control group. Thus, it is possible that the mean years of education may affect the comparisons between schizophrenic patients and normal controls.

RSS and RT-crossover

In the data from our previous and present work, we did not identify any patients with psychiatric disease in which the RSS was similar to that of schizophrenic patients.[4,8,9,15] Not only chronic and acute schizophrenic patients but also those in remission can be distinguished on RSS from patients with depression, neurosis, methamphetamine psychosis, temporal lobe epilepsy, frontal lobe lesions and normal controls. Thus, we consider that the RSS in the EEM test may be specific to schizophrenia.

In the EEM test the RSS is obtained from eye movements that occur in response to an examiner's question 'Are there any other differences?'. The subjects explore the figure again and try to search for differences. The RSS may reflect the visual behavior of a subject who wants to check or confirm their response induced by the interaction between the subject and the examiner, and therefore, the RSS may be an indicator of an interpersonal response. As for the RSS, Kojima *et al.* described the following.[4] According to Neisser's theory of the perceptual cycle, at each moment the viewer has expectations of certain kinds of information, which are readily accepted if they are available. It is postulated that the subject must frequently and actively explore the visual field by moving the eyes or head to make the information in the field available. These explorations are dictated by the anticipatory schemata. The anticipatory schemata are considered to be related to mental attitude: the desire to obtain more information from the visual field.[16] The lower RSS in schizophrenic patients seems to indicate a dysfunction of the anticipatory schemata. We propose that the RSS may reflect the information processing of the brain in relation to the anticipatory schemata in the interpersonal response.

Data from several studies are consistent with the assertion that RT-crossover abnormalities are found in the majority of process schizophrenic patients. Almost all of the available literature suggests that a high rate of process schizophrenic patients shows the RT-crossover.[6,10,11,14,17] These findings suggest that the crossover phenomenon may be a marker for process schizophrenia.

Shakow accounted for this mechanism using his segmental set theory.[18] RT-crossover is the phenomenon in which schizophrenic patients have slower RT in a regular series than in an irregular series. Ordinarily, the consistency of the preparatory intervals in the regular series should give an individual an advantage and lead to faster RT than in the irregular series. Schizophrenic patients, however, are not able to take advantage of such regularity information, and thus they perform poorly. Schizophrenic patients have difficulty in keeping up a state of readiness for response to a coming stimulus. In order to deliver the optimal response to the stimulus, an individual has to focus on the relevant aspects of the defined situation; that is, the individual must maintain a high readiness to respond. But schizophrenic patients are affected by irrelevant aspects of the stimulus surroundings, which prevent focusing on the main stimulus. Schizophrenia patients cannot extract the relevant aspects for optimal response; hence they have difficulty in maintaining a readiness to respond. The mechanism for maintaining this readiness is directed by the major set. The major set reflects the readiness of subjects to recognize stimuli, and is the primary and principal layer of information processing. Shakow proposed that schizophrenic patients are characterized by a failure to maintain an adequate major set.[18]

There is one point we would like to emphasize. According to the Neisser theory, it appears that the anticipatory schemata are similar to the readiness reflected by the major set.[16] Therefore, we consider that these two theories (Neisser's perceptual cycle and Shakow's major set) are similar in concept. In the present study, associations were found between the RSS of the EEM test and the RT-crossover score of the RT test in the schizophrenic group. If the assumption that the RSS of the EEM test reflects the anticipatory schemata and that the crossover phenomenon of the RT test reflects the major set, is correct, it is reasonable to propose that the RSS is associated with the crossover phenomenon. Moreover, based on our previous data, we consider that the EEM test parameters except for the RSS may not relate to the anticipatory schemata in the interpersonal response.[4] Thus it is also reasonable to consider that the EEM test parameters except for the RSS are not strongly associated with the crossover phenomenon. But from the psychological explanation of the P300 amplitude, it may also relate to the anticipatory schemata or the major set.[3,5] In the present study there was no association between the P300 amplitude and the crossover phenomenon. The P300 amplitude also did not relate to the RSS.

Hence the information processing reflected by the P300 amplitude may be distinct from that of the RSS or the cross-over phenomenon. These findings, however, should be interpreted cautiously, and additional studies are needed to confirm these considerations for the P300 amplitude.

In the present study we detected two fundamental information processing abnormalities in schizophrenic. These two abnormalities are in accordance with the former theory. Because it is considered that abnormalities of information processing are among the most important symptoms of schizophrenia, the present results may contribute to elucidation of the pathophysiological signature of schizophrenia. But the present sample size was not very large; thus the present findings should be interpreted cautiously; and additional studies with a larger sample are needed to confirm the findings. Moreover, we did not estimate psychiatric symptoms of patients using a scale for assessment of symptoms. Thus, further limitation of the study are that we were not able to present the severity of subjects or a relationship between the physiological tests and psychiatric symptoms.

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Regular Article

Impairment of exploratory eye movement in schizophrenia patients and their siblings

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Aims: Previous family, adoption and twin studies of schizophrenia have shown that genetic factors contribute significantly to the risk of schizophrenia. The aim of the present study was therefore to investigate whether exploratory eye movement (EEM) abnormalities are related to the genetic markers linked to schizophrenia.

Methods: Twenty-three probands with schizophrenia, 23 of their healthy siblings (23 proband–sibling pairs), and 43 unrelated normal controls performed EEM tasks. Two parameters were measured: (i) number of eye fixations in responsive search (NEFRS) and (ii) responsive search score (RSS).

Results: Abnormalities in NEFRS and RSS were more frequent in schizophrenia probands than in their unaffected siblings and in normal controls, and were also more frequent in the healthy siblings than in normal controls. Thus, the EEM test performances of the healthy siblings were intermediate between those of the probands with schizophrenia and those of normal controls.

Conclusion: Abnormalities of the EEM test parameters may be related to the genetic etiology of schizophrenia. The use of EEM parameters as an endophenotype for schizophrenia may facilitate linkage and association studies in schizophrenia.

Key words: etiology, exploratory eye movement, genetic factors, schizophrenia, siblings.

PREVIOUS FAMILY, ADOPTION and twin studies of schizophrenia have indicated that genetic components contribute significantly to the development of schizophrenic disorder. The mode of inheritance in schizophrenia, however, is complex. In addition, schizophrenia probably has etiologic heterogeneity, including locus heterogeneity, in genetic-associated cases of schizophrenia.[1–4] The conflicting results of recent linkage studies involving schizophrenia as the phenotype may be due to the complexity of genetic transmission.[5,6] Current findings of genetic studies in schizophrenia cannot completely account for the genetic factors of schizophrenia. One approach to resolving this issue is to search for a biological marker that fulfils the following criteria: (i) characteristic of schizophrenia; and (ii) related to the genetic predisposition to schizophrenia. Such an indicator may facilitate linkage analysis of schizophrenia.[7] Linkage analysis with such a biological marker of schizophrenia may lead to identification of chromosomal loci for susceptibility to schizophrenia.

Our group previously developed a method to study eye movements while subjects viewed geometric figures, called the exploratory eye movement (EEM) test.[8–10] We have obtained responsive search scores (RSS) for the EEM test. In previous studies we did not identify any patients with psychiatric diseases in

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whom the RSS was similar to that of schizophrenia patients. RSS abnormalities were found only in schizophrenia patients.[8–10] Moreover, we conducted a worldwide collaborative EEM study to analyze the stability of parameters of EEM. The EEM tests were performed at seven World Health Organization collaborative centers in six countries. The RSS of patients with schizophrenia were significantly lower than those of depressed patients or healthy controls in all centers.[10] Thus, we believe that RSS may be a candidate indicator of schizophrenia.

The aim of the present study was to investigate whether EEM abnormalities are related to genetic vulnerability to schizophrenia. For that purpose, this project was designed to compare EEM test data between schizophrenia probands, their healthy siblings, and normal controls. We investigated the possibility that the EEM test can assist with the clarification of genetic components in schizophrenia.

METHODS

Subjects

Twenty-three probands with schizophrenia, 23 of their healthy siblings (23 proband–sibling pairs), and 43 unrelated normal controls participated in this study. All probands met the DSM-IV criteria for schizophrenia. The schizophrenia probands (14 men and nine females) had a mean age of 29.3 ± 9.1 years; mean duration of illness was 5.2 ± 4.6 years; mean age at onset was 24.0 ± 5.8 years. All probands were receiving an average daily dosage of 9.3 ± 7.1 mg of a neuroleptic medication equivalent to haloperidol, and were also taking anti-cholinergic drugs. The probands were 10 inpatients and 13 outpatients at Nihon University Hospital in Tokyo or one of three affiliated hospitals (two in Tokyo; one in Chiba Prefecture close to Tokyo). The schizophrenia probands were subclassified into DSM-IV categories: disorganized type ($n = 3$), paranoid type ($n = 15$), residual type ($n = 2$), and undifferentiated type ($n = 3$). We performed the EEM test on the probands during a period when they were not suffering from acute symptoms. All probands in the present study cooperated with the tests and understood the investigator's instructions clearly.

