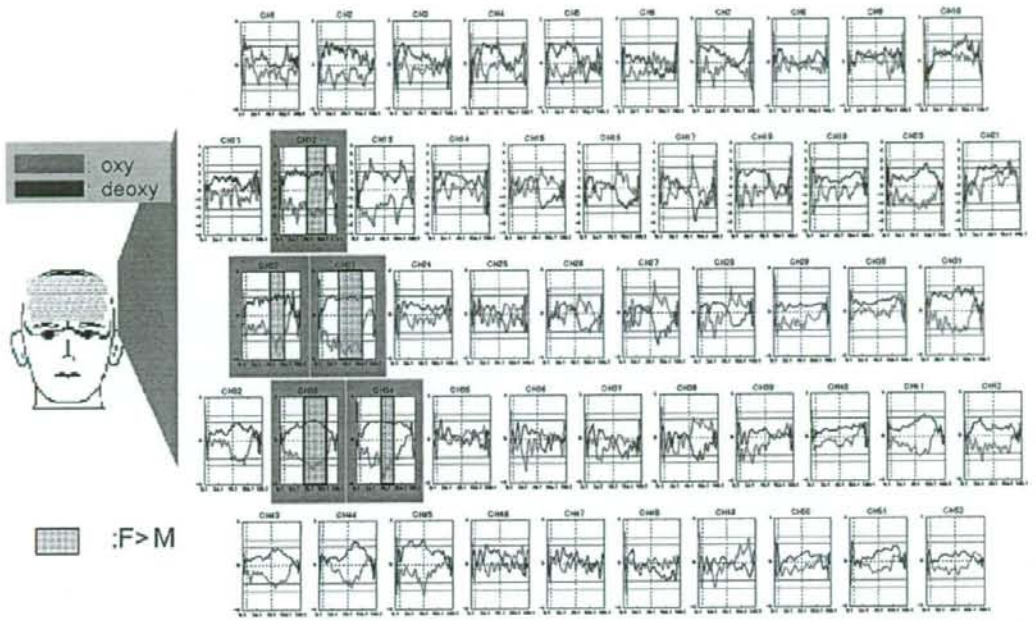

Fig. 3. *t*-value graphs of [oxy-Hb] comparison between the groups during emotional activation. F, female group; M, male group. The blue and red lines in each *t*-graph correspond to the statistical significance levels of 5% and 1%, and when the [oxy-Hb] changes in the females were significantly larger than those in the males, the times with significant differences in each graph are marked red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

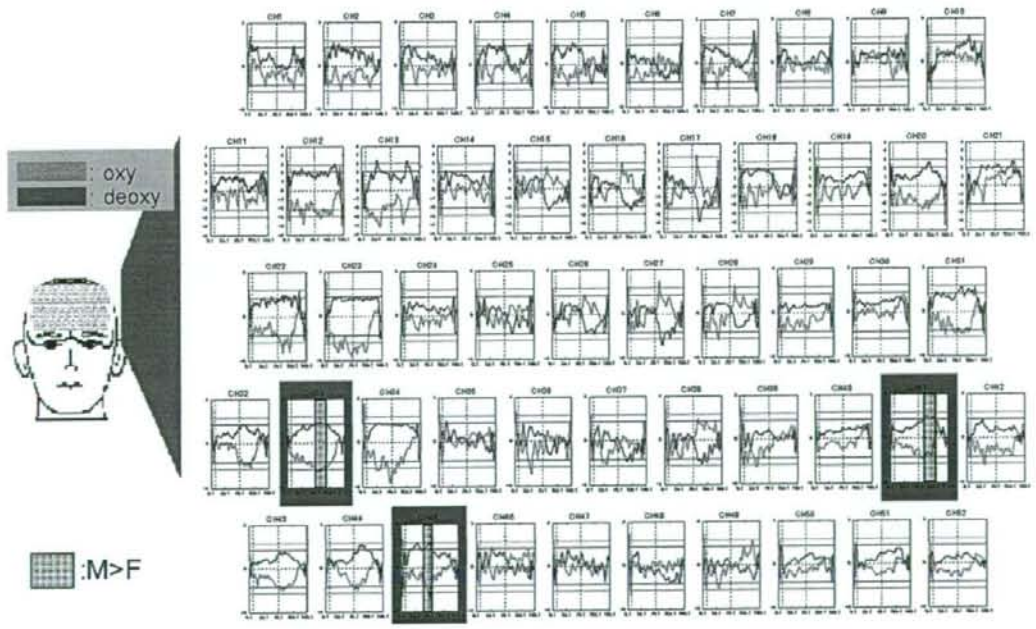

Fig. 4. *t*-value graphs of [deoxy-Hb] comparison between the groups during emotional activation. F, female group; M, male group. The blue and red lines in each *t*-graph correspond to the statistical significance levels of 5% and 1%, and when the [deoxy-Hb] changes in the males were significantly larger than those in the females, the times with significant differences in each graph are marked blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

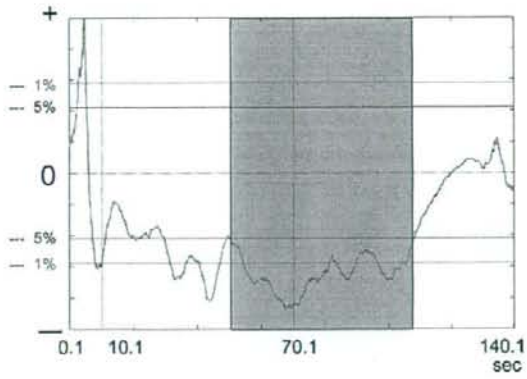

Fig. 5. Typical pattern of gender difference about [oxy-Hb] on a single channel (ch23) enlarged figure in Fig. 3. The *t*-value graph of [Oxy-Hb] consecutively reached a significance level of 5% during red marked period. In this channel, negative *t*-value was found, which indicated that [oxy-Hb] change of female subjects was larger than that of males. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

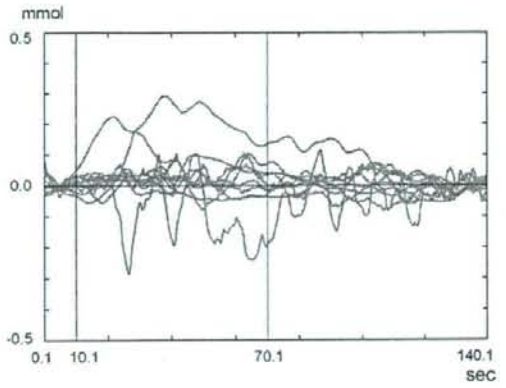

Fig. 6. All female subjects' superimposed figure about [oxy-Hb] in the significant channel (ch23).

