of [^{11}C]DAA1106 BP_{ND} of total cortical regions between normal controls (NC) and patients with schizophrenia (S).

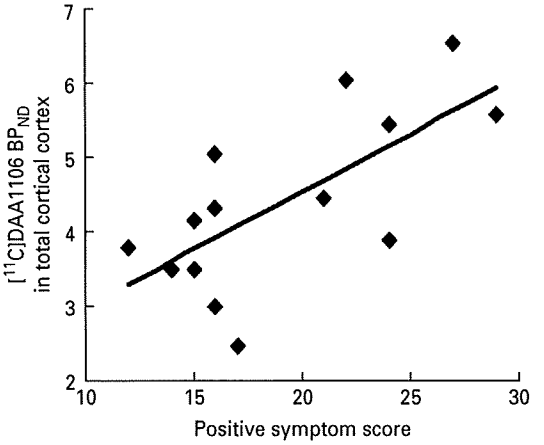

Fig. 4. Positive correlation between [^{11}C]DAA1106 BP_{ND} in the total cortical region and positive symptom scores in the Positive and Negative Syndrome Scale.

Total cortical regions

There was no significant difference of BP_{ND} values in total cortical regions between patients with schizophrenia and normal controls (Fig. 3). Significant correlation was found with the positive symptom scores ($p=0.006$) (Fig. 4). There was no significant correlation with other symptom scores (negative, general, and total symptom scores). Total cortical regions were correlated with duration of illness ($p=0.020$) (Fig. 5) and duration of drug treatment ($p=0.023$). BP_{ND} of total cortical regions was not correlated with chlorpromazine-equivalent doses.

There was no significant change of BP_{ND} values in total cortical regions with age in normal controls, but significant changes of BP_{ND} values with age were observed in total cortical regions of the patients with schizophrenia ($p=0.018$).

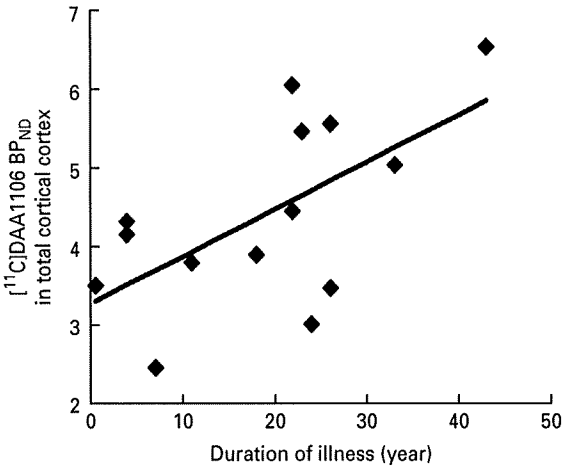

Fig. 5. Positive correlation between [^{11}C]DAA1106 BP_{ND} in the total cortical region and duration of illness.

Discussion

In this study, [^{11}C]DAA1106 binding, which was considered to correspond to the density of PBR, was not different between the patients with chronic schizophrenia and normal controls. A recent study demonstrated that [^{11}C]PK11195 binding increased in total grey matter in patients with acute-onset schizophrenia (van Berckel *et al.* 2008). Another recent study reported that [^{11}C]PK11195 binding in the hippocampus was significantly increased in patients with schizophrenia during acute psychosis, while there was no significant difference in other regions compared with normal controls (Doorduin *et al.* 2009). To understand the difference in the results between the present study and the two [^{11}C]PK11195 studies, several factors, such as the use of different radioligands and different patient groups, should be taken into consideration. Although PK11195 fully displaced the [^{3}H]DAA1106 binding (Chaki *et al.* 1999), a high concentration of PK11195 was required for this displacement. This suggested that the binding domain for DAA1106 contains an extra component that does not interact efficiently with PK11195 (Chaki *et al.* 1999). The mean age of patients with schizophrenia enrolled in the present study was higher (44 yr in 14 patients) than those in the two [^{11}C]PK11195 studies (24 yr in 10 patients, and 31 yr in seven patients). Most of the patients in the present study were at the chronic stage.

Within the patient group, [^{11}C]DAA1106 binding had a significant correlation with the positive symptom score of PANSS, a finding that might be in line

with those recent findings with [^{11}C]PK11195. The present results might indicate that the activated neuroimmune system was related to the pathophysiology of schizophrenia at the chronic stage.

In previous MRI volumetric research in schizophrenia, volume reduction in the brain has been reported in patients with chronic schizophrenia (Shenton *et al.* 2001). However, in the present study, there was no significant difference in the volume of ROIs by ANOVA, and total cortical ROI by Student's *t* test between the patients and normal controls (data not shown). Thus, the insignificance of the difference of [^{11}C]DAA1106 binding between the patients and normal controls is not related to the partial volume effect due to brain atrophy.

