detector probes as 'channel'. It is supposed that the machine, in which the source-detector spacing is 3.0 cm, measures points at 2–3 cm depth from the scalp, that is, the surface of the cerebral cortex (Hock et al., 1997; Okada and Delpy, 2003a,b; Toronov et al., 2001). The probes of the NIRS machine were fixed with thermoplastic 3 × 11 shells, with the lowest probes positioned along the Fp1-Fp2 line according to the international 10–20 system used in electroencephalography. The time needed for this fixation is usually less than 5 min, which is less-demanding for the subjects. The 52 measuring areas are labeled ch1-ch52 from the right-posterior to the left-anterior. This arrangement of the probes can measure [Hb] from bilateral prefrontal (approximately dorsolateral [Brodmann's area (BA) 9, 46], ventrolateral [BA 44, 45], and frontopolar [BA 10]) and superior temporal cortical surface regions (Fig. 1, panel A). The correspondence of the probe positions and the measuring areas on the cerebral cortex was confirmed by superimposing the measuring positions on a magnetic resonance image of a three-dimensionally reconstructed cerebral cortex of a representative subject (Fig. 1, panels B–E).

The time resolution of the NIRS machine was set at .1 s. [Hb] changes were analyzed using the first-order correction to exclude task-unrelated changes during the verbal fluency task. The pre-task baseline was determined as the mean across the last 10 s of the pre-task period and the post-task baseline was determined as the mean across the last 5 s of the post-task period, and a linear fitting was performed based on the data between the two baselines. Moving average methods were applied to remove short-term motion artifacts in the analyzed data (moving average window: 5 s). Grand mean waveforms averaged across subjects were created separately for type of [Hb] and for each group. The moving average methods cannot correct all the artifacts and the most researchers qualitatively judge and remove the data with significant

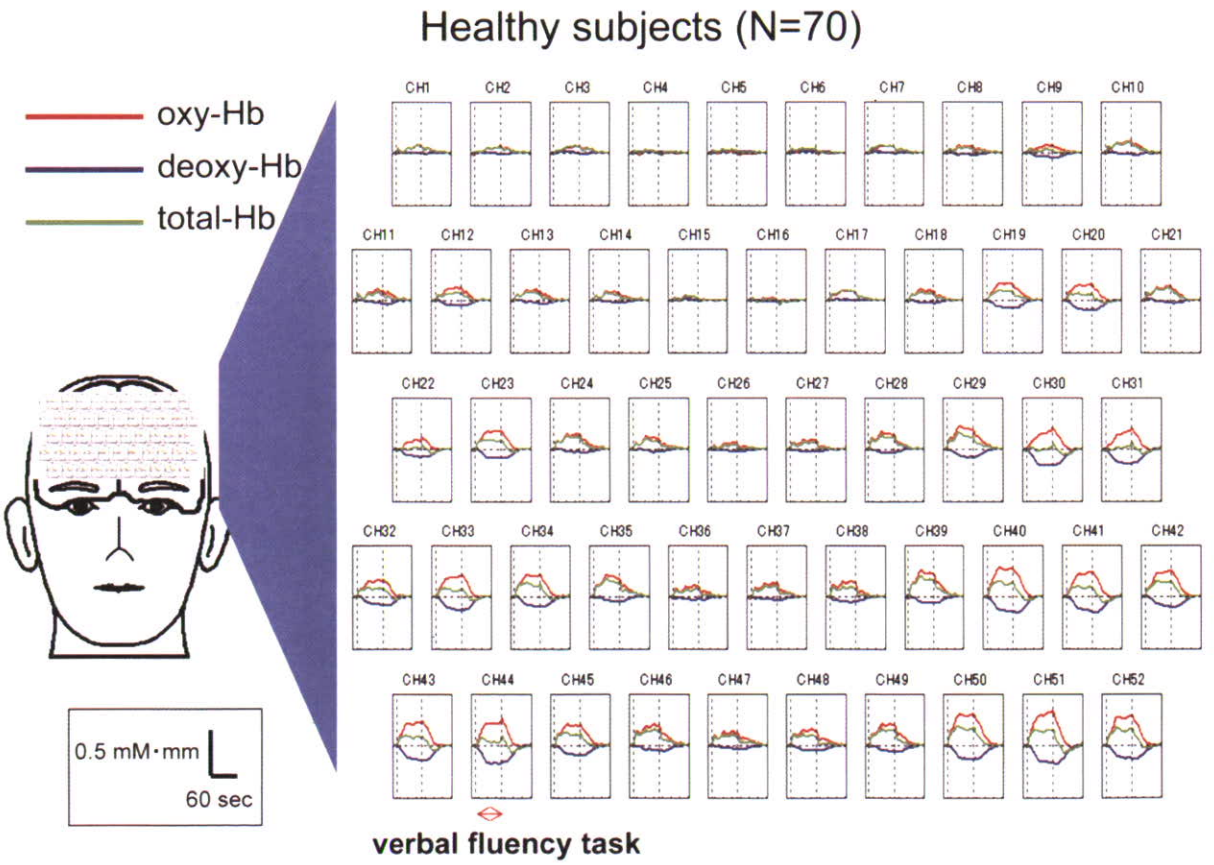

Fig. 2. Grand average waveforms in healthy subjects (N = 70). Oxy-, deoxy-, and total-hemoglobin concentration changes during cognitive activation are presented as grand average waveforms in 52 channels in red, blue, and green lines, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

artifacts; however, it remains subjective (Sato et al., 2006). Thus, we developed an algorithm to quantitatively evaluate the artifacts which enables a fully automatic rejection of data with artifacts (see Supplementary material I for details) separately for each channel; i.e., the number of averaged subjects varied across channels (schizophrenia: $N=30$–53 [mean, 43.8; SD, 5.4]; healthy subjects: $N=34$–67 [mean, 58.1; SD, 6.7]; percentage: schizophrenia, 80.7%; healthy subjects, 84.4%, n.s.).

