Table 2. EEM, P300 and RT tests (Spearman's δ) in schizophrenia

		P300		RT			
		LAT	AMP	SRT	SI	IRT-cross	CV
EEM	NEF	−0.29	0.15	−0.37	−0.33	−0.11	−0.08
	TESL	−0.18	0.32	−0.39	−0.33	−0.43	0.02
	CSS	0.02	0.01	−0.23	−0.17	−0.04	−0.10
	RSS	−0.09	0.19	−0.16	−0.08	−0.56*	0.26
P300	LAT			0.12	0.14	0.26	0.18
	AMP			−0.22	−0.23	−0.15	−0.30

*$P < 0.01$.

AMP, amplitude; CSS, cognitive search score; CV, coefficient of variation; EEM, exploratory eye movement; IRT-cross, index of reaction time crossover; LAT, latency; NEF, number of eye fixations; RSS, responsive search score; RT, reaction time; SI, set index; SRT, simple reaction time; TESL, total eye scanning length.

EEM test was significantly negatively correlated with the IRT-crossover of the RT test in the schizophrenia group ($\delta = -0.56$, $n = 34$, $P = 0.00066$). There was no significant correlation with respect to other parameters of the three tests (EEM, P300 and RT) in the schizophrenic patients. In reference to the normal controls, we also found no significant correlations between the results of the three tests.

EEM, P300, RT and medications

Relationships between EEM, P300, RT variables and the dosage of a haloperidol equivalent neuroleptic medication were tested using Spearman rank-order correlation test to investigate the medication effects. There were no significant correlations between the parameters of EEM, P300 and RT and the dosage of a haloperidol equivalent neuroleptic medication.

DISCUSSION

Schizophrenic patients and normal controls

In the present study all EEM, P300 and RT tests parameters in the schizophrenic group differed significantly from those in the control group. The present findings are consistent with previous studies in that we were able to replicate abnormalities in EEM, P300 and RT tests in schizophrenic patients.[3–5,8,9] As already noted, we inspected our data in detail and set an optimal cut-off point between schizophrenic patients and normal controls. Hence, it is reasonable to propose that the schizophrenic group was significantly different from the normal control group in the RT-crossover. Moreover, concerning the mean years of education, the schizophrenic group education level was significantly lower than that of the normal

Table 3. EEM, P300 and RT tests (Spearman's δ) in normal controls

		P300		RT			
		LAT	AMP	SRT	SI	IRT-cross	CV
EEM	NEF	0.21	0.02	−0.07	−0.09	0.12	0.21
	TESL	0.40	−0.28	0.13	0.12	0.04	0.06
	CSS	0.18	0.06	−0.22	−0.18	0.24	−0.07
	RSS	0.14	0.11	−0.34	−0.15	−0.08	−0.21
P300	LAT			−0.08	0.02	0.03	−0.15
	AMP			−0.24	−0.20	0.04	0.03

AMP, amplitude; CSS, cognitive search score; CV, coefficient of variation; EEM, exploratory eye movement; IRT-cross, index of reaction time crossover; LAT, latency; NEF, number of eye fixations; RSS, responsive search score; RT, reaction time; SI, set index; SRT, simple reaction time; TESL, total eye scanning length.

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.

REFERENCES

1 Frith CD. *The Cognitive Neuropsychology of Schizophrenia*. Lawrence Erlbaum Association Publishers, London, 1992.

2 Andreasen NC. *Schizophrenia: From Mind to Molecule*. American Psychiatric Press, Washington, DC, 1994.

3 Braff DL. Information processing and attention dysfunctions in schizophrenia. *Schizophr. Bull.* 1993; **19**: 233–259.

4 Kojima T, Matsushima E, Ando K *et al.* Exploratory eye movements and neuropsychological tests in schizophrenic patients. *Schizophr. Bull.* 1992; **18**: 85–94.

5 Friedman D, Squires-Wheeler E. Event-related potentials (ERPs) as indicators of risk for schizophrenia. *Schizophr. Bull.* 1994; **20**: 63–74.

6 Nuechterlein KH. Reaction time and attention in schizophrenia: A critical evaluation of the data and theories. *Schizophr. Bull.* 1977; **3**: 373–436.

7 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. American Psychiatric Association, Washington, DC, 1994.

8 Kojima T, Matsushima E, Nakajima K *et al.* Eye movements in acute, chronic and remitted schizophrenics. *Biol. Psychiatry* 1990; **27**: 975–989.

9 Kojima T, Matsushima E, Ohta K *et al.* Stability of exploratory eye movements as a marker of schizophrenia: A WHO multi-center study. *Schizophr. Res.* 2001; **52**: 203–213.

10 Steffy RA, Galbraith KJ. A comparison of segmental set and inhibitory deficit explanations of the crossover pattern in process schizophrenic reaction time performance. *J. Abnorm. Psychol.* 1972; **83**: 227–233.

11 DeAmicis LA, Cromwell RL. Reaction time crossover in process schizophrenic patients, their relatives, and control subjects. *J. Nerv. Ment. Dis.* 1979; **167**: 593–600.

12 Rodnick EH, Shakow D. Set in the schizophrenic as measured by a composite reaction time index. *Am. J. Psychiatry* 1940; **1**: 214–225.

