convulsion was frequently observed in the Psychosis-Epilepsy patients, comparable to that of Epilepsy-Psychosis patients (Kanemoto et al., 2001). Whereas Epilepsy-Psychosis patients tend to develop epilepsy earlier (Adachi et al., 2002), our Psychosis-Epilepsy patients developed epilepsy in comparatively advanced age (late 20s). This appeared to be partly due to our operational criteria of Psychosis-Epilepsy with which epilepsy occurred after the development of psychosis, that is, mostly after adolescence (Weinberger, 1987). Because epilepsy types are strongly associated with the brain development, our Psychosis-Epilepsy patients did not have child-onset epilepsies, such as West syndrome or Lennox-Gastaut syndrome and benign epilepsy of childhood with centrotemporal electroencephalography (EEG) foci (BECCT). Similar to newly developed adult epilepsies (Collaborative Group for the Study of Epilepsy, 1992; Cockerell et al., 1997; MacDonald, 2001), the Psychosis-Epilepsy patients showed a low frequency of seizures that were easily controlled with first-line AED treatment. Psychosis-Epilepsy patients may have characteristics of adult-onset epilepsy.

When disregarding the dichotomy, the time interval between the onset of psychosis and that of epilepsy in both groups distributed continuously (Fig. 1A). Such a continuous distribution was consistent with the other findings in this study that there was no particular difference in clinical characteristics between Psychosis-Epilepsy and Epilepsy-Psychosis. These findings did not fully support the distinction of Psychosis-Epilepsy and Epilepsy-Psychosis as two separate groups. Slater (1969) defined psychosis after the development of epilepsy as epileptic psychosis. Although Slater et al. (1963) investigated epilepsy patients with psychosis regardless of the chronological order of their occurrence, theoretically including those with psychosis antedating epilepsy, they did not find any with Psychosis-Epilepsy in their 69 patients studied. Given that the proportion of Psychosis-Epilepsy is smaller than Epilepsy-Psychosis even in our study with a fairly large number of subjects, this might have been due to their small sample size. Alternatively, the study subjects of Slater et al. might not have reflected the whole patient cohort with epilepsy and psychosis owing to their study design. There may be vulnerabilities common in all patients who have both epilepsy and psychosis regardless of the chronological order of the two conditions.

Limitations of the study should be acknowledged. The number of patients, in particular those with Psychosis-Epilepsy, was insufficient for analyzing the effect of multiple variables on either epilepsy or psychosis. Despite a consecutive registration to the database, a selection bias toward patients with intractable epilepsies in our tertiary, specialist clinics may not be negligible. Although data were collected in a prospective manner, past events that had taken place before the patients transferred to the study institutions, such as earlier treatment, could not be fully evaluated. Because the current study included only patients with multiple seizures, it is uncertain whether our findings could apply to those with a single seizure.

In the current study, Psychosis-Epilepsy patients had many features in common with Epilepsy-Psychosis patients. The two groups cannot be clearly delineated by the timing of development of epilepsy. Indeed, some patients have genetic vulnerabilities to both psychoses and seizures (Waziri et al., 1996; Hyde & Weinberger, 1997; Adachi et al., 2010; Craddock & Owen, 2010b). Among these patients, psychosis may develop either antedating or postdating the development of epilepsy. Studies in a larger sample size will clarify these vulnerabilities in more detail. Furthermore, future studies are needed to explore psychopathology and symptomatology for remodeling of clinical entities in psychoses in patients with epilepsy.

Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

None of the authors has any conflict of interest to disclose.

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Regular Article

Relationships between exploratory eye movement dysfunction and clinical symptoms in schizophrenia

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Aim: Many psychophysiological tests have been widely researched in the search for a biological marker of schizophrenia. The exploratory eye movement (EEM) test involves the monitoring of eye movements while subjects freely view geometric figures. Suzuki *et al.* (2009) performed discriminant analysis between schizophrenia and non-schizophrenia subjects using EEM test data; consequently, clinically diagnosed schizophrenia patients were identified as having schizophrenia with high probability (73.3%). The aim of the present study was to investigate the characteristics of schizophrenia patients who were identified as having schizophrenia on EEM discriminant analysis (SPDSE) or schizophrenia patients who were identified as not having schizophrenia on EEM discriminant analysis (SPDNSE).

Methods: The data for the 251 schizophrenia subjects used in the previous discriminant-analytic study were analyzed, and the demographic or symptomatic characteristics of SPDSE and SPDNSE were investigated. As for the symptomatic features, a factor analysis of the Brief Psychiatric Rating Scale (BPRS) rating from the schizophrenia subjects was carried out.

Results: Five factors were found for schizophrenia symptoms: excitement/hostility; negative symptoms; depression/anxiety; positive symptoms; and disorganization. SPDSE had significantly higher factor scores for excitement/hostility, negative symptoms and disorganization than SPDNSE. Furthermore, the BPRS total score for the SPDSE was significantly higher than that for the SPDNSE.

Conclusion: SPDSE may be a disease subtype of schizophrenia with severe symptoms related to excitement/hostility, negative symptoms and disorganization, and EEM parameters may detect this subtype. Therefore, the EEM test may be one of the contributors to the simplification of the heterogeneity of schizophrenia.

Key words: biological marker, clinical symptoms of schizophrenia, exploratory eye movement, heterogeneity, schizophrenia.

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MANY PSYCHOPHYSIOLOGICAL TESTS have been performed in the search for a biological marker for schizophrenia.[1,2] Event-related potentials (ERP), P300,[3] P50[4] and mismatch negativity (MMN),[5,6] prepulse inhibition (PPI),[7,8] saccadic and smooth pursuit eye movements[9–12] and working memory tasks[13,14] have been widely researched. Moreover, many researchers have focused on abnormalities of working memory as an endophenotype for schizophrenia in molecular genetic studies.[15,16]

We have studied eye movements while subjects freely viewed geometric figures; this is called the exploratory eye movement (EEM) test. In most previous studies, only schizophrenia patients have consistently shown disturbances of EEM.[17–25] Moreover, the parents and siblings of schizophrenia patients had EEM dysfunctions.[26,27] In addition, EEM demonstrated a significant linkage to chromosome 22q11.[28] Chromosome 22q11 is one of the most interesting regions in the genetic etiology of schizophrenia. Microdeletions at chromosome 22q11 cause velocardio-facial syndrome (VCFS/DiGeorage syndrome: DGS), and patients with VCFS have a high risk of schizophrenia.[29,30] Furthermore, there is strong evidence that this deletion is a risk factor for schizophrenia in a genome-wide association study (GWAS) using copy number variants (CNV).[31] Therefore, we believe that EEM disturbance may be a biological marker of schizophrenia, in addition to the aforementioned physiological defects.

On the basis of these findings, we considered that the EEM test might be useful for the clinical diagnosis of schizophrenia as well. Suzuki *et al.* carried out a discriminant analysis between schizophrenia patients and non-schizophrenia subjects in a large sample using EEM test data.[32] EEM performance was recorded in 251 schizophrenia patients and 389 non-schizophrenia subjects (111 patients with mood disorder; 28 patients with neurotic disorder; 250 normal controls). As a result, 184 of the 251 clinically diagnosed schizophrenia patients were identified as having schizophrenia (sensitivity, 73.3%); and 308 of the 389 clinically diagnosed non-schizophrenia subjects were identified as non-schizophrenic (specificity, 79.2%). Based on this finding, we propose that the EEM test might be useful for the clinical diagnosis of schizophrenia.

In the discriminant-analytic study,[32] we were interested in characteristics of the schizophrenia patients who were identified as having schizophrenia on EEM discriminant analysis (SPDSE), or those who were identified as not having schizophrenia on EEM discriminant analysis (SPDNSE). Many researchers have indicated the potential heterogeneity of schizophrenia.[33–37] Hence, the EEM parameters may be able to detect different subtypes of schizophrenia. In the present study, to clarify the features of SPDSE and SPDNSE, we reanalyzed that data,[32] and focused on the demographic or symptomatic characteristics. If the characteristics of SPDSE and SPDNSE are clarified, further knowledge regarding the heterogeneity of schizophrenia may be yielded. Therefore, in the present study we discuss the features of SPDSE and SPDNSE and a further application of EEM for scientific research into schizophrenia.

METHODS

Subjects

Two hundred and fifty-one schizophrenia patients participated in the discriminant-analytic study (paranoid type, 65.3%; hebephrenic type, 15.9%; catatonic type, 1.2%; undifferentiated type, 5.2%; residual type, 9.6%; simple type, 1.6%; and unspecified type, 1.2%).[32] The patients were in/outpatients recruited from multiple centers, eight university hospitals and three affiliated hospitals. Diagnoses were made by one experienced psychiatrist according to the ICD-10 criteria for research at each university or hospital.[38] The demographic characteristics of the subjects were as follows: age, 37.9 ± 11.3 years; gender (M/F), 157/94; and duration of illness, 14.5 ± 13.1 years. The patients who had a history of alcohol abuse or illicit substance abuse, or head injury were excluded from the study; also excluded were those with convulsive, neurologic or ophthalmologic disorders.

