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

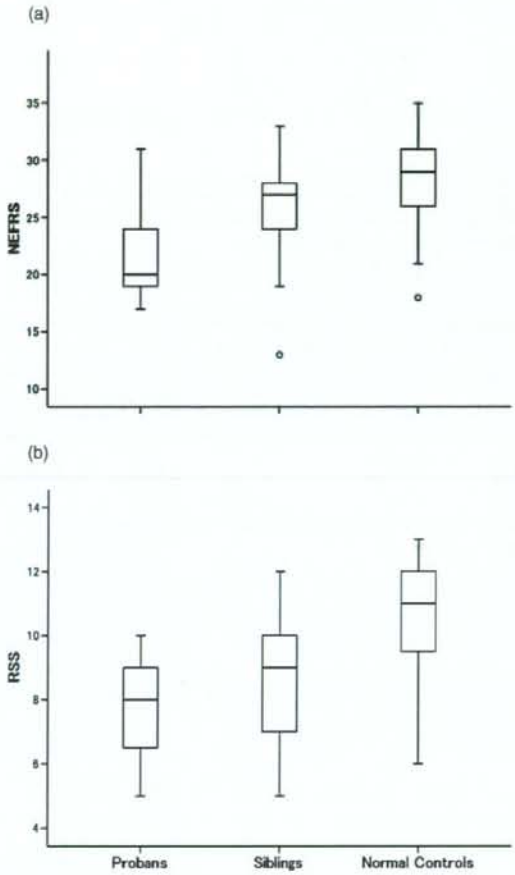

Figure 3. Boxplots of (a) number of eye fixations in responsive search (NEFRS) and (b) responsive search score (RSS). (○) Outliers in the boxplot of the NEFRS. Outliers are >1.5 box lengths from the end of the box (SPSS manual).

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, non-schizophrenic 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 non-schizophrenic 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.

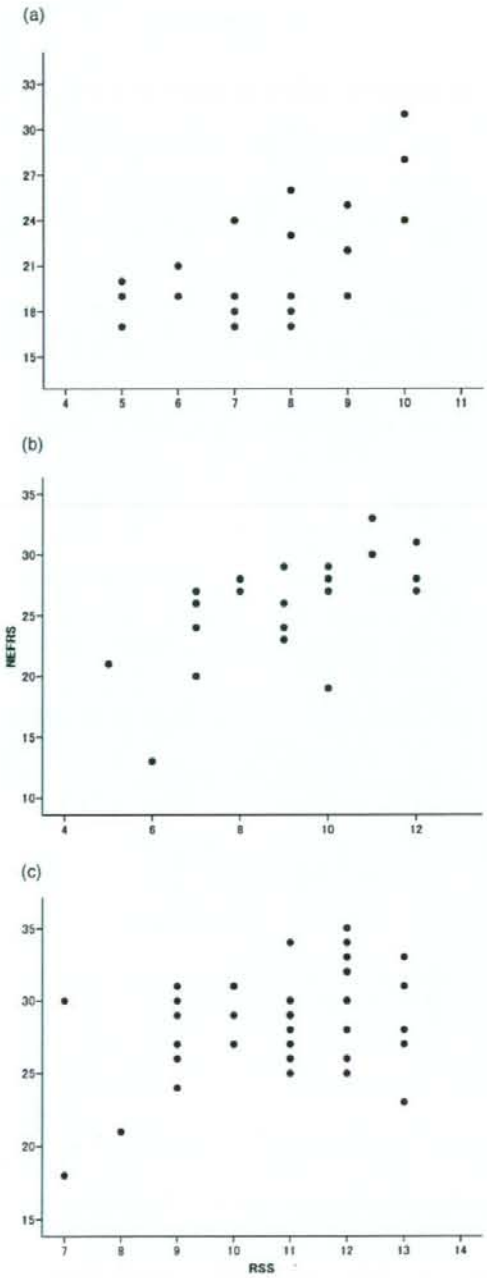

Figure 4. Correlation between the number of eye fixations in responsive search (NEFRS) and responsive search score (RSS) in (a) probands ($\rho = 0.53$, $n = 23$, $P = 0.0095$), (b) siblings ($\rho = 0.62$, $n = 23$, $P = 0.0016$) and (c) normal controls ($\rho = 0.34$, $n = 43$, $P = 0.025$).