The normal siblings (10 men and 13 women) had a mean age of 30.9 ± 12.3 years. The goal of this project was to research one non-psychotic sibling for each proband. Whenever possible, the healthy sibling chosen was of the same sex and nearest in age to the proband from each family. The unrelated normal controls (22 men and 21 women) had a mean age of 34.7 ± 12.2 years. The controls were selected from healthy volunteers among hospital staff, students from Nihon University, and members of Tokyo-based drug companies. The healthy siblings and normal controls had no specific history of mental illness according to DSM-IV criteria and had never received psychiatric medications. In addition, the normal controls had no history of psychotic illness in their first-degree family members.

The schizophrenia probands, their healthy siblings, and the normal controls were matched for age and sex. None of the probands, their healthy siblings, or the normal controls had evidence of substance or alcohol abuse or organic brain pathology. The diagnosis of the probands, their healthy siblings, and the normal controls was based on structured clinical interviews for DSM-IV. Each face-to-face interview was conducted by two experienced interviewers. After the nature of the study had been fully explained, written informed consent was obtained from the probands, their siblings, and the normal controls.

Exploratory eye movement

The EEM procedure followed that used by Kojima *et al.*[8] The subjects were asked to sit on a stool equipped with a nac VIII-type Eye Mark Recorder (nac, Tokyo, Japan), a device that detects corneal reflection of infrared light. Three repeats of an original horizontal S-shaped motifs (Fig. 1a,c,e) and two S-shaped motifs that differed slightly from the original one (Fig. 1b,d) were projected individually onto a screen positioned 1.5 m directly in front of the subject's eyes. The width of each of these projected geometric figures was 90 cm, and the height was 75 cm (angle of sight was 33° horizontally and 27.5° vertically). Figure 1 illustrates the sequence of events in the EEM test, which was done in the following steps. First, each subject was directed to view the motif carefully because he/she would be asked to draw it later. The subject was then shown the original S-shaped motif (original motif: OM, Fig. 1a) for 15 s. Immediately after viewing it, the subject was asked to draw the OM from memory. Second, the subject was instructed to compare the OM (Fig. 1a) with a subsequent motif and was then shown a slightly different motif with one bump in a different position (bump in different position motif: BDPM, Fig. 1b) for 15 s;

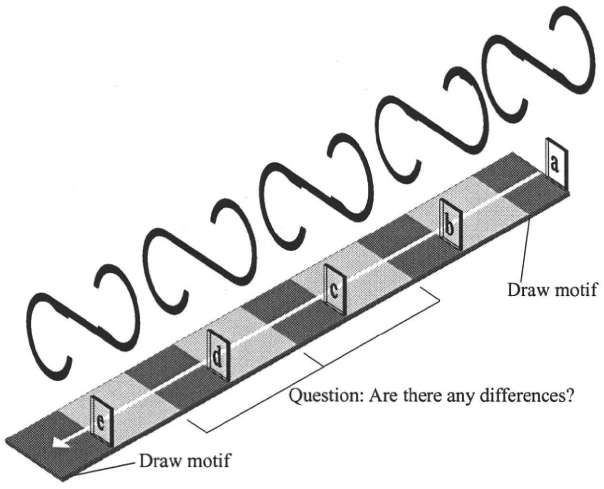

Figure 1. Sequence of events in the exploratory eye movement (EEM) test. (a,c,e) Repeats of the original motif; (b,d) slightly different motifs from the original one (motif b has one bump in a different position; motif d has no bumps). The EEM test proceeded from motif (a) to motif (e).

after 15 s had elapsed and with the BDPM still visible, the subject was asked whether it differed from the OM and if it did, how it differed; after the subject had replied and while the BDPM was still being shown, he/she was asked, 'Are there any other differences?' This question was repeated until the subject stated there were no further differences. Step 2 was repeated with the OM (Fig. 1c) and with a motif without bumps (no bump motif: NBM, Fig. 1d). Third, the subject was told to look at a projection of the OM (Fig. 1e) again for 15 s and to draw it again.

EEM tests during all steps were recorded on videotape with the eye mark recorder. These tapes were analyzed with a computerized system (eye movement analyzing software for Windows developed by our group). Eye fixations that focused on the same position for at least 200 ms were taken as real eye fixations. Movements of two degrees or more of sight were considered eye movements. In the present study we ascertained the following two measures: number of eye fixations in responsive search (NEFRS) and responsive search score (RSS). The actual NEFRS and RSS of a normal control subject are presented in Fig. 2.

Number of eye fixations in responsive search

The NEFRS is the number of eye fixations during the first 5 s immediately after the final question ('Are there any other differences?') when the subjects look at the BDPM (Fig. 1b) and the NBM (Fig. 1d). The NEFRS is the total number: BDPM result (Fig. 2a) + NBM result (Fig. 2b). In Fig. 2 the NEFRS of one control subject is shown: 30 (15 + 15).

Responsive search score

The BDPM and NBM were each divided into seven sections. Figure 2 shows the seven sections relevant to RSS scoring. The number of sections upon which the subject's eye fixed at least once was counted during

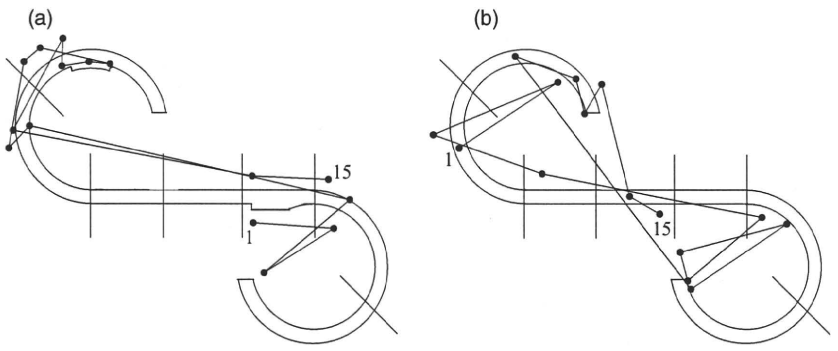

Figure 2. Number of eye fixations in responsive search (NEFRS) and the responsive search score (RSS) of a normal control subject. (a) and (b) are slightly different from the original motif (Fig. 1a). Figure 2(a) has a bump in a different position motif (BDPM). Figure 2(b) has no bump motif (NBM). The fixation points and movement sequences are represented by closed circle dots and lines. First and last fixation points are numbered 1 and 15 respectively. The NEFRS of this subject is 30 (15 + 15). These motifs are separated into seven sections for scoring of RSS. The RSS of this subject is 11 (5 + 6).

the first 5 s immediately after the final question while the subjects looked at the BDPM and the NBM. The RSS is the total score: BDPM result (Fig. 2a) + NBM result (Fig. 2b). As shown in Fig. 2, the RSS of one of the controls was 11 (5 + 6).

The NEFRS is a new parameter in the EEM test. The RSS has been developed by our group. The RSS is not raw eye movement data. Hence, it can be suggested that we are not able to obtain comprehensive information from the data of eye movements when we use only the RSS as the EEM parameter. For this reason, in the present study we added the NEFRS as a new item in the EEM test.

Statistical analysis

Based on the distribution of scores, the present data did not meet the criteria for normality. Therefore, comparisons of the three groups were performed using Wilcoxon matched-pair signed-ranks test for proband group versus sibling group pairwise comparisons of each EEM parameter, and the Mann-Whitney *U*-test for comparisons of proband group versus normal control group and sibling group versus normal control group according to previous studies.[11,12] An association between the two EEM test parameters was investigated using Spearman rank-order correlational test. Statistical significance was set at $P < 0.05$ (two-tailed). Statistical analysis was carried out with SPSS for Windows, version 14.0 (SPSS, Chicago, IL, USA).