2.4. Statistical analysis

For data analysis using parametric statistical tests, obtained [Hb] data of each channel were averaged across the two time segments (pre-task baseline and task period). We focused on [oxy-Hb] here, since [oxy-Hb] change is assumed to more directly reflect cognitive activation than [deoxy-Hb] change as shown by a stronger correlation with blood-oxygenation level-dependent signal measured by fMRI (Strangman et al., 2002b), although the analysis of [deoxy-Hb] was also shown (see Supplementary material II for details). First, at each channel, the mean [Hb] for the pre-task baseline period and that for the task period were compared using Student's paired t-test in order to confirm the statistically significant increase associated with the verbal fluency task. Since we performed 52 paired t-tests, the correction for multiple comparisons was made using false discovery rate (FDR) (two-tailed; we set the value of q specifying the maximum FDR to .05, so that there are no more than 5% false positives on average (Singh and Dan, 2006)). Next, the mean [Hb] changes during the 60-s task period were compared between the two groups for each channel by Student's t-test (two-tailed was used since task-load-dependent hypo- or hyperperfusion of prefrontal cortex in schizophrenia was found in previous literature; FDR correction for multiple comparisons [52 channels] was applied). As a confirmatory analysis, we performed the same group comparison of the performance-matched (50 healthy controls: mean, 15.8 [SD=3.5]; 50 schizophrenia patients: mean, 15.0 [SD=4.1]; $t[2,98]=1.02$, $P=.31$, n.s.) and premorbid

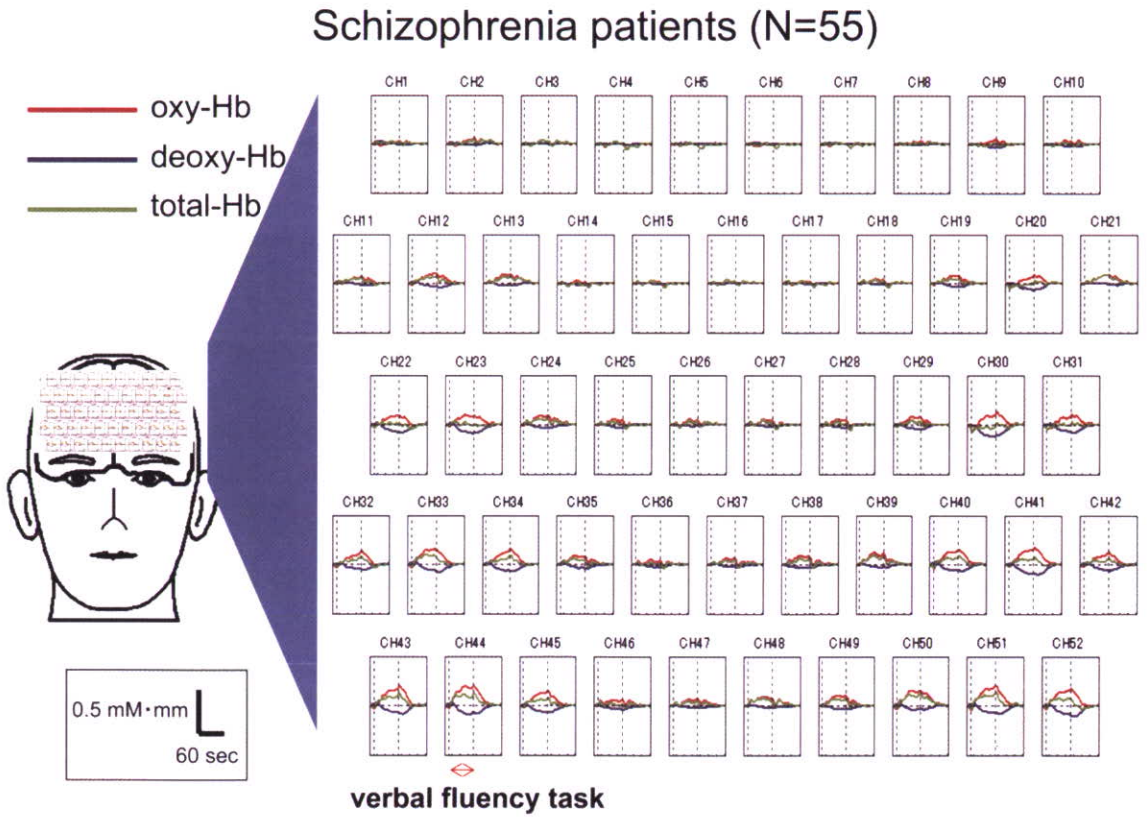

Fig. 3. Grand average waveforms in schizophrenia patients ($N=55$). Oxy-, deoxy-, and total-hemoglobin concentration changes during cognitive activation are presented as grand average waveforms in 52 channels in red, blue, and green lines, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

IQ-matched (48 healthy controls: mean, 106.8 [SD= 9.0]; 48 schizophrenia patients: mean, 104.9 [SD=10.1]; $t[2,94]=.96$, $P=.34$, n.s.) samples. Third, for analysis in time course of [Hb] change, the slope of the first 5-s during the task period were compared between the two groups for each channel by Student's paired t-test (two-tailed; FDR correction for multiple comparisons [52 channels] was applied).

For the schizophrenia group, Pearson's correlation coefficients were calculated for a relationship between the mean [Hb] changes during the task period and the GAF and PANSS scores for each channel. Degrees of freedom varied across the channels due to the artifact rejection procedure explained above. Since we sought to explore which regions of the brain showed more association with clinical assessment, we did not use the multiple correction; rather, we performed multiple correlational analyses for each channel and evaluated the graduation of the r values that reached a significance level of $P<.05$ over the frontotemporal regions (Fig. 5).

Additionally, we performed correlational analysis of [Hb] and age, duration of illness, dose of medication in the schizophrenia group. Statistical analysis was performed using SPSS 10.1.3J software (Tokyo, Japan).

3. Results

3.1. Test for significance in [Hb] change during activation period relative to baseline

The grand averaged waveforms of [oxy-Hb], [deoxy-Hb], and [total-Hb] during cognitive activations in healthy controls and schizophrenia patients were shown in Figs. 2 and 3.

A significant increase in [oxy-Hb] changes occurred during the task period relative to the pre-task baseline at 43 channels (ch7-14, ch17-25, ch27-52; FDR-corrected P: .001 to .041) in healthy controls and at 23 channels (ch12-13, ch19, ch24, ch29, ch32-35, ch37-45, ch48-52;