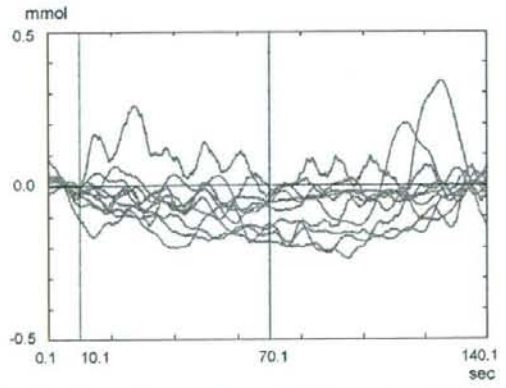

Fig. 7. All male subjects' superimposed figure about [oxy-Hb] in the significant channel (ch23).

premotor cortex activity. Wild et al. (2001) reported that females were more susceptible to emotion contagion than males. Also, Risch et al. (1981) reported that females are more likely to show depression and anxiety. Taken together with the present findings this might reflect a gender difference of vulnerability to emotion-related psychiatric disorders such as depression and anxiety disorder. These results lead to the possibility that, with further investigation, NIRS measurement might play a role in the development of gender-specific evaluation and treatment of psychiatric disorders.

4.2. Ventrolateral prefrontal activation in emotional task

Ventrolateral PFC (BA 44, 45 and 47; also known as inferior frontal gyrus) is thought to be involved in emotional processing. Activations in the right mid-ventrolateral prefrontal cortex were found to be correlated with the correct retrieval conditions for negative words (Kuchinke et al., 2006). In the present study a significant difference in [deoxy-Hb] between males and females was identified in bilateral ventrolateral PFC. Therefore, these results suggest that ventrolateral cortical function is involved in emotional processes, at least with respect to response to fearful facial expressions.

4.3. NIRS analysis along the time-course of the task

NIRS signals analysis along the time-course in the ventrolateral PFC (Figs. 3 and 4), revealed a larger gender difference emerging from the mid to the end of the task period. In the female subjects, a small [oxy-Hb] increase was found in the early task period and the activation persisted during the whole task. In contrast, male subjects showed no significant [oxy-Hb] increase at any stage of the task, but did show a [oxy-Hb] decrease in the latter half of the task period.

These gender differences of [oxy-Hb] might have been exposed by the task design. In this study, subjects were not told anything about the expression of the faces they would be shown, and were instructed only to answer whether each face was male or female. They were not told explicitly that they would encounter fearful faces and that the pictures would be change from neutral to fearful faces at the start point of the task period and change again from fearful to neutral faces at the end of the task. Subjects were also not alerted to the start or end of the task period, so it might have taken some time before they consciously noticed the facial expressions, if indeed they did at all. Therefore, any prefrontal hemodynamic response to viewing fearful faces might have been slow and small. Several studies (Laura et al., 2002) showed females are more sensitive to negative emotional stimuli than males. Gender difference in brain activation in the present study was in line with the results of earlier research. In this study, most male subjects might not have been aware of the change in the facial expressions presented, whereas female subjects might possibly have recognized the fearful facial expression either consciously or unconsciously. This gender difference in emotional sensitivity to facial recognition might be reflected in gender difference in NIRS signals, especially during the latter half of the task period. These findings highlight the ability of NIRS to analyze temporal changes in the pattern of hemodynamic response.

4.4. Right brain hemisphere activation in gender difference

According to both the right-hemisphere hypothesis (Borod et al., 1998) and valence hypothesis (Ahern et Schwartz, 1979), negative emotions are thought to be processed by the right hemisphere of the brain. The right-hemisphere hypothesis

postulates that the right hemisphere is dominant for the expression and perception of emotion, regardless of valence (i.e., pleasantness level). The valence hypothesis, however, states that the right hemisphere is dominant for negative/unpleasant emotions and the left hemisphere is dominant for positive/pleasant emotions (Borod et al., 1998). In the present study, the channels with significant gender difference of [oxy-Hb] response to the fearful stimuli were situated approximately in the right ventrolateral PFC and premotor cortex. Our results of significant gender difference in right prefrontal hemodynamic response are therefore consistent with these two hypotheses.

4.5. Limitations

There are some limitations in this study. First, NIRS cannot detect activities in deeper structures such as limbic regions that are also thought to be key to emotional process. However, in considering clinical application and translational approach, this limitation might be compensated by the several advantages of NIRS apparatus, such as non-invasiveness, convenience, portability and restraint-free environment. Second, multi-channel NIRS has lower spatial resolution relative to functional MRI. However, Okamoto et al. (2004) demonstrated that the NIRS channels that are set according to the international 10-20 system used in electroencephalography can identify the difference between gyri or Brodmann' areas. Third, subjective ratings for the degree of response to the fearful stimuli were not available in the present study.

5. Conclusions

The effects of gender difference and trait anxiety on prefrontal hemodynamic response to emotional stimuli were investigated by multi-channel NIRS, a non-invasive functional neuroimaging technique. This study demonstrated that, during the fearful facial expression task, women showed significantly greater activation in the right ventrolateral prefrontal area than did men. Further, [deoxy-Hb] change in the frontopolar region was significantly associated with trait anxiety in the combined sample. These results suggest that gender and trait anxiety have an effect on individual variability of NIRS signals in response to an emotional task. Our observation may provide useful information to establish NIRS assessment as a useful objective tool for monitoring clinical status in psychiatric disorders on an individual basis.

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Reduced frontopolar activation during verbal fluency task in schizophrenia: A multi-channel near-infrared spectroscopy study

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Abstract

Functional neuroimaging studies to date have shown prefrontal dysfunction during executive tasks in schizophrenia. However, relationships between hemodynamic response in prefrontal sub-regions and clinical characteristics have been unclear. The objective of this study is to evaluate prefrontal hemodynamic response related to an executive task in schizophrenia and to assess the relationship between activation in the prefrontal sub-regions and clinical status. Fifty-five subjects with schizophrenia and age- and gender-matched 70 healthy subjects were recruited for this case-control study in a medical school affiliated hospital in the Tokyo metropolitan area, Japan. We measured hemoglobin concentration changes in the prefrontal (dorsolateral, ventrolateral, and frontopolar regions) and superior temporal cortical surface area during verbal fluency test using 52-channel near-infrared spectroscopy, which enables real-time monitoring of cerebral blood volumes in the cortical surface area under a more restraint-free environment than positron emission tomography or functional magnetic resonance imaging. The two groups showed distinct spatiotemporal pattern of oxy-hemoglobin concentration change during verbal fluency test. Schizophrenia patients were associated with slower and reduced increase in prefrontal activation than healthy controls. In particular, reduced activations of the frontopolar region, rather than lateral prefrontal or superior temporal regions, showed significant positive correlations with lower global assessment of functioning scores in the patient group, although task performance was not significantly associated with the scores. These results suggest that reduced frontopolar cortical activation is associated with functional impairment in patients with schizophrenia and that near-infrared spectroscopy may be an efficient clinical tool for monitoring these characteristics.