RESULTS

Group comparisons (probands, siblings, controls) based on the EEM test parameters

For visualization of data, boxplots (sometimes called box-and-whiskers plots) of the NEFRS and RSS are presented in Fig. 3. The boxplot describes the distribution and dispersion of a variable, showing its median, quartiles and outliers. The box shows the quartiles; and a line in the box is the median. Whiskers at the ends of the box present the distance from the end of the box to the largest and smallest observed values that are <1.5 box lengths from either end of the box (SPSS manual). As shown in the boxplots, the NEFRS and RSS are lower in schizophrenia probands than in their unaffected siblings or in normal controls, and are also lower in healthy siblings than in normal controls. The scores of the

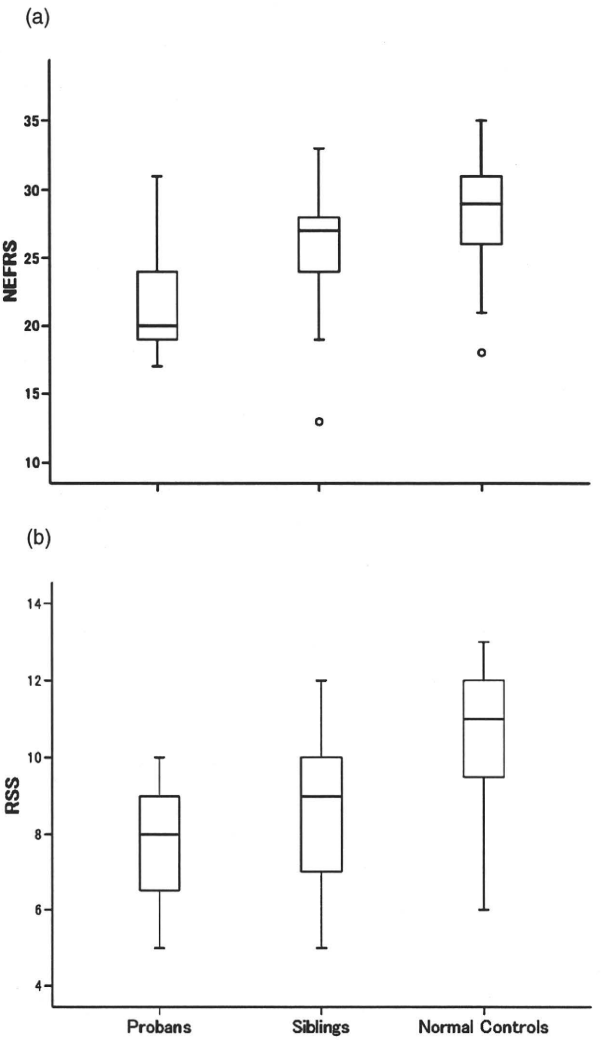

Figure 3. Boxplots of (a) number of eye fixations in responsive search (NEFRS) and (b) responsive search score (RSS). (○) Outliers in the boxplot of the NEFRS. Outliers are >1.5 box lengths from the end of the box (SPSS manual).

healthy siblings were intermediate between those of the probands with schizophrenia and those of normal controls.

Table 1 shows the results of EEM tests for the three groups. NEFRS was significantly lower in the schizophrenia probands than in their healthy siblings ($z = -3.09$, $P = 0.0020$) or in the unrelated normal controls ($z = -5.40$, $P < 0.0001$). Moreover, the NEFRS was significantly lower in the healthy siblings than in the normal controls ($z = -2.47$, $P = 0.0137$). The probands had significantly lower RSS than that of their siblings ($z = -2.38$, $P = 0.0173$) or that of the

Table 1. EEM test parameters (mean ± SD)

	Schizophrenia probands ($n = 23$)	Healthy siblings ($n = 23$)	Normal, unrelated controls ($n = 43$)	Probands vs controls		Probands vs siblings		Siblings vs controls	
				z	P	z	P	z	P
NEFRS	21.4 ± 3.8	25.8 ± 4.4	28.5 ± 3.6	−5.40	<0.0001	−3.09	0.0020	−2.47	0.0137
RSS	7.5 ± 1.7	8.9 ± 2.0	10.7 ± 1.7	−5.39	<0.0001	−2.38	0.0173	−3.44	0.0006

Probands vs controls, Mann–Whitney *U*-test probands vs siblings, Wilcoxon matched-pair signed-ranks test; siblings vs controls, Mann–Whitney *U*-test.
EEM, exploratory eye movement; NEFRS, number of eye fixations in responsive search; RSS, responsive search score.

normal controls ($z = -5.39$, $P < 0.0001$). In addition, the siblings had significantly lower RSS than that of the normal controls ($z = -3.44$, $P = 0.0006$). There were significant differences between the probands, their siblings, and the normal controls in the NEFRS and the RSS.

Relationship between the two parameters of the EEM test

Figure 4 illustrates the Spearman correlations between the NEFRS and the RSS. The NEFRS were significantly positively correlated with the RSS in all groups ($\rho = 0.53$, $n = 23$, $P = 0.0095$ in probands; $\rho = 0.62$, $n = 23$, $P = 0.0016$ in siblings; $\rho = 0.34$, $n = 43$, $P = 0.025$ in controls).

Relationship between NEFRS, RSS, and medication

Relationship between NEFRS, RSS, and the dosage of a haloperidol-equivalent neuroleptic medication were examined on Spearman rank-order correlational test to investigate medication effects. There were no significant correlations between NEFRS, RSS, and dosage (NEFRS, $\rho = -0.28$, $n = 19$, $P = 0.37$; RSS, $\rho = 0.06$, $n = 19$, $P = 0.80$).

DISCUSSION

The principal findings of the present study are that abnormalities of EEM test parameters are more frequent in schizophrenia probands than in their unaffected siblings or in normal controls, and are also more frequent in healthy siblings than in normal controls. The EEM test performances of the healthy siblings were intermediate between those of the probands with schizophrenia and those of the normal controls.

EEM studies of schizophrenia patients have indicated consistent disturbances. In our previous and present investigation we did not identify any normal individuals or patients with other psychiatric diseases in whom the RSS was similar to that of schizophrenia patients. Not only chronic and acute schizophrenia patients but also those in remission can be distinguished on RSS from patients with depression, neurosis, methamphetamine psychosis, temporal lobe epilepsy, and frontal lobe lesions, and from normal controls.[8–10,13,14] The present findings are consistent with those of previous studies in that we were able to replicate abnormalities in the EEM test in schizophrenia patients. Thus, we believe that the RSS in the EEM test may be specific to schizophrenia and may be a predictor for schizophrenia.

Because the NEFRS is a new parameter, there are no previous studies that have investigated differences of the NEFRS between schizophrenia patients, nonschizophrenic psychosis patients and normal controls. Thus, the present results do not prove that the NEFRS is specific to schizophrenia. In the present study, we did confirm that there is a significant difference between schizophrenia patients and normal controls. Further investigation is needed to examine the possible presence of NEFRS abnormalities in nonschizophrenic psychosis. If NEFRS is not specific to schizophrenia, it cannot be presumed to be an indicator of genetic vulnerability to schizophrenia. RSS, however, is scored from the NEFRS (Fig. 2), and there were significant correlations between NEFRS and RSS in all groups. The correlation coefficient of the control group was lower than that of the proband or sibling group, but there was a marginal correlation between the NEFRS and the RSS even though the correlation coefficient was low in the control group. Therefore, based on the evidence that the RSS may be specific to schizophrenia, it is possible that the NEFRS may also be one of the characteristics of schizophrenia.