13 Bohannon WE, Strauss ME. Reaction time crossover in psychiatric out-patients. *Psychiatry Res.* 1983; **9**: 17–22.

14 Strauss ME, Bohannon WE, Kaminsky MJ *et al.* Simple reaction time crossover occurs in schizophrenic outpatients. *Schizophr. Bull.* 1979; **5**: 612–615.

15 Matsushima E, Koiima T, Ohbayashi S *et al.* Exploratory eye movements in schizophrenic patients and patients with frontal lobe lesions. *Eur. Arch. Psychiatry Clin. Neurosci.* 1992; **241**: 210–214.

16 Neisser U. *Cognition and Reality*. W. H. Freeman, San Francisco, 1987.

17 Bellissimo A, Steffy RA. Redundancy-associated deficit in schizophrenic reaction time performance. *J. Abnorm. Psychol.* 1972; **80**: 299–307.

18 Shakow D. Segmental set: A theory of the formal psychological deficit in schizophrenia. *Arch. Gen. Psychiatry* 1962; 6: 1–17.

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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;

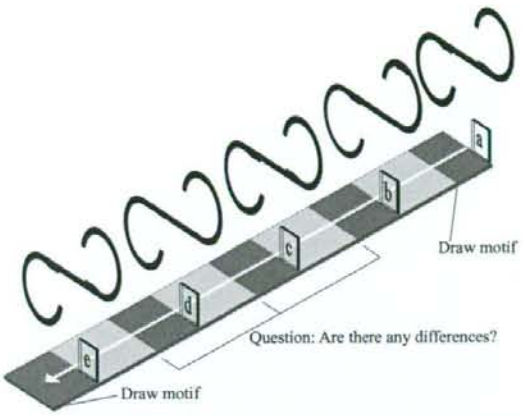

Figure 1. Sequence of events in the exploratory eye movement (EEM) test. (a,c,e) Repeats of the original motif; (b,d) slightly different motifs from the original one (motif b has one bump in a different position; motif d has no bumps). The EEM test proceeded from motif (a) to motif (e).

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

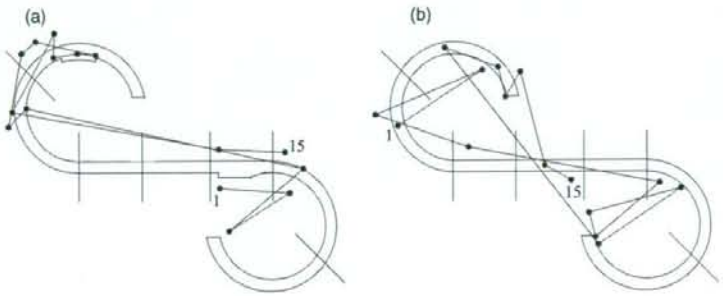

Figure 2. Number of eye fixations in responsive search (NEFRS) and the responsive search score (RSS) of a normal control subject. (a) and (b) are slightly different from the original motif (Fig. 1a). Figure 2(a) has a bump in a different position motif (BDPM). Figure 2(b) has no bump motif (NBM). The 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 healthy siblings were intermediate between those of the probands with schizophrenia and those of normal controls.

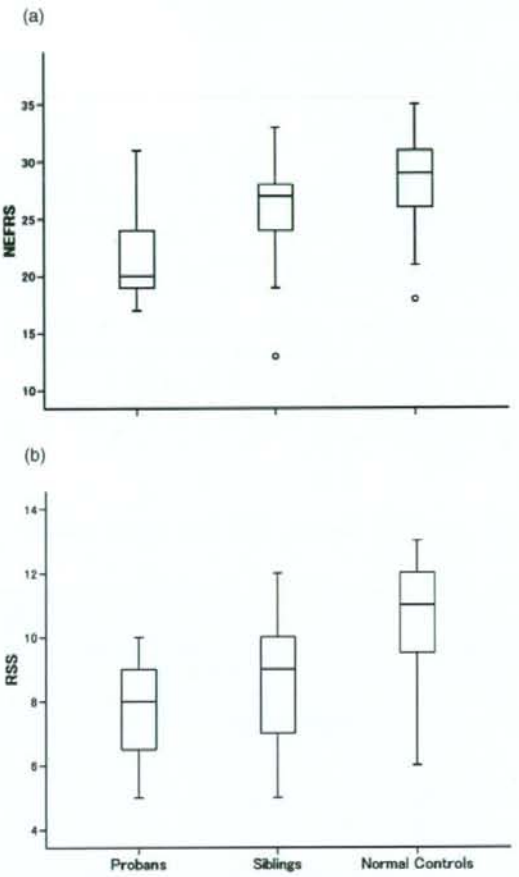

Figure 3. Boxplots of (a) number of eye fixations in responsive search (NEFRS) and (b) responsive search score (RSS). (○) Outliers in the boxplot of the NEFRS. Outliers are >1.5 box lengths from the end of the box (SPSS manual).

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.