The clinical symptoms of the schizophrenia patients were assessed using the Brief Psychiatric Rating Scale (BPRS),[39] which yielded an average total score of 41.5 ± 13.3. All BPRS ratings were done by one experienced psychiatrist in each university or hospital. Of the 251 patients with schizophrenia, 249 received neuroleptic medication. The average daily dosage is expressed as a haloperidol equivalent of 13.9 ± 10.7 mg.[40] This study was approved by the Ethics Committees of the eight universities. Written informed consent was obtained from all participants, after the procedures and possible risks of the study were fully explained.

Procedure

A standard test of the EEM using a digital eye-mark recording system (nac Image Technology, EMR-NS, Tokyo, Japan) was performed. An eye camera that detected corneal reflection of infrared light to identify eye movements, and a 15-in LCD monitor (1024 × 768 pixels) that displayed target figures for the EEM tasks (Fig. 1) were included in this system. According to the following method, three horizontal S-shaped figures (an original target figure and two figures slightly different from the original target figure) were individually displayed on the LCD monitor (Fig. 1). First, the retention task: the subject was shown the original S-shaped figure (Fig. 1a) for 15 s. Next, the comparison task: the subject was instructed to compare a figure with the original figure (Fig. 1a); they were then shown a figure slightly different from the original one, which had one bump in a different position (Fig. 1b), for 15 s. After 15 s had elapsed and with the figure still in view, the subject was asked whether it differed from the original figure and, if it did, how it differed. After the subject had replied and while the figure was still being shown, he/she was asked 'Are there any other differences?' The comparison task was then repeated with a figure without bumps (Fig. 1c).

In the digital eye-mark recording system, the detected eye movements were automatically analyzed by a digital computerized EEM analyzer. Consequently, four parameters emerged: number of eye fixations (NEF), total eye scanning length (TESL), mean eye scanning length (MESL) and responsive search score (RSS). The NEF, TESL and MESL were based on data of eye movements that occurred during 15 s of the retention task. In the comparison task, the RSS was based on data of eye movements that occurred for 5 s immediately after the question: 'Are there any other differences?' More detailed descriptions of the EEM test equipment and method are given in our previous studies.[17,20,32]

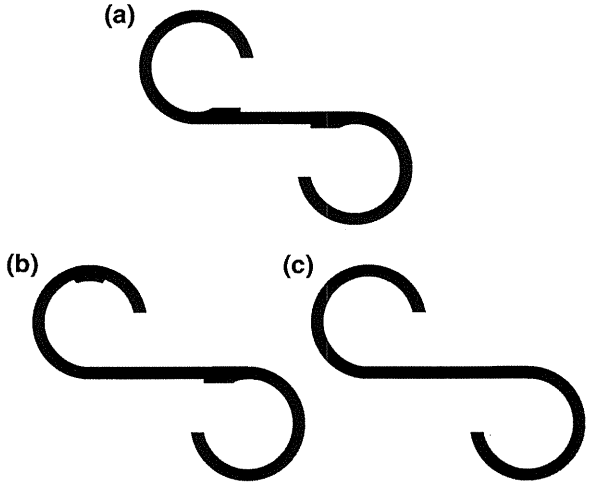

Figure 1. (a) Original target figure; (b,c) two figures slightly different from the target.

In our previous study, 184 of the 251 clinically diagnosed schizophrenia patients were identified as having schizophrenia on discriminant analysis using the EEM parameters (SPDSE).[32] The remaining 67 schizophrenia patients were identified as not having schizophrenia (SPDNSE). Table 1 lists the background data of the SPDSE and SPDNSE. In the present study we compared demographic and symptomatic characteristics of SPDSE with those of SPDNSE.

Statistical analysis

Group differences on the demographic and symptomatic data were assessed using the *t*-test or the χ^2 test. For group comparison of the symptomatic data, scores for factors extracted by factor analysis of BPRS ratings and BPRS total scores were used. In the factor analysis, we conducted a principal component analysis with orthogonal rotation (Varimax method) according to previous studies.[41–43] Moreover, based on prior studies, factors with eigenvalues >1.0 were considered to be meaningful.[41,43] All statistical analyses were performed using SPSS for Windows version 17.0. The statistical significance was set at $P < 0.05$ (two-tailed).

RESULTS

Group comparisons (SPDSE vs SPDNSE) of demographic characteristics

There were no significant differences for age, sex, duration of illness or drug dosage between SPDSE and SPDNSE.

Group comparisons (SPDSE vs SPDNSE) of subtypes and clinical symptoms

There were no significant differences for the subtypes between SPDSE and SPDNSE.

Table 1. Subject characteristics

	SPDSE ($n = 184$)	SPDNSE ($n = 67$)
Age (years), mean ± SD	38.0 ± 12.6	37.7 ± 12.0
Gender (M/F)	112/72	45/22
Duration of illness (years), mean ± SD	14.6 ± 13.9	14.3 ± 10.8
Equivalent dose of haloperidol (mg), mean ± SD†	14.4 ± 11.1	12.5 ± 9.7
Subtype, *n* (%)		
Paranoid	120 (65.3)	44 (65.6)
Hebephrenic	30 (16.3)	10 (14.9)
Catatonic	3 (1.6)	0 (0)
Undifferentiated	9 (4.9)	4 (6.0)
Residual	18 (9.8)	6 (9.0)
Simple	3 (1.6)	1 (1.5)
Unspecified	1 (0.5)	2 (3.0)

†In each group (SPDSE or SPDNSE), one patient did not receive neuroleptic medication, respectively.
SPDSE, schizophrenia patients identified as having schizophrenia on exploratory eye movement (EEM) discriminant analysis; SPDNSE, schizophrenia patients identified as not having schizophrenia on EEM discriminant analysis.

Factor analysis of BPRS items

Table 2 lists the factors and factor loadings derived using principal component analysis of BPRS rating. The principal component analysis extracted five factors that accounted for 70.0% of the variance. Based on previous studies, BPRS items with factor loadings >0.5 were considered to load on the

Table 2. Factors and factor loadings derived in BPRS principal component analysis

	Factor				
	1	2	3	4	5
BPRS items					
Somatic concern	0.033	0.080	0.615	0.505	−0.074
Anxiety	0.184	0.123	0.727	0.272	−0.126
Emotional withdrawal	0.070	0.879	0.139	0.043	0.140
Conceptual disorganization	0.401	0.298	0.113	0.356	0.629
Guilt feelings	0.091	−0.085	0.670	−0.157	0.487
Tension	0.416	0.404	0.543	0.106	−0.126
Mannerisms and posturing	0.383	0.457	0.178	0.339	0.393
Grandiosity	0.736	−0.115	0.133	0.124	0.158
Depressive mood	0.192	0.287	0.722	0.041	−0.058
Hostility	0.783	0.077	0.213	0.210	−0.118
Suspiciousness	0.477	0.126	0.273	0.546	−0.111
Hallucinatory behavior	0.246	0.171	0.045	0.805	0.067
Motor retardation	0.004	0.850	0.179	0.159	0.083
Uncooperativeness	0.677	0.432	−0.057	0.122	0.086
Unusual thought content	0.276	0.170	0.133	0.734	0.322
Blunted affect	0.021	0.857	0.083	0.168	0.160
Excitement	0.778	−0.023	0.195	0.218	0.153
Disorientation	−0.034	0.241	−0.241	0.056	0.659
Variance explained (total = 70.0%)†	17.5	17.5	14.1	12.6	8.4

†Cumulative or percentage of variance explained is rounded off; therefore, the cumulative percentage is not identical to the sum of each percentage. Underline, BPRS items with factor loadings >0.5.
BPRS, Brief Psychiatric Rating Scale.

Table 3. Mean factor scores and BPRS total score (mean ± SD)

	SPDSE ($n = 184$)	SPDNSE ($n = 67$)	t (d.f. = 249)	z
Factor				
1 Excitement/hostility	0.09 ± 1.07	−0.25 ± 0.74		−2.16*
2 Negative symptoms	0.10 ± 1.01	−0.27 ± 0.93	−2.57*	
3 Depression/anxiety	−0.03 ± 1.03	0.07 ± 0.92	0.70	
4 Positive symptoms	0.03 ± 1.03	−0.07 ± 0.92	−0.71	
5 Disorganization	0.08 ± 1.03	−0.21 ± 0.89	−2.06*	
BPRS total score (mean ± SD)	43.08 ± 13.48	37.51 ± 12.10	−2.98*	

*$P < 0.05$.