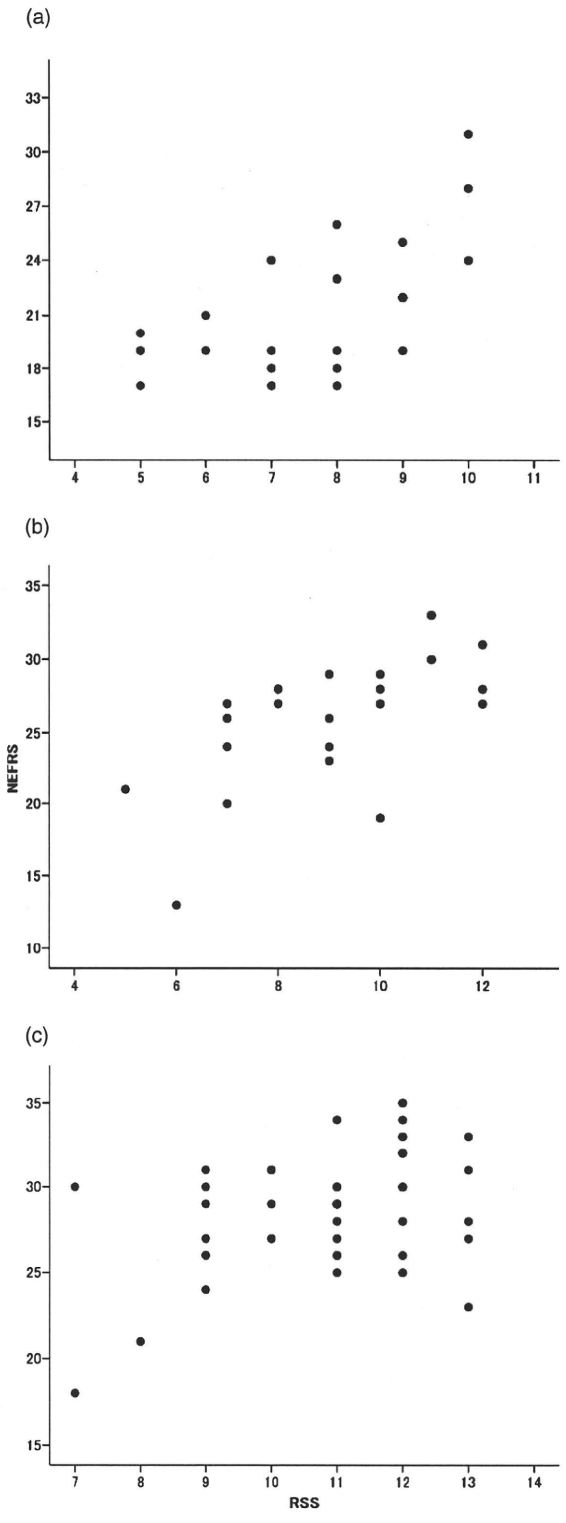

Figure 4. Correlation between the number of eye fixations in responsive search (NEFRS) and responsive search score (RSS) in (a) probands ($\rho = 0.53$, $n = 23$, $P = 0.0095$), (b) siblings ($\rho = 0.62$, $n = 23$, $P = 0.0016$) and (c) normal controls ($\rho = 0.34$, $n = 43$, $P = 0.025$).

From the fact that siblings share 50% of their genes on average, the present findings indicate that the NEFRS and the RSS may relate to genetic liability to schizophrenia. But siblings also share many environmental features with the schizophrenia probands. Therefore, it is possible that the NEFRS and the RSS may reflect environmental factors. From our previous data and the present study, however, we propose that each of the EEM test parameters may be a trait indicator.[8,9,13,15] It seems likely that genetic factors influence the NEFRS and the RSS more potently than do environmental factors.

According to these discussions, the NEFRS and the RSS may be an intermediate phenotype of schizophrenia, and may be useful for linkage studies of schizophrenia. We found a significant linkage to chromosome 22q11.2-q12.1 in our previous linkage study using the NEFRS as an endophenotype for schizophrenia.[16] Chromosome 22q11 is one of the most interesting regions for schizophrenia. Several studies have found that adults with 22q11 microdeletions have a high risk of schizophrenia, and suggested linkage between 22q11 and schizophrenia.[17,18] Moreover, there are several candidate genes for schizophrenia, for example *COMT*, *PRODH* and *ZDHHC8* and so on, in this area.[17,18] Therefore, based on the fact that the NEFRS is linked to 22q11, we also consider that the NEFRS may be characteristic of schizophrenia, and be related to genetic predisposition to schizophrenia.

In the light of abnormalities of brain function in schizophrenia, we investigated brain activation during a visual exploration task that was similar to the EEM task using functional magnetic resonance imaging (fMRI) in schizophrenia patients and normal controls. The normal control subjects had activations at the bilateral thalamus and the left anterior medial frontal cortex. In contrast, the schizophrenia subjects had activations at the right anterior cingulate gyrus, but no activations at the thalamus and the left anterior medial frontal cortex.[19] These findings indicate that the RSS abnormality of schizophrenia may be associated with dysfunctions of the thalamus, frontal cortex or cingulate gyrus.

In conclusion, we suggest that the present EEM test parameters may be markers of genetic predisposition to schizophrenia. In the future, the EEM test may facilitate advances in linkage and association studies of schizophrenia. Mapping EEM abnormalities to a specific chromosome, and finding an association between EEM deficits and a candidate gene for

schizophrenia may yield further knowledge concerning genetic influences on schizophrenia.

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Low serum levels of brain-derived neurotrophic factor and epidermal growth factor in patients with chronic schizophrenia

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Abstract

Neurotrophic factors (NFs) play a pivotal role in the development of the central nervous system. They are thus also suspected of being involved in the etiology of schizophrenia. Previous studies reported a decreased level of serum brain-derived neurotrophic factor (BDNF) in schizophrenia, whereas the association of epidermal growth factor (EGF) with this illness remains controversial. Using a two-site enzyme immunoassay, we conducted the simultaneous measurement of serum BDNF and EGF levels in a group of patients with chronic schizophrenia ($N=74$) and a group of normal controls matched in age, body mass index, smoking habit and sex ($N=87$). We found that, compared to normal controls, patients with chronic schizophrenia exhibited lower serum levels of both BDNF and EGF across all ages examined (21–59 years). The serum levels of BDNF and EGF were negatively correlated in the controls ($r=-0.387$, $P=0.0002$) but not in the patients. Clinical parameters such as duration of illness and psychiatric rating scale also showed no robust correlations with the NF levels. Collectively, these results suggest that pervasive, abnormal signaling of NFs underlies the pathophysiology of chronic schizophrenia.

Keywords: Brain-derived neurotrophic factor; Epidermal growth factor; Neurotrophic factor; Schizophrenia

1. Introduction

Accumulating evidence from previous pharmacological, neuroimaging, genetic and postmortem studies has suggested that the etiology of schizophrenia should be viewed as a combination of genetic background and environmental factors, resulting in maldevelopment of the central nervous system and impaired neurotransmissions (Lewis and Gonzalez-Burgos, 2006; Nawa et al., 2000; Nawa and Takei, 2006; Rapoport et al., 2005; Ross et al., 2006; Stephan et al., 2006).

Neurotrophic factors (NFs) play a pivotal role in the survival, growth and differentiation of distinct populations of neurons. Among NFs, brain-derived neurotrophic factor (BDNF) is synthesized predominantly in

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neurons and is widely distributed in the brain, the highest expression having been identified in the hippocampus and cerebral cortex (Ernfors et al., 1990; Hofer et al., 1990; Wetmore et al., 1990). It has been suggested that BDNF possesses a potential role in promoting the function and survival of cholinergic, dopaminergic, serotonergic and GABAergic neurons (Connor and Dragunow, 1998). Another NF, epidermal growth factor (EGF), also serves as a neurotrophic molecule to stimulate the proliferation, migration and differentiation of neuronal cells, and influences synaptic plasticity, including hippocampal long-term potentiation (Ishiyama et al., 1991; Xian and Zhou, 1999). EGF has been suggested to be involved especially in the growth and survival of midbrain dopaminergic neurons (Alexi and Hefti, 1993; Casper et al., 1991; Casper and Blum, 1995; Ventrella, 1993). Thus, dysfunction in the BDNF and/or EGF systems may contribute to impairment in brain development, neuroplasticity and synaptic connectivity, leading eventually to the manifestation of schizophrenic syndrome. In fact, genetic manipulation of BDNF or neonatal perturbation of EGF signaling in mice has been reported to cause behavioral abnormalities often observed in psychiatric disorders (Chen et al., 2006; Futamura et al., 2003; Mizuno et al., 2004).

