

(1.33 or more) described in the initial report for each SNP (Chumakov et al., 2002). Genotype and allele frequencies of eight SNPs located in the *G72* gene and the flanking regions are shown in Table 1. Genotyping completeness ranged from 98.5% (M15) to 99.5% (M12). No deviation from HWE was detected in cases and controls (data not shown). Significant differences in the genotype frequency of M22 ($\chi^2=6.75$, $P=0.034$) and in the allele frequencies of M22 ($\chi^2=5.48$, $P=0.019$), M23 ($\chi^2=4.72$, $P=0.030$) and M24 ($\chi^2=4.35$, $P=0.037$) between patients and controls were observed. However, the associations did not survive after correction for multiple testing (the effective number of independent marker loci: 6.0, M22 allelic association: $P=0.11$ after SNPSpD correction). There was no allelic or genotypic association of the other five SNPs with schizophrenia.

Haplotype analysis showed associations in two-marker haplotypes: M15–M19 (global $P=0.03$) and M19–M20 (global $P=0.04$), three-marker haplotype: M14–M15–M19 (global $P=0.03$), four-marker haplotype: M12–M14–M15–M19 (global $P=0.03$), and five-marker haplotype: M14–M15–M19–M20–M22 (global $P=0.03$) (Table 2). This weak evidence for association became negative after correction for multiple testing (22 independent global test, M15–M19 haplotypic association: $P=0.66$ after Bonferroni correction).

M22 showed a strong LD with M24 and a moderate LD with M23 in controls, and similar LD results were obtained in patients (Supplementary Fig. 1). The LD pattern of our data was similar to that of other ethnic groups in the previous studies. The two strong LD structures (Block 1 and Block 2) observed in the present study were similar to those observed in previous studies (Detera-Wadleigh and McMahon, 2006; Li and He, 2007; Ma et al., 2006).

4. Discussion

The purpose of the present study was to investigate the association between *G72* polymorphisms and schizophrenia in a large Japanese population, comparable to the sample size included in the meta-analysis of Li and He (2007). Eight *G72* SNPs, which have been associated with schizophrenia in previous studies, were examined in case-control subjects (Detera-Wadleigh and McMahon, 2006; Li and He, 2007; Shi et al., 2008). We failed to replicate the association of any *G72* polymorphism (M12, M14, M15, M19, M20, M22, M23 and M24) with schizophrenia after correction for multiple testing. Power analysis showed that our subjects had sufficient power (>0.99) to detect an effect of the odds ratio (1.33 or more) for each SNP shown in the original study. The findings of the power calculation did not support the hypothesis that the eight SNPs in the *G72* gene are associated with schizophrenia in Japanese population.

Nominal associations of the alleles, M22, M23 and M24, and the genotype of M22 with schizophrenia in this study were no longer positive after correction for multiple testing. However, we discuss the direction of the association in the SNPs, as there are considerable discrepancies among studies. These three SNPs are located from 25.8 Kb to 54.8 Kb 3' downstream of the last exon (exon 5) of *G72*. These SNPs form a highly strong LD block whose pattern is similar among different ethnic groups. The first study by Chumakov et al. reported that the minor allele G of M22 was less frequent in

Canadian patients with schizophrenia (31%) than in controls (40%). However, the following studies have indicated the reverse direction of the association of M22 compared with the original study (patients vs. controls: Ma et al.; 47.5 vs. 39.1 in Scottish samples, 41.1 vs. 34.6 in Chinese samples). Our results indicated that the minor allele G of M22 was enriched in patients with schizophrenia (26.0) than in control (23.6). Consistent to the discrepancies among studies (different direction in European populations and same direction in Asian population), recent meta-analysis has reported significant evidence for the association of M22 with schizophrenia in Asian population, but not in European population (Ma et al., 2006; Shi et al., 2008). The direction of association of M23 has shown significant heterogeneity between individual studies in European populations (C allele at M23, patients vs. controls: Chumakov et al., 2002; 43 vs. 51 in Canadian samples, 40 vs. 49 in Russian sample, Korostishevsky et al., 2006; 37.7 vs. 56.7 in Ashkenazi Jewish samples, Schumacher et al., 2004; 53 vs. 46 in German samples, Ma et al., 2006; 59.3 vs. 46.4 in Scottish samples) (Shi et al., 2008). Our results (patients vs. controls: 43.6 vs. 41.2) were consistent with the direction of the association of M23 in an Asian population (Shi et al., 2008; 53.3 vs. 52.4), although the statistical significance was not evident. The direction of association of M24 among the previous studies was identical (patients vs. controls: Chumakov et al., 2002; 55 vs. 47 in Canadian samples, Schumacher et al., 2004; 56 vs. 50 in German samples). We firstly examined a possible association of M24 with schizophrenia in an Asian population and did not find association with schizophrenia (the frequency of the T allele at M24 in patients: 49.8 and controls: 47.4). This suggests that M24 is not likely to have ethnic heterogeneity between Asian and European populations.