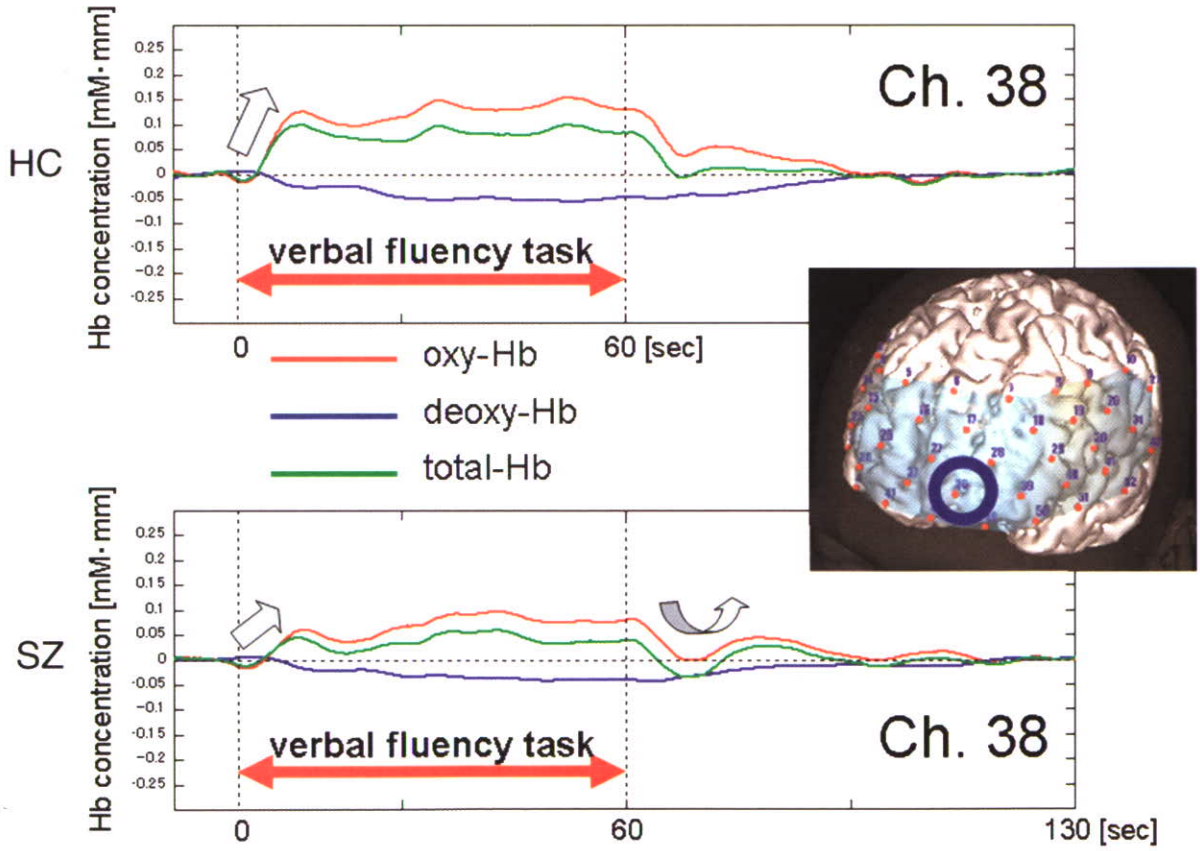

Fig. 4. The differential time course of [Hb] changes in healthy subjects and schizophrenia patients. The differential time course of [Hb] changes between healthy controls (HC; $N=70$) and schizophrenia patients (SZ; $N=55$) were indicated in a representative channel (channel 38; left frontopolar region).

FDR-corrected P: .001 to .022) in schizophrenia patients.

3.2. Group comparison

Schizophrenia patients were associated with significantly lower [oxy-Hb] increase than healthy subjects at 20 channels (ch 17-18, ch24-25, ch28-29, ch 35-40, ch42, ch46-52; FDR-corrected P: .001 to .019). The statistical conclusion did not significantly change when the task-performance-matched sample (significance found at 37 channels [ch1, ch3, ch7-8, ch10, ch14, ch17-21, ch24-25, ch27-32, ch34-43, ch45-52; FDR-corrected P: .001 to .036) or the premorbid IQ-matched sample [significant found at 33 channels (ch7-10, ch14, ch17-21, ch24-25, ch28-29, ch31-32, ch34-43, ch45-47, ch49-52; FDR-corrected P: .001 to .031) were compared.

3.3. Time course of [oxy-Hb] change

The [oxy-Hb] slope of the first 5-s in the task period was significantly steeper in healthy subjects than those in schizophrenia patients at 33 channels (ch1, ch3, ch5, ch10, ch12-13, ch17-18, ch20-21, ch23-25, ch28-32, ch34-35, ch38-47, ch49-52; FDR-corrected P: .001 to .031). Fig. 4 indicates the differential time course between healthy subjects and schizophrenia patients in a representative channel (ch38; left frontopolar region). In healthy subjects, the [oxy-Hb] rapidly increased at the beginning of the verbal fluency task, remained at the activated level during the task and gradually decreased after the end of the task. In contrast, the [oxy-Hb] in schizophrenia patients showed more gradual and lower increase during the task period, and began to decrease immediately after the end of the task, then followed by an inefficient re-increase during the post-task period. These differential patterns were similar to the findings reported by Suto et al. (2004) using a similar protocol.

3.4. Correlational analysis

In schizophrenia patients, the mean [oxy-Hb] changes showed a significantly positive correlation with GAF scores in 10 channels (ch13: r=.34, P=.04; ch16: r=.29, P=.05; ch24: r=.29, P=.04; ch25: r=.40, P=.004; ch26: r=.32, P=.02; ch27: r=.30, P=.04; ch36: r=.38, P=.007; ch37: r=.29, P=.05; ch38: r=.38, P=.007; ch47: r=.32, P=.04), with the highest correlations located approximately in the frontopolar (BA 10) and right dorsolateral (BA 9, 46) regions (Fig. 5), although task performance during verbal fluency test was not significantly correlated with GAF scores.

The mean [oxy-Hb] changes during the task period were not significantly correlated with premorbid IQ or task performance for any channels in either group. The mean [oxy-Hb] changes also showed no significant correlation with clinical variables including duration of illness or dose of medication in the schizophrenia group, except for a significant correlation with age at channel 21 (r=−.35, P=.02). Correlations with PANSS scores were found in a few channels: positive (ch23: r=.37, P=.03; ch33: r=.38, P=.01; ch38: r=.33, P=.02; ch49: r=.31, P=.03); negative (none); general psychopathology (ch12: r=−.32, P=.04; ch25; r=−.36, P=.01; ch27: r=−.32, P=.03; ch36: r=−.35, P=.01; ch47: r=−.32, P=.03), which did not converge on specific sub-regions or in consistent directions.