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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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 electrooculogram (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.[8] 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

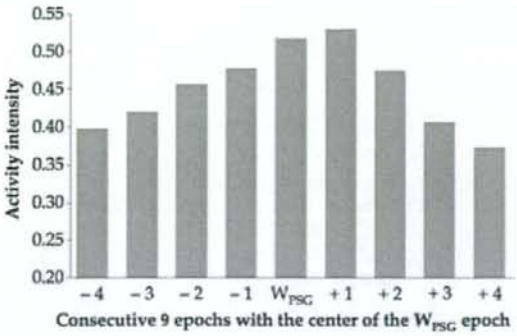

Figure 1 Activity intensity distribution before and after the scored epoch. The mean activity intensity recorded by the Lifecorder (LC) for nine consecutive epochs (18 min) centered at the W_{PSG} epoch. Vertical bars indicate the activity intensity. The mean activity intensity bars formed an inverted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

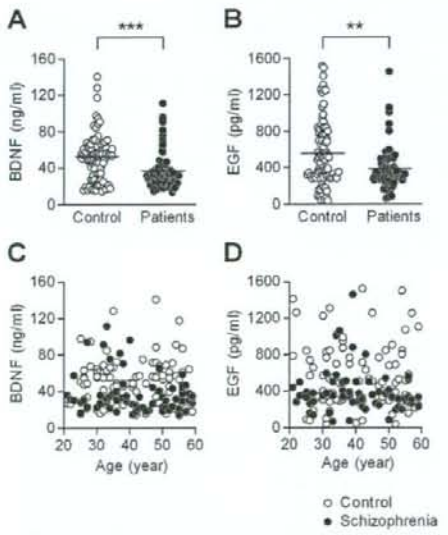

Fig. 1. Serum levels of (A) BDNF and (B) EGF measured by two-site enzyme immunoassay in normal controls ($N=87$) and patients with chronic schizophrenia ($N=74$). Compared with controls, patients exhibited lower serum levels of both neurotrophic factors (BDNF, $^{***}P<0.001$; EGF, $^{**}P<0.01$). Horizontal 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

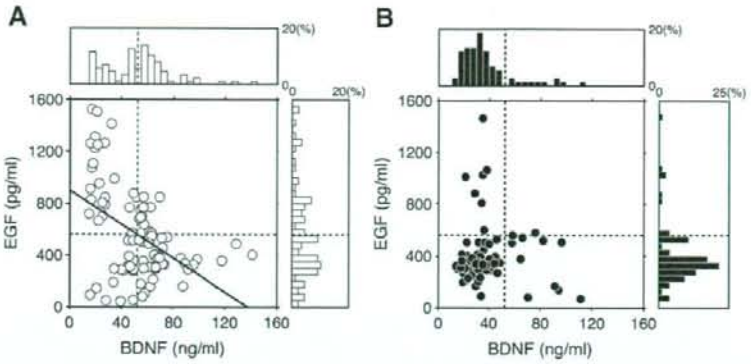

Fig. 2. Relation between the serum levels of BDNF and EGF measured simultaneously in (A) normal controls and (B) chronic schizophrenia patients. For controls, serum levels of the two neurotrophic factors were negatively correlated as shown by the line ($r=-0.387$, $P=0.0002$). The histograms above and 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).

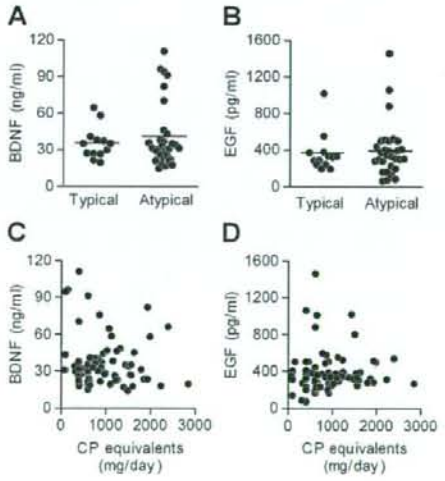

Fig. 3. Effects of antipsychotic drugs on serum (A) BDNF and (B) EGF levels. For both neurotrophic factors, no significant differences were seen between patients taking typical ($N=13$) and atypical ($N=31$) antipsychotic drugs. Horizontal lines indicate the mean levels. Antipsychotic dosages in chlorpromazine 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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ORIGINAL PAPER

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Exploratory eye movement dysfunction as a discriminator for schizophrenia

A large sample study using a newly developed digital computerized system

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Abstract In our previous studies, we identified that exploratory eye movement (EEM) dysfunction appears to be specific to schizophrenia. The availability of a biological marker specific to schizophrenia would be useful for clinical diagnosis of schizophrenia. Consequently, we performed the discriminant analysis between schizophrenics and non-schizophrenics on a large sample using the EEM test data and examined an application of the EEM for clinical diagnosis of schizophrenia. EEM performances were recorded in 251 schizophrenics and 389 non-schizophrenics (111 patients with mood disorders, 28 patients with neurotic disorders and 250 normal controls). The patients were recruited from eight university hospitals and three affiliated hospitals. For this study with a large sample, we developed a new digital computerized version of the EEM test, which automatically handled large amounts of data. We measured four parameters: number of eye fixations (NEF), total eye scanning length (TESL), mean eye scanning length (MESL) and responsive search score (RSS). These parameters of schizophrenics differed significantly from those of the other three groups.

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