Previous studies have reported alterations of BDNF and EGF levels in several brain regions as well as in serum of patients with schizophrenia, although the reported changes varied among the studies (Tables 1 and 2). Postmortem studies have shown elevated BDNF levels in

Table 1
Previous studies on BDNF levels of patients with schizophrenia

Authors (Year)	Origin of sample	Controls		Patients			Remarks
		Number	Concentration*	Number	Concentration*	Level**	
Takahashi et al. (2000)	Postmortem Brain	22	100***	14	170***	↑	In anterior cingulate
		13	100***	13	230***	↑	In hippocampus
Durany et al. (2001)	Postmortem brain	11	1.68±0.21	11	2.70±0.40	↑	In frontal cortex
			1.59±0.22		2.93±0.53	↑	In parietal cortex
			1.39±0.18		2.80±0.40	↑	In temporal cortex
			1.34±0.16		2.91±0.60	↑	In occipital cortex
			4.84±0.61		2.70±0.42	↓	In hippocampus
Weickert et al. (2003)	Postmortem brain	19	100***	12	60***	↓	In prefrontal cortex
Toyooka et al. (2002)	Serum	35	11.4±7.7	34	6.3±3.4	↓	Number of platelets was decreased
Pirildar et al. (2004)	Serum	22	26.8±9.3	22	14.19±8.12 (pretreatment)	↓	
					14.53±2.93 (posttreatment)	↓	
Tan et al. (2005)	Serum	45	9.9±4.3	81	7.3±2.6	↓	Correlation with PANSS negative ($r=-0.307$, $P=0.005$)
Zhang et al. (2007)	Serum	37 (male)	9.7±4.5	91 (male)	7.1±2.2	↓	Correlation with BMI gain in females ($r=-0.453$, $P=0.008$)
		13 (female)	9.0±4.4	33 (female)	5.9±2.3	↓	
Grillo et al. (2007)	Serum	25	0.17±0.03	24 (typicals)	0.10±0.05	↓	Correlation with clozapine dose ($r=0.643$, $P=0.002$)
				20 (clozapine)	0.13±0.04	↓	No correlation with age at onset and duration of illness
Shimizu et al. (2003)	Serum	40	28.5±9.1	25 (medicated)	27.9±12.3	n.s.	No correlation with age at onset and duration of illness
				15 (drug-naïve)	23.8±8.1		
Huang and Lee (2006)	Serum	96	14.17±6.86	126	14.20±6.92	n.s.	Catatonia group ($N=7$) showed decreased BDNF levels No correlation with age at onset
Present Study	Serum	87	52.2±25.3	74	37.1±20.4	↓	

*Data indicate mean±SD of brain (ng/ml protein) and serum (ng/ml). **As compared with BDNF levels of normal controls. *** % control.
BDNF, Brain-Derived Neurotrophic Factor; PANSS, Positive and Negative Syndrome Scale; BMI, Body Mass Index; n.s., not significant.

Table 2
Previous studies on EGF levels of patients with schizophrenia

Authors (Year)	Origin of sample	Controls		Patients			Remarks
		Number	Concentration*	Number	Concentration*	Level**	
Futamura et al. (2002)	Postmortem brain	12	6.3±2.0	14	4.8±2.0	↓	In prefrontal cortex
		16	3.8±1.5	14	2.0±0.9	↓	In striatum
	Serum	45	392±344	45 (medicated)	125±80.8	↓	
		14	554±350	6 (drug-free)	167±100	↓	
Hashimoto et al. (2005)	Serum	40	411±217	25 (medicated)	481±241	n.s.	Correlation with BPRS (r=0.434, P=0.005)
				15 (drug-naïve)	331±226		
Present Study	Serum	87	560.7±357.1	74	395.5±231.7	↓	

*Data indicate mean±SD of brain (pg/ml protein) and serum (pg/ml). **As compared with EGF levels of normal controls.
EGF, Epidermal Growth Factor; BPRS, Brief Psychiatric Rating Scale; n.s., not significant.

the anterior cingulate, hippocampus (Takahashi et al., 2000) and cerebral cortex (Durany et al., 2001), whereas decreases in BDNF levels in the hippocampus (Durany et al., 2001) and prefrontal cortex (Weickert et al., 2003) have also been reported. In the serum of treated patients, BDNF levels have been found to be decreased (Grillo et al., 2007; Pirildar et al., 2004; Tan et al., 2005; Toyooka et al., 2002; Zhang et al., 2007). Yet, other studies have shown that the serum BDNF level in patients was not significantly different from that in normal controls (Huang and Lee, 2006; Shimizu et al., 2003). As for EGF, its protein levels were found to be decreased in the prefrontal cortex and striatum of postmortem schizophrenic brains (Futamura et al., 2002). The serum EGF level was markedly reduced in patients with schizophrenia in one report (Futamura et al., 2002), whereas in another report, there was no difference between patients and normal controls (Hashimoto et al., 2005). Taking these conflicting results together, it is clear that the issue of NF levels in patients with schizophrenia requires further study.

Compared to postmortem studies, measurement of serum NFs has the obvious clinical advantage of being available from blood samples that can be drawn from living subjects as frequently as necessary. BDNF is produced in various peripheral tissues, such as retina, muscle and platelets (Radka et al., 1996), in addition to the central nervous system as described above. EGF is excreted by the pituitary gland and peripheral tissues including salivary and Brunner's gland of the gastrointestinal system (Plata-Salamán, 1991). Thus, the origins of BDNF and EGF in serum are not yet completely understood. Importantly, however, serum BDNF levels reportedly correlate with BDNF concentrations in the central nervous system (Karege et al., 2002). It has also been reported that the expression of EGF is impaired in both central and peripheral organs of patients (Futamura et al., 2002). Therefore, the serum levels of both NFs might reflect the pathophysiology and possibly the clinical outcome of schizophrenia.

In the present study, we measured the serum levels of both BDNF and EGF simultaneously in individual subjects by using a two-site enzyme immunoassay, and we examined their association with the clinical parameters of patients with schizophrenia.

2. Methods and materials

2.1. Subjects

Two groups of subjects, 74 patients with schizophrenia and 87 control subjects, participated in this study. The patients were recruited from inpatients and outpatients of Asai Hospital. Diagnoses were made by I.I., Y.O., and the attending psychiatrists on the basis of a review of their charts and a conventionally semi-structured interview. All patients also met the DSM-IV criteria for schizophrenia. Their symptoms were evaluated by Global Assessment of Functioning (GAF) and Brief Psychiatric Rating Scale (BPRS). All patients had been receiving antipsychotic drugs. Mean antipsychotic dose was 936.6±588.8 mg/day in chlorpromazine equivalents. Antipsychotic drugs administered to patients were risperidone (N=31), olanzapine (N=23), quetiapine (N=16), levomepromazine (N=15), chlorpromazine (N=14), haloperidol (N=13), zotepine (N=10), perospirone (N=7), sulpiride (N=6), sultopride (N=4), bromperidol, propericyazine (N=3 each), fluphenazine (N=2), nemonapride, perphenazine, timiperone (N=1 each). Of the patients, 23 were receiving monotherapy.

Healthy normal control subjects with no history of psychiatric disorders were recruited from the local community. There was no significant difference in age (P=0.160), body mass index (BMI) (P=0.920), sex ratio (P=0.867) and smoking habit (P=0.955) between

the two groups. Their detailed demographic data are summarized in Table 3. The present study was approved by the ethics committees of all participating institutes. After complete explanation of the study, written informed consent was obtained from all subjects.

2.2. Two-site enzyme immunoassay for BDNF and EGF

The concentrations of BDNF and EGF proteins were measured by two-site enzyme immunoassay (Futamura et al., 2002; Nagano and Suzuki, 2003). Blood samples were obtained between 10:00 and 16:00 at Asai Hospital. Samples were collected into tubes without anticoagulant and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 7 min and then stored at −80 °C until use. EIA titer plates (FluoroNunc Module, Nunc A/S, Roskilde, Denmark) were coated with primary polyclonal antibodies against BDNF (Promega, Madison, WI) or EGF (Oncogene, San Diego, CA) overnight and then blocked with EIA buffer (50 mM Tris [pH 7.5], 0.5 M NaCl, 0.3% Triton X-100, 0.4% gelatin and 0.4% bovine albumin) at 4 °C for more than 3 h. One hundred microliters of diluted serum (in duplicate) or each NF standard (1–1000 pg; in triplicate) for BDNF (Chemicon, Temecula, CA) or EGF (PeproTech, London, UK) in EIA buffer was placed into each well, and the plates were then incubated at room temperature for 7 h. After three washes with Wash-buffer (EIA buffer without bovine serum albumin), 100 μl of biotinylated antibody against human BDNF (Genzyme-Techne, Minneapolis, MN) or human EGF (R&D, Minneapolis, MN) in EIA buffer was added to the wells, and the plates were incubated for 12–18 h at room temperature. The biotinylated secondary antibody bound to BDNF or EGF was detected by incubation with streptavidin-β-galactosidase (Roche Diagnostics, Mannheim, Germany) at room temperature for 3 h. Unbound enzyme was removed by extensive washes with Wash-buffer followed by phosphate-buffered saline free of calcium and magnesium. Then, β-galactosidase activity in each well was measured by incubation with a substrate, 200 μM 4-methylumbelliferyl β-D-galactoside (Sigma, St. Louis, MO) in 50 mM sodium phosphate (pH 7.3) and 10 mM $MgCl_2$. The reaction proceeded in a dark at room temperature for 3 h, and the amount of fluorescent products was monitored by Spectraflour Plus microplate reader (Tecan, Männedorf, Switzerland) with excitation and emission wavelengths of 360 nm and 465 nm, respectively. A standard curve was obtained for each assay in a range of 1–1000 pg of recombinant BDNF or EGF. Serum NFs were measured simultaneously, as far as possible, with several standard samples to minimize inter-assay difference. The intra-assay coefficient of variation was less than 3%. There was no significant cross-reactivity among other neurotrophic factors for BDNF (Nagano and Suzuki, 2003) and the EGF family members of EGF (data not shown). The assays were all performed in a blinded fashion.