BPRS, Brief Psychiatric Rating Scale; SPDSE, schizophrenia patients identified as having schizophrenia on exploratory eye movement (EEM) discriminant analysis; SPDNSE, schizophrenia patients identified as not having schizophrenia on EEM discriminant analysis.

respective factor.[41,43] Consequently, we summarized the five factors as follows: factor 1 loaded heavily in grandiosity, hostility, uncooperativeness and excitement; factor 2 had heavy loadings in emotional withdrawal, motor retardation and blunted affect; factor 3 loaded heavily in somatic concern, anxiety, guilt feelings, tension and depressive mood; factor 4 had heavy loadings in somatic concern, suspiciousness, hallucinatory behavior and unusual thought content; factor 5 loaded heavily in conceptual disorganization and disorientation. Accordingly, we interpreted the five factors as having the following dimensions: factor 1, excitement/hostility (17.5% of total variance); factor 2, negative symptoms (17.5%); factor 3, depression/anxiety (14.1%); factor 4, positive symptoms (12.6%); and factor 5, disorganization (8.4%).

Group comparisons (SPDSE vs SPDNSE) of factor scores

Table 3 lists the mean factor scores of the five factors for SPDSE and SPDNSE. SPDSE had significantly higher scores of excitement/hostility ($P = 0.005$), negative symptoms ($P = 0.011$) and disorganization ($P = 0.040$) than SPDNSE. Furthermore, the BPRS total score of SPDSE was significantly higher than that of the SPDNSE ($P = 0.003$). For the excitement/hostility factor, the Levene test for equality of variance did not show homoskedasticity between the two groups. Therefore, the P-value for the excitement/hostility factor was based on an unequal-variance t-value. In order to confirm the result of the excitement/hostility factor, we also performed the non-parametric test, Mann–Whitney U-test. Consequently, SPDSE also demonstrated significantly higher scores of excitement/hostility than SPDNSE on non-parametric analysis ($P = 0.031$).

DISCUSSION

Suzuki *et al.* performed discriminant analysis between schizophrenia patients and non-schizophrenia subjects using the EEM test data.[32] As a result, 184 of the 251 clinically diagnosed schizophrenia patients were identified as having schizophrenia (sensitivity, 73.3%). In the present study, results of the factor analysis of BPRS ratings from the aforementioned 251 schizophrenia subjects produced five factors of symptoms (excitement/hostility; negative symptoms; depression/anxiety; positive symptoms; and disorganization). Excitement/hostility, negative symptoms and disorganization were more predominant in the 184 SPDSE subjects compared to the SPDNSE subjects. Furthermore, the BPRS total score of the SPDSE was significantly higher than that of the SPDNSE. Consequently, the SPDSE group may consist of patients with severe schizophrenia, and the severity of symptoms in SPDSE was found to be due mainly to excitement/hostility, negative symptoms and disorganization.

Evidence for five dimensions in schizophrenia symptoms was found in the present study. Many studies have proposed similar five-factor structures.[41–47] In these studies, the Positive and Negative Syndrome Scale (PANSS) has been used as the symptom rating scale. In contrast, the present data were based on the BPRS. All items of the BPRS, however, are included in the PANSS.[39,48] Therefore, it

is possible that the present findings reflect the past studies of the factor analysis using PANSS items. Consequently, although items included for each factor in previous studies and the present study were not identical, the present findings of the factor analysis are distinctly similar to previous factor-analytic study results. Thus, we consider that the present five-factor structure may be meaningful for the symptomatology of schizophrenia. The PANSS, however, is more informative than the BPRS, therefore the present study may be limited by this issue.

In the present study, demographic data, age, sex, duration of illness and drug dosage for SPDSE and SPDNSE were not significantly different. But there were significant differences for symptom, excitement/hostility, negative symptoms and disorganization between SPDSE and SPDNSE. In our previous study, EEM parameters were not influenced by the demographic data.[27,32] Moreover, one of the EEM parameter, RSS, which was principally used in the discriminant analysis of SPDSE, was associated with negative symptoms.[17] Altogether, we believe that differences between SPDSE and SPDNSE in the EEM may relate to symptoms of schizophrenia, but not demographic data, sex, age, course of illness or medication.

With regard to the ICD-10 subtypes, we also did not find significant differences between SPDSE and SPDNSE. This finding seems to conflict with the significant differences of the BPRS scores between the two groups. Lykouras *et al.* investigated relationships between the DSM-III-R schizophrenia subtypes and the PANSS scores.[49] As a result, paranoid type was associated with positive symptoms, and disorganized type linked to negative symptoms. In addition to disorganized type, however, catatonic type related to negative symptoms. Moreover, based on the DSM-IV-TR, the schizophrenia symptoms have been divided into three dimensions.[50] However, past reports and the present study propose that schizophrenia may be symptomatically more complex.[41–47] This has also been indicated by Wolthaus *et al.*[47] In this way, subtypes and dimensions of the diagnostic criteria are often not consistent with those of the symptomatic rating scales. There is, however, a possible limitation to the present study. Although we discussed diagnoses using the ICD-10 criteria and the BPRS scores in detail, inter-rater and intra-rater reliabilities for those were not formally assigned. Consequently, if they were formally assigned, the ICD-10 subtypes might coincide with the BPRS scores.

Based on the present findings, SPDSE may be associated with excitement/hostility, negative symptoms and disorganization in the present five symptomatic dimensions. Accordingly, SPDSE may have three different dimensions; but it can also be said that SPDSE may be a schizophrenia subtype characterized by these three dimensions. The present findings may indicate that there is a putative subtype of schizophrenia with severe symptoms related to excitement/hostility, negative symptoms and disorganization. Furthermore, the EEM abnormality may be a biological marker for this subtype of schizophrenia. There is another point worth making. As mentioned here, the EEM parameter, RSS was associated with negative symptoms.[17] Thus, negative symptoms may be the most specific of the three dimensions to the subtype.

In addition to the schizophrenia patients, their parents and siblings also had EEM dysfunction.[26,27] Therefore, we considered that the EEM abnormality may be an intermediate phenotype of schizophrenia, and may be useful for linkage studies of schizophrenia. Indeed, we found a significant linkage to chromosome 22q11.2–12.1 in our previous linkage study using EEM impairment as an endophenotype of schizophrenia.[28] Chromosome 22q11 is one of the most interesting regions for the etiology of schizophrenia. Moreover, in this area, there are several candidate genes for schizophrenia, for example COMT, PRODH and ZDHHC8, and so on.[29,30]

Many researchers have presented positive linkage and association findings with schizophrenia, but initial findings have often not been replicated.[30] One of the most significant causes of conflicting results in the present molecular genetic studies of schizophrenia may be the potential heterogeneity of schizophrenia. Several investigators have suggested that schizophrenia is not a single disease entity but may reflect common symptomatology caused by several distinct genetic abnormalities.[33–37] As mentioned here, the EEM deficits are linked to chromosome 22q11. If the EEM parameters are associated with a schizophrenia subtype with severe symptoms related to excitement/hostility, negative symptoms and disorganization, chromosome 22q11 and genes of 22q11 may relate to this subtype. In this manner, if we are able to find a new subtype using the EEM disturbances, and clarify the heterogeneity of schizophrenia, then linkage or association studies for schizophrenia using the subtype may yield further knowledge regarding the genetic influences on schizophrenia.

In conclusion, we have found evidence for the existence of five dimensions of schizophrenia symptoms: excitement/hostility; negative symptoms; depression/anxiety; positive symptoms; and disorganization. Schizophrenia patients with EEM abnormalities (SPDSE) may have severe symptoms related to excitement/hostility, negative symptoms and disorganization. In light of the heterogeneity of schizophrenia, SPDSE may be a disease subtype of schizophrenia with the aforementioned symptomatic features; and the EEM parameters may detect this subtype. Therefore, EEM may be one of the contributors to the simplification of the heterogeneity of schizophrenia. Consequently, we may apply EEM to other scientific studies as an endophenotype for schizophrenia.

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LETTER TO THE EDITOR

Norepinephrine in the brain is associated with aversion to financial loss

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Understanding the molecular mechanism of extreme or impaired decision-making observed in neuropsychiatric disorders, such as pathological gambling and attention-deficit hyperactivity disorder (ADHD), could contribute to better assessment and the development of novel pharmacological therapies for those disorders. Typically, most people show a disproportionate distaste for possible losses compared with equal-sized gains. This human *in vivo* molecular imaging study has demonstrated that individuals with lower thalamic norepinephrine transporters (NET) showed more exaggerated aversion to financial loss.

Empirical and field studies suggest that losses have greater impact than equivalent gains.[1] For example, a typical person would only accept a two-outcome gamble in which $50 could be lost if the possible gain is $100, twice as large. This greater sensitivity to losses than to gains is called 'loss aversion' and substantial individual differences in it have been observed in many behavioral studies.[1,2] In psychiatric populations, pathological gambling showed diminished sensitivity to monetary loss itself and, more specifically, patients with ADHD and psychopaths showed reduced sensitivity to the magnitude of monetary loss.[3,4]

Recent functional magnetic resonance imaging and lesion studies have shown that the prefrontal cortex (PFC), striatum and amygdala are involved in loss aversion.[5,6] However, little is known about modulatory neurotransmission in this phenomenon. There is circumstantial evidence that NE may be important for loss aversion. Central NE blockade by propranolol reduced sensitivity to the magnitude of possible losses from gambles.[7] A recent psychophysiological study demonstrated that arousal is associated with loss aversion.[8] We utilized positron emission tomography (PET) scans with (S,S)-[^{18}F]FMeNER-D$_2$ to investigate the relationship between central NET and loss aversion. A NET-rich region available to PET imaging with this ligand is the thalamus. The amygdala and PFC are also innervated by NE, but relatively low expression of NET prevented reliable measurement of their NET binding in the current study. We expected that NET in the thalamus would mediate loss aversion.