3.5. Comparison between high- and low-social functioning group in schizophrenia

To confirm the relationship between prefrontal cortical activation and social functioning, we divided patients with schizophrenia into high- and low-social functioning groups by the GAF median value of 52. Student's t-test was used to compare [Oxy-Hb] change between 28 high social functioning group (14 male and 14 female) and 27 low-social functioning group (15 males and 12 females). Potential confounding factors such as age, gender, task performance and premorbid IQ were matched between the two condition groups (not

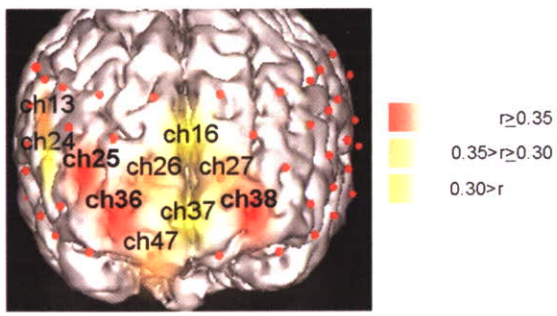

Fig. 5. The cortical distribution of a significant correlation between oxy-hemoglobin changes and global assessment of functioning (GAF) scores. The channels with a significant correlation (Pearson's correlation; P<.05) between the mean [oxy-Hb] changes and GAF scores were indicated with colored area. To illustrate the graduation of the correlation coefficients over the prefrontal cortical surface area, channels with $r \geq .35$ were colored in red, $.35 > r \geq .30$ in orange, and r<.30 in yellow. These areas approximately correspond to frontopolar region (BA 10) and right dorsolateral region (BA 9 and 46). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

described). We found a significant difference in the [oxy-Hb] change between the two groups in the channels located in frontopolar and right dorsolateral prefrontal regions (A significant difference at 5 channels; ch13 (right DLPFC), $P=.018$; ch25 (right DLPFC), $P=.037$; ch26 (right FPPFC), $P=.048$; ch38 (left FPPFC), $P=.029$; ch39 (left FPPFC), $P=.050$).

4. Discussion

Using a 52-channel NIRS with a wide coverage over the prefrontal cortical surface area, it was shown that [oxy-Hb] change during verbal fluency test was significantly slower and smaller in schizophrenia patients as compared with age- and gender-matched healthy subjects, which was not explained by difference in task performance or premorbid IQ. Furthermore, this smaller [oxy-Hb] change following cognitive activation was significantly associated with severer functional impairment in the schizophrenia patients, although the relationship between GAF score and verbal fluency task performance was not significant. And, the regions that showed significant association with the global assessment of functioning were relatively localized in frontopolar regions (BA 10). These results suggest that reduced frontopolar cortical activation associated with executive tasks may be associated with functional impairment in schizophrenia and that NIRS may offer promise as a non-invasive clinical method for evaluating these differential patterns in schizophrenia patients.

4.1. Prefrontal sub-regions

The present study has segregated specific regions in the prefrontal cortex associated with functional impairment in patients with schizophrenia. Petrides' model proposed that ventrolateral prefrontal regions (BA 44/45) are involved in simple short-term operation, whereas mid-dorsal regions perform high-level executive or working memory operations, such as monitoring, reasoning and planning (Petrides, 1994; 1995; Owen, 1997). Fletcher and Henson (2001) attributed ventrolateral and dorsolateral activations to the updating/maintenance of information and to the selection/manipulation/monitoring of that information, respectively. In contrast, recent studies have shed light upon an important role of frontopolar regions (also known as anterior prefrontal cortex) (BA 10), which has been relatively less recognized in functional neuroimaging studies, in higher-order integrative prefrontal function (Ramnani and Owen, 2004). Interestingly, area 10 has been suggested to have enlarged and become specialized during hominid evolution by comparative studies of humans and apes (Semendeferi et al., 2001). Frontopolar regions might provide higher level of control to coordinate ventrolateral and dorsolateral functions in order to maximize task performance, or to achieve these goals (Koechlin et al., 1999; Fletcher and Henson, 2001; Braver and Bongiolatti, 2002). Christoff and Gabrieli (2000) proposed that frontopolar activations become recruited when internally generated information needs to be evaluated. Areas 9/10 are also involved in selecting among competing candidate responses (Desmond et al., 1996; Thompson-Schill et al., 1997).

4.2. Verbal fluency task and prefrontal cortex

In the present NIRS study, the verbal fluency test recruited widespread regions of the prefrontal cortical surface area and superior temporal regions, which is in accordance with previous studies using fMRI and PET (Elfgren and Risberg, 1998; Cabeza and Nyberg, 2000). The verbal fluency test not only requires retrieval of items from long-term memory storage but also concurrently requires working memory capacity to hold the already-generated words, maintenance of cognitive effort, and inhibition of inappropriate response (Henry and Crawford, 2004). This characteristic of the task demands may recruit frontopolar regions as well as lateral prefrontal cortex. Social daily activities require complex operations of working memory, executive function and memory retrieval that including monitoring, reasoning, organizing, selecting and planning, rather than simple short-term operations. Burgess et al. (2000) noted that the high-level of executive control associated with the frontopolar region is likely to be a vital component of everyday life. Considering these observations together, it may be reasonable to postulate that the smaller activations observed in the frontopolar regions during verbal fluency test in the present study were associated with severer functional impairment in schizophrenia.

Our study replicated the findings of reduced prefrontal activation during the letter version of the verbal fluency test in schizophrenia patients (Curtis et al., 1998; Suto et al., 2004). However, neuropsychological studies on Western populations have suggested that the category version of the verbal fluency test is more severely impaired than the letter version of the verbal fluency test in schizophrenia (Bokat and Goldberg, 2003). However, Japanese patients with schizophrenia have been shown to have a similar degree of impairments in both tasks (Sumiyoshi et al., 2004). Future studies should conduct an NIRS measurement during letter and category fluency test and investigate the relationship with functional outcome in Japanese patients with schizophrenia.

4.3. Limitations

Some comment upon methodological considerations is necessary. First, the continuous-wave NIRS enables measurement of Hb concentration changes not as absolute values but as measures relative to pre-task baseline. Therefore we cannot empirically rule out the possibility that the present findings may be due to a difference in prefrontal blood volume during the pre-task period (i.e., *hyper*perfusion in the pre-task period in schizophrenia). However, PET studies have found significant *hypo*perfusion during the resting state in the frontal areas of schizophrenia as compared to healthy controls (Hill et al., 2004). More recently, a near-infrared time-resolved spectroscopy study replicated a hypoperfusion in the resting state in patients with schizophrenia (Hoshi et al., 2006). Thus, decreased activation during the cognitive task was not likely to be due to a saturated hemodynamic state in the pre-task baseline in schizophrenia. Second, although we did not find a significant correlation between [oxy-Hb] change and dose of medication, we cannot fully rule out the possible effect of antipsychotics in the observed prefrontal activation in schizophrenia patients. Third, our study design was cross-sectional and used chronic patients. Investigations into longitudinal relationship between NIRS and functional outcome should be an important next step. Fourth, spatial resolution for detecting hemodynamic response from the scalp surface using NIRS is lower than that of fMRI and PET. Future investigations should conduct a simultaneous measurement of NIRS and fMRI, which is technically possible (Strangman et al., 2002b), using a cognitive task directly segregating frontopolar, dorsolateral, and ventrolateral prefrontal cortex (Koechlin et al., 1999).