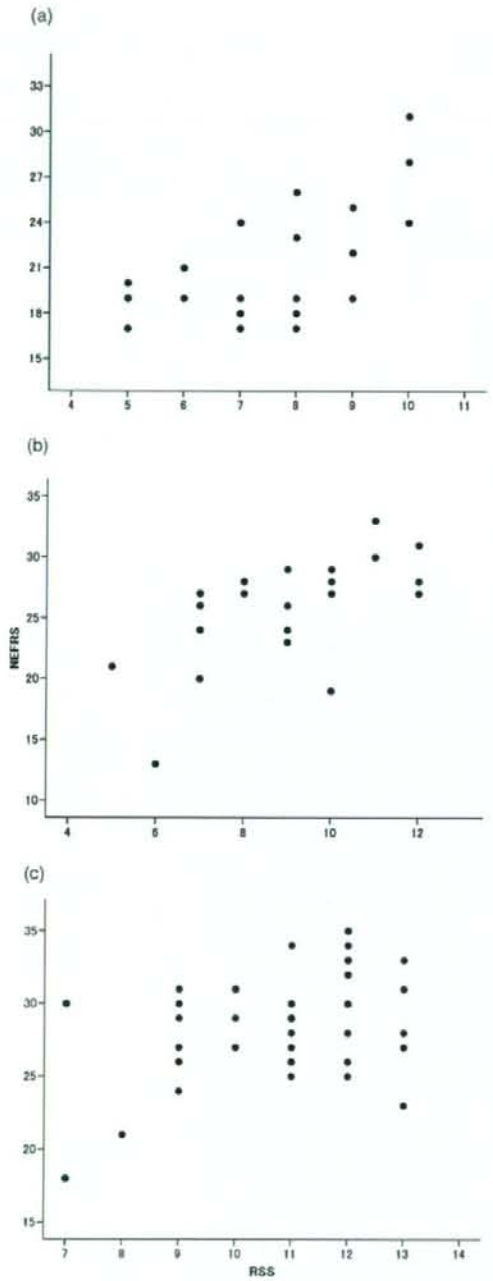

Figure 4. Correlation between the number of eye fixations in responsive search (NEFRS) and responsive search score (RSS) in (a) probands ($\rho = 0.53$, $n = 23$, $P = 0.0095$), (b) siblings ($\rho = 0.62$, $n = 23$, $P = 0.0016$) and (c) normal controls ($\rho = 0.34$, $n = 43$, $P = 0.025$).

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.

REFERENCES

[1] Gottesman II. *Schizophrenia Genetics: The Origins of Madness*, 1st edn. WH Freeman and Company, New York, 1991.

[2] Kendler KS, Diehl SR. The genetics of schizophrenia: A current, genetic-epidemiologic perspective. *Schizophr. Bull.* 1993; **19**: 261–285.

[3] Moldin SO, Gottesman II. At issue: Genes, experience, and chance in schizophrenia: Positioning for the 21st century. *Schizophr. Bull.* 1997; **23**: 547–561.

[4] Tsuang MT, Faraone SV. The case for heterogeneity in the etiology of schizophrenia. *Schizophr. Res.* 1995; **17**: 161–175.

[5] Pulver AE. Search for schizophrenia susceptibility genes. *Biol. Psychiatry* 2000; **47**: 221–230.

[6] Tsuang MT. Schizophrenia: Genes and environment. *Biol. Psychiatry* 2000; **47**: 210–220.

[7] Freedman R, Adler LE, Leonard S. Alternative phenotypes for the complex genetics of schizophrenia. *Biol. Psychiatry* 1999; **45**: 551–558.

[8] Kojima T, Matsushima E, Ando K *et al.* Exploratory eye movements and neuropsychological tests in schizophrenic patients. *Schizophr. Bull.* 1992; **18**: 85–94.

[9] Matsushima E, Kojima T, Ohta K *et al.* Exploratory eye movement dysfunctions in patients with schizophrenia: Possibility as a discriminator for schizophrenia. *J. Psychiatr. Res.* 1998; **32**: 289–295.

[10] Kojima T, Matsushima E, Ohta K *et al.* Stability of exploratory eye movements as a marker of schizophrenia: A WHO multi-center study. *Schizophr. Res.* 2001; **52**: 203–213.

[11] Ismail B, Cantor-Graae E, McNeil TF. Neurological abnormalities in schizophrenic patients and their siblings. *Am. J. Psychiatry* 1998; **155**: 84–89.

[12] Ismail B, Cantor-Graae E, McNeil TF. Minor physical anomalies in schizophrenic patients and their siblings. *Am. J. Psychiatry* 1998; **155**: 1695–1702.

[13] Kojima T, Matsushima E, Nakajima K *et al.* Eye movements in acute, chronic, and remitted schizophrenics. *Biol. Psychiatry* 1990; **27**: 975–989.

[14] Matsushima E, Kojima T, Ohbayashi S *et al.* Exploratory eye movements in schizophrenic patients and patients with frontal lobe lesions. *Eur. Arch. Psychiatry Clin. Neurosci.* 1992; **241**: 210–214.

[15] Kojima T, Potkin SG, Kharazmi M *et al.* Limited eye movement patterns in chronic schizophrenic patients. *Psychiatry Res.* 1989; **28**: 307–314.

[16] Takahashi S, Ohtsuki T, Yu SY *et al.* Significant linkage to chromosome 22q for exploratory eye movement dysfunction in schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 2003; **123**: 27–32.