In all, 19 healthy male volunteers participated in PET scans for quantification of NET in the brain. Brain radioactivities were measured with scanning from 0 to 90 min, followed by scanning from 120 to 180 min. The region-of-interest was set on the bilateral thalamus. NET binding in the thalamus was calculated by the area-under-the-curve ratio method using the PMOD software package (PMOD Technologies, Zurich, Switzerland). An integration interval of 120–180 min was used in this method because specific binding reaches a peak during this period of PET measurement (Supplementary Information). Loss aversion parameters were determined outside the PET scanner. Participants were presented mixed gambles that had a 50% chance of losing a fixed amount of X and a 50% chance of gaining Y. The amounts of possible gain Y to make up for a 50% chance of losing X were determined by a staircase procedure (Supplementary Information), yielding an estimate of loss aversion λ from $Y = \lambda \times X$. A median of λ was 3.01 (range: 0.98–9.98). Mean binding potential of (S,S)-[^{18}F]FMeNER-D$_2$ in the thalamus was 0.57 ± 0.10. There was a strong negative correlation between λ and NET binding in the thalamus (Figure 1).

Although NE has been implicated in arousal, recent studies also suggest that NE affects processing of salient information.[9] Neurons of the locus coeruleus (LC), the major source of NE in the brain, is phasically evoked by salient or emotional stimuli, and phasic LC activation also increases NE release in target sites.[9] Increasing NE tone by NE reuptake inhibitor improves detection of emotional stimuli,[10] and blockade of central NE by propranolol attenuates the sensitivity to the magnitude of possible losses.[7]

A recent study showed that, on average, physiological arousal response to losses was greater than to equivalent gains.[8] This means that losses are more emotionally laden and salient than equivalent gains. The study also reported that individuals with greater arousal response to losses versus gains tend to be more loss aversive.[8] Thus, our finding suggests that individuals with low NET might show an enhanced effect of NE released by salient stimuli due to low re-uptake, and consequently show pronounced emotional or arousal response to losses relative to gains. Due to radioligand limitations, we could not test the amygdala and PFC, which are innervated by NE and implicated in loss aversion. Thalamic NET might be an indirect mediator of the relationship between NE transmission and loss aversion. It stands to reason that careful interpretation is needed, and future investigation will be required. In any event, we believe that this novel finding could provide new

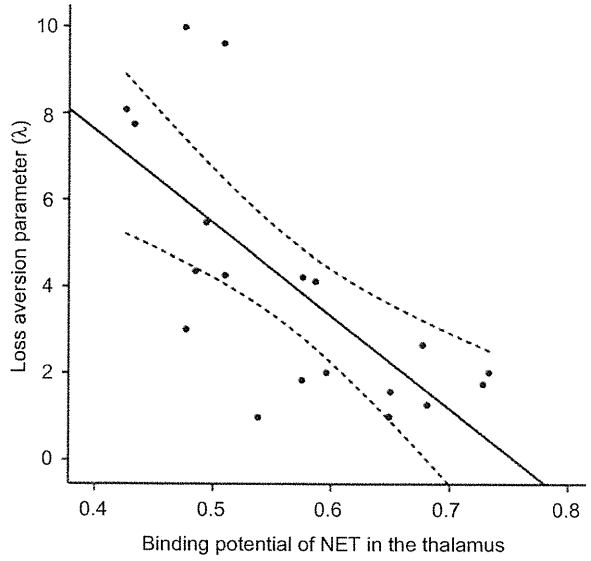

Figure 1 Correlation between loss aversion parameters and norepinephrine transporters (NET) binding in the thalamus. Plots and regression line of correlation between λ and Binding potential of the thalamus ($R=-0.71$, $P<0.001$). The dashed lines are 95% confidence interval boundaries.

perspectives on altered decision making observed in neuropsychiatric disorders.

Conflict of interest

The authors declare no conflict of interest.

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Effect of radiolabeled metabolite elimination from the brain on the accuracy of cerebral enzyme activity estimation using positron emission tomography with substrate tracers

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ABSTRACT

Cerebral enzyme activity can be quantified using positron emission tomography (PET) in conjunction with a radiolabeled enzyme substrate. We investigated the relationship between the elimination rate (k_{el}) of tracer metabolites from the brain and the precision of target enzyme activity estimation (k_3). An initial simulation study indicated that the precision of k_3 estimates was highly dependent on k_{el}, and was characterized by several kinetic parameters including the ratio of k_{el} and the efflux rate (k_2) of authentic tracer ($\beta \equiv k_{el}/k_2$). The optimal tracer condition for high sensitivity was found to be $\beta<0.1$. To verify the simulation results, we performed a PET study with a single monkey using two PET tracers, *N*-[^{18}F]fluoroethylpiperidin-4-ylmethyl acetate ([^{18}F]FEP-4MA) and *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A). Both of these substrate type tracers were developed for measuring cerebral acetylcholinesterase activity. There was good retention of the radioactive metabolite of [^{11}C]MP4A in the brain ($k_{el}=0.0036\pm0.0013$ min^{-1}, $\beta=0.028$), whereas that of [^{18}F]FEP-4MA was eliminated from the brain ($k_{el}=0.012\pm0.0010$ min^{-1}, $\beta=0.085$). A non-linear least square analysis for simultaneous estimation of all parameters showed that the precision of the k_3 estimate for [^{18}F]FEP-4MA was as high (7.4%) as that for [^{11}C]MP4A (10%). These results indicate that tracers with metabolites that are eliminated from the brain at a slow rate ($\beta<0.1$) may be useful for the quantitative measurement of target enzyme activity.

Introduction

Cerebral enzyme activity can be quantified using positron emission tomography (PET) in conjunction with a radio-labeled enzyme substrate. Fig. 1 depicts a postulated compartmental model of a tracer based on the rationale of enzyme-mediated trapping. In this model, k_3 represents the enzyme-mediated first-order metabolic rate of the tracer, and can be estimated by kinetic analysis of the time activity curve (TAC) in the brain, as measured with PET. *N*-[^{11}C] Methylpiperidin-4-yl acetate ([^{11}C]MP4A), an acetylcholinesterase substrate, has been clinically used for the measurement of cerebral acetylcholinesterase activity based on this rationale (Shinotoh et al., 2004).

An effective tracer based on radioactive metabolite trapping must possess several properties, including a high specificity for the target enzyme, a moderate metabolic rate, and high blood brain barrier permeability. In terms of the second property, it has been reported that the ratio of the metabolic rate (k_3) and the efflux rate (k_2) of a given tracer, i.e. $\alpha \equiv k_3/k_2$ (the kinetic parameter proposed by Lassen et al., 1988), can affect the reliability of data in static and dynamic analysis (Fukushi et al., 1993; Koeppe et al., 1994, 1999). In terms of the third property, it is expected that a high blood brain barrier permeability of the tracer metabolite is not a desirable quality, unlike the case for the authentic tracer. The influx of metabolite into the brain makes it difficult to estimate k_3, and the elimination of metabolite from the brain would be expected to reduce the radioactivity in the brain. Thus, an effective radioactive metabolite should have hydrophilic properties to limit membrane permeability. However, even hydrophilic metabolites can be extruded from the brain by an efflux transporter. *N*-[^{11}C]Methylhalopurine derivatives, glutathione S-transferase substrates, are metabolized to form hydrophilic glutathione conjugates (Okamura et al., 2007, 2009). Glutathione conjugates are extruded from the brain by an efflux transporter, but they do not enter the brain from the blood. Similarly, the hydrophilic

Abbreviations: PET, positron emission tomography; TAC, time activity curve; MP4A, *N*-methylpiperidin-4-yl acetate; FEP-4MA, *N*-fluoroethylpiperidin-4-ylmethyl acetate; CV, coefficient of variation; NLS, non-linear least square; TLC, thin layer chromatography; PMP, *N*-methylpiperidin-4-yl propionate; %ID, percentage injected dose.