Further, the difficulty in making a real-time measurement of the accurate differential pathlength factor (DPF) in vivo is one of the major considerations regarding data accuracy of NIRS method. In this continuous-wave NIRS system, "hemoglobin concentration change*DPF" (ΔC*L) is calculated as a solution to the simultaneous equations based on the modified Beer–Lambert law.

It should be also noted that controversies exist regarding DPF in NIRS measurement. Some researchers have estimated the DPF value by one-channel time-resolved NIRS system and have incorporated it into the calculation of the modified Beer–Lambert law. However, if one uses a one-channel time-resolved NIRS system, one could detect the sum of 'partial optical pathlengths within the cerebral and extracerebral tissues' in another session, but could not make a real-time measurement of the precise 'optical pathlength within the cerebral tissue' (Hoshi, 2003). Since commonly used NIRS systems employ the multiple wavelengths, the incorporation of one constant DPF value of a certain wavelength estimated from one-channel time-resolved NIRS system into the calculation of the modified Beer–Lambert law in all the multi-channels would not necessarily mean the improvement of accuracy. It is for this reason that we examined the NIRS signals including DPF (ΔC*L) with clinical evaluation in schizophrenia, according to the previous researches that have reported the results of ΔC*L closely agreed with various clinical data (Fallgatter et al., 1997; Kameyama et al., 2006; Matsuo et al., 2002; Suto et al., 2004).

Meanwhile, Zhao et al. (2002) used a Monte Carlo simulation to report the estimated DPF in various brain regions and suggested that the estimated DPF variation in the forehead region of adult humans was regarded as roughly homogeneous (in accordance with Ferrari et al., 1993). Also, from a practical point of view, the characteristics of time course pattern in the NIRS signals (ΔC*L) of the prefrontal cortex was found to be significantly different between mental disorder groups and healthy control group during verbal fluency task, but not during motor activation task (finger tapping that is cognitively less-demanding task) (Kameyama et al., 2006; Suto et al., 2004). These results suggest that only the difference of DPF could not account for the between-group difference in the NIRS signals (ΔC*L) of the prefrontal cortex during verbal fluency task.

However, to improve the accuracy of NIRS data, when feasible, the technology for the real-time measurement of the estimated DPF at each channel and the incorporation into the calculation of the modified Beer–Lambert law would be an issue for the future NIRS study.

4.4. Conclusions

In conclusion, our study suggested reduced hemodynamic response in frontopolar sub-region of prefrontal cortex during executive task and its relationship with functional impairment in patients with schizophrenia. NIRS may be a candidate biological marker for objectively monitoring the functional level in schizophrenia which may be potentially useful not only for clinicians, but also for consumers and families with severe mental illness such as schizophrenia.

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Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Ryu Takizawa, Kiyoto Kasai, Masato Fukuda designed the study and wrote the protocol. Ryu Takizawa and Kiyoto Kasai undertook the statistical analysis. Ryu Takizawa, Kiyoto Kasai, Yuki Kawakubo, and Kohei Marumo conducted data acquisition. Ryu Takizawa, Kiyoto Kasai, Shingo Kawasaki, and Hidenori Yamasue analyzed the data. Ryu Takizawa and Kiyoto Kasai wrote the first draft of the manuscript, and the other authors revised it critically for important intellectual content. All authors have approved the final version of the manuscript.

Conflict of interest

Drs. Kasai, Kawasaki, and Fukuda have potential conflict of interest (please see below for details). Other authors have no relevant conflict of interest.

Dr. Kiyoto Kasai: Since July 31, 2003 through present, the University of Tokyo and The Research and Developmental Center, Hitachi Medical Corporation has had an official contract for a collaborative study on clinical application of near-infrared spectroscopy in psychiatric disorders, which has been approved by the Research Promotion Office, University of Tokyo Hospital. The principal investigator of this study is Kiyoto Kasai. For this study, Hitachi Medical Corporation provided a project grant (JPY 300,000/year) and material support (temporary rental of a near-infrared spectroscopy (Optical Topography) machine, ETG-4000).

Dr. Shingo Kawasaki: His contribution to this study was in part thorough his role as an employee of Hitachi Medical Corporation. Since May 17, 2002 through present, Gunma University and Hitachi Group (Advanced Research Laboratory, Hitachi Ltd. and the Research and Developmental Center, Hitachi Medical Corporation) have had the official contract for a collaborative study on clinical application of near-infrared spectroscopy in psychiatric disorders. The principal investigator of this study is Masato Fukuda. For this study, Hitachi Group provides a project grant (JPY 1,000,000–1,500,000/year) and material support (temporary rental of a near-infrared spectroscopy (Optical Topography) machine, ETG-4000). Since July 31, 2003 through present, Tokyo University and Hitachi Medical Corporation (Application Development Office, Optical Topography Group) have had an official contract for a collaborative study on clinical application of near-infrared spectroscopy in psychiatric disorders. The principal investigator of this study is Kiyoto Kasai. For this study, Hitachi Medical Corporation provided a project grant (JPY 300,000/year) and material support (temporary rental of a near-infrared spectroscopy (Optical Topography) machine, ETG-4000).

Dr. Masato Fukuda: Since May 17, 2002 through present, Gunma University and Hitachi Group (Advanced Research Laboratory, Hitachi Ltd. and The Research and Developmental Center, Hitachi Medical Corporation) has had an official contract for a collaborative study on clinical application of near-infrared spectroscopy in psychiatric disorders. The principal investigator of this study is Masato Fukuda. For this study, Hitachi Group provided a project grant (JPY 1,000,000–1,500,000/year) and material support (temporary rental of a near-infrared spectroscopy (Optical Topography) machine, ETG-4000).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2007.10.025.

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ORIGINAL PAPER

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Reduced planum temporale volume and delusional behaviour in patients with schizophrenia

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☐ **Abstract** The structural abnormality of planum temporale (PT), a part of the superior temporal heteromodal association cortex involved in auditory and language processing, has been implicated in the pathophysiology of schizophrenia. However, its relationship to clinical manifestations remains unclear. Magnetic resonance images were obtained from 17 right-handed Japanese men with schizophrenia and from 22 age-, handedness-, and parental socioeconomic-status-matched healthy Japanese men in order to manually evaluate grey matter volumes of Heschl's gyrus (HG) and PT. Psychiatric symptoms were assessed using positive and negative syndrome scale among the patients. Compared with healthy participants, patients with schizophrenia were associated with a statistically significant PT grey matter volume reduction without left or right lateralization, whereas HG volume was preserved. Smaller right PT volume was significantly correlated with more severe delusional behaviour in the patients. Previous investigations have focused on smaller-than-normal left PT in the pathophysiology of schizophrenia; however, the present results suggest a possible role of the right PT, which is involved in social cognition such as understanding the intentions of others, in the production of psychotic experiences in patients with schizophrenia.