Recently, a large scale genome-wide association study (GWAS) using 479 cases and 2937 controls reported that 12 SNPs (odds ratios ranging 1.27–2.06, $P < 1 \times 10^{-5}$) were associated with schizophrenia in the first analysis (O'Donovan et al., 2008). The subsequent replication studies using 16,726 of total subjects showed the evidence for association with three of the 12 SNPs ($P < 1 \times 10^{-5}$) (O'Donovan et al., 2008). They reported the odds ratios 1.12–1.16 in the three positive SNPs in the overall analysis (O'Donovan et al., 2008). The sample size in the first analysis of the GWAS had power (>0.85) to detect an effect of the odds ratio (1.33 or more) for each SNP in the original *G72* study. However, association between SNPs in *G72* and schizophrenia was not detected in the study. Failure to detect association signal does not provide conclusive exclusion of any given gene identified so far. The discrepancies among previous studies and the present study might be explained by differences in ethnic heterogeneity, phenotypic heterogeneity or study designs, such as sample size and case-control versus family-based association study. Factors like incomplete coverage of common variants, inadequate power, allelic and locus heterogeneity could all affect our ability to detect genetic association.

In conclusion, the present study did not support a strong association of the *G72* gene with schizophrenia in a Japanese population. Three SNPs and several haplotypes gave nominal evidence for association and this did not survive correction for multiple testing. *G72* is not likely to be a major susceptibility gene for schizophrenia in this Japanese population.

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Contributors

R. Hashimoto supervised the entire project, collected the data, wrote the manuscript, was critically involved in the design, analysis and interpretation of the data and was responsible for performing the literature review. K. Ohi was critically involved in the collection and analysis of the data, and contributed to the editing of the final manuscript and contributed intellectually to the interpretation of the data. Y. Yasuda, T. Yoshida, H. Takahashi, N. Iike, M. Fukumoto, H. Takamura, M. Iwase, K. Kamino, R. Ishii, H. Kazui, R. Sekiyama, Y. Kitamura, M. Azechi, K. Ikezawa, R. Kurimoto, E. Kamagata, H. Tanimukai, S. Tagami, T. Morihara, M. Ogasawara, M. Okochi, H. Tokunaga, S. Numata, M. Ikeda, T. Ohnuma, T. Fukunaga, T. Tanaka, T. Kudo, S. Ueno, H. Arai, T. Ohmori, N. Iwata, N. Ozaki and M. Takeda were heavily involved in the collection of the majority of the data and contributed intellectually to the interpretation of the data. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2009.01.019.

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Impaired regional hemodynamic response in schizophrenia during multiple prefrontal activation tasks: A two-channel near-infrared spectroscopy study