[17] Kirov G, O'Donovan MC, Owen MJ. Finding schizophrenia genes. *J. Clin. Invest.* 2005; **115**: 1440–1448.

[18] Arinami T. Analyses of the associations between the genes of 22q11 deletion syndrome and schizophrenia. *J. Hum. Genet.* 2006; **51**: 1037–1045.

[19] Nemoto Y, Matsuda T, Matsuura M *et al.* Neural circuits of eye movements during performance of the visual exploration task, which is similar to the responsive search score task, in schizophrenia patients and normal subjects. *J. Nihon Univ. Med. Ass.* 2004; **63**: 352–359 (in Japanese).

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ORIGINAL ARTICLE

Newly developed waist actigraphy and its sleep/wake scoring algorithm

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Abstract

The purpose of this study was to formulate an algorithm for assessing sleep/waking from activity intensities measured with a waist-worn actigraphy, the Lifecorder PLUS (LC; Suzuken Co. Ltd., Nagoya, Japan), and to test the validity of the algorithm. The study consisted of 31 healthy subjects (M/F = 20/11, mean age 31.7 years) who underwent one night of simultaneous measurement of activity intensity by LC and polysomnography (PSG). A sleep(S)/wake(W) scoring algorithm based on a linear model was determined through discriminant analysis of activity intensities measured by LC over a total of 235 h and 56 min and the corresponding PSG-based S/W data. The formulated S/W scoring algorithm was then used to score S/W during the monitoring epochs (2 min each, 7078 epochs in total) for each subject. The mean agreement rate with the corresponding PSG-based S/W data was 86.9%, with a mean sensitivity (sleep detection) of 89.4% and mean specificity (wakefulness detection) of 58.2%. The agreement rates for the individual stages of sleep were 60.6% for Stage 1, 89.3% for Stage 2, 99.2% for Stage 3 + 4, and 90.1% for Stage REM. These results demonstrate that sleep/wake activity in young to middle-aged healthy subjects can be assessed with a reliability comparable to that of conventional actigraphy through LC waist actigraphy and the optimal S/W scoring algorithm.

Key words: actigraphy, polysomnography, sleep/wake scoring algorithm, sleep-waking, waist-worn.

INTRODUCTION

An actigraphy is a small lightweight device for non-invasive and continuous monitoring of human rest/activity (sleep/wake) cycles.[1,2] The most commonly used actigraphy in current sleep research is a unit that is worn on the non-dominant wrist like a wristwatch for continuous measurement of forearm motor activity. The actigraphy unit generally consists of a piezoelectric accelerometer and a memory for storing the measured values for a specific time epoch, typically from 1 s to several minutes.

Algorithms using the activity level measured by the actigraphy to determine whether the person wearing the unit is awake or asleep during the time epoch have been developed for use with individual actigraphy units.[3–5] Studies to date investigating the agreement rate of polysomnography (PSG) and various actigraphy units in

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healthy adults have reported a very high agreement rate of 85 to 96% between the two methods with use of the optimal specific sleep/wake scoring algorithm.[3–7]

Although actigraphy is suitable for assessment of sleep/wake activity during a specific time epoch, it cannot be used independently for confirmation or diagnosis of sleep disturbances because, contrary to PSG, it does not allow for collection of data on electro-oculogram (EOG), electromyogram (EMG), electrocardiogram (ECG), and breathing function during sleep.[7] On the other hand, it has a distinct advantage over PSG in that it allows for continuous recording of rest/activity (sleep/wake state) over long periods of time outside of the sleep lab with minimal disruption to the subject's normal life. It is therefore commonly used in human sleep physiology research and clinical studies in patients with insomnia and circadian rhythm sleep disorders.[6] Future beneficial applications of actigraphy include sleep disturbance screening in a large number of subjects and evaluation of the effectiveness and side effects of drug and non-drug therapies requiring continuous assessment of sleep/wake activity. Inexpensive multipurpose devices providing a favorable cost–benefit balance in the clinical setting are, however, necessary to realize these new potential applications. There have been a few previous studies that assessed sleep/wake activity using an actigraphy placed on the trunk[8,9] and the head[10] because the current mainstream wrist-worn actigraphy unit cannot be readily used in individuals with upper dystaxia, individuals with involuntary movement such as finger tremors, and children and dementia patients who may inadvertently interfere with the device. Most are also not waterproof and cannot thus readily be used in individuals whose work involves handling of water. So actigraphy units that can be worn on body sites other than the wrist, such as the trunk, are still needed.

We therefore focused our research on an inexpensive activity monitor that is worn around the waist to measure activity as a new actigraphy option in sleep research and sleep medicine. In our study, data obtained from healthy adults was used to formulate an algorithm to score sleep/waking measured by waist actigraphy and test the validity of the algorithm.