☐ **Key words** schizophrenia · MRI · planum temporale · superior temporal gyrus · delusion

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Introduction

In the past decade, accumulated evidence has suggested that schizophrenia is a brain disorder with frontal and temporal lobe abnormalities even at brain morphological level (reviewed in refs. [15, 21, 32]). Schizophrenia is also clinically characterized by psychotic experiences such as auditory hallucinations and delusions (reviewed in ref. [7]). Since these characteristic clinical manifestations might originate from brain pathology, the relationship between brain structural abnormality and psychotic experiences have been examined by numerous previous studies (e.g. [33]). However, previous literature is inconsis-

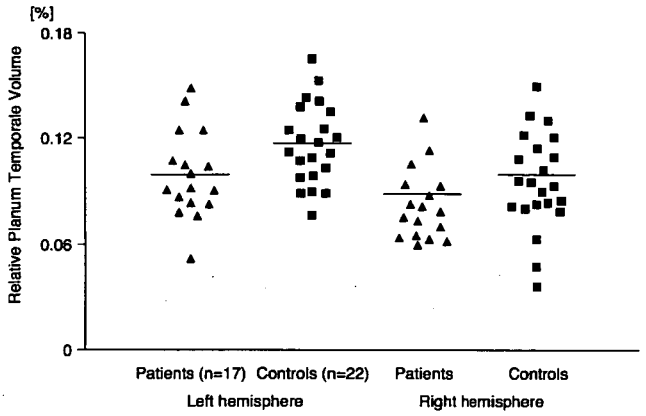

Fig. 1 Left and right volumes of the planum temporale for patients with schizophrenia (n = 17) and controls (n = 22). Horizontal lines indicate means

tent on the neuroanatomical correlates of psychotic experience.

The temporal lobe has long been a focus of interest with regard to the origins of positive symptoms (reviewed in ref. [26]. In previous studies, reduced grey matter volume of the superior temporal gyrus (STG) (e.g. [2, 12, 16, 27, 33, 40]) as well as that of Heschl's gyrus (HG) and planum temporale (PT) [4, 11, 17, 20, 27, 28, 34, 36, 42] have been consistently demonstrated in patients with schizophrenia. It has been also suggested that the volume reduction of these superior temporal structures was a specific feature of patients with schizophrenia, compared with patients with affective psychosis [11, 12, 16, 17].

The STG is further decomposed into the HG, which mainly contains primary auditory cortex, and the PT, which lies in the most posterior portion of STG and contains auditory association and Wernicke's language area in left hemisphere. Based on the prior knowledge of their contributions to auditory and language function in humans, HG and PT have been thought as candidates for the neural basis of language-related psychotic symptoms such as auditory hallucinations and thought disorder in patients with schizophrenia (Fig. 1).

Our previous study showed a significant association between left smaller PT volume and reduced phonetic mismatch strength in left hemisphere [40, 42]. Based on the finding, it was predicted that structural abnormalities of PT could underlie the functional abnormalities of fundamental language-related processing such as thought disorder in schizophrenia. In line with the hypothesis, Sumich et al. [35] employed "delusional behaviour", a composite score of delusions, grandiosity, suspiciousness and unusual thought disorder, to examine functional correlates of structural abnormality of PT in patients with first episode psychosis.

Several previous studies have also reported a relationship between positive symptoms and STG structural abnormalities (e.g. [2, 20, 23, 33]).

However, a comparable number of studies have reported no significant results regarding the symptom–structure correlation (e.g. [11, 30, 31]). The present study was thus designed to examine the following issues. First, it was expected that volume reduction of left PT would be replicated in Japanese men with schizophrenia. Since previous studies have reported sex dimorphism [14, 19, 39] and ethnic differences [37, 43] in brain structures, the current study population was limited to Japanese males. Second, the relationship between the positive symptoms and the volume of HG and PT was investigated.

Method

Participants

Demographic data of participants are shown in Table 1. Seventeen male right-handed in- and out patients with schizophrenia were recruited from the Department of Neuropsychiatry, the University of Tokyo Hospital, Japan. Handedness were determined using the Edinburgh Inventory [25]; a laterality index of >0.8 was the cut-off for right-handedness. Diagnosis of schizophrenia was determined for each patient according to DSM-IV [1] criteria through the Structured Clinical Interview for DSM-IV Axis I Disorder (SCID-I) Clinical Version [5]. Psychiatric symptoms were evaluated using the Positive and Negative Syndrome Scale (PANSS; [18]. The

Table 1 Demographic characteristics of study participants

	Patients with schizophrenia (n = 17)		Controls (n = 22)		t-test	
Variables	Mean	SD	Mean	SD	t-value	p
Age (range)	29.5	5.5	29.1	2.8	0.29	n.s.
Education (years)	13.1	1.9	17.0	1.0	8.46	<0.01
SES[a]	4.2	0.9	1.6	0.5	11.6	<0.001
Parental SES[a]	2.5	0.6	2.4	0.6	0.62	n.s.
Neuroleptic dose[b] (mg/day)	429.8	337.2	–	–		
Onset of illness (years)	20.6	4.8	–	–		
Duration of illness (years)	8.8	4.9	–	–		
Positive symptoms	13.8	5.7	–	–		
Negative symptoms	17.8	2.9	–	–		
General psychopathology	33.8	6	–	–		

[a] Socioeconomic status, assessed using the Hollingshead. Higher scores indicate lower status
[b] Based on chlorpromazine equivalents

interview for symptom evaluation and diagnosis was done by a trained psychiatrist (H.Y.) within three days prior to MRI scanning. The schizophrenia subtypes present in the sample were: catatonic (n = 2), paranoid (n = 3), undifferentiated (n = 1), and residual (n = 11). Twelve patients received typical neuroleptics only; 2 received risperidone only; 3 received both.

Twenty-two right-handed age-, gender and parental socioeconomic status (SES)-matched healthy participants were employed as controls. SES was assessed using the Hollingshead scale [13]. There were no significant differences between the patients with schizophrenia and controls in age and parental SES, although the patients had significantly fewer years of education and lower socioeconomic status than controls (Table 1).

No participants in the present study were included in our previous study employing a different MRI acquisition sequence to measure the grey matter volumes of HG and PT [42]. To test the validity of intracranial volume measurement using optimized voxel-based morphometry (VBM) compared with the intensity based semi-automated method employed in our previous study [40], the MRI images were obtained from a completely different sample of 50 adult participants (see Sect. "MRI processing" for details).