Table 3
Demographic data of patients with schizophrenia and normal controls

		Schizophrenia (N=74)	Control (N=87)
Gender (M/F)		39/35	47/40
Age		41.9±11.1	39.8±10.7
BMI (kg/m^2)*		23.6±4.7	23.1±2.1
Atopic dermatitis (presence/absence)		1/22	3/31
Smoking habit (presence/absence)		11/12	16/18
Age at onset		22.2±6.9	
Duration of illness (years)		19.6±11.2	
Number of hospitalizations		4.4±3.6	
Total duration of hospitalization (years)		8.8±9.5	
Chlorpromazine equivalents (mg/day)		936.6±588.8	
GAF**		39.7±10.9	
BPRS**	Total	43.8±15.5	
	Positive	11.0±4.6	
	Negative	9.8±4.6	

BMI, Body Mass Index; GAF, Global Assessment of Functioning; BPRS, Brief Psychiatric Rating Scale. All data were reported as mean±SD. *, N=44 for schizophrenia and N=34 for control. **, N=33.

2.3. Statistical analysis

NF levels and demographic data of the subjects were reported as mean±SD. The Mann–Whitney U test was employed for group comparisons. Linear relationship between two variables was examined by Spearman rank correlation coefficients. Pearson chi-square test was used for comparing sex ratio and smoking habit between the controls and patients, and between low and high-BDNF groups in the controls. $P<0.05$ was considered statistically significant.

3. Results

3.1. Serum BDNF and EGF levels

Both serum BDNF and EGF levels in schizophrenia patients and normal controls were measured by two-site enzyme immunoassay. The mean serum BDNF level

of patients was significantly lower than that of controls (37.1±20.4 and 52.2±25.3 ng/ml in patients and controls, respectively; $P=0.00003$; Fig. 1A). The mean serum EGF level was also significantly lower in patients than in controls (395.5±231.7 vs. 560.7±357.1 pg/ml; $P=0.002$; Fig. 1B).

The relation between serum NF levels and age was examined. The age of both patient and control groups ranged from 21 to 59 years. As shown in Fig. 1C (BDNF), Fig. 1D (EGF) and Table 4, there were no significant correlations between serum NF levels and age in either group.

Because both BDNF and EGF were measured simultaneously within the same individuals, the correlation between serum BDNF and EGF was examined in each group. In the controls, a negative correlation between BDNF and EGF levels was found ($r=-0.387$, $P=0.0002$; Fig. 2A). In contrast, there was no significant correlation between the serum BDNF and EGF levels in the patients ($P=0.161$, Fig. 2B).

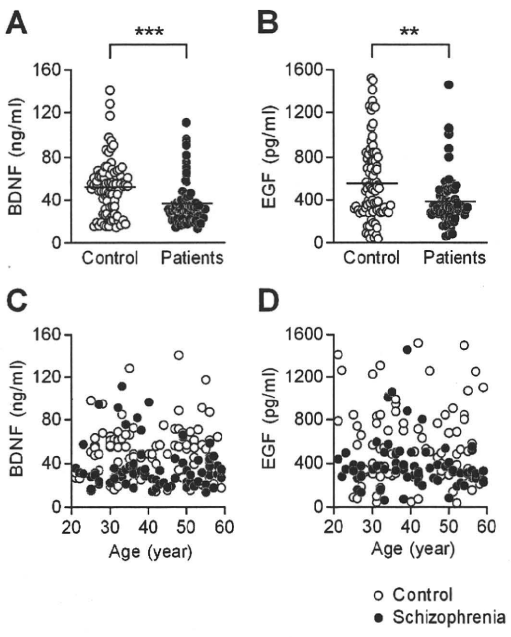

Fig. 1. Serum levels of (A) BDNF and (B) EGF measured by two-site enzyme immunoassay in normal controls ($N=87$) and patients with chronic schizophrenia ($N=74$). Compared with controls, patients exhibited lower serum levels of both neurotrophic factors (BDNF, ***$P<0.001$; EGF, **$P<0.01$). Horizontal lines indicate the mean levels. Distributions of serum (C) BDNF and (D) EGF levels in controls (open circles) and patients (filled circles) with age. No significant correlation was observed between NF levels and age (21–59 years) in the two groups. BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor.

Table 4
Correlations between levels of neurotrophic factors and clinical parameters in patients with schizophrenia

Clinical parameters		N	BDNF		EGF	
			r	P	r	P
Age		74	−0.031	0.795	−0.227	0.053
Age at onset		74	0.303	0.009	0.052	0.644
Duration of illness		74	−0.196	0.098	−0.281	0.016
CPZ-EQ (mg/day)		74	0.051	0.520	0.079	0.327
BMI (kg/m^2)		44	0.171	0.267	−0.088	0.569
GAF		33	0.024	0.843	−0.076	0.727
BPRS	Total	33	−0.099	0.588	0.349	0.046
	Positive	33	−0.189	0.303	0.347	0.047
	Negative	33	0.102	0.558	0.127	0.468

CPZ-EQ, Chlorpromazine Equivalents; BMI, Body Mass Index; GAF, Global Assessment of Functioning; BPRS, Brief Psychiatric Rating Scale.

Since the distribution of BDNF in the control group appeared bimodal as shown in Fig. 2A, we examined whether the low-BDNF group (40 ng/ml of BDNF as a tentative threshold for the dichotomy; $N=26$) and high-BDNF group ($N=61$) differed in their biological parameters. Statistical analyses revealed that there were no significant differences in their BMI ($P=0.627$), age ($P=0.959$), sex ratio ($P=0.654$), and smoking habit ($P=0.464$).

3.2. Correlation of serum BDNF and EGF levels with clinical parameters

Overall, clinical parameters did not exhibit robust correlations with the BDNF and EGF levels ($P>0.05/10$ [$=0.005$], corrected for multiple comparisons in Table 4 and Fig. 2B), although age at onset was marginally correlated with the BDNF level ($r=0.303$, $P=0.009$). We also analyzed the effects of BMI and smoking habit on NF levels. There were no significant correlations between serum NF levels and BMI in patients ($P=0.267$ for BDNF, $P=0.569$ for EGF, $N=44$) or in controls ($P=0.687$ for BDNF, $P=0.697$ for EGF, $N=34$). In addition, NF levels were not significantly different between the presence ($N=11$ for patients, $N=16$ for controls) and absence ($N=12$ for patients, $N=18$ for controls) of smoking habit in patients ($P=0.735$ for BDNF, $P=0.132$ for EGF) and in controls ($P=0.569$ for BDNF, $P=0.593$ for EGF).

3.3. Type of antipsychotic drugs and neurotrophic factor levels

Thirteen patients had been taking one or more typical antipsychotic drugs, while thirty-one other patients had been taking only atypical antipsychotic drugs. We found

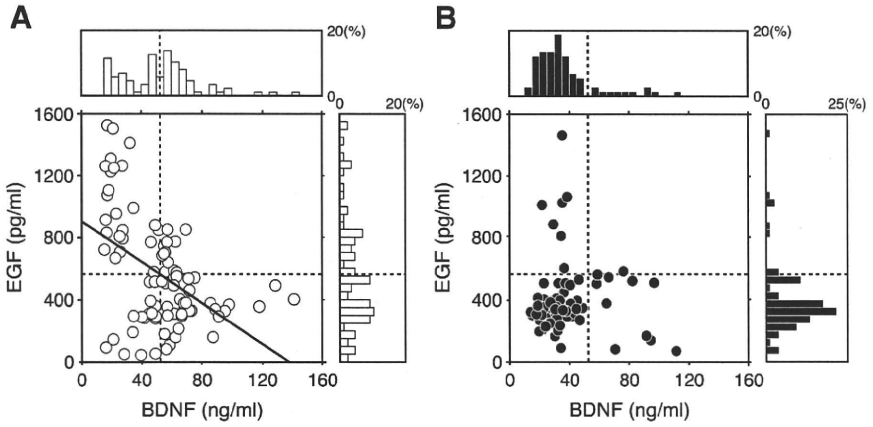

Fig. 2. Relation between the serum levels of BDNF and EGF measured simultaneously in (A) normal controls and (B) chronic schizophrenia patients. For controls, serum levels of the two neurotrophic factors were negatively correlated as shown by the line ($r=-0.387$, $P=0.0002$). The histograms above and on the right of the main plots show the fractions of subjects that fall into particular intervals of serum BDNF (in steps of 5 ng/ml) and EGF (in steps of 50 pg/ml) levels, respectively. In both histograms, dotted lines represent the mean levels of BDNF (52.2 ng/ml) and EGF (560.7 pg/ml) of normal controls, respectively. BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor.

that the levels of both BDNF and EGF did not differ between the patients taking typical and atypical antipsychotic drugs ($P>0.05$, Fig. 3A and B). In addition, there was no significant correlation between the chlorpromazine equivalents of medication and serum NF levels (Fig. 3C and D; Table 4).