Keywords: Schizophrenia; Near-infrared spectroscopy (NIRS); Frontopolar prefrontal cortex; Verbal fluency test; Global assessment of functioning (GAF)

1. Introduction

Neuroimaging studies have identified schizophrenia as being associated with dysfunction of the prefrontal cortex (Callicot et al., 2000; Carter et al., 1998; Curtis et al., 1998), an area involved in almost all high-level cognitive functions such as working memory, memory

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retrieval, executive function, and language (Cabeza and Nyberg, 2000). Moreover, recent advances in neuroscience have sought to clarify functional segregation in the prefrontal cortical surface areas such as dorsolateral, ventrolateral, and frontopolar (anterior frontal) regions (Daw et al., 2006; Fletcher and Henson, 2001; Fox et al., 2006). The ventrolateral and dorsolateral sub-regions are involved in the updating/maintenance of information and to the selection/manipulation/monitoring of that information, respectively (Fletcher and Henson, 2001). In contrast, frontopolar cortex (BA 10), which has been suggested to have enlarged and become specialized during hominid evolution (Semendeferi et al., 2001), provides higher level of control to coordinate ventrolateral and dorsolateral functions in order to maximize task performance (Koechlin et al., 1999; Fletcher and Henson, 2001; Braver and Bongiolatti, 2002), which has led to the idea that frontopolar region is likely to have a vital role in achieving high-order executive control in everyday life (Burgess et al., 2000). However, it remains unclear which specific sub-regions of the prefrontal cortex is most directly associated with clinical characteristics in schizophrenia.

An independent line of work has suggested that cognitive deficits as indexed by neuropsychological assessments are more tightly coupled with social functioning in patients with schizophrenia than positive or negative symptoms (Green, 1996; Green et al., 2000; Green et al., 2004; Flashman and Green, 2004). Whereas progress has been made for an association between psychosocial impairment and electrophysiological measures such as P300, N200b, and mismatch negativity event-related potentials (Ikebuchi et al., 1996; Iwanami et al., 1999; Kawakubo and Kasai, 2006; Light and Braff, 2005; Ohno et al., 2000) and brain morphological measures (Ho et al., 2003; Milev et al., 2003; Prasad et al., 2005; Staal et al., 2001; Wassink et al., 1999), the relationships between functional hemodynamic response in the sub-regions of the prefrontal cortex and clinical characteristics in schizophrenia has been unclear. These research questions may be an important step towards developing an objective monitoring tool and ultimately an effective intervention strategy for cognitive and social dysfunction in schizophrenia.

Multi-channel near-infrared spectroscopy (NIRS), a recently developed functional neuroimaging technology, enables the non-invasive detection of spatiotemporal characteristics of brain function (Strangman et al., 2002a, 2003; Boas et al., 2004; Huppert et al., 2006). NIRS has enabled non-invasive and bedside measurement of the concentrations of oxy-hemoglobin ([oxy-Hb]) and deoxy-hemoglobin ([deoxy-Hb]), which are assumed to reflect the regional cerebral blood volume (rCBV). While functional brain imaging methodologies such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have an excellent spatial resolution, they are limited in that they require large apparatuses which prevents their use in a bedside setting for diagnostic and treatment purposes. In contrast, NIRS is a neuroimaging modality that, for the following reasons is especially suitable for psychiatric patients (Matsuo et al., 2003a). First, because NIRS is relatively insensitive to motion artifact, it can be applied to experiments that might cause some motion of the subjects such as vocalization. Second, the subject can be examined in a natural sitting position, without any surrounding distraction. Third, the cost is much lower than other neuroimaging modalities and the set-up is very easy. Fourth, the high temporal resolution of NIRS is useful in characterizing the time course of prefrontal activity of psychiatric disorders (Kameyama et al., 2006; Suto et al., 2004). Accordingly, NIRS has been used to assess brain functions in many psychiatric disorders, including schizophrenia, bipolar disorder, depression, dementia, post traumatic stress disorder, and pervasive developmental disorders (Fallgatter et al., 1997; Hock et al., 1997; Kameyama et al., 2006; Kubota et al., 2005; Kuwabara et al., 2006; Matsuo et al., 2003a, b, 2004; Shinba et al., 2004; Suto et al., 2004).

A previous study (Suto et al., 2004) showed reduced [oxy-Hb] changes in the prefrontal cortex during verbal fluency task in patients with schizophrenia using a NIRS machine with insufficient coverage of important sub-regions of the prefrontal cortex such as ventrolateral portions. The purpose of the present study was to use an NIRS machine with a wide coverage of the prefrontal cortex in order to investigate more precisely the relationship between activity in the prefrontal sub-regions and the clinical characteristics in a larger group of patients with schizophrenia.

2. Materials and methods

2.1. Subjects

Fifty-five patients with schizophrenia and 70 age- and gender-matched healthy subjects participated in the study (Table 1). All the participants were right-handed according to the Edinburgh Inventory (Oldfield, 1971) and were native Japanese speakers.