In this study, normal controls showed no age effects on [^{11}C]DAA1106 binding in any region. This is in line with the report with [^{11}C]PK11195 binding except the thalamus, where [^{11}C]PK11195 binding was reported to increase with age (Cagnin *et al.* 2001). This might be due to different radioligands or different age ranges between the two studies (24–55 yr in this study and 32–80 yr in the [^{11}C]PK11195 study). On the other hand, [^{11}C]DAA1106 binding was found to increase with age in patients with schizophrenia. Schizophrenia has been considered to be progressive in functional disability and morphological changes (Lieberman *et al.* 2001; Mathalon *et al.* 2001; Saijo *et al.* 2001). The present results of the positive correlation among [^{11}C]DAA1106 binding, duration of illness, and age might suggest that the progressive change occurs at the glial reaction level.

A recent meta-analysis showed that some cytokines such as IL-1RA, sIL-2R, and IL-6 are increased in schizophrenia (Potvin *et al.* 2008). PBR has been considered to modulate the release of pro-inflammatory cytokines in the CNS. PBR was reported to modulate the release of the inflammatory molecules NO and tumour necrosis factor-alpha (TNF-α) (Wilms *et al.* 2003). A PBR ligand, PK11195, has been reported to inhibit lipopolysaccharide-induced expressions of COX-2 and TNF-α in human microglia (Choi *et al.* 2002). Immunomodulatory drugs such as cyclooxygenase-2 (COX-2) inihibitors have been reported to show beneficial effects in schizophrenia (Muller & Schwarz, 2008). The combination of risperidone and COX-2 inhibitor has been reported to show superiority over risperidone alone in positive symptoms and PANSS total scores (Akhondzadeh *et al.* 2007). On the other hand, cytokines such as IL-2 and IL-6 are reported to increase after olanzapine and clozapine treatment (Kluge *et al.* 2009). The present results of PBR binding in the patients with schizophrenia might be in accord with the previous reports of cytokines.

A recent report demonstrated that PBR expression was not confined to microglia but was inducible in nervous tissue cells of neuroepithelial origin (Ji *et al.* 2008). Thus, PBR binding might also arise from astrocytes and other non-microglial elements. Schizophrenia patients with high S100B serum concentration, considered to indicate astrocyte activation, were reported to have cognitive dysfunction compared with patients with low S100B serum concentration (Pedersen *et al.* 2008). DAA1106 binding in patients with schizophrenia might also be related to the change in PBR on astrocytes.

In a post-mortem study, a subgroup of the patients with schizophrenia who committed suicide had increased microglial densities, although microglial HLA-DR expression in the patients with schizophrenia was not different from normal controls (Steiner *et al.* 2008). Microglial activation has been suggested to be interpretable as a consequence of presuicidal stress (Avital *et al.* 2001; Lehmann *et al.* 2002).

Although BP_{ND} of total cortical regions was not correlated with chlorpromazine-equivalent doses in the present study, some antipsychotics were reported to have anti-inflammatory effects (Kato *et al.* 2007; Kowalski *et al.* 2003, 2004; Labuzek *et al.* 2005; Zheng *et al.* 2008). The effect of antipsychotics on DAA1106 binding remains to be studied.

There are several confounding factors in the present study. First, the number of subjects was relatively small. Further larger-scale studies will be needed to confirm the present results. Second, all the patients were under different kinds of antipsychotic treatment. Further study is needed with drug-naive patients and patients under well-controlled drug treatment. Third, the PANSS scores of patients were higher as the duration of the illness was longer and age increased. This might reflect a possible subgroup of treatment-resistant patients.

In conclusion, we found no significant differences in PBR binding between the brains of patients with schizophrenia and those of normal control subjects, unlike recent reports with [^{11}C]PK11195 (van Berckel *et al.* 2008; Doorduin *et al.* 2009). Nevertheless, PBR binding in the patients with schizophrenia was correlated with positive symptoms, disease duration and age. The present results suggest that the glial reaction process might be involved in the pathophysiology of schizophrenia. Although the correlations should be interpreted with caution, these results at least suggest that additional studies are warranted in order to determine whether baseline

differences exist between patients with schizophrenia and healthy subjects, as well as to reveal the biological meanings of the correlations with disease parameters.

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Statement of Interest

None.

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Decreased binding of [^{11}C]NNC112 and [^{11}C]SCH23390 in patients with chronic schizophrenia

Jun Kosaka [a,b], Hidehiko Takahashi [a], Hiroshi Ito [a], Akihiro Takano [a], Yota Fujimura [a], Ryohei Matsumoto [a], Shoko Nozaki [a], Fumihiko Yasuno [a], Yoshiro Okubo [c], Toshifumi Kishimoto [b], Tetsuya Suhara [a,*]

[a] Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan
[b] Department of Psychiatry, Nara Medical University, 840, Shijo-cho, Kashihara-shi, Nara 634-8521, Japan
[c] Department of Neuropsychiatry, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan

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ABSTRACT

Aims: Abnormality of cognitive function in schizophrenia has been suggested to be related to dopamine D_1 receptor. However, the results of previous positron emission tomography (PET) studies of dopamine D_1 receptor in schizophrenia were not consistent.
Main methods: In this study, six patients with schizophrenia in severe residual phase with chronic antipsychotic treatment and twelve healthy age-matched controls participated. Two different radioligands, [^{11}C]NNC112 and [^{11}C]SCH23390, for dopamine D_1 receptor were used on the same subjects. Binding of the ligands was measured by PET, and statistical analysis was performed using one-way analysis of covariate (ANCOVA) with age as covariate.
Key findings: Good correlations between binding potential values (BP_{ND}) and age were observed in all regions of interest (ROIs) with both ligands. ANCOVA with age as covariate of BP_{ND} values of all ROIs revealed that the patient group showed significantly lower BP_{ND} value compared with the control group in both ligands.
Significance: In patients with chronic schizophrenia in severe residual phase with chronic antipsychotic treatment, the binding potential values of both ligands were significantly lower in the striatum and cortical regions than those of healthy controls.

Introduction

Schizophrenia is a chronic illness characterized by positive, negative, cognitive and affective symptoms (Schultz and Andreasen 1999). Although a positive symptom is characteristic of schizophrenia in the acute phase, the characteristic symptoms in the severe residual phase are negative symptom and cognitive dysfunction. The dopamine hypothesis is widely accepted for the pathophysiology of schizophrenia. Regarding dopamine receptors, the density of dopamine D_1 receptor in the cortical region is several times higher than that of dopamine D_2 receptor (Lidow et al. 1998). Abnormality of cognitive function in schizophrenia has been suggested to be related to dopamine function in the prefrontal cortex (Sawaguchi and Goldman-Rakic 1991). Dopamine D_1 receptor plays important roles in cognitive function such as working memory (Goldman-Rakic, 2000). One postmortem study has reported low dopamine D_1 receptors in the striatum in patients with schizophrenia (Hess et al. 1987), but no significant change has been reported in other studies (Seeman et al. 1987; Czudek and Reynolds 1988; Knable et al. 1994). In vivo PET studies reported decreased (Okubo et al. 1997), unaltered (Karlsson et al. 2002), and increased (Abi-Dargham et al. 2002) binding of D_1 receptor in patients with schizophrenia compared with control subjects. Those results were possibly influenced by parameters of the particular patient populations including duration of illness, symptoms and medications. In addition, differences in radioligand [^{11}C]SCH23390 (Okubo et al. 1997; Karlsson et al. 2002) and [^{11}C]NNC112 (Abi-Dargham et al. 2002) were suggested to account for inconsistent PET findings. Furthermore, subjects were medication-free or -naïve patients with schizophrenia in the prodromal, acute or active phase, and the duration of untreated illness may have influenced the difference in dopamine D_1 receptor binding in previous human PET studies.

The purpose of the present study was to compare the dopamine D_1 receptor binding of chronic patients with schizophrenia in severe residual phase with chronic antipsychotic treatment to that of healthy controls in the striatum and extrastriatal regions using both [^{11}C]SCH23390 and [^{11}C]NNC112 in the same subjects.

Materials and methods

Subjects

Six patients with schizophrenia, 1 female and 5 males aged 46.5 ± 8.2 years (mean ± SD), participated in this study (Table 1). All patients

* Corresponding author. Tel.: +81 43 206 3251; fax: +81 43 253 0396.
E-mail address: suhara@nirs.go.jp (T. Suhara).

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Table 1
Clinical characteristics of patients.

Patient no.	Age (years)	Gender	Diagnosis (DSM-IV)	Dose of sulpiride (mg)	Duration of illness (years)	PANSS			
						Positive	Negative	General	Total
1	32	M	295.30	1200	15	16	21	30	67
2	45	F	295.30	600	28	25	26	49	100
3	47	M	295.30	1200	8	18	23	44	85
4	47	M	295.30	1000	25	23	26	47	96
5	52	M	295.10	1200	5	13	33	46	92
6	56	M	295.60	600	29	20	43	59	122
Mean ± SD	46.5 ± 8.2			966.7 ± 294.4	18.3 ± 10.5	19.2 ± 4.4	28.7 ± 8.1	45.8 ± 9.4	93.7 ± 18.1

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; PANSS, Positive and Negative Scale for Schizophrenia; M, Male; F, Female.

met the criteria of the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) for diagnosis of schizophrenia. The diagnosis was assessed by Structured Clinical Interview for DSM-IV by three psychiatrists. The patients underwent general medical and laboratory evaluation. Organic brain disease was ruled out by CT, T1-weighted magnetic resonance (MR) images, and electroencephalogram.

Prior to this study, they had been prescribed antipsychotics during the periods indicated as 'duration of illness' in Table 1. In chlorpromazine equivalents, daily doses ranged from 200 mg to 606 mg and mean dose was 384 ± 139 mg/day (Inagaki and Inada 2006).