METHODS

Features of waist actigraphy

An inexpensive activity monitor that is worn around the waist (Lifecorder PLUS [LC]; Suzuken Co. Ltd., Nagoya, Japan; ¥14800 = €100 = $128) was used to measure activity level during sleep. The LC was originally developed for measurement of daytime physical-activity level and has been used for the assessment of physical-activity-related energy expenditure.[11,12] The LC measures acceleration along the longitudinal axis every 4 s with an internal piezoelectric accelerometer and classifies the intensity into 11 levels from 0 to 9 (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9) every 2 min.[11] Level 0 (corresponding to <0.06 G) denotes immobility and Levels 0.5 to 9 (corresponding to ≥0.06 G) denote subtle to strong movements. The cut-off point of activity intensity (the acceleration value) for each level is not provided by the manufacturer. It is possible to continuously record the activity intensity level with the time information for at least 2 months. After the completion of measurement, the recorded activity intensity data can be downloaded to a personal computer through a USB cable. The scoring algorithm was formulated from these data.

Experimental subjects

The study consisted of 31 healthy adults (20 males and 11 females with a mean age of 31.6 ± 10.4 years). Monitoring was performed by the Sleep Electroencephalography Lab at Aoki Hospital and the Sleep/Biological Rhythm Monitoring Unit of the National Institute of Mental Health of the National Center of Neurology and Psychiatry. Subjects underwent simultaneous continuous monitoring of intensity of physical movement during sleep by PSG and LC. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry. Subjects were informed of the purposes and methods of the study and gave written consent to participate in the investigation.

PSG and LC recordings

The PSG consisted of measurement of a standard electroencephalogram (C3-A2, C4-A1, O1-A2, O2-A1), EOG, chin EMG, ECG, breathing function, and tibialis anterior EMG data every 30 s. The Polymate 1524 (TEAC Corporation, Tokyo, Japan) and Comet PSG (Grass-Technologies, RI, USA) were used for the PSG. The sleep stage (Stage 1, Stage 2, Stage 3 + 4, Stage REM or Stage wake) was then determined every 30 s according to the rules of Rechtschaffen and Kales.[13] Four consecutive 30-s intervals of sleep stage data were used to assess sleep/wake state every 2 min to correspond with the intervals with LC data. When four consecutive data contained two or more of Stage wake, the data set was classified as wake (W_{PSG}) according to the definition

adopted the previous studies.[14–16] All other data sets were classified as sleep (S_{PSG}). Furthermore, S_{PSG} was subclassified as Stage REM, Stage 1, Stage 2, or Stage 3 + 4, according to the most frequent sleep stage in the data set (e.g. when S_{PSG} contained two or more Stage 1 data, it was classified as Stage 1). However, when S_{PSG} contained two of two different stages, the priority order (Stage REM → Stage 1 → Stage 2 → Stage 3 + 4) was used (e.g. when S_{PSG} contained two Stage 1 and two Stage REM, it was classified as Stage REM).

Formulation of an algorithm for assessing sleep/waking

A S/W scoring algorithm for LC was newly formulated by the discriminant analysis. The data used for the development were the datasets of S_{PSG} (=0) and W_{PSG} (=1) corresponding to the LC exercise intensities obtained from 7078 epochs obtained from 31 subjects on 31 nights over a total of 235 h and 56 min.

Taking the S/W algorithm for the present actigraphy into account, we assume the five-dimension linear model that incorporates the exercise intensities during 10 min with the center of the time epoch of interest. The activity intensities 4 min before the scored epoch, 2 min before the scored epoch, during the scored epoch, 2 min after the scored epoch, and 4 min after the scored epoch were represented by x_1, x_2, x_3, x_4, and x_5, respectively. A linear discriminant function was given as the following equation for an arbitrary set of weight coefficients of a_1, a_2, a_3, a_4, and a_5.

$$z = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_5$$

Where the variable of z can be used as the discriminant score to classify a set of activity intensities into the stage of S_{LC} or W_{LC}.

The above discriminant function was determined by the discriminant analysis. Supposing that the LC activity intensity in sleeping status and in waking status are categorized in class 1 and 2, respectively, and the number of the datasets in each class is set to n_1 and n_2, the i-th ($i = 1$ to n_k) variable in class k ($k = 1, 2$), $z_i^{(k)}$ is given as

$$z_i^{(k)} = a_1x_{1i}^{(k)} + a_2x_{2i}^{(k)} + a_3x_{3i}^{(k)} + a_4x_{4i}^{(k)} + a_5x_{5i}^{(k)}.$$

The variation of $\{z_i^{(k)}\}$ is represented by the total sum of squares, S_T, which can be decomposed to the between sum of squares, S_B, and the within sum of the squares, S_W ($S_T = S_B + S_W$).

$$S_T = \sum_{k=1}^{2}\sum_{i=1}^{n_k}(z_i^{(k)} - \bar{z})^2$$

$$S_B = \sum_{k=1}^{2} n_k(\bar{z}^{(k)} - \bar{z})^2$$

$$S_W = \sum_{k=1}^{2}\sum_{i=1}^{n_k}(z_i^{(k)} - \bar{z}^{(k)})^2.$$

Since the better discriminability between the two classes using z is equivalent to the increase of the ratio of correlation, $\eta^2 = S_B / S_T$, the set of weight coefficients, $\hat{a}_1$, $\hat{a}_2$, $\hat{a}_3$, $\hat{a}_4$, $\hat{a}_5$, that gives the maximum η^2 can be calculated by the following equations:

$$\begin{bmatrix} s_{11} & s_{12} & s_{13} & s_{14} & s_{15} \\ s_{21} & s_{22} & s_{23} & s_{24} & s_{25} \\ s_{31} & s_{32} & s_{33} & s_{34} & s_{35} \\ s_{41} & s_{42} & s_{43} & s_{44} & s_{45} \\ s_{51} & s_{52} & s_{53} & s_{54} & s_{55} \end{bmatrix} \begin{bmatrix} \hat{a}_1 \\ \hat{a}_2 \\ \hat{a}_3 \\ \hat{a}_4 \\ \hat{a}_5 \end{bmatrix} = \begin{bmatrix} \bar{x}_1^{(1)} - \bar{x}_1^{(2)} \\ \bar{x}_2^{(1)} - \bar{x}_2^{(2)} \\ \bar{x}_3^{(1)} - \bar{x}_3^{(2)} \\ \bar{x}_4^{(1)} - \bar{x}_4^{(2)} \\ \bar{x}_5^{(1)} - \bar{x}_5^{(2)} \end{bmatrix}.$$

Where $\bar{x}_j^{(k)}$ is the average of the j-th variable in class k, $s_{jj'}$ is the within covariance between the j-th and j'-th variables. They are evaluated by

$$\bar{x}_j^{(k)} = \frac{1}{n_k}\sum_{i=1}^{n_k} x_{ji}^{(k)}$$

$$s_{jj'} = \frac{1}{n_1 + n_2 - 2}\sum_{k=1}^{2}\sum_{i=1}^{n_k}(x_{ji}^{(k)} - \bar{x}_j^{(k)})(x_{j'i}^{(k)} - \bar{x}_{j'}^{(k)}).$$

S/W agreement rate

The S/W scoring algorithm was used to determine the S_{LC}/W_{LC} state from the activity intensity data in a total of 7078 epochs in the 31 subjects, and the agreement rate with the corresponding S_{PSG}/W_{PSG} results was calculated by subject and sleep stage. The agreement rate with the PSG-based sleep epochs (sensitivity) and agreement rate with the PSG-based wakefulness epochs (specificity) were also calculated by subject. SPSS version 11.5 was used for the statistical analysis (SPSS Japan Inc., Tokyo, Japan). Results were expressed as mean ± SD.

RESULTS

S/W scoring algorithm

The following S/W scoring algorithm was derived from the results of discriminant analysis of the activity

Table 1 Sleep parameters scored by polysomnography (PSG) and Lifecorder (LC) data

Sleep parameters	PSG	LC	Significance
Sleep efficiency (%)	90.2 ± 9.6 (61.8–99.1)	86.8 ± 11.1 (44.1–100.0)	$t(60) = 1.26$, $P = 0.21$
Total sleep time (min)	406.6 ± 78.9 (179.3–587.0)	376.3 ± 76.3 (208.0–586.0)	$t(60) = 1.53$, $P = 0.13$
Wake after sleep onset (min)	45.2 ± 48.3 (3.67–232.7)	59.9 ± 68.5 (0–388.0)	$t(60) = 0.98$, $P = 0.33$

Table 2 Decision parameters of S/W prediction algorithm for the Lifecorder

			Number of epochs
Agreement rates (%)	Overall	86.9 ± 8.9	7078
	Stage W	58.2 ± 30.4	819
	Stage 1	60.6 ± 26.2	427
	Stage 2	89.3 ± 10.6	3694
	Stage 3 + 4	99.2 ± 2.1	838
	Stage REM	90.1 ± 17.5	1300
Sensitivity (%)		89.4 ± 10.6	
Specificity (%)		58.2 ± 30.4	
Percentage of S_{PSG} epochs misscored as W_{LC} (%)		10.6 ± 10.6	
Percentage of W_{PSG} epochs misscored as S_{LC} (%)		41.8 ± 30.4	

S, sleep; W, wakefulness.

intensity data and PSG-based sleep/wake data from the total 7078 epochs obtained from 31 subjects:

$$z = 0.635x_1 + 0.427x_2 + 0.701x_3 + 0.805x_4 + 0.718x_5$$

where $z \geq 1$ indicates wakefulness (W_{LC}) and $z < 1$ indicates sleep (S_{LC}).

The linear discriminant function was transformed in advance by using linearity of the discriminant function in such a way that the threshold (z) becomes 1. Here, x_1, x_2, x_3, x_4, and x_5, indicate the activity intensity 4 min before the scored epoch, 2 min before the scored epoch, during the scored epoch, 2 min after the scored epoch, and 4 min after the scored epoch.

Validity of the S/W scoring algorithm

The sleep parameters derived from PSG and the LC activity intensity data are shown in Table 1. Sleep efficiency, total sleep time, and wakefulness after sleep onset were each derived from PSG and the LC activity intensity data (Table 1). No statistically significant differences were observed between PSG and the LC in any of the sleep parameters.

Table 2 shows the sleep/wake agreement rates between the LC and PSG, and the sensitivity and specificity of the LC. The overall agreement rate between the LC and PSG in the 31 subjects was 86.9 ± 8.9%. By sleep stage, the Stage 1 agreement rate was low at approximately 60%, but the Stage 2, Stage REM, and Stage 3 + 4 agreement rates were high at approximately 90% for Stage 2 and Stage REM and close to 100% for Stage 3 + 4.