The patients were recruited among outpatients and inpatients at the University of Tokyo Hospital. Diagnosis of schizophrenia was made through the Structured Clinical Interview for DSM-IV Axis I Disorders (First

Table 1
Clinical characteristics of the study groups

	Patients with schizophrenia (N=55)		Healthy subjects (N=70)		Group difference P value
	Mean	SD	Mean	SD	
Age, year	40.1	11.1	37.4	13.6	.22
Gender, women/men	26/29		34/36		.89[a]
Handedness	92.4	17.3	92.9	16.3	.85
Education, year	14.7	2.5	15.6	2.0	.022
Self socio-economic status (SES)	3.4	1.1	2.0	.6	<.001
Parental SES	2.5	.7	2.3	.7	.18
Estimated premorbid IQ	102.3	12.2	108.3	9.8	.006
Number of words generated	14.3	4.6	17.3	4.4	<.001
Age at onset, years	26.4	8.7	NA		
Duration of illness, years	13.8	10	NA		
PANSS					
Positive	16.7	5.6	NA		
Negative	21.6	6.4	NA		
General psychopathology	38.2	7.9	NA		
Global Assessment of Functioning (GAF)	47.2	12.9	NA		
Medication					
Chlorpromazine equivalent dose, mg/day	778	655	NA		
Diazepam equivalent dose, mg/day	13.4	18.5	NA		
Biperiden equivalent dose, mg/day	3.2	2.3	NA		

Abbreviations: IQ, Intelligence Quotient; PANSS, Positive and Negative Symptom Scale; NA, not applicable.
[a] Chi-square test was used for testing group difference. Otherwise, t-test was used.

et al., 1997) by an experienced psychiatrist (K.K.). For screening of healthy subjects, SCID non-patient edition (SCID-NP) was used. On the same day as the near-infrared spectroscopy (NIRS) experiment, psychiatric symptoms and the level of social functioning were evaluated by one psychiatrist (K.K.) using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and the Global Assessment of Functioning scores (GAF) (American Psychiatric Association, 1994), respectively, without knowledge of the NIRS data. At the time of the study, the patients with schizophrenia were on medication with antipsychotics and/or anxiolytics and/or antiparkinsonian agents. Socioeconomic status (SES) and parental SES were assessed using the Hollingshead scale (Hollingshead, 1965). Premorbid IQs were estimated using Japanese version of the National Adult Reading Test (Matsuoka et al., 2006) (Table 1). The reliability of the GAF as an assessment of social functioning was confirmed based on the high correlation between GAF scores and total scores on the Japanese version of Life Skills Profile (N=55, r=.61, P<.001) (Parker et al., 1991; Japanese version, Hasegawa et al., 1997).

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 alcohol/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 Hospital of Tokyo University approved this study. All subjects gave written informed consent according to the Declaration of Helsinki after a complete explanation of the study.

2.2. Activation task

[Hb] changes were measured during a cognitive activation task. Each participant sat on a comfortable chair with their eyes open throughout the measurements. The subjects were instructed to minimize movement such as head movements, strong biting and eye blinking during the NIRS measurements, for they can produce artifacts or changes in cerebral perfusion unrelated to the task.

The cognitive activation task included a 30-s pre-task baseline, a 60-s verbal fluency task (letter version), and a 70-s post-task baseline. The verbal fluency test was chosen, because it has been often used for cognitive activation in NIRS studies, and previous reports showed measurable prefrontal activation during the letter fluency task in healthy subjects (Herrmann et al., 2003, 2006; Kameyama et al., 2004). This procedure was similar to that of Suto et al. (2004), Ito et al. (2005) and Kameyama

et al. (2006) except for the use of a 70-s post-task baseline instead of their 60 s, to enable a more complete return of [Hb] change to the baseline in the post-task period.

For the pre- and post-task baseline periods, the subjects were instructed to repeat Japanese vowels (/a/, /i/, /u/, /e/ and /o/) aloud. This was intended to correct the data during the fluency task for activation due to vocalization.

During the verbal fluency task period, they were instructed to generate as many Japanese words beginning with a designated syllable as possible, which is commonly used in Japanese letter version of the verbal fluency task since Japanese words inevitably begin with a vowel or consonant-vowel syllable. The three initial syllables (first; /to/, /a/, or /na/, second; /i/, /ki/, or /se/, third; /ta/, /o/, or /ha/) were presented in the order which was counterbalanced among the subjects and changed every 20 s during the 60-second task to reduce the time during which the subjects remained silent. The subjects were instructed by an auditory cue at the start and end of the task and when the syllable was changed. Because the number of words generated was not significantly different among the three initial syllables (one-way repeated measures ANOVA; $F[2, 123]=1.28$, $P=.28$, n.s.), the total of correct words generated during verbal fluency tasks was defined as a measure of task performance (Table 1).

2.3. NIRS measurement

The 52-multi-channel NIRS machine (ETG-4000, Hitachi Medical Co.) measures relative changes of [oxy-Hb] and [deoxy-Hb] using two wavelengths (695 nm and 830 nm) of infrared light based on the modified Beer–Lambert law (Yamashita et al., 1996). The [total-Hb] was calculated as the sum of [oxy-Hb] and [deoxy-Hb]. In this system, these [Hb] values include differential pathlength factor (DPF). The distance between pairs of source-detector probes was set at 3.0 cm and we defined each measuring area between pairs of source-

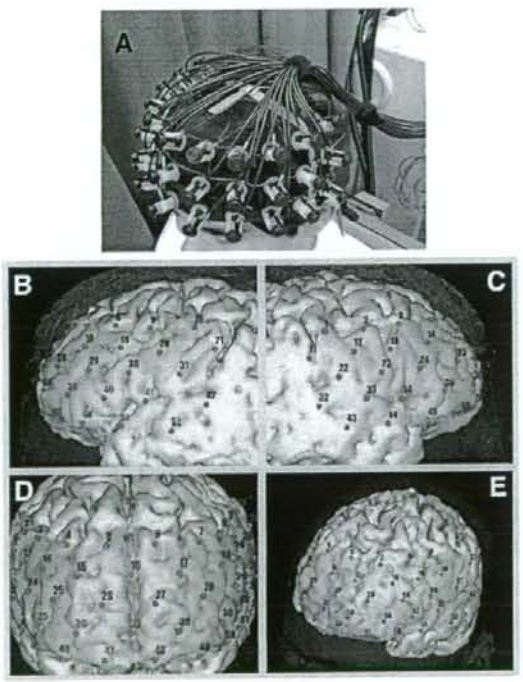

Fig. 1. The probe setting and measurement points of 52-channel near-infrared spectroscopy (NIRS). Panel A: the probes with thermoplastic 3×11 shells were placed over a subject's bilateral frontal regions. Panels B–E: the 52 measuring positions of the NIRS machine are superimposed on 3D-reconstructed cerebral cortical surface from magnetic resonance imaging of a representative subject. The channel numbers are indicated above the measuring points.