In all patients, the previously used antipsychotic drugs were changed to sulpiride, a selective dopamine D_2/D_3 receptor antagonist without affinity to dopamine D_1 receptor. PET scans were performed after a washout period of at least three weeks after changing to sulpiride. Sulpiride was maintained at the same dosage during the washout period. Because of extrapyramidal side effects, two patients were administered a relatively low dose of sulpiride (600 mg), although there had been no exacerbation of their psychic symptoms. All patients underwent clinical ratings of their psychopathology using the positive and negative syndrome scale (PANSS; Kay et al. 1987), and the following cognitive function tests: Wisconsin Card Sorting Test (Heaton 1981) to evaluate executive function, Stroop test (Cohen and Servan-Schreiber 1992) and *n*-back tasks (2-back minus 0-back using letters as stimulus; Cohen et al. 1994; Owen et al. 2005) to evaluate working memory.

The healthy control sample consisted of 6 females and 6 males, age-matched at 42.8 ± 8.5 years. Based on unstructured psychiatric screening interviews, none had a history of neurological or psychiatric illness. Organic brain disease was ruled out by T1-weighted MRI.

The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. After providing a complete explanation of the study, written informed consent was obtained from all subjects.

PET and MRI procedures

All patients except patient #6 (Table 1) underwent both PET scans using [^{11}C]NNC112 and [^{11}C]SCH23390 on the same day. Patient #6 and twelve healthy controls underwent each of the PET scans with [^{11}C]NNC112 and [^{11}C]SCH23390 within several days. The PET system ECAT EXACT HR+ (CT1-Siemens, Knoxville, TN) was used for all PET studies. The system provides 63 planes with a 15.5 cm axial field of view. After a transmission scan with a ^{68}Ge–^{68}Ga source, a bolus of [^{11}C]NNC112 or [^{11}C]SCH23390 was rapidly injected into the antecubital vein with a 20-ml saline flush. Injected radioactivity and specific radioactivity were 220.5 ± 9.25 MBq and 140.0 ± 64.1 GBq/μmol for patients in the [^{11}C]NNC112 studies, 215.0 ± 14.1 MBq and 152.5 ± 50.6 GBq/μmol for controls in the [^{11}C]NNC112 studies, 200.2 ± 15.9 MBq and 59.7 ± 15.5 GBq/μmol for patients in the [^{11}C]SCH23390 studies, and 220.5 ± 18.1 MBq and 68.6 ± 11.0 GBq/μmol for controls in the [^{11}C]SCH23390 studies, respectively.

Radioactivity in the brain was measured by a series of scans for 90 min for [^{11}C]NNC112 or 60 min for [^{11}C]SCH23390, starting immediately after the injection. During image acquisition, the subjects were instructed to lie quietly with their eyes closed and earplugs in place. Image reconstruction was performed with a Hanning filter with a cut-off frequency of 0.4, a value experientially determined for the purpose of noise reduction, resulting in a final spatial resolution of 7.5 mm FWHM (full width at half maximum).

T1-weighted MR images were acquired on Philips Gyroscan NT, 1.5 T (Philips Medical Systems, Best, The Netherlands). Scan parameters were 1-mm-thick 3D images with a transverse plane (repetition time, TR/echo time, TE 21/9.2 ms, flip angle 30°, matrix 256 × 256, field of view (FOV) 256 × 256), yielding 196 contiguous slices of the head.

PET data analysis

Regions of interest (ROIs) were manually drawn on the transverse slices from each subject's PET summation images referred from MRI images coregistered to the reconstructed PET images. ROIs were set to cover 3 adjacent slices for the striatum including both the caudate nucleus and the putamen, anterior cingulate, cerebellum, temporal cortex and frontal cortex including the superior frontal gyrus, middle frontal gyrus, and inferior frontal gyrus, which roughly corresponds to dorsolateral prefrontal cortex. The sets of ROIs for each section were transferred to the corresponding PET images, and time–activity curves (TACs) were obtained. The TACs of each region were analyzed using a simplified reference tissue model in a least-squares manner, in which the cerebellum was used as reference tissue (Lammertsma and Hume 1996). This procedure produced the binding potential (BP_{ND}; Innis et al. 2007) value.

Statistical analysis

Statistical analysis of the regional BP_{ND} obtained from patients with schizophrenia and healthy control subjects was performed using one-way analysis of covariance (one-way ANCOVA) with age as covariate using SPSS for Windows 16.0.2J (SPSS Inc, Chicago, Illinois, USA 2008), and post hoc Bonferroni correction was used for multiple comparisons. p value $< 0.05/4 = 0.0125$ was considered significant.

Results

Table 1 lists the clinical profiles of the patients. The average duration of illness after schizophrenia diagnosis was 18.3 years. Scores of the two cognitive functional tests are shown in Table 2, and significant group effects were found in each cognitive function test. Because four patients, #2, #3, #5 and #6, were not able to do *n*-back task (2 back), results were not shown in Table 2.

Significant correlations between BP_{ND} and age were observed in patients with [^{11}C]NNC112 (frontal cortex, $r = -0.924$, $p = 0.004$; striatum, $r = -0.981$, $p = 0.001$), controls with [^{11}C]NNC112 (striatum, $r = -0.886$, $p < 0.001$) and controls with [^{11}C]SCH23390 (frontal cortex, $r = -0.757$, $p = 0.004$; striatum, $r = -0.700$, $p = 0.011$). Trend

Table 2
Cognitive task scores of patients.