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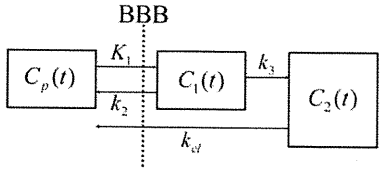

Fig. 1. Two-tissue compartment model with four parameters, for an incomplete trapping irreversible tracer. It can be seen that uptake of the metabolites of a given tracer from the arterial plasma to the brain cannot occur. C_1 and C_2 represent the concentration of the authentic and metabolized tracer in the brain, respectively. C_p represents the concentration of the authentic tracer in arterial plasma. K_1, k_2, k_3 and k_{el} represent the rate parameters in the model, corresponding to the influx rate constant, the efflux rate constant, the metabolic rate constant and the elimination rate constant, respectively.

metabolite of *N*-[^{18}F]fluoroethylpiperidin-4-ylmethyl acetate ([^{18}F]FEP-4MA), an acetylcholinesterase substrate, is extruded from the brain, but does not enter the brain from the blood (Kikuchi et al., 2010). In addition, the efflux rate of the [^{11}C]MP4A metabolites from the brain were low but not zero. However, it remains unclear how the extent of elimination of the tracer metabolites affects the sensitivity of a tracer for detecting changes in target enzyme activity (i.e. k_3 parameter changes).

In this study, we investigated the relationship between the kinetic properties of tracers in terms of the elimination of tracer metabolites, in addition to tracer α-value, and the precision of k_3 estimates using a simulation technique. In addition, we validated our simulation results with a dynamic PET experiment in a monkey using [^{11}C]MP4A and [^{18}F]FEP-4MA as model tracers.

Materials and methods

Theoretical basis

General

When the metabolites of a tracer in the blood do not enter the brain, in contrast to the elimination of the metabolite from the brain, the kinetics of such a tracer can be described by a two-tissue compartment model with four parameters (Friberg et al., 1994; Fig. 1). The time-course of the concentration of total radioactivity in the brain, which is the sum of authentic and metabolized tracer ($C_t = C_1 + C_2$), is expressed as:

$$C_t = K_1 \cdot e^{-(k_2+k_3)t} \otimes C_p + \frac{K_1 k_3}{k_2 + k_3 - k_{el}} \left(e^{-k_{el}t} - e^{-(k_2+k_3)t}\right) \otimes C_p \quad (1)$$

where K_1 represents the influx rate constant, k_2 represents the efflux rate constant, k_3 represents the metabolic rate constant, k_{el} represents the elimination rate constant of tracer metabolites, and C_p represents the concentration of an authentic tracer in arterial plasma.

β-value as kinetic parameter

It has been reported that the ratio of the metabolic rate constant (k_3) and efflux rate constant (k_2), i.e. α-value, determines the sensitivity of irreversible tracers (Koeppe et al., 1999). Besides the tracer α-value, we must consider the kinetic effects of metabolite elimination on tracer sensitivity, when the metabolite is eliminated from the brain. The Eq. (1) can be rewritten as follows:

$$C_t = K_1 \left(\frac{1-\beta}{1+\alpha-\beta} e^{-(k_2+k_3)t} + \frac{\alpha}{1+\alpha-\beta} e^{-k_{el}t}\right) \otimes C_p \quad (2)$$

where, $\alpha \equiv k_3/k_2, \beta \equiv k_{el}/k_2$.

The time-course of concentration of total radioactivity is characterized by two coefficients and two time constants ($1/k_2 + k_3$ and $1/k_{el}$). Both the coefficients can be described by an α- and a β-value. On the other hand, the C_t may also be characterized by the ratio of the two time constants, i.e. γ-value ($\equiv k_{el}/(k_2 + k_3)$). Thus, we considered that the two kinetic parameters, the β- and the γ-value, of a given tracer would affect the precision of k_3 estimation. However, we mainly focused on the β-value, because the optimal β condition was found to be more important than the γ condition in terms of tracer sensitivity. As such, we felt that the γ-value should be dealt with separately (see Supplementary data for details).

Simulation study

Generation of TACs for simulations

We performed a Monte Carlo simulation study. Noise-free-TACs for a target were generated using Eq. (1) with the given K_1, k_2, k_3 and k_{el} parameter values and a typical [^{11}C]MP4A plasma input curve from a healthy human subject (Iyo et al., 1997; Namba et al., 1999). The dynamic sequence was set as follows: 3×20 s, 3×40 s, 1×60 s, 2×180 s, 5×360 s, 2×600 s. Based on a previous report (Logan et al., 2001), additive noise for simulated TAC was generated by the following equation:

$$\sigma_i = \varepsilon \sqrt{\frac{C_i}{\Delta t_i \cdot e^{-\lambda t_i}}} \times (xx) \quad (3)$$

where ε indicates the scale factor that determines noise level, t_i indicates the mid-scan time, Δt_i indicates the scan duration time, C_i indicates noise-free simulated radioactivity concentration at frame number i, λ indicates the ^{11}C decay constant, and (xx) are pseudo random numbers from a Gaussian distribution N(0,1). To generate the TACs for the simulations (simulated TACs), the random noise derived from Eq. (3) was added to each time point of the noise-free TAC. The scale factor was adjusted as the coefficient of variation (CV(%) = SD/mean×100) of the k_3 parameter, being approximately 10% when the k_{el}-value is 0.00001 min^{-1}, based on data from a previous PET study using [^{11}C]MP4A in human cortex (Nagatsuka et al., 2001). A weighted non-linear least square (NLS) analysis using the Marquardt algorithm (Levenberg, 1944; Marquardt, 1963) was performed to estimate k_3 from a simulated TAC.

Effect of tracer α-value on precision of k_3 estimation

The simulation study was performed to examine the effect of the α-value of a given tracer on the precision of a k_3 estimate. Simulated TACs were generated using the values of rate constants, set as follows: $K_1 = 0.54$ mL g^{-1} min^{-1}, $k_2 = 0.13$ min^{-1}, based on the previous human PET study using MP4A (Iyo et al., 1997). The k_3 parameter was altered so that the α-value varied from 0.001 to 10 under each β condition altered in five levels; 0, 0.00008, 0.08, 0.4 and 0.8 min^{-1} (corresponding to k_{el} conditions; 0, 0.00001, 0.01, 0.05 and 0.1 min^{-1}). The NLS analysis was performed to estimate four rate constants (K_1, k_2, k_3 and k_{el}) simultaneously from a simulated TAC. These processes were repeated to obtain over 300 parameter sets under every k_{el} condition, and the CV and the bias of the estimated parameters were calculated. In addition, we performed the same simulation for an irreversible tracer with the kinetics described by the two-tissue compartment model with three parameters (K_1, k_2 and k_3).

Precision and bias of k_3 estimate from simultaneous estimation of four rate constants

Simulated TACs were generated using the values of rate constants; $K_1 = 0.54$ mL g^{-1}min^{-1}, $k_2 = 0.13$ min^{-1}, $k_3 = 0.079$ min^{-1} based on a previous human PET study for MP4A (Iyo et al., 1997). The k_{el}-value was altered so that the β-value was changed from about 0.00008 to 0.8 as described above. The NLS analysis was performed to estimate simultaneously four rate constants (K_1, k_2, k_3 and k_{el}) from a simulated

TAC. The CV of k_3 estimation was calculated in the same manner as described above.

Monkey PET study

[^{11}C]MP4A, [^{18}F]FEP-4MA and the alcoholic metabolite of [^{18}F]FEP-4MA were prepared as described previously (Namba et al., 1999; Kikuchi et al., 2005). Physostigmine was purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). All other chemicals were of reagent grade or better, and were available commercially.

A male monkey (*Macaca mulatta*, 20 years old, 7 kg) served as the subject in this experiment, and was maintained and handled in accordance with the guidelines of the National Institute of Radiological Sciences (NIRS). The present study was approved by the Animal Ethics Committee of NIRS, Chiba, Japan.

PET scans were performed using a high-resolution SHR-7700 PET camera (Hamamatsu Photonics, Japan; 31 transaxial slices 3.6 mm (center-to-center) apart, a 33.1-cm field of view, 111.6-mm axial field of view, spatial resolution of 2.6 mm full width at half maximum) designed for laboratory animals. The monkey was fixed on a chair in an unanesthetized condition throughout the PET session, and was immobilized with a fixation device to ensure accuracy of repositioning. After transmission scans for attenuation correction using a ^{68}Ge–^{68}Ga source, a dynamic scan in enhanced 2D mode was performed for 60 min. A dynamic sequence of 3×20 s, 2×30 s, 4×60 s, 2×180 s, and 5×480 s scans was used. One mL of each tracer solution, [^{11}C]MP4A (780 MBq) and [^{18}F]FEP-4MA (330 MBq), was infused via the crural vein for 1 min. Emission data were reconstructed with a 4.0-mm Hanning filter. Concentrations of radioactivity (%ID/mL) in the occipital cortex, cerebellum and striatum were measured, and these corresponded to regions with low, middle and high acetylcholinesterase activity, respectively. In the occipital cortex, six regions of interest (ROIs) were also sampled. To confirm the extent to which penetration of the alcoholic [^{18}F]FEP-4MA metabolite from the blood to the brain occurred, a dose of the metabolite (230 MBq) was administrated to the monkey and the concentration of radioactivity in the whole brain was determined.