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

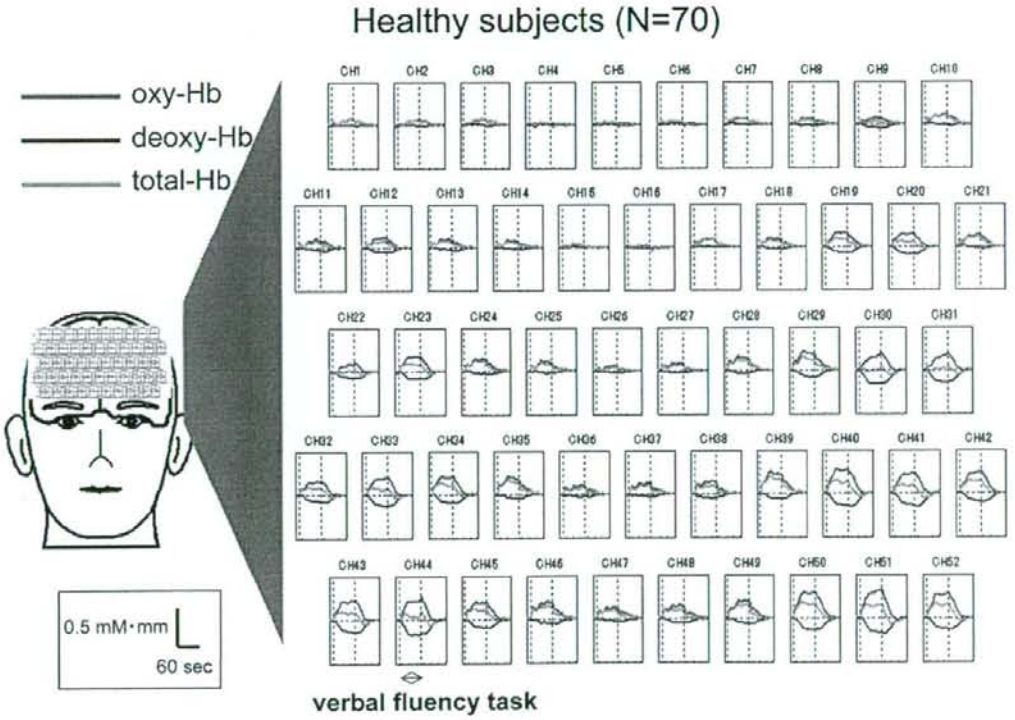

Fig. 2. Grand average waveforms in healthy subjects (N=70). Oxy-, deoxy-, and total-hemoglobin concentration changes during cognitive activation are presented as grand average waveforms in 52 channels in red, blue, and green lines, respectively. (For interpretation of the references to color in this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

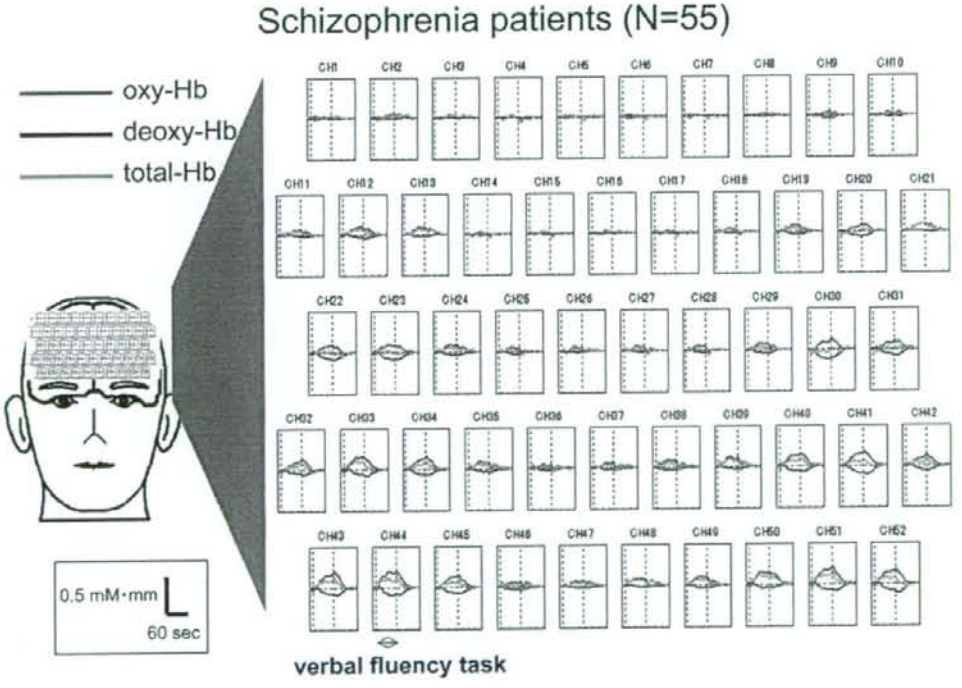

Fig. 3. Grand average waveforms in schizophrenia patients (N=55). Oxy-, deoxy-, and total-hemoglobin concentration changes during cognitive activation are presented as grand average waveforms in 52 channels in red, blue, and green lines, respectively. (For interpretation of the references to color in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

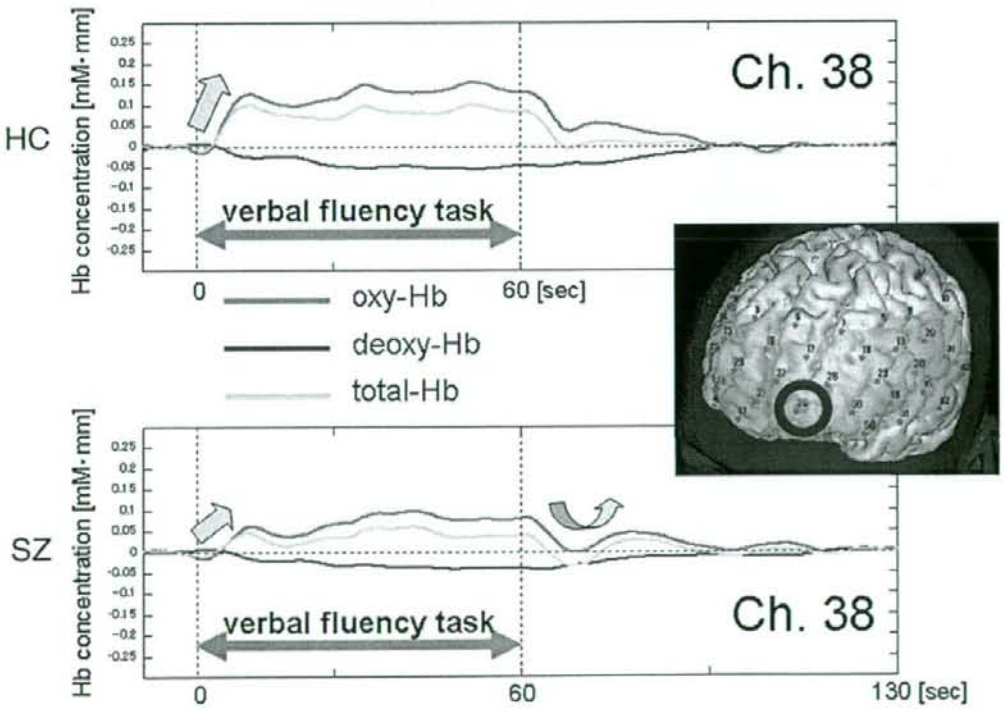

Fig. 4. The differential time course of [Hb] changes in healthy subjects and schizophrenia patients. The differential time course of [Hb] changes between healthy controls (HC; $N=70$) and schizophrenia patients (SZ; $N=55$) were indicated in a representative channel (channel 38; left frontopolar region).