Patient no.	W-CST			Stroop test	
	Category	PEN	DMS	Error	Time score
1	6	0	0	0	17.4
2	2	9	5	11	46.6
3	1	7	3	1	7.4
4	5	1	1	0	5.4
5	2	14	0	2	68
6	Incapable	Incapable	Incapable	2	75
Mean ± SD	3.2 ± 2.2	6.2 ± 5.8	1.8 ± 2.2	2.7 ± 4.2	36.6 ± 30.8
Controls					
Mean ± SD	4.7 ± 1.6	1.4 ± 2.0	0.8 ± 1.4	0.8 ± 1.2	5.6 ± 4.0

W-CST, Wisconsin card sorting test; PEN, errors of nelson; DMS, difficulty in maintaining set.

level correlations were observed in other regions and patients with [^{11}C]SCH23390.

All BP_{ND} values of both ligands are shown in Fig. 1 and summarized in Table 3. ANCOVA with age as covariate ($df = 1,15$) of BP_{ND} values of all ROIs revealed that the patient group showed significantly lower BP_{ND} value compared with the control group in both ligands ([^{11}C]NNC112: temporal cortex, $F = 26.24$, $p < 0.001$; striatum, $F = 60.08$, $p < 0.001$; anterior cingulate cortex, $F = 9.14$, $p = 0.009$; frontal cortex, $F = 42.96$, $p < 0.001$, [^{11}C]SCH23390: temporal cortex, $F = 34.68$, $p < 0.001$; striatum, $F = 25.46$, $p < 0.001$; anterior cingulate cortex, $F = 8.91$, $p = 0.009$; frontal cortex, $F = 37.60$, $p < 0.001$). There was significant correlation between average BP values of [^{11}C]NNC112 weighted by ROI size and that of [^{11}C]SCH23390 ($r = 0.859$; $BP_{NNC} = 0.613\ BP_{SCH} + 0.0414$).

There was no significant correlation between BP_{ND} values and doses of antipsychotic drugs and between BP_{ND} values and PANSS scores for positive symptom, negative symptom, general symptom and total score in any of the brain regions.

Discussion

Both [^{11}C]NNC112 and [^{11}C]SCH23390 bindings in the striatum and cortical regions of patients with schizophrenia in severe residual phase were significantly lower compared with healthy controls. In previous PET studies of patients with schizophrenia who were antipsychotics-naïve or -free, BP of [^{11}C]SCH23390 was decreased (Okubo et al. 1997) or unchanged (Karlsson et al. 2002), and was increased when measured by [^{11}C]NNC112 (Abi-Dargham et al. 2002). Several differences in those studies have been discussed, including those regarding duration of illness, medications, race, severity of symptoms and radioligands. Guo et al. (2003) reported different characteristics of in vivo binding of the two radioligands in rat brain, increased [^{11}C]NNC112 binding and decreased [^{3}H]SCH23390 binding, following subchronic dopamine depletion with reserpine. But the inconsistent results cannot be explained solely by the difference of radiotracers, and demographics of patients might have been contributing factors.

Although [^{11}C]SCH23390 and [^{11}C]NNC112 are selective radioligands for dopamine D_1 receptor, both ligands have some affinity for 5-