Approximately 0.5 mL of arterial blood was drawn from the artery cannula into 1 mL heparinized syringes, and the blood samples were immediately transferred into tubes containing the cholinesterase inhibitor physostigmine (0.1 mg in 10 μL saline). The blood samples were drawn at 15 s, 32 s, 41 s, 56 s, 65 s, 76 s, 100 s, 120 s, 144 s, 170 s, 191 s, 215 s, 245 s, 280 s, 311 s, 340 s, 397 s, 457 s, 523 s, 583 s, 642 s, and 1,245 s after starting [^{11}C]MP4A administration, and 26 s, 37 s, 50 s, 57 s, 69 s, 93 s, 117 s, 135 s, 159 s, 191 s, 209 s, 237 s, 300 s, 367 s, 424 s, 482 s, 541 s, 607 s, 901 s, and 1,207 s after starting [^{18}F]FEP-4MA administration. Then, 50 μL of plasma samples, which was obtained by centrifuging the blood sample at 10,000×*g* for 10 min, was mixed with 0.1 mL of ethanol and centrifuged at 10,000×*g* for 10 min. A portion of the plasma sample was subjected to thin layer chromatography (TLC) with a silica-gel plate (silica gel 60 F254; Merck Ltd., Tokyo, Japan) and a mixture of ethyl acetate:iso-propanol:28% ammonia (15:5:1 volumes for [^{11}C]MP4A, 100:10:1 volumes for [^{18}F]FEP-4MA) as a developing solvent. The fraction of the authentic compounds in the plasma sample was detected quantitatively using radio-TLC analysis (BAS 5000, FUJIFILM Co., Tokyo, Japan). In addition, radioactivity in 150 μL of each intact plasma sample was measured with a gamma counter (Wizard; PerkinElmer Co., Ltd. Kanagawa, Japan).

Each rate constant was estimated by NLS analysis in the same manner as performed for the simulation. The data of authentic tracer in blood plasma were fitted by a multiexponential function as previously reported (Namba et al., 1999). The time delay between PET measurement and arterial plasma measurement was corrected using a method described by Iida et al. (1988). The blood volume in the brain was fixed at 3% (Tsukada et al., 1999). Standard errors (SE) of estimated k_3 parameters, uncertainty of the parameter value because of fitting error, were calculated using the covariance matrix (Carson, 1986).

Results

The effect of tracer α-value on the precision of a k_3 estimate

We investigated how tracer α-value affected k_3 precision under different k_{el} conditions. The results revealed that tracer α-value was one of the critical factors determining k_3 precision (Fig. 2A), and the optimal α-value with the maximal precision of k_3 parameter was around 0.5. The high precision of k_3 estimates was maintained when the tracer α-value was within a range of approximately 0.2 to 1.0 (optimal α range). The tracer β-value strongly affected the precision of the k_3 estimate (Fig. 2A). The tracer α-value also affected TAC sensitivity for k_3 changes (Fig. 2B). In addition, when the two-tissue compartment model with three parameters was used in the analysis (i.e. using an irreversible tracer), the optimal α-value was slightly reduced to a lower value (around 0.2) compared with that using the two-tissue compartment model with four parameters (around 0.5; Fig. 2A). The precision of the k_3 estimate in irreversible tracers was higher than that in incomplete trapping irreversible tracers throughout the whole α range. The difference between the k_3 precision of an irreversible tracer and an incomplete one was decreased with increases in tracer α-value.

Effect of tracer β-value on precision and bias of estimated k_3

The precision of k_3 estimates was highly dependent on the β-value of a given tracer (Fig. 3A). The precision of k_3 was primarily determined by the β-value rather than the k_{el}-value of a given tracer alone (Supplementary Fig. A), and was drastically decreased when the β-value was over 0.1 (Fig. 3A, Supplementary Fig. A). The tracer β-value was also found to be a critical factor in the sensitivity of TAC. The change in TAC shapes with a 30% decreased k_3 became larger as the β-value decreased (Fig. 3B). The change in TAC shapes became obscure as the β-value increased, especially over around 0.1 of the β-value (Fig. 3B). The positive bias of the k_3 estimate was drastically increased when the tracer β-value was over approximately 0.4 (Fig. 3A).

Monkey PET study

Fig. 4 shows TACs for both [^{18}F]FEP-4MA and [^{11}C]MP4A in the cerebellum, striatum and occipital cortex. The proportion of regional uptake of both tracers in the three areas was: striatum>cerebellum>cortex, indicating the relative acetylcholinesterase activity in the different regions. The differences in the TAC values for [^{11}C]MP4A at the later phase in the different regions (Fig. 4B) was larger than those for [^{18}F]FEP-4MA (Fig. 4A). On the other hand, the radioactivity in the monkey brain remained low during the observation period when the alcoholic metabolite of [^{18}F]FEP-4MA was administrated (Fig. 4A).

Fig. 5 shows TACs for each of [^{18}F]FEP-4MA and [^{11}C]MP4A in the arterial plasma and occipital cortex. Both tracers disappeared from the arterial blood within 15 min of the injection (Fig. 5A). In the early phase after intravenous injection, the uptake of [^{18}F]FEP-4MA into brain tissue was higher than that of [^{11}C]MP4A (Fig. 5B). For [^{18}F]FEP-4MA, the K_1 and k_{el}-values were larger than those observed for [^{11}C]MP4A (Table 1). In particular, the k_{el}-value of [^{18}F]FEP-4MA was more than three times as large as the k_{el}-value for [^{11}C]MP4A.

The k_{el}-value for [^{18}F]FEP-4MA (0.012 min^{-1}) was relatively low compared with the k_2-values, resulting in a low β-value (=0.085). The precision of k_3 parameter was high for [^{18}F]FEP-4MA (7.4%) and [^{11}C]MP4A (10%).

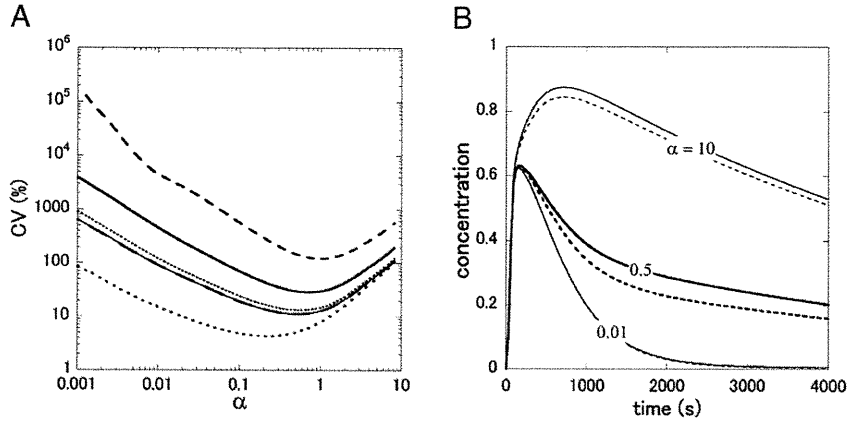

Fig. 2. Dependency of k_3 precision (A) and TAC sensitivity for k_3 change (B) on tracer α-value. (A) The vertical axis represents the coefficient of variation (CV(%)) of k_3 parameter and horizontal represents logarithms of the tracer α-value. In this calculation, K_1 and k_2 are constants ($K_1 = 0.54$, $k_2 = 0.13$). Each plotted line estimated using two-tissue compartment model with four parameters corresponds to the conditions of tracer β-values; 0 (bold broken line), 0.00008 (thin solid line), 0.08 (thin dotted line), 0.4 (bold solid line), 0.8 (bold broken line). The former two graphs are almost same. The bold dotted line corresponds to the curve estimated using two-tissue compartment model with three parameters in the case of $\beta = 0$. (B) Three pairs of TACs for incomplete trapping irreversible tracer with different α-values (0.01, 0.5, 10) are calculated using Eq. (1) with the same input function and rate parameter set (K_1, k_2, k_3, β) = (0.54, 0.13, C, 0.08; solid line) or (0.54, 0.13, 0.7×C, 0.08; broken line). C indicates the k_3-value corresponding to each α-value.

Discussion

The present study investigated how the incomplete trapping of tracer metabolites in a target tissue affects the sensitivity of detecting the activity of a target enzyme (i.e. the k_3 parameter), using both simulations and a monkey PET study. In addition to the α-value, we used the β-value as kinetic parameter related to the extent of the elimination of tracer metabolites. The α-value refers to the ratio of metabolic rate to the efflux rate of authentic tracer, and determines the sensitivity of an irreversible tracer (Koeppe et al., 1999). In contrast, the β-value is the ratio of elimination rate to efflux rate. Theoretical models predict that the β-value also affects the precision of k_3 estimates: when the tracer β-value changes to 1, estimation of the k_3 parameter becomes impossible. As such, testing this value involved in the k_{el}-value may help to elucidate the effects of the elimination of the tracer metabolite on the precision of k_3 estimates.