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

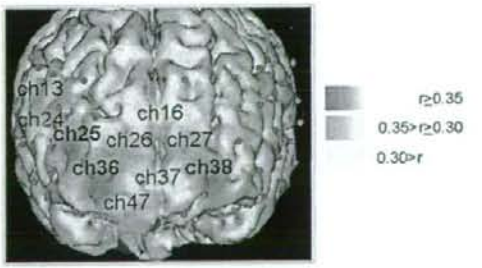

Fig. 5. The cortical distribution of a significant correlation between oxy-hemoglobin changes and global assessment of functioning (GAF) scores. The channels with a significant correlation (Pearson's correlation; P<.05) between the mean [oxy-Hb] changes and GAF scores were indicated with colored area. 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 middorsal 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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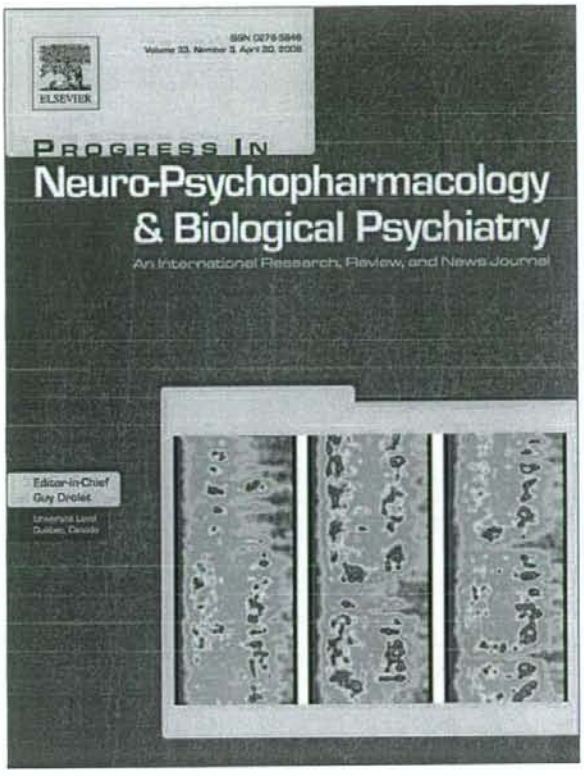

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Association between sigma-1 receptor gene polymorphism and prefrontal hemodynamic response induced by cognitive activation in schizophrenia

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ABSTRACT

The molecular biological role of the sigma-1 receptor (Sig-1R) has attracted much attention. Evidence suggests that the Sig-1R engaged in modulating NMDA and dopamine receptors is involved in the pathophysiology of schizophrenia and the mechanism of psychotropic drug efficacy. However, whether the Sig-1R genotype affects brain function in schizophrenia *in vivo* remains unknown. We investigated the association between Sig-1R functional polymorphism (Gln2Pro) and brain function in schizophrenia. The subjects were 40 patients with schizophrenia and 60 healthy controls, all right-handed, who gave written informed consent to participate. Signals, detected from prefrontal regions by 52-channel near-infrared spectroscopy (NIRS) during cognitive activation, were compared between two Sig1-R genotype subgroups (Gln/Gln individuals and Pro carriers) matched for age, gender, premorbid IQ and task performance. The prefrontal hemodynamic response of healthy controls during the verbal fluency task was higher than that of patients with schizophrenia. For the patients with schizophrenia, even after controlling the effect of medication, the [oxy-Hb] increase in the prefrontal cortex of the Gln/Gln genotype group was significantly greater than that of the Pro carriers (false discovery rate corrected $p<0.05$). Clinical symptoms were not significantly different between the two Sig-1R genotype subgroups. These differences were not significant in the healthy controls. This is the first functional imaging genetics study that implicated the association between Sig-1R genotype and prefrontal cortical function in schizophrenia *in vivo*. Our findings also suggest that the prefrontal hemodynamic response assessed by noninvasive and less demanding NIRS is a useful intermediate phenotype for translational research in schizophrenia.

1. Introduction

Sigma-1 receptors (Sig-1Rs), which are unique endoplasmic reticulum (ER) proteins, have attracted much attention since their basic molecular mechanism was progressively clarified (Hayashi and Su, 2007). Many studies have suggested that Sig-1Rs have a robust cell-protective property and regulate neuritogenesis, IP3R-mediated Ca^{2+} signaling, learning and memory in nervous systems (Hashimoto and Ishiwata, 2006; Hayashi and Su, 2001, 2004; Maurice and Lockhart, 1997; Spruce et al., 2004; Vagnerova et al., 2006). Sig-1Rs are also engaged in modulating *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (Shimazu et al., 2000; Urani et al., 2002) that have a knock-on effect in regulating dopamine (Moison et al., 2003). These findings suggest that Sig-1Rs may be involved in the brain pathophysiology of schizophrenia and in the mechanism of psychotropic drug efficacy (Hayashi and Su, 2004; Hashimoto, 2006; Hashimoto et al., 2007).

From the perspective of the pharmacological treatment of schizophrenia, the improvement of neurocognitive deficits that are a core feature of schizophrenia by treatment with several atypical antipsychotic drugs generally remains limited and the development of more effective agents remains necessary (Lehman et al., 1995). Most research findings support the hypothesis that the hypofunction of glutamate neurotransmission via NMDA receptors is involved in the pathophysiology of schizophrenia, particularly neurocognitive deficits (Goff and Coyle, 2001; Hashimoto, 2006). In an animal study, the

Abbreviations: CI, confidence interval; CPZ, chlorpromazine; DL, dorsolateral; ER, endoplasmic reticulum; FDR, false discovery rate; FP, frontopolar; Gln, Glutamine; NIRS, Near-infrared spectroscopy; PFC, Prefrontal cortex; Pro, Proline; Sig-1R, Sigma-1 receptor; SNPs, single nucleotide polymorphisms; VL, ventrolateral.