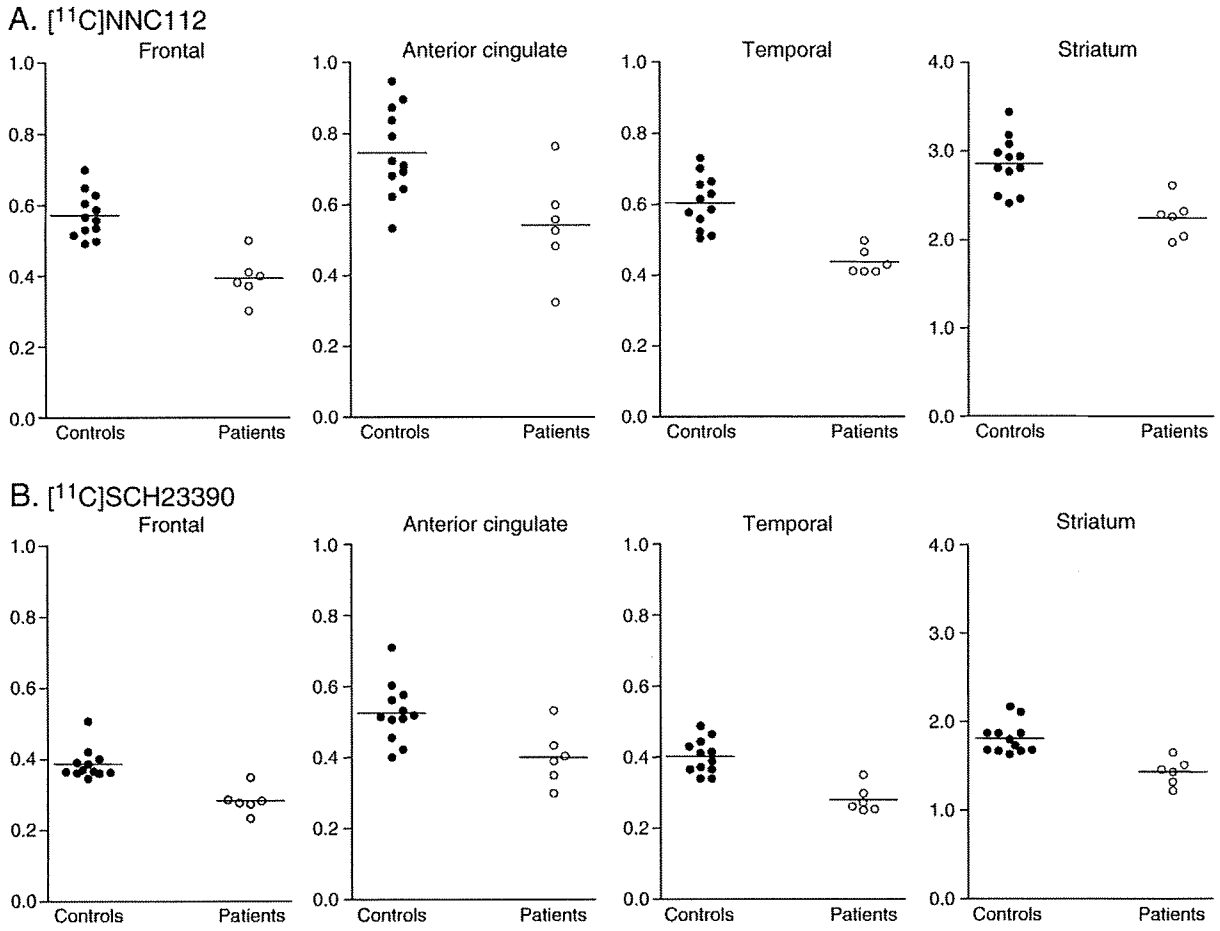

Fig. 1. BP_{ND} values of all subjects in both ligands [^{11}C] NNC112 and [^{11}C]SCH23390. Filled circles represent controls and open circles represent patients. A. BP_{ND} measured by [^{11}C] NNC112; B. BP_{ND} measured by [^{11}C]SCH23390. The horizontal line represents the group mean. In all ROIs, statistically significant differences were observed between patients with schizophrenia and healthy controls (one-way ANCOVA with age as covariate, $p < 0.0125 = 0.05/4$).

Table 3
[^{11}C] NNC112 and [^{11}C]SCH23390 binding potential.

Region	[^{11}C]NNC112				[^{11}C]SCH23390			
	Controls ($n=12$)	Patients ($n=6$)	p value	Reduction (%)	Controls ($n=12$)	Patients ($n=6$)	p value	Reduction (%)
Frontal cortex	0.57 ± 0.064	0.39 ± 0.065	<0.001*	31.2	0.39 ± 0.043	0.28 ± 0.037	<0.001*	26.7
Anterior cingulate	0.75 ± 0.12	0.54 ± 0.14	0.009*	27.2	0.53 ± 0.083	0.40 ± 0.079	0.009*	23.5
Temporal cortex	0.61 ± 0.074	0.44 ± 0.037	<0.001*	27.7	0.40 ± 0.048	0.28 ± 0.038	<0.001*	29.9
Striatum	2.85 ± 0.31	2.25 ± 0.23	<0.001*	21.4	1.83 ± 0.18	1.45 ± 0.15	<0.001*	20.9

Data are mean ± SD.
* $p<0.0125$ (=0.05/4, Bonferroni corrected) ANCOVA with age as covariate ($df=1,15$).

HT_{2A} receptor (Slifstein et al. 2007). However, Okubo et al. (2000) reported no difference in binding in the prefrontal cortex using [^{11}C]N-methylspiperone as ligand for 5-HT2 receptor in the same schizophrenia patients who showed lower binding with [^{11}C]SCH23390 (Okubo et al. 1997) and a non-significant trend towards decreased binding. In this study, all patients were medicated with only sulpiride as antipsychotic drug. Sulpiride is a selective dopamine D_2 antagonist and has negligible affinity to dopamine D_1 receptor in vivo (Farde et al. 1989). All antipsychotics of the patients were changed to sulpiride. Even though sulpiride had no direct affinity to dopamine D_1 receptor, these patients had been receiving long-term chronic antipsychotic treatment. Several studies of primates have reported that chronic administration of dopamine D_2 receptor antagonist decreased the density of dopamine D_1 receptor (Lidow and Goldman-Rakic 1994; Lidow et al. 1997), although one animal study has reported that there was no influence of chronic medication on dopamine D_1 receptor density (Sanci et al. 2002). Hirvonen et al. (2006) reported a widespread reduction of D_1 receptor binding in the brain in patients with schizophrenia, which was associated with antipsychotic medication dose. However, we did not find a correlation between them, possibly due to a lack of variance in antipsychotic dose.