In the simulation study, we found that the tracer α-value was still the critical kinetic parameter for determining k_3 precision with incomplete trapping irreversible tracers, and precision became high when the α-value was within the range of approximately 0.2 to 1.0 regardless of the tracer β-value. The optimal range with incomplete trapping irreversible tracers was found to be slightly higher than that of irreversible tracers. In addition, the results of the simulation revealed that the optimal α-value of irreversible tracers was around 0.2, in accord with previous reports that the precision of k_3 estimation is high in irreversible tracers when the α-value is within the range of 0.1–0.3 for [^{11}C]PMP (Koeppe et al., 1999) and 0.14–0.6 for [^{11}C] clorgyline (Logan et al., 2002). When the α-value of a given tracer was far from the optimal α-value, the TAC of the tracer became insensitive to k_3 change. When a tracer α-value is extremely low, the change in TAC shape corresponding to k_3 change becomes small, resulting in a k_3 estimate with low precision. A decrease of k_3 precision is also caused under high α conditions, because the net incorporation of the tracer with a high α-value into a target tissue will be dependent not on metabolic rate (k_3), but on a blood flow (K_1) (i.e. a delivery limitation effect).

We conducted a simulation study to investigate how the tracer β-value (or k_{el}-value) affects k_3 precision. We found that the precision of k_3 estimates was highly dependent on the k_{el}-value. Specifically, precision was largely determined by the β-value of the tracer: when

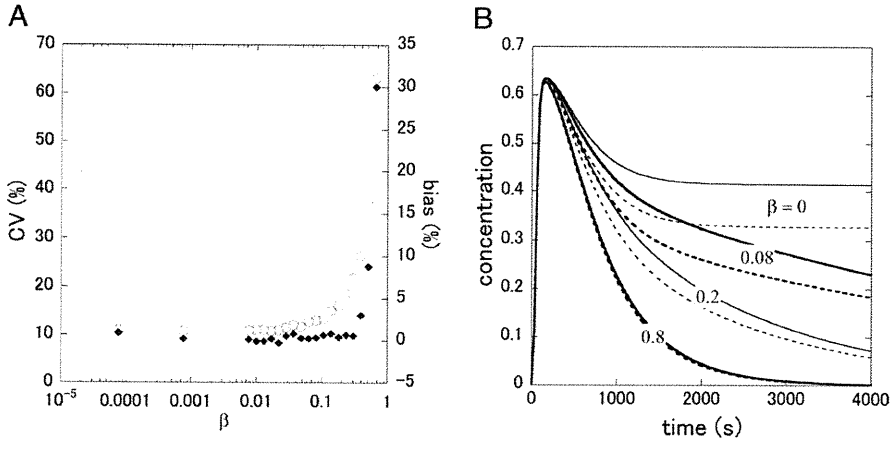

Fig. 3. Dependency of precision and bias of k_3 estimate (A) and dependency of TAC sensitivity for k_3 change (B) on the β-value of a tracer. (A) The vertical axis on the left side represents the CV of the k_3 parameter. The vertical axis on the right side represents the k_3 bias, and the horizontal axis represents logarithms of the β-value of the tracer. Open circles indicate k_3 precision and closed diamonds indicate k_3 bias. (B) Dependency of TAC sensitivity for k_3 change on β-values of a tracer. Four pairs of TACs for incomplete trapping irreversible tracer with different β-values are calculated using Eq. (1) with the same input function and rate parameter set (K_1, k_2, k_3) = (0.54, 0.13, 0.079; solid line) or (0.54, 0.13, 0.7×0.079; broken line) and different β-values (0, 0.08, 0.2, and 0.8).

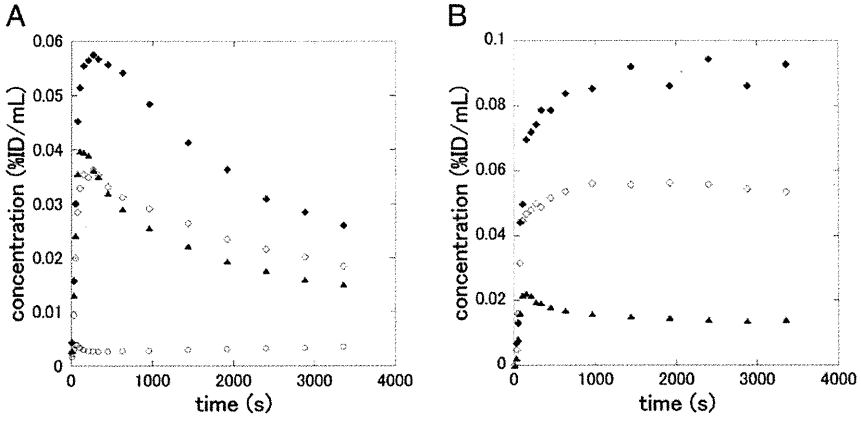

Fig. 4. The TACs for [^{18}F]FEP-4MA (A) and [^{11}C]MP4A (B) in the occipital cortex (closed triangle), cerebellum (open diamond) and striatum (closed diamond) of the monkey. The open circles in panel A indicate the TAC of the alcoholic metabolite of [^{18}F]FEP-4MA in the brain, which was infused via the crural vein in the same manner as in the [^{18}F]FEP-4MA experiment. All data represent decay corrected measured values.

the tracer β-value was over 0.1, k_3 precision was drastically decreased. The boundary value was not dependent on the k_2- and k_3-value of a tracer (data not shown). The decrease of k_3 precision with the increase of the tracer k_{el}-value may be attributed not only to the decrease of the total counts of radioactivity due to the reduction of the retention of the tracer, but also to the decrease of TAC sensitivity due to the tracer β-value being over 0.1. On the other hand, the extent of change in TAC shape with k_3 change was increased when the β-value was close to 0, suggesting that the β-value of a given tracer is preferred as low as possible for static analysis. These results indicate that, in addition to the α-value, the β-value is an important factor in determining tracer sensitivity.

To validate our simulation results, dynamic PET scans were performed in a monkey using two model tracers, [^{18}F]FEP-4MA and [^{11}C]MP4A. The biochemical and kinetic properties of both tracers have been well characterized. The finding that the uptake of [^{18}F]FEP-4MA into a brain was higher than that of [^{11}C]MP4A may reflect the higher lipophilicity of [^{18}F]FEP-4MA (Kikuchi et al., 2005). The α-value of [^{18}F]FEP-4MA was within the optimal α-value range ($\alpha = 0.70$). In [^{18}F]FEP-4MA, the values of the parameter reflecting the elimination of tracer metabolite were relatively low; (β, k_{el}) = (0.085, 0.012). These properties of [^{18}F]FEP-4MA resulted in high k_3 precision (SE = 7.4%). The high uptake of [^{18}F]FEP-4MA and slow decay of ^{18}F compared with ^{11}C may also be contributed to the high precision (Supplementary Fig. B). The precision of k_3 estimation was also high in [^{11}C]MP4A (SE = 10%) with (α, β) = (0.79, 0.028). These results indicate that the availability of a tracer with metabolite elimination should be determined by both the α- and β-values. On the basis of these results, we propose that the β-value, rather than k_{el}-value, is a fundamental kinetic parameter for determining the precision of k_3 estimation.

Conclusions

Our results revealed that the precision of k_3 estimation for a tracer is determined by both its α- and β-value; within the optimal α range of approximately 0.2–1, and with optimal values of $\beta < 0.1$. The incompleteness of the trapping of a tracer metabolite lowers the tracer sensitivity to changes in k_3. However, when the elimination rate is relatively low compared with the k_2-value (i.e. k_{el}-values satisfying the optimal β condition) a tracer is expected to show high sensitivity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.neuroimage.2011.02.030.

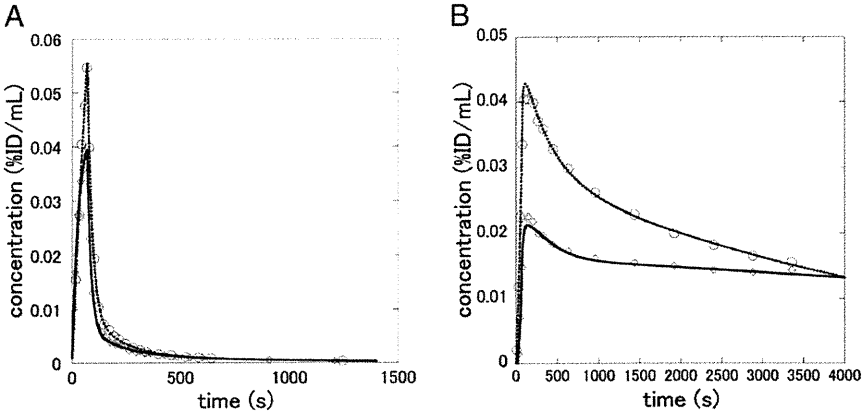

Fig. 5. The TACs of authentic [^{18}F]FEP-4MA and [^{11}C]MP4A in arterial plasma (A), and the TACs for the both tracers in occipital cortex (B) in a monkey. Open circles and open diamonds indicate the decay corrected measured values for [^{18}F]FEP-4MA and [^{11}C]MP4A, and the dotted and solid lines show fitted curves for both, respectively.