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agonistic action to Sig-1Rs was reported to be involved in the improvement of phencyclidine-induced neurocognitive deficits (Hashimoto et al., 2007). Interestingly, fluvoxamine, a Sig-1R agonist, was also reported to be effective for the treatment of negative symptoms of schizophrenia compared with other selective serotonin reuptake inhibitors (Silver, 2004). Therefore, Sig-1R agonists are expected to be effective for the treatment of neurocognitive deficits and negative symptoms in schizophrenia.

Cloning studies (Hanner et al., 1996) have shown that identical Sig-1Rs are 223-amino-acid proteins that reside primarily in the ER and are widely expressed in organs of the immune, reproductive, endocrine and central nervous systems. The gene for human Sig-1R is located on human chromosome 9, band p13, a region reported to be associated with some psychiatric disorders. In postmortem studies, Sig-1Rs in the brain have been reported to be significantly reduced in patients with schizophrenia (Tam and Zhang, 1988; Weissman et al., 1988).

In genetic association studies, the first study (Ishiguro et al., 1998) detected two functional polymorphisms of the Sig-1R genotype: GC-241-240TT and Gln2Pro; they reported a significant association between Sig-1R genotype and schizophrenia. Subsequent studies, however, reported no such significant association (Ohmori et al., 2000; Satoh et al., 2004). Another functional polymorphism, T-485A, was found to be associated with alcoholism (Miyatake et al., 2004). However, the question of whether the Sig-1R genotype affects brain function in patients with schizophrenia remains to be answered. To investigate this effect *in vivo*, we employed an imaging genetics paradigm and a relatively new functional neuroimaging technology, near-infrared spectroscopy (NIRS), to assess brain cortical function noninvasively.

Some neuroimaging techniques have limitations in that the act of measurement places subjects in an uncomfortable and unnatural setting, e.g., lying in a supine position in a narrow gantry with the head position fixed during the entire examination. In contrast, multichannel NIRS enables the noninvasive and less demanding measurement of the spatiotemporal characteristics of brain function under natural conditions, in a sitting position in a well-lit room. The usefulness and limitations of NIRS have been discussed widely in previous literatures (Hoshi, 2003; Obrig and Villringer, 2003; Strangman et al., 2002a). NIRS is restraint-free, easy to use, portable, and noninvasive and entails almost no running cost, which is advantageous not only for neuroimaging research but also for clinical application and translational approach. Because Sig-1R genotype is a potential candidate genetic variant that may partly account for individual differences in the efficacy of pharmacological psychiatric treatment, if an association is found between NIRS signals and functional polymorphisms in the Sig-1R gene, NIRS signals could be potentially used as a biological marker to aid in the evaluation and prediction of response to medication.

In human cortical neuron (HCN-1A) cells, a gene-reporter assay showed that, relative to that of the GC-241-240/Gln2 haplotype, the transcription activity of the TT-241-240/Pro2 haplotype was reduced by 33.3% (Miyatake et al., 2004), which suggested that GC-241-244TT/Gln2Pro polymorphism might be related to changes in transcriptional regulation (Ishiguro et al., 1998) and the expression of Sig-1R gene mRNA (Uchida et al., 2005) in human cerebral cortex. In addition, many preclinical studies have demonstrated that selective agonists of Sig-1Rs affect high-order brain functions such as learning, memory, cognition and mood (Hayashi and Su, 2004), which suggests that Sig-1Rs are also involved in prefrontal cognitive function. Therefore, this study aims to investigate the association between Sig-1R genotype and prefrontal hemodynamic response in healthy controls and patients with schizophrenia using noninvasive and less demanding NIRS with a wide coverage of the prefrontal cortex (PFC).

2. Methods

2.1. Subjects

The subjects included 40 stably maintained outpatients with chronic schizophrenia and 60 healthy controls (Table 1). All the participants were native Japanese speakers and right-handed according to Oldfield's Edinburgh Inventory (Oldfield, 1971). Socioeconomic status (SES) was assessed using the Hollingshead Index of social position (Hollingshead, 1965). Premorbid IQs were estimated using the Japanese version of National Adult Reading Test (Matsuoka et al., 2006) (Table 1).

Table 1
Clinical characteristics of study groups.

	Patients with Schizophrenia ($N=40$)					Healthy controls ($N=60$)					Group difference
	Gln/Gln ($N=20$)		Pro carriers ($N=20$)		t-test p-value	Gln/Gln ($N=30$)		Pro carriers ($N=30$)		t-test p-value	t-test p-value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		
Age, years	40.7	11.3	38.1	8.1	.42	31.0	6.6	31.5	5.8	.76	<.00
Gender, women/men	10/10		12/8		.53[a]	13/17		10/20		.43[a]	.10[a]
Handedness[b]	91.2	20.0	92.5	19.6	.83	92.0	33.1	97.0	8.3	.42	.57
Education, years	14.6	2.6	14.4	2.9	.86	17.5	1.9	17.1	1.8	.44	<.00
Socioeconomic status (SES)[c]	3.2	1.3	3.8	0.9	.10	1.5	0.6	1.7	0.6	.27	<.00
Estimated premorbid IQ[d]	100.2	11.2	100.0	15.5	.90	111.6	6.4	110.0	8.7	.36	<.00
Task performance	14.4	4.8	13.8	5.4	.69	16.4	4.9	17.2	3.8	.50	<.01
Age at onset, years	27.1	9.2	26.5	7.8	.83	NA		NA			
Duration of illness, years	13.9	10.6	11.2	8.1	.40	NA		NA			
PANSS											
Positive	17.1	6.5	14.5	3.4	.12	NA		NA			
Negative	20.8	7.5	20.7	6.7	.97	NA		NA			
General psychopathology	39.3	8.8	38.0	7.7	.61	NA		NA			
Medication											
Chlorpromazine equivalent dose, mg/day	440.2	370.0	1048.9	644.1	.00	NA		NA			
Diazepam equivalent dose, mg/day	7.8	7.5	13.0	17.5	.24	NA		NA			
Biperiden equivalent dose, mg/day	2.3	2.1	4.1	2.2	.01	NA		NA			

Abbreviations: IQ, Intelligence Quotient; PANSS, Positive and Negative Symptom Scale; NA, not applicable.
[a] Chi-square test was used for testing group difference in gender distribution. Otherwise, *t*-test was used.
[b] Assessed using Oldfield's Edinburgh Inventory.
[c] Assessed using Hollingshead Index of social position.
[d] Estimated using Japanese version of National Adult Reading Test.