The patients in this study were in a very severe residual phase according to the deficits in the cognitive test scores (Table 2) and the high total scores of PANSS despite the low positive symptom scores (Table 1). Some studies have reported regional structural brain abnormalities of gray matter in the striatum and extrastriatal regions of schizophrenia patients with chronic antipsychotic treatment (Jernigan et al. 1991; Tamagaki et al. 2005). In this study, since we confirmed that there was no significant difference between the volume of each ROI in patients and that of controls, we measured the gray matter volume ratio in each ROI. The results revealed no significant difference between the gray matter volume in patients and that of controls in each ROI (data not shown). The values of reduction in BP_{ND} shown by percentage (Table 3) seemed considerably larger than the reduction of gray matter. However, the effect of brain gray matter reduction cannot also be ruled out.

Our results indicated lower dopamine D_1 receptor binding in schizophrenia patients with chronic antipsychotic treatment measured by different radioligands, [^{11}C]NNC112 and [^{11}C]SCH23390. However, as the small sample size was a distinct limitation of this study, a larger study population will be necessary to more definitively examine the relation between dopamine D_1 receptor binding and factors such as duration of illness and severity of symptoms.

Conflict of interest statement

There are no conflicts of interests.

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

Sayaka Aritake-Okada [a,b,*], Makoto Uchiyama [c], Hiroyuki Suzuki [a], Hirokuni Tagaya [d], Kenichi Kuriyama [e], Masato Matsuura [b], Kiyohisa Takahashi [f], Shigekazu Higuchi [a], Kazuo Mishima [a]

[a] Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry,Tokyo, Japan
[b] Department of Life Sciences and Bio-informatics, Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Tokyo, Japan
[c] Department of Neuropsychiatry, Nihon University School of Medicine, Tokyo, Japan
[d] Department of Health Science, School of Allied Health Sciences, Kitasato University, Japan
[e] Department of Adult Mental Health, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan
[f] Japan Foundation for Neuroscience and Mental Health, Tokyo, Japan

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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

* Corresponding author at: Department of Psychophysiology, Institute of Mental Health, National Center of Neurology and Psychiatry, Ogawa-higashicho 4-1-1, Kodaira, Tokyo 187-8502, Japan. Tel.: +81 42 346 2014; fax: +81 42 346 2072.
E-mail address: sayaca@ncnp.go.jp (S. Aritake-Okada).

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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,

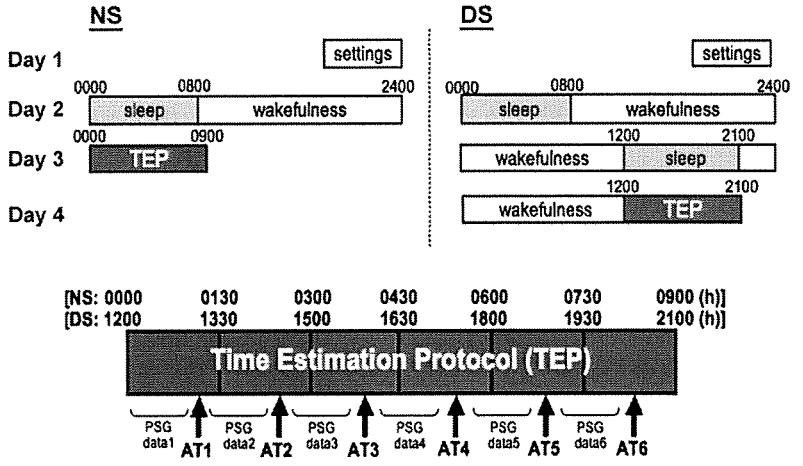

Fig. 1. Time estimation protocol (TEP). TEP was conducted between 00:00 h and 09:00 h (nighttime sleep: NS) or 12:00 h and 21:00 h (daytime sleep: DS). The 9-h polysomnography (PSG) recording periods were divided into six 90-min periods. We woke the participants and conducted a structured interview once 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