The exclusion criteria for both groups were neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, a history of electroconvulsive therapy, and substance abuse or addiction. An additional exclusion criterion for the control group was a history of psychiatric disease in themselves or a family history of axis I disorder in their first-degree relatives. The ethical committee of the University of Tokyo Hospital approved this study. After a complete explanation of the study to the participants, written informed consent was obtained.

☐ MRI acquisition

The methods of MRI acquisition were described in detail elsewhere [40, 41]. Briefly, MRI data were obtained on a 1.5-Tesla scanner (General Electric Signa Horizon Lx version 8.2, GE Medical Systems, Milwaukee, WI, USA). For manual measurement of brain structures, a three-dimensional (3D) spoiled gradient recalled acquisition with steady state (SPGR) sequence was used. Of note, this sequence affords better contrast between the grey matter and white matter than the fast-SPGR sequence in our earlier study evaluating grey matter volumes of HG and PT [42]. The repetition time was 35 ms, the echo time 7 ms with one repetition, the nutation angle 30°, the field of view 24 cm, and the matrix 256 × 256 (192) × 124. A trained neuroradiologist (Ha.Ya. or O.A.) evaluated the MRI scans and found no gross abnormalities in any participants. Magnetic field inhomogeneity in our scanner was monitored with daily basic quality control, and has been stable over the MR acquisition time for this study.

☐ MRI processing

The HG and PT grey matter regions of interest (ROIs) were outlined manually using a software package for medical image analysis (3D Slicer; software available at http://www.slicer.org) without knowledge of diagnosis. The landmarks to delineate HG and PT grey matter were similar to those described in elsewhere [11, 17] and the same as that employed in our previous fast-SPGR study [42]. Briefly, HG was first identified in the axial plane, a demarcation that helped pinpoint the location of HG on coronal images. In most cases, HG represented a single transverse convolution. In cases where more than one transverse convolution was present, we followed the literature definition; when multiple convolutions originated medially from a common stem, all were defined as HG (the sulcus (i) between these convolutions represents the sulcus intermedius of Beck); when they originated separately from the retroinsular regions, only the most anterior gyrus was labeled as HG, and more posterior gyri were identified as PT. The posterior border of HG (Heschl's sulcus) defined the anterior border of PT. Posteriorly, PT grey matter was traced on coronal images to the end of the Sylvian fissure, and the grey matter of the ascending ramus of the Sylvian fissure was also included. Thus, our definition of the PT included PT proper and its parietal extension. Once drawn, both HG and PT ROIs could be viewed in any plane and as a 3-dimensional object, for any further editing.

For measuring intracranial content (ICC), total grey matter, white matter, and cerebrospinal fluid, volumes were calculated from the optimized-VBM procedure [8]. Then ICC was calculated by summing up total grey matter, white matter, and cerebrospinal fluid volume. To validate this method, the ICCs of the pooled 50 participants were measured by both the optimized VBM and intensity-based semi-automated segmentation procedure using ANAYZE PC 3.0 [40]. The inter-method reliability was high (intraclass correlation coefficient = 0.96).

In most cases, HG represented a single transverse convolution (left: 88% of patients with schizophrenia, 91% of control participants; right: 88% of patients with schizophrenia, 77% of control participants). In the cases of more than one transverse convolution, we followed the definition used in the previous study [17]. The prevalence of double HG, multiple transverse gyri from a common stem arising separately, was not significantly different between groups (left HG: $[\chi^2] = 0.07$, $p = 0.78$; right HG: $[\chi^2] = 0.78$, $p = 0.38$).

For interrater reliability of the volumetric measurements, two raters (S.Y. and H.Y.), blind to group membership, independently traced ROIs. Ten cases were selected at random, and the raters traced ROIs on every slice. The intraclass correlation coefficient was 0. 97/0.91 for left/right HG grey matter, 0.91/0.92 for left/right PT grey matter, respectively. Intrarater reliability, computed using all of the slices from one randomly selected brain and measured by one rater (S.Y.) at two separate times (approximately 6 months apart), was >0.97 for all structures.

☐ Statistical analysis

Group comparison

The relative volumes [(absolute ROI volume)/(ICC) × 100] were used as the dependent variable as is the standard method for MRI studies in schizophrenia (e.g. [3, 16, 17, 33]). We employed a repeated measure ANOVA with 1 between-subject factor (group: schizophrenia, controls) and 2 within-subject factors (hemisphere: left and right; region: HG and PT). Once a significant group-by-region or group-by-region-by-hemisphere interaction was found, follow-up analyses using repeated measures ANOVA separately for each region (HG or PT) were performed.

Correlational analysis

The association between the relative volume of ROI, which showed a significant group difference, and the severity of psychiatric symptoms was tested with Spearman's rank correlation in the patients group. In the correlation analysis, positive, negative and general psychopathology scores of PANSS were used. Delusional behaviour score was also used to investigate the relationship between unreality symptoms and the volumetric variables. Delusional behaviour was scored as the sum of P1 (delusions), P5 (grandiosity), P6 (suspiciousness) and G9 (unusual thought content), based on Sumich et al. [35]. Since our approach was hypothesis-driven, a statistically significant level was defined as $p < 0.05$ without Bonferroni correction.

Results

☐ Volumes of ROIs

The absolute and relative volumes for HG and PT in the two groups were displayed in Table 2. The repeated-measures ANOVA of relative volumes did not

Table 2 Volumetric measures

Variables		Patient schizophrenia with (n = 17) Mean	Controls (n = 22) SD	Repeated measure ANOVA (follow-up analyses) Mean	SD	F	p
Absolute volume (ml)							
Intracranial contents		1680.4	142.2	1660.5	98.9		
HG	Left hemisphere	1.38	0.27	1.27	0.41		
	Right hemisphere	1.09	0.29	1.20	0.36		
PT	Left hemisphere	1.66	0.42	1.93	0.39		
	Right hemisphere	1.37	0.34	1.57	0.43		
Relative volume (%)							
HG	Left hemisphere	0.083	0.016	0.076	0.023	0.01	0.930
	Right hemisphere	0.064	0.016	0.072	0.019		
PT	Left hemisphere	0.099	0.025	0.116	0.023	6.50	0.015
	Right hemisphere	0.082	0.020	0.095	0.027		

HG: Heshl's gyrus, PT: Planum temporale

Table 3 Relationships between anatomical measures and psychotic symptoms

	Left PT		Right PT	
	Relative volume	Absolute volume	Relative volume	Absolute volume
Positive symptoms	0.19	0.30	−0.46**	−0.48**
Negative symptoms	0.14	0.12	0.20	0.17
General symptoms	−0.28	−0.34	−0.08	−0.21
Delusional behaviour	−0.02	0.08	−0.49*	−0.55*