The patients were recruited from among outpatients at the University of Tokyo Hospital. The diagnosis of schizophrenia was made through a Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First et al., 1997) by an experienced psychiatrist (K.K.). SCID non-patient edition (SCID-NP) was used screening healthy controls. On the same day as the NIRS measurement, psychiatric symptoms were evaluated by one psychiatrist (K.K.) using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), without knowledge of the NIRS data. During the study, the patients with schizophrenia were on medication with antipsychotics and/or anxiolytics and/or antiparkinsonian agents.

The exclusion criteria for both diagnostic 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 alcohol/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 ethics committee of the University of Tokyo Hospital approved this study (receipt number: 630). All the subjects gave written informed consent in accordance with the Declaration of Helsinki after a complete explanation of the study.

2.2. Activation task

The activation task was similar to that employed in our previous study (Takizawa et al., 2008), and is fully described in Supplementary Information (1). Briefly, NIRS signal ([Hb]) changes were measured during a verbal fluency task (letter version), which included a 30-s pretask baseline, a 60-s activation period, and a 70-s posttask baseline. During the activation period, the subjects were instructed to generate as many Japanese words beginning with a designated syllable as possible. For the pre- and posttask baseline periods, the subjects were instructed to simply repeat Japanese vowels out loud. The total number of correct words generated during the 60-s activation period was defined as a measure of task performance (Table 1).

Out of many neuropsychological tasks that revealed neurocognitive deficits in patients with schizophrenia (Fioravanti et al., 2005), the verbal fluency task was chosen in the present study for the following reasons. Among the executive tasks involving prefrontal cortex, verbal fluency is one of the most robust tasks that show distinct difference in performance between schizophrenia patients and controls (large effect size > 1.0) (Bokat and Goldberg, 2003). We have sought to establish best cognitive activation tasks for NIRS, and previously found that verbal fluency is the most reliable task showing prominent and wide-spread frontotemporal activation in normal subjects that can be easily differentiated from that in schizophrenia patients (Suto et al., 2004; Takizawa et al., 2008). One of the reasons may be that 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).

2.3. NIRS measurement

A full explanation of the NIRS apparatus and measurement procedure was given in our previous study (Takizawa et al., 2008) and appears in Supplementary Information (1). Briefly, we used a 52-channel NIRS system (ETG-4000, Hitachi Medical Co.). The arrangement of the probes can measure [Hb] from the bilateral prefrontal cortical area (e.g., dorsolateral [DL; Brodmann's area (BA) 9,46], ventrolateral [VL; BA 44,45,47], and frontopolar [FP; BA 10]) and superior temporal cortical surface regions, which was supported by a multisubject study of anatomical cranio-cerebral correction via the international 10–20 system(Okamoto et al., 2004). The setting up of the apparatus usually takes less than 5 min including the audiovisual on-screen instructions, and our version of the verbal fluency task takes less than 3 min, which is less demanding for participants. The time resolution of NIRS was set at 0.1 s. Grand mean waveforms averaged across subjects were calculated separately for each type of [Hb] and for each group.

2.4. Genotyping

Genomic DNA was extracted from leukocytes using a standard method. The Sig-1R Gln2Pro, GC-241-240TT and T-485A polymorphisms were genotyped using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, California, USA). This study was performed using ethnically homogeneous individuals (only of Japanese descent).

2.5. Statistical analysis

For data analysis using parametric statistical tests, the obtained [Hb] data from each channel were averaged across two time segments (pretask baseline and task period). We focused on [oxy-Hb] here, since the [oxy-Hb] increase is assumed to more directly reflect cognitive activation than [deoxy-Hb] decrease as shown by its stronger correlation with the blood-oxygenation-level-dependent signal measured by fMRI (Strangman et al., 2002b) and by the results of animal work (Hoshi et al., 2001), although the analysis of [deoxy-Hb] is also presented.

First, to confirm the increase associated with the verbal fluency task in the healthy controls, the mean [Hb] changes for the pretask baseline period and that for the task period were compared for each channel using Student's paired t-test (two-tailed). False discovery rate (FDR) correction for multiple comparisons [52 channels] was applied. We set the value specifying the maximum FDR to 0.05, so that there are no more than 5% false positives on average (Singh and Dan, 2006).

Second, t-tests for differences in potential confounding factors, such as age, gender, handedness, education, premorbid IQ, task-performance, clinical symptoms, and dose of medication were performed between the two Sig-1R genotype subgroups in the healthy controls and in patients with schizophrenia, respectively.

Third, the first 5-s slope and mean [Hb] changes during the 60-s task period between the two Sig-1R genotype subgroups were compared in each group. NIRS signal changes were compared between the two genotype subgroups at each channel by two-tailed Student's t-test. p-values were adjusted by FDR correction for multiple comparisons [p<FDR 0.05; 52 channels]. Two-tailed analysis was used because task-load-dependent hypo- and hyperperfusions of the prefrontal cortex in the patients with schizophrenia relative to those in the healthy controls have been reported in previous literature (Brahmbhatt et al., 2006). When channels were considered significant after FDR correction, we showed their effect size (Cohen's d) and 95% confidence interval (CI). Statistical analysis was performed using SPSS 10.1.3J software (Tokyo, Japan).

3. Results

3.1. Genotype determination

Because GC-241-240TT and Gln2Pro are in nearly complete linkage disequilibrium with each other (Miyatake et al., 2004) and all the subjects were T carriers of T-485A polymorphism, we focused on Gln2Pro polymorphism in this study. The genotypic distributions of the three genotypes in the Sig-1R gene are as follows: in healthy controls, Gln/Gln 30 (50.0%), Gln/Pro 26 (43.3%), Pro/Pro 4 (6.7%); in patients with schizophrenia, Gln/Gln 20 (50.0%), Gln/Pro 13 (32.5%), Pro/Pro 7 (17.5%). The distributions of the three genotypes followed the Hardy–Weinberg equilibrium in both diagnostic groups. The sample size of the Pro/Pro genotype was too small to provide sufficient statistical power and to draw a conclusion. Thus, the three genotypes in the Sig-1R gene were classified into two subgroups: the genotypes with Pro allele ('Pro carriers') and the Gln/Gln genotype ('Gln/Gln