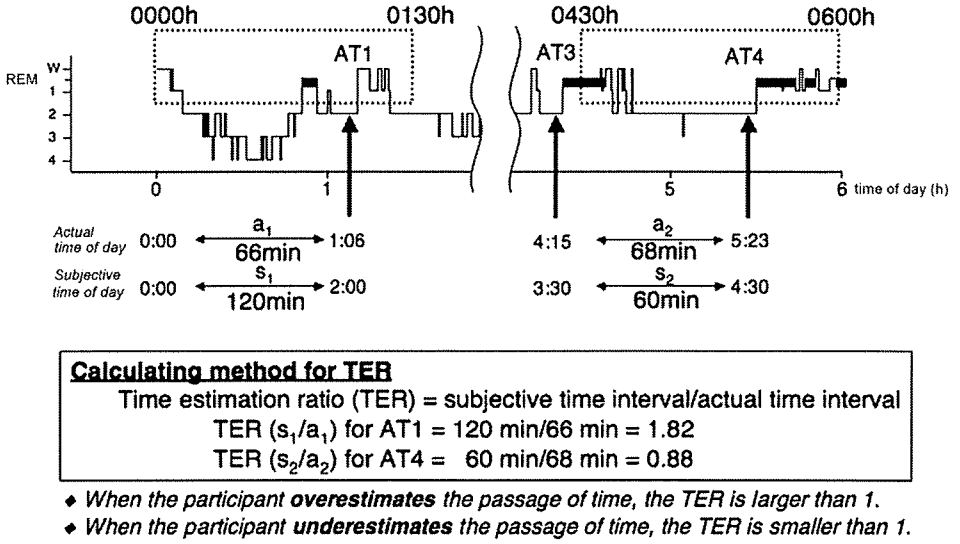

Fig. 2. Time estimation ratio (TER). Subjective time interval in both experiments was defined as the time difference between subjective times of the day, which were obtained at successive awakening trials (ATs). The actual time interval was defined as the actual time difference between successive ATs. 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

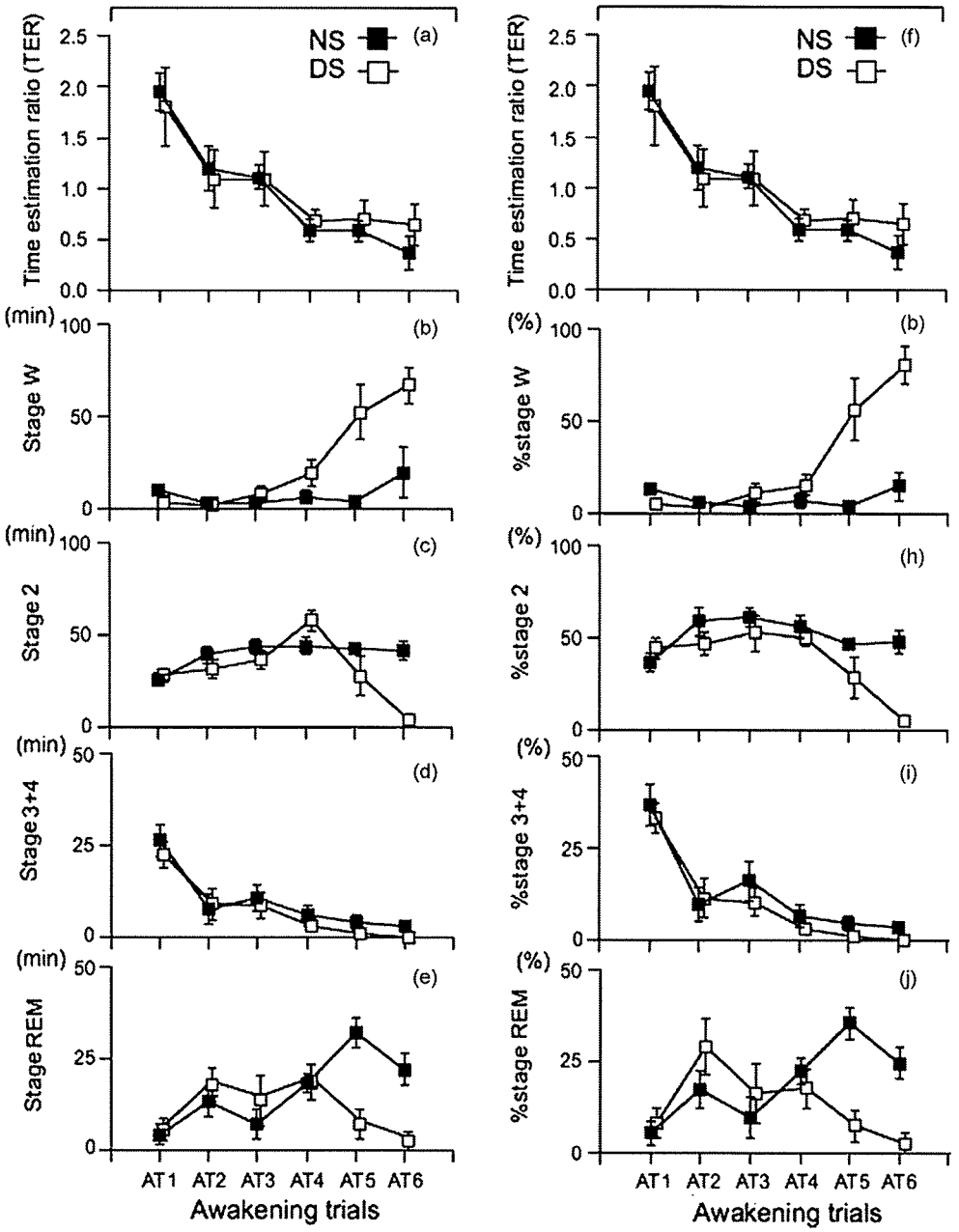

Fig. 3. (a–j) Time course of the mean time estimation ratio (TER) and the amounts (left panel) and the percentages (right panel) of sleep stages. Filled and open circles represent the data in nighttime sleep (NS) and daytime sleep (DS) experiments, respectively. The horizontal axes indicate the AT number. 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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