* $p < 0.05$, ** $p < 10$
PT: Planum temporale

show a significant main effect of diagnosis (F [1, 37] = 3.11, p = 0.086); however, there was a significant group-by-region interaction (F [1, 37] = 4.56, p = 0.039). There was no group-by-hemisphere (F [1, 37] = 0.49, p = 0.49) or group-by-region-by-hemisphere interaction (F [1, 37] = 3.26, p = 0.079). Then we conducted follow-up analyses separately for HG and PT. For HG, there was no main effect of group (F [1, 37] = 0.01, p = 0.93). We found a group-by-hemisphere interaction for HG (F [1, 37] = 4.70, p = 0.037); however, this was not considered significant due to a lack of significant group-by-hemisphere or group-by-region-by-hemisphere interaction in the main analysis. For PT, there was a significant main effect of group (F [1, 37] = 6.50, p = 0.015), whereas there was no significant group-by-hemisphere interaction (F [1, 37] = 0.19, p = 0.67). These results suggest a total (left + right), that is, unlateralized, reduction of PT grey matter volume, and HG grey matter volume in patients with schizophrenia comparable with that of the control participants.

☐ Correlational analyses

The results from the correlational analyses are summarized in Table 3. The relative volume of right PT showed a significant negative correlation with the score of delusional behaviour (R = −0.49, p = 0.047).

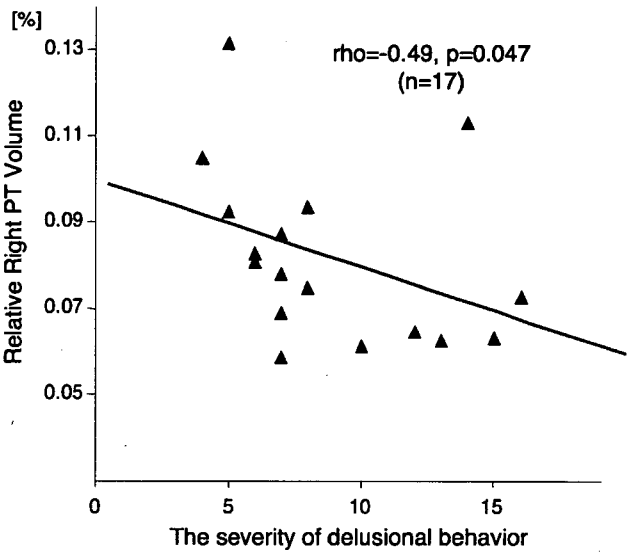

Fig. 2 Scatter plots depicting correlations between the right PT volume and the severity of delusional behaviour

Of note, the statistical conclusion remained the same when the absolute volume was used (R = −0.55, p = 0.023). The relative (R = −0.46, p = 0.061) and absolute (R = −0.48, p = 0.051) volumes of right PT were negatively correlated with the positive symptom severity, although the significance level of the correlations remained at the trend level. There were no significant correlations between the left PT volume and severity of psychiatric symptoms (Fig. 2).

Discussion

The present study identified a statistically significant PT grey matter volume reduction without left or right lateralization in male patients with schizophrenia compared with healthy controls. In contrast, HG volume did not differ significantly between the patient

group and the control group. Furthermore, the smaller right PT volume was associated with more severe delusional behaviour in the patients.

The observed pattern of reduced PT and preserved HG in our study is generally in line with those of previous studies examining chronic [20, 42] and first episode patients with schizophrenia [34], although two studies examining samples of first-episode schizophrenia [11, 17] have found reduced HG volume as well as the PT volume reduction. However, our findings of non-lateralized reduction of PT were in contrast to previous studies reporting left-lateralized PT volume reduction [11, 20, 42], which deserves further discussion. Although the etiology of brain lateralization remains unclear, gender [19], culture and/or ethnicity [22] have been implicated in the normal hemispheric lateralization. The normal sexual dimorphism of superior temporal cortices has been reported by previous studies [10, 14, 19]. Given that all participants in the present study were males, in contrast to our previous study [42], a possible interaction between gender and diagnosis might account for the discrepancy in findings related to laterality. In addition, the posited role of the STG in the right hemisphere in functions such as discourse comprehension, discourse production, understanding metaphors, and humour [24] may be related to differences in culture and language. Therefore, cultural and/or ethnic differences might affect the pathophysiology of right STG in patients with schizophrenia. However, the pure ethnicity as well as pure gender of the current study sample might contribute to the clarity of the current findings. In line with this notion, a recent study [36] reported bilateral PT volume reduction in Japanese patients with schizophrenia compared with healthy Japanese individuals, although they reported that volume reduction was greater in left PT than in right PT. Furthermore, the patients with schizophrenia in the present study consisted mainly of chronically treated patients. Although the volumes of STG structures did not show any significant correlation with duration of illness or neuroleptic dose, the findings in the present study cannot totally rule out the possibility of subtle morphological change associated with chronic illness [16, 17] and medication [9]. Taken together, ethnicity, gender, chronic illness course and their possible interactions might contribute to the discrepancy of findings related to laterality between previous studies and the present investigation.

In the present study, the smaller grey matter volume of right PT showed significant correlation with the more severe delusional behaviours within the patient group. Although previous findings have emphasized the importance of left, but not right PT in the pathophysiology of schizophrenia (e.g. [2, 20, 23]), the present results indicate that right hemisphere PT may play a role in the production of psychotic experiences in patients with schizophrenia. Recently, a possible role of the right STG in human social cognition has received attention, whereas the left STG is a well-known center for language function. In order to compete and survive in our highly organized society, it is important to have the ability to recognize and relate to other members of the community [29]. In other words, human survival has depended to a large extent on accurate social judgment. Winston et al. [38] found that right superior temporal cortex showed enhanced signal change during explicit trustworthiness judgments in a recent functional MRI study. Although speculative, impaired social cognition such as intention reading in schizophrenia is at least in part mediated through right STG abnormalities, which may underlie the basis for production of delusional symptoms.

Supporting this speculation, Mitchell and Crow [24] suggest an important role of the right STG in discourse comprehension, discourse production, and understanding metaphors, indirect requests, humour and emotional prosody and its abnormality in schizophrenia. In addition, there is further supportive evidence of a right hemisphere link with delusional misidentification syndromes [6].

Conclusion

In conclusion, the present study demonstrated a significant PT grey matter volume reduction without hemispheric lateralization in male patients with schizophrenia compared with matched healthy controls. The smaller right PT volume further showed a significant association with more severe delusional behaviour in the patient group. These results suggest an important role of right hemisphere PT, which is involved in social cognition such as intention reading, in the generation of delusional behaviours in patients with schizophrenia.

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

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Abstract

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

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

1. Introduction

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

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

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

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

Table 1
Previous studies on BDNF levels of patients with schizophrenia

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

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

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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

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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

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