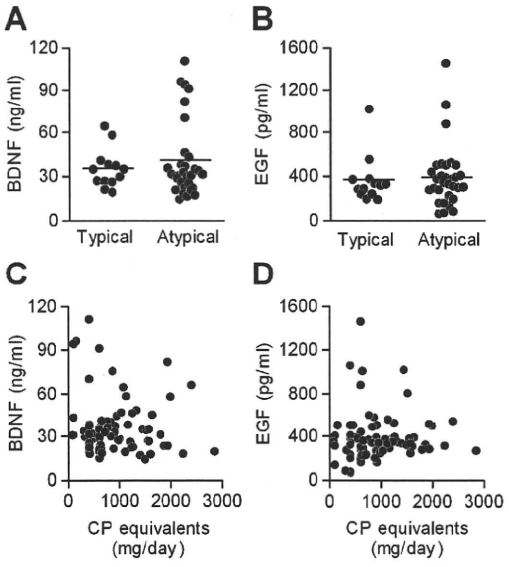

Fig. 3. Effects of antipsychotic drugs on serum (A) BDNF and (B) EGF levels. For both neurotrophic factors, no significant differences were seen between patients taking typical ($N=13$) and atypical ($N=31$) antipsychotic drugs. Horizontal lines indicate the mean levels. Antipsychotic dosages in chlorpromazine equivalents were correlated neither (C) with serum BDNF nor (D) with EGF levels ($N=74$). BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; CP, chlorpromazine.

We also analyzed the effects of anticholinergic drugs on the NF levels. Thirty-five patients had been taking anticholinergic drugs including biperiden and trihexyphenidyl in combination with antipsychotic drugs. NF levels were not significantly different between the patients with (BDNF, 37.9±20.1 ng/ml; EGF, 395.8±225.0 pg/ml; $N=35$) and without (BDNF, 36.3±20.9 ng/ml; EGF, 395.3±240.5 pg/ml; $N=39$) anticholinergic drugs ($P=0.626$ for BDNF, $P=0.475$ for EGF).

4. Discussion

4.1. Lower serum BDNF and EGF levels in schizophrenia

As summarized in Tables 1 and 2, previous studies have mostly reported low serum BDNF levels (Grillo et al., 2007; Pirildar et al., 2004; Tan et al., 2005; Toyooka et al., 2002; Zhang et al., 2007), while changes in the serum EGF level have remained a matter of controversy (Futamura et al., 2002; Hashimoto et al., 2005). In the present study, at least, it was clearly shown that most of the chronic schizophrenia patients had lower serum levels of EGF as well as BDNF. Mean serum BDNF values were 37.1 and 52.2 ng/ml in patients and controls, respectively, in the present study. These values were higher than those in several other reports, but, as can be seen in Table 1, BDNF levels varied considerably among the studies reported. Such differences may be due to the antibodies used against neurotrophic factors, the methods of measurement, and the sampling conditions. Actually, the

values in the present study fell into a range similar of values to those in the reports adopting similar methods (Toyooka et al., 2002). In addition, this decrease in NFs was observed in patients regardless of age, ranging from the early 20s to the late 50s. This observation was consistent with previous reports showing no correlation between age and serum BDNF levels (Grillo et al., 2007; Huang and Lee, 2006; Toyooka et al., 2002), lending credence to the hypothesis that schizophrenia is the behavioral outcome of aberration in the neurodevelopmental processes.

In the present work, the simultaneous measurement of NFs revealed a significant negative correlation between serum BDNF and EGF levels in controls (Fig. 2A), whereas there was no correlation between the two NF levels in patients (Fig. 2B), possibly reflecting their low levels of both BDNF and EGF. The fact that no control subjects showed high serum levels of both BDNF and EGF is of particular interest. Neurite outgrowth from EGF-responsive stem cell-derived neurons can be enhanced by treatment with BDNF (Shetty and Turner, 1999), while BDNF reportedly induced the down-regulation of EGF receptors (Huang et al., 1988). In addition, the co-application of transforming growth factor-alpha, a member of the EGF family, with BDNF blocked the BDNF-triggered up-regulation of AMPA receptor expression and currents (Namba et al., 2006). Thus, complementary roles of both factors may underlie the normal development of the nervous system. In other words, chronic schizophrenia may represent a state deficient in NF-regulated neural functions, leading eventually to various mental malfunctions.

The origins of serum BDNF and EGF are not yet completely understood. EGF reportedly enters the brain through the blood-brain barrier (BBB) in mouse (Pan and Kastin, 1999). BDNF is reported to be transported across the BBB in normal mouse (Pan et al., 1998) and rats with cerebral ischemia (Schäbitz et al., 2000), while another report has argued that the transport of BDNF is negligible (Sakane and Pardridge, 1997). EGF and BDNF are produced in various peripheral tissues (Plata-Salamán, 1991; Radka et al., 1996), in addition to the central nervous system as described above. Nevertheless, the serum levels of NFs can be used as clinical markers, since they show different distributions between patients and controls, as shown in previous studies as well as in the present study.

4.2. Clinical parameters and neurotrophic factors

We failed to find any clinical parameters that demonstrated robust correlation with the two NF levels. As shown in Tables 1 and 2, previous reports also examined the correlation between clinical parameters and NF levels: the BDNF level was correlated with the negative symptom subscore of the Positive and Negative Syndrome Scale (Tan et al., 2005); the serum EGF level was significantly correlated with the BPRS score (Hashimoto et al., 2005). Although the reasons for the discrepancy between the previous and present results are unclear, differences in demographic characteristics of the patients (such as age at onset, illness duration, sample size, distribution of BPRS score, and dosage of antipsychotic drugs) might provide at least a partial explanation.

Other factors than psychiatric parameters have been reported to affect serum BDNF levels. BMI (Suwa et al., 2006) and age (Ziegenhorn et al., 2007) showed positive and negative correlation with BDNF levels, respectively. Patients with atopic dermatitis have higher levels of serum BDNF in association with the severity of symptoms (Raap et al., 2005; Namura et al., 2007), while smokers have lower values as compared with non-smokers (Kim et al., 2007). We could not completely rule out the possibility that these factors affected the values in the present study, since data could not be obtained from all participants. However, the limited data suggested that neither BMI nor smoking habit affected neurotrophic levels in patients or controls.

4.3. Types of antipsychotic drugs and serum neurotrophic factor levels

In the present study, the NF levels were not correlated with any types or dosages of medications. Although Grillo et al. (2007) found a significant correlation between the BDNF level and clozapine dosage, other investigators found no significant correlation between BDNF (Hori et al., 2007; Shimizu et al., 2003; Tan et al., 2005; Toyooka et al., 2002; Zhang et al., 2007) or EGF level (Futamura et al., 2002) and antipsychotic dosages. In addition, treatment with olanzapine for 8 weeks (Hori et al., 2007) or antipsychotic drugs (risperidone for most patients) for 6 weeks (Pirildar et al., 2004) did not alter BDNF levels in blood. It was recently suggested that the effects of atypical and typical antipsychotic drugs on the BDNF level were different. In animal experiments, haloperidol, a typical antipsychotic drug, decreased the BDNF expression in the hippocampus, whereas atypical antipsychotics did not affect or even up-regulated this expression (Bai et al., 2003; Chlan-Fourney et al., 2002; Parikh et al., 2004). In addition, atypical antipsychotics, but not haloperidol, stimulated neurogenesis in the subventricular zone of the rat brain (Wakade et al., 2002). Clinically, chronic treatment with haloperidol, but not olanzapine, was associated with a significant reduction in gray matter volume in schizophrenia patients with first-

episode psychosis (Lieberman et al., 2005). However, the present study failed to show that the type of drug affects either the BDNF or the EGF serum level. This observation might indicate a limitation concerning the measurement of serum NFs for predicting their function in the brain. Nevertheless, the serum levels of NFs could be used as clinical markers from the viewpoint that they are independent of the type of medication used.