Table 1
Rate constants of occipital cortex in monkey.

	Rate constants (mean ± SD)				
	K_1	k_2	k_3	k_{el}	α-value
	$mLg^{-1}min^{-1}$	min^{-1}			
[^{18}F]FEP-4MA	0.65 ± 0.055	0.14 ± 0.026	0.10 ± 0.017 (SE 7.4%)	0.012 ± 0.0010	0.70
[^{11}C]MP4A	0.47 ± 0.023†	0.13 ± 0.012	0.10 ± 0.014 (SE 10%)	0.0036 ± 0.0013†	0.79

Table shows averaged values of six ROIs in occipital cortex of a single monkey. Standard errors (SE) of k_3 estimates calculated from the mean TAC of six ROIs are presented in parentheses. $^{\dagger}P<0.01$ for each rate parameter for [^{18}F]FEP-4MA vs. that for [^{11}C]MP4A in unpaired, two-tailed Student's t-tests.

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Review Article

Functional significance of central D1 receptors in cognition: beyond working memory

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The role of dopamine D1 receptors in prefrontal cortex function, including working memory, is well acknowledged. However, relatively little is known about their role in other cognitive or emotional functions. We measured both D1 and D2 receptors in the brain using positron emission tomography in healthy subjects, with the aim of elucidating how regional D1 and D2 receptors are differentially involved in cognitive and emotional functions beyond working memory. We found an inverted U-shaped relation between prefrontal D1 receptor availability and Wisconsin Card Sorting Test performance, indicating that too little or too much D1 receptor stimulation impairs working memory or set shifting. In addition, variability of D1 receptor availability in the amygdala and striatum was related to individual differences in emotional responses and decision-making processes, respectively. These observations suggest that the variability of available D1 receptors might be associated with individual differences in brain functions that require phasic dopamine release. An interdisciplinary approach combining molecular imaging of dopamine neurotransmission with cognitive neuroscience and clinical psychiatry will provide new perspectives for understanding the neurobiology of neuropsychiatric disorders such as schizophrenia, addiction and Parkinson's disease, as well as novel therapeutics for cognitive impairments observed in them.
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Positron emission tomography imaging of D1 and D2 receptors and working memory

Because dopamine D1 receptors in the prefrontal cortex (PFC) are several times more abundant than D2 receptors (Hall *et al*, 1994), the relationship between D1 receptors and PFC functions has been widely investigated. Sawaguchi and Goldman-Rakic (1994) showed that local administration of D1 receptor antagonists into PFC induced impairment in working memory task in nonhuman primate. In human, Müller *et al* (1998) reported that systemic administration of a mixed D1/D2 agonist facilitated working memory while the selective D2 agonist had no effect, indicating that the dopaminergic modulation of working memory processes is mediated primarily via D1 receptors.

In contrast to D1 receptors, relatively less attention has been paid to the role of prefrontal D2 receptors in cognitive functions partly because their density in extrastriatal regions is very low (Suhara *et al*, 1999). It was reported that blockade of D2 receptors in PFC did not impair working memory in nonhuman primate (Sawaguchi and Goldman-Rakic, 1994), but some human studies reported that systemic administration of D2 agonist or antagonist modulated cognitive functions that are subserved by PFC (McDowell *et al*, 1998; Mehta *et al*, 1999). We measured both D1 and extrastriatal D2 receptor availabilities (binding potentials), indices proportional to receptor density, using [^{11}C]SCH23390 and [^{11}C]FLB457 positron emission tomography (PET), respectively, in healthy male subjects, and aimed to

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elucidate how regional D1 and D2 receptors are differentially involved in neurocognitive performance including frontal lobe functions. Receptor availability is defined as receptors that are available to be bound by the radiotracer. This means receptors that are available for stimulation by released endogenous dopamine.

A body of animal studies has indicated that stimulation of D1 receptors in PFC produces an inverted U-shaped dose–response curve, such that too little or too much D1 receptor stimulation impairs PFC functions (Cools and D'Esposito, 2011; Goldman-Rakic *et al*, 2000; Williams and Castner, 2006). Therefore, we conducted quadratic regression analysis to reveal the putative 'U-shaped' relation between D1 receptor availability in PFC and PFC function. Although standard linear regression analysis revealed a trend-level negative correlation between D1 receptor availability in PFC and total error of the Wisconsin Card Sorting Test (WCST), a test requiring working memory and set-shifting abilities, a quadratic regression model better predicted the relation (Takahashi *et al*, 2008). That is, we found a significant 'U-shaped' relation between D1 receptor availability in PFC and total error of WCST (because total error of WCST is a negative measure of frontal lobe function, the relation is not 'inverted'; Figures 1A and 1B). However, neither linear nor quadratic relation was found between D2 receptor availability in PFC and any neuropsychological measures.

Primal animal studies indicated that stimulation of D1 receptors in PFC produces an inverted U-shaped response in working memory, with the response being optimized within a narrow range of D1 receptor stimulation (Castner and Goldman-Rakic, 2004; Goldman-Rakic *et al*, 2000; Lidow *et al*, 2003; Seamans and Yang, 2004; Vijayraghavan *et al*, 2007). Subsequent human studies have investigated the effect of a functional polymorphism in the catechol O-methyltransferase gene, which has been shown to modulate the prefrontal dopamine level, on prefrontal function. Catechol O-methyltransferase gene contains a common polymorphism, a valine (Val)-to-methionine (Met) substitution at codon 158 (Val158Met). The Val allele is associated with higher activity, whereas the Met allele is associated with lower enzymatic activity. Consequently, Val carriers have a lower level of extracellular dopamine in PFC. A PET study using [^{11}C]NNC112 has demonstrated that Val carriers show significantly higher cortical D1 receptor availability than Met carriers, and the authors suggested a mechanism in which a lower level of extracellular dopamine in PFC induces upregulation of D1 receptors in Val carriers (Slifstein *et al*, 2008). Val carriers show lower performance and increased (inefficient) PFC activation during completion of cognitive tasks related to PFC functions (WCST and N-back task) (Egan *et al*, 2001; Goldberg *et al*, 2003). It was reported that amphetamine challenge in Val carriers induced improvement in the performance of WCST and decreased (efficient) PFC activation during N-back task, whereas that in Met carriers caused deterioration in the performance of WCST and increased (inefficient) PFC activation, indicating that too little or too much dopamine signaling would impair PFC functions, although these studies could not identify the receptor subtype that has a central role in this effect (Mattay *et al*, 2003; Williams-Gray *et al*, 2007).

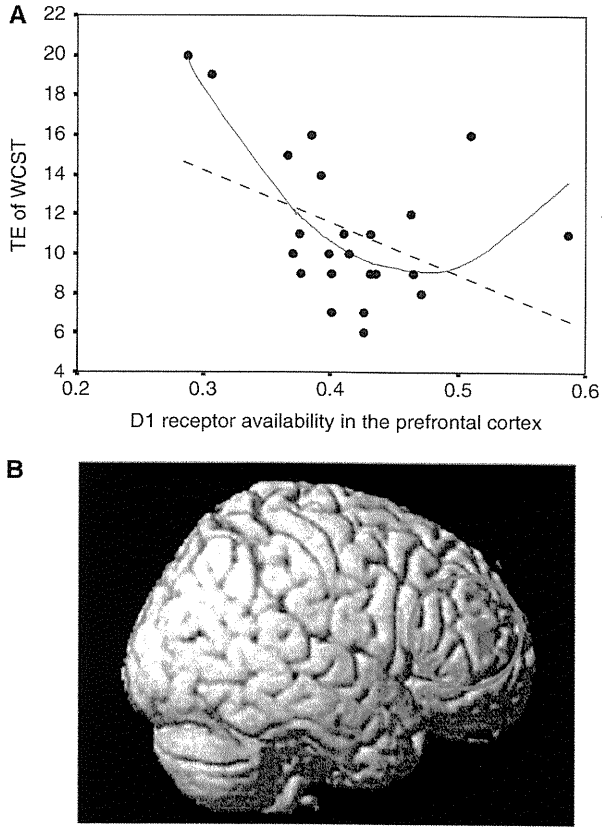

Figure 1 Quadratic (inverted U-shaped) relation between D1 receptor availability in prefrontal cortex (PFC) and performance of Wisconsin Card Sorting Test (WCST). (**A**) Region of interest (ROI) analysis revealed a significant quadratic regression between D1 receptor availability in PFC and total error (TE) of WCST. Red solid line: quadratic regression, black broken line: linear regression. (**B**) Statistical parametric mapping (SPM) analysis also revealed significant quadratic regression between prefrontal D1 receptor availability and total error of WCST.

We first showed an inverted U-shaped relation between D1 receptors in PFC and executive function including working memory in normal healthy subjects (Takahashi *et al*, 2008). An inverted U-shaped response has been suggested based on cognitive and behavioral studies, but the exact physiological mechanism of this effect has not yet been fully understood. A recent monkey electrophysiology study has demonstrated a neuron-level mechanism that constitutes the inverted U-shaped response