The S/W scoring algorithm had a mean sensitivity (S detection) of 89.4 ± 10.6% and a mean specificity (W detection) of 58.2 ± 30.4%. In other words, 10.6 ± 10.6% of S_{PSG} were misscored as W_{LC} and 41.8 ± 30.4% of W_{PSG} were misscored as S_{LC}.

Activity intensity distribution before and after the scored epoch

Figure 1 shows the mean activity intensity recorded by the LC for nine consecutive epochs (18 min) centered at the W_{PSG} epoch (averaged for a total of W_{PSG} 819 epochs obtained from 31 subjects). The mean activity intensity recorded by the LC peaked just after the W_{PSG} epoch.

DISCUSSION

In the study, an S/W scoring algorithm for the LC was formulated through linear-based discriminant analysis of the corresponding longitudinal "PSG-based sleep/wake state" and "LC-recorded activity intensity" data in 7078 epoch recordings in 31 subjects over a total of

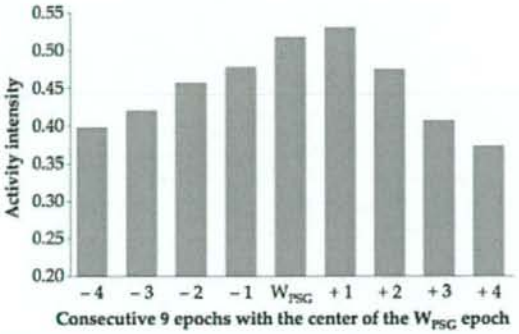

Figure 1 Activity intensity distribution before and after the scored epoch. The mean activity intensity recorded by the Lifecorder (LC) for nine consecutive epochs (18 min) centered at the W_{PSG} epoch. Vertical bars indicate the activity intensity. The mean activity intensity bars formed an inverted U-shape and peaked just after the W_{PSG} epoch.

235 h and 56 min. Comparison of the S/W activity determined from the LC data through the S/W scoring algorithm and the comparable activity determined from the PSG data through the rules of Rechtschaffen and Kales showed a mean agreement rate of approximately 87% in the 31 subjects. This rate is comparable to the 85 to 96% agreement rates obtained with conventional actigraphy units and their S/W scoring algorithms.[3–7] The LC and its S/W scoring algorithm yielded a high agreement rate of 90% or greater for Stage 2 and Stage 3 + 4 deep sleep and REM sleep, as well as an approximately 60% agreement rate for W_{PSG}, which is higher than that yielded by conventional algorithms. In order to examine the superiority of the five-dimensional model over the three-, seven-, or nine-dimensional models, we assumed linear models which incorporate the activity intensities during intervals of 6, 14, and 18 min centered at the time epoch of interest. The total agreement rates of the algorithms for the three-, five-seven-, and nine-dimensional models were 82.9%, 86.9% 86.0%, and 87.3%, respectively. Finally, we adopted the algorithm of the five-dimensional model since the agreement rate appeared to become saturated for models with more than five-dimensions. These findings show that when used with the S/W scoring algorithm developed in the study, the LC is a useful sleep assessment device with equivalent S/W identification capacity to conventional actigraphy systems.

Silent awakeness has been generally difficult to detect through actigraphic S/W assessment, in which it may be misscored as sleep, resulting in a pattern of overassessment of total sleep time and sleep efficiency compared to PSG-based assessment.[4,16,17] The LC and the S/W scoring algorithm derived in this study did not, however, result in a pattern of over-identification of S_{LC}, but contrarily yielded lower total sleep time and sleep efficiency values than the S_{PSG}/W_{PSG} assessment (Table 1). The specificity of the S/W scoring algorithm for the LC (58.2%) is in fact higher than that for conventional actigraphy units and their S/W scoring algorithms (40.6 *vs* 44%),[4,17] demonstrating that the S/W scoring algorithm for the LC developed in the study allows for more accurate identification of W_{LC}.

The S/W detection algorithm for wrist actigraphy used in a previous study assigned the highest weighting coefficient to the scored epoch.[4] However, in the S/W scoring algorithm for the LC, the highest weighting coefficient was assigned to the period immediately following the scored epoch. In fact, the mean activity intensity recorded by the LC peaked just after the W_{PSG} epoch (Fig. 1), and the delayed increase in truncal movement after awakening characterized the highest weighting coefficient assigned immediately after the scored epoch.

The LC is worn on the trunk while the conventional actigraphies used to be worn on the non-dominant wrist.[3–7] This may be related to the high specificity of the LC and its S/W scoring algorithm. The different application sites mean that S/W activity is assessed through different types of movement during sleep, either extremity or trunk movement (which are often independent),[18,19] which may produce the differences in assessment noted above. The LC and its S/W scoring algorithm investigated in the current study may more accurately detect silent awakeness due to the sensitivity to small movements of the torso during sleep and a resulting higher composite variable z value.