In conclusion, we showed herein that patients with chronic schizophrenia have lower serum levels of both BDNF and EGF across all ages, possibly reflecting pervasive abnormal signaling of NFs underlying the pathophysiology of schizophrenia. A future study should investigate NFs of patients with schizophrenia before pharmacological intervention or those undergoing the first-episode of the disease, thereby addressing whether this overall reduction in NFs is a common characteristic in the symptomatology of schizophrenia.

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Contributors

Y.I. measured the concentrations of BDNF and EGF proteins, analyzed the data and wrote the manuscript. N.Y. undertook the statistical analyses of whole data including neurotrophic factor levels and demographical data, and wrote the manuscript. M.N. developed the two-site enzyme immunoassay for BDNF and EGF and measured the concentrations of BDNF and EGF proteins. I.I, T.T and T.Y recruited the subjects for this project and collected blood samples. Y.O and H.S designed and supervised the whole study and wrote the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Gaze-triggered orienting is reduced in chronic schizophrenia

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Abstract

Patients with schizophrenia have been reported to demonstrate subtle impairment in gaze processing, which in some cases indicates hypersensitivity to gaze, while in others, hyposensitivity. The neural correlate of gaze processing is situated in the superior temporal sulcus (STS), a major portion of which is constituted by the superior temporal gyrus (STG), and may be the underlying dysfunctional neural basis to the abnormal gaze sensitivity in schizophrenia. To identify the characteristics of gaze behavior in patients with chronic schizophrenia, in whom the STG has been reported to be smaller in volume, we tested 22 patients (mean duration of illness 29 years) in a spatial cueing paradigm using two central pictorial gaze cues, both of which effectively triggered attentional orienting in 22 age-matched normal controls. Arrow cues were also employed to determine whether any compromise in schizophrenia, if present, was gaze-specific. Results demonstrated that schizophrenic subjects benefit significantly less from congruent cues than normal subjects, which was evident for gaze cues but not for arrow cues. This finding is suggestive of a relatively gaze-specific hyposensitivity in patients with chronic schizophrenia, a finding that is in line with their clinical symptomatology and that may be associated with a hypoactive STS.

Keywords: Ambiguous stimulus; Arrow; Biological motion; Spatial cueing; Superior temporal gyrus; Superior temporal sulcus

1. Introduction

Schizophrenia is a neuropsychiatric disorder that can be disabling due to a variety of socio-cognitive impairments. One of its most intriguing symptoms is an abnormal sensitivity to gaze. In a typical course of schizophrenia, the acutely ill patient often expresses complaints of 'always being watched', reflecting heightened sensitivity to gaze. As the course becomes chronic, however, the patient tends to be more and more withdrawn, and hyposensitivity to gaze takes place. This is often observed through the patient's gaze behavior; he/she becomes very reluctant to engage in mutual eye contact. Some previous studies have highlighted this hyper/hyposensitivity to gaze. For example, schizophrenic subjects have been demonstrated to be impaired in the discrimination of whether gaze is looking at self or not (Rosse et al., 1994; Hooker and Park, 2005) in the face of an intact right/left discrimination (Franck et al., 1998); to have reduced fixation on prominent facial features such as the eyes when viewing faces (Phillips and David, 1997); and to show very early attentional orienting in response to

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gaze and head direction (Langdon et al., 2006). The neural correlate of such gaze processing is located in the superior temporal sulcus (STS) region, through animal studies (Campbell et al., 1990; Perrett et al., 1992), human activation studies (Puce et al., 1998; Wicker et al., 1998; Hoffman and Haxby, 2000; Hooker et al., 2003; Pelphrey et al., 2003; Kingstone et al., 2004), and a recent neuropsychological case (Akiyama et al., 2006a). Here, gaze cognition is considered one component in a wider range of biological motion understanding, which is essential in social interaction (Allison et al., 2000). It coincides that the schizophrenic brain has been reported repeatedly to show significantly smaller superior temporal gyrus (STG) volume (Rajarethinam et al., 2000, 2004; Onitsuka et al., 2004), which constitutes the upper bank of the STS. There is a possibility that the hyper/hyposensitivity to gaze in schizophrenic subjects might actually have a brain-based origin, for example, in a dysfunctional STS.

One way of testing behavior toward gaze is a gaze-cued target detection test. Friesen and Kingstone (1998) have applied Posner's spatial cueing paradigm (1980), using centrally presented pictorial gaze direction as a cue in detecting peripheral targets. Normal subjects demonstrated significantly faster target detection when cued by gaze direction congruent to the target location, compared with incongruently cued targets, in a non-predictive condition. Subsequently, a number of studies have confirmed the nature of gaze to strongly orient the viewer's attention in its direction (Driver et al., 1999; Zorzi et al., 2003). The congenital, or peri-natal patient group of autism, in which the STG has also been reported to be dysfunctional (Ohnishi et al., 2000; Zilbovicius et al., 2000; Boddaert et al., 2004; Pelphrey et al., 2005), and whose cardinal symptom is a deficit in reciprocal gaze interaction, has been studied recently with this spatial cueing paradigm. This patient group has demonstrated an absence of gaze-triggered orienting in a non-predictive condition (Ristic et al., 2005). Schizophrenia also has some common fundamental features, and as is the case for autism, its pathogenesis is far from elucidated. Investigation of the performance of schizophrenic subjects in such a paradigm would offer insight into the generation of the hyper/hyposensitivity toward gaze, as well as have some implications concerning the neural basis of such symptoms.

In this report, we have investigated the behavior toward gaze in a group of relatively uniform, long-term, unremitted schizophrenic subjects (mean duration of illness 29 years), using three different stimuli as directional cues. Given the well-documented concreteness in schizophrenic visual processing (Silverstein et al., 2000; Vianin et al., 2002; Uhlhaas et al., 2005), two gaze stimuli (ambiguous rectangular eyes, concrete elliptical eyes) were employed so as not to let very subtle compromise go unnoticed, if present. Arrow signs, which like gaze, have distinct directional property but no biological significance, have also been extensively studied in spatial cueing paradigms in normal (Tipples, 2002; Friesen et al., 2004), autistic (Senju et al., 2004), and schizophrenic subjects (Bustillo et al., 1997), and were used in this experiment as well for comparison with gaze cues. This comparison of behavior toward gaze versus arrows would give us an opportunity to determine whether any detected compromise in schizophrenia was specific to gaze, or represented a more generalized deficit. As hypo-arousal to gaze has been the clinical impression in chronic schizophrenia, we hypothesized that this patient group would demonstrate a distinct behavior pattern attributable to gaze hyposensitivity. Additionally, in relation to the documented schizophrenic volume decrease of the STG, which has been implicated in biological motion processing, we expected that such hyposensitivity would be specific to gaze in comparison to arrows.

2. Experiment 1

2.1. Methods for Experiment 1

2.1.1. Subjects

Twenty-two clinical participants were recruited from a psychiatric hospital in the suburbs of Tokyo. The inclusion criteria were a DSM-IVdiagnosis of schizophrenia (American Psychiatric Association, 1994), a duration of illness longer than 10 years, a history of multiple hospitalizations for acute psychosis, and currently undergoing treatment with neuroleptics. The exclusion criteria were an acute relapse within a year, mental retardation, and a neurological deficit. Twenty-

Table 1
Demographic data

	Schizophrenia (N=22)	Normal controls (N=22)
Age	51.2±7.2	51.2±11.3
Gender	M 17, F 5	M 12, F 10
Handedness	R 21, L 1	R 20, L 2
Education (years)	12.5±1.7	14.5±2.8
Duration of illness (years)	28.9±9.3	
Neuroleptic dosage (HP-mg)	12.8±6.5	
Inpatient/outpatient	15/7	
PANSS score		
Positive symptoms	20.2±5.5	
Negative symptoms	21.0±3.6	
Total	82.3±12.5	

HP-mg; haloperidol-equivalent milligram.