There are several issues that require further exploration with respect to use of the LC as a novel option for sleep assessment. First, the time epoch of S/W scoring algorithms for conventional actigraphy is often 1 min or less.[3,5,14] The time epoch for the LC used in this study is 2 min, leading to the assumption that devices with higher temporal resolution may result in higher agreement rates. Although it is more expensive (¥37 000 = €230 = $350), there is an LC that is programmable to 4-s time epochs. It would therefore be of merit to formulate an S/W scoring algorithm for this LC to determine whether it yields a higher agreement rate. Second, the S/W scoring algorithm formulated in the study uses the data from the scored time epoch as well as the data from the two epochs (4-min interval) immediately prior

and immediately after to scoring S/W. This means that activity intensity data prior to onset of sleep will be included in the scoring formula for the scored time epoch unless at least 4 min have passed from the onset of sleep on PSG. This complicates detection of differences in sleep latency of the order of several minutes. Accordingly, sleep latency was not analyzed in this study. This perhaps poses a constraint to the use of the LC in studies and tests requiring accurate evaluation of sleep latency. It is expected that development of LCs with higher temporal resolution and their S/W scoring algorithms will solve this issue.

In the current study, an S/W scoring algorithm for the LC was formulated from the data of young to middle-aged healthy adults and the validity of the algorithm was tested. Other potential useful applications of the inexpensive LC include sleep disorder screening in a large number of individuals. In the future, it will be necessary to determine whether the high agreement rates can also be obtained when the LC and its S/W scoring algorithm are used to assess sleep/wake activity in subjects from different age groups, including children and the elderly, and in patients with common sleep disorders, such as insomnia and sleep respiratory disturbances.

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REFERENCES

1 Kupfer DJ, Foster FG. Sleep and activity in a psychotic depression. *J. Nerv. Ment. Dis.* 1973; **156:** 341–8.
2 Kripke DF, Mullaney DJ, Messin S, Wyborney VG. Wrist actigraphic measures of sleep and ryhthms. *Electroencephalogr. Clin. Neurophysiol* 1978; **44:** 674–6.
3 Webster JB, Kripke DF, Messin S, Mullaney DJ, Wyborney G. An activity-based sleep monitor system for ambulatory use. *Sleep* 1982; **5:** 389–99.
4 Cole RJ, Kripke DF, Gruen W, Mullaney DJ, Gillin JC. Automatic sleep/wake identification from wrist activity. *Sleep* 1992; **15:** 461–9.
5 Jean-Louis G, Kripke DF, Cole RJ, Assmus JD, Langer RD. Sleep detection with an accelerometer actigraph: comparisons with polysomnography. *Physiol. Behav.* 2001; **72:** 21–8.
6 Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. *Sleep* 2003; **26:** 342–92.
7 Acebo C, Lebourgeois MK. Actigraphy. *Respir. Care Clin. N. Am.* 2006; **12:** 23–30.
8 Kochersberger G, McConnell E, Kuchibhatla MN, Pieper C. The reliability, validity, and stability of a measure of physical activity in the elderly. *Arch. Phys. Med. Rehabil.* 1996; **77:** 793–5.
9 Paavonen EJ, Fjallberg M, Steenari MR, Aronen ET. Actigraph placement and sleep estimation in children. *Sleep* 2002; **25:** 235–7.
10 Spivak E, Oksenberg A, Catz A. The feasibility of sleep assessment by actigraph in patients with tetraplegia. *Spinal Cord.* 2007; **45:** 765–70.
11 Kumahara H, Schutz Y, Ayabe M *et al.* The use of uniaxial accelerometry for the assessment of physical-activity-related energy expenditure: a validation study against whole-body indirect calorimetry. *Br. J. Nutr.* 2004; **91:** 235–43.
12 Saito N, Yamamoto T, Sugiura Y, Shimizu S, Shimizu M. Lifecorder: a new device for the long-term monitoring of motor activities for Parkinson's disease. *Intern. Med.* 2004; **43:** 685–92.
13 Rechtschaffen A. A manual of standardized terminology, technique and scoring system for sleep stages of human subjects. Los Angeles, CA: Brain Information Service/ Brain Research Institute, UCLA. 1968.
14 Sadeh A, Sharkey KM, Carskadon MA. Activity-based sleep-wake identification: an empirical test of methodological issues. *Sleep* 1994; **17:** 201–7.
15 Lotjonen J, Korhonen I, Hirvonen K, Eskelinen S, Myllymaki M, Partinen M. Automatic sleep-wake and nap analysis with a new wrist worn online activity monitoring device vivago WristCare. *Sleep* 2003; **26:** 86–90.
16 Paquet J, Kawinska A, Carrier J. Wake detection capacity of actigraphy during sleep. *Sleep* 2007; **30:** 1362–9.
17 de Souza L, Benedito-Silva AA, Pires ML, Poyares D, Tufik S, Calil HM. Further validation of actigraphy for sleep studies. *Sleep* 2003; **26:** 81–5.
18 Van Hilten JJ, Middelkoop HA, Kuiper SI, Kramer CG, Roos RA. Where to record motor activity: an evaluation of commonly used sites of placement for activity monitors. *Electroencephalogr. Clin. Neurophysiol.* 1993; **89:** 359–62.
19 Middelkoop HA, van Dam EM, Smilde-van den Doel DA, Van Dijk G. 45-hour continuous quintuple-site actimetry: relations between trunk and limb movements and effects of circadian sleep-wake rhythmicity. *Psychophysiology* 1997; **34:** 199–203.

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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