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ABSTRACT

In schizophrenia, dysfunction of the prefrontal cortex (PFC), regarded as a core feature of the disease, has been investigated by different neuroimaging methods. Near infrared spectroscopy (NIRS), a novel neurophysiological method, is being increasingly used in the investigation of frontal dysfunction in schizophrenia. However, NIRS measurements during multiple frontal activation tasks have been rarely reported. The purpose of this study was to compare hemodynamic changes in the PFC between patients with schizophrenia and healthy controls during four different types of frontal lobe tasks using a 2-channel NIRS system. Thirty patients with schizophrenia and thirty age- and gender-matched healthy controls were enrolled in this study. In both groups, changes in oxygenated hemoglobin concentration ($\Delta[\text{oxyHb}]$) at the bilateral forehead were measured during Verbal fluency test letter version (VFT-letter), VFT category version, Tower of Hanoi (TOH), the Sternberg and Stroop tasks. Regarding $\Delta[\text{oxyHb}]$ in PFC, a diagnosis group effect was found for VFT-letter and TOH. Significant negative correlation was found between left $\Delta[\text{oxyHb}]$ during TOH and negative and cognitive symptom scores in schizophrenia patients. Right $\Delta[\text{oxyHb}]$ during TOH also showed significant negative correlation with cognitive symptoms scores. No significant correlation between $\Delta[\text{oxyHb}]$ and clinical characteristics were observed during VFT-letter. These findings suggest that among a battery of frontal lobe tasks administered to schizophrenia patients, VFT-letter and TOH are more sensitive to detect PFC activation, as indicated by $\Delta[\text{oxyHb}]$ using a 2-channel NIRS. Taken together, these findings and those of previous neuroimaging studies suggest that VFT-letter and TOH might represent possible candidate physiological markers of prefrontal dysfunction in schizophrenia, though extensive testing in clinical settings will be necessary.

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1. Introduction

Schizophrenia is a mental disorder emerging in adolescence that is typically characterized by hallucinations and delusions as well as emotional and social dysfunction. Recently, marked cognitive impairments, predominantly in memory, attention, and executive functions have been described in patients with

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schizophrenia and are regarded as being independent of psychiatric symptoms (Heinrichs and Zakzanis, 1998). In fact, cognitive impairments in multiple domains are considered important features of the psychopathology of schizophrenia and have been reported in association with social functions, quality of life, and prognosis of social life (Harvey et al., 1998). These cognitive impairments are related to dysfunction of several areas in the brain in schizophrenia including the prefrontal area. Previous studies on schizophrenia have reported poor performance of neuropsychological tests that assess prefrontal function such as the Verbal Fluency Test (VFT), the Wisconsin Card Sorting Test (WCST – measuring conversion of the concept and flexibility of reaction), and the Stroop task (measuring attention and inhibition, see Ma et al., 2007). Several functional MRI (fMRI) studies indicated involvement of the prefrontal cortex (PFC) in the WCST in healthy controls (Alvarez and Emory, 2006), and decreased activation of the PFC in schizophrenia (Ragland et al., 2007). Assessing prefrontal function is therefore essential to elucidate the schizophrenia pathophysiology.

Near-infrared spectroscopy (NIRS), a novel neuroimaging method, is increasingly used in investigating psychiatric disorders. This method exploits the property of near-infrared light penetrating into tissues where it is absorbed by hemoglobin depending on the oxygenation state of the hemoglobin (Jobsis, 1977). Using different infrared wavelengths, it is thus possible to measure relative changes in oxygenated hemoglobin concentration ([oxyHb]) and deoxygenated hemoglobin ([deoxyHb]), Hoshi, 2003; Soul and du Plessis, 1999). It is well established that oxygen consumption, regional cerebral blood response (rCBR), and oxygenated hemoglobin supply are increased in the highly activated neural regions (Hoshi et al., 2001; Fox and Raichle, 1986).

Compared to other neuroimaging methods such as fMRI and PET, NIRS measurement is quite simple, which is advantageous in a clinical setting. NIRS is non-invasive in nature, portable, has a low running cost and it is available for continuous and repetitive measurements, albeit with the limitation of low spatial resolution and the inability to examine deep brain structures. Since several studies have successfully utilized NIRS in various psychiatric disorders such as schizophrenia, bipolar disorder, and dementia (Richter et al., 2007; Kameyama et al., 2006; Suto et al., 2004; Fallgatter et al., 1997), there are growing expectations for clinical NIRS applications in the neuropsychiatric area.

Several NIRS studies have reported a significantly smaller increase in [oxyHb] in the PFC in schizophrenia during execution of frontal lobe tasks like the VFT or the Random Number Generation Task (Takizawa et al., 2008; Ehliis et al., 2008; Hoshi et al., 2006; Folley and Park, 2005; Kubota et al., 2005; Watanabe and Kato, 2004; Shinba et al., 2004; Suto et al., 2004; Fallgatter and Strik, 2000; Okada et al., 1994). However, only few frontal lobe tasks have been assessed in these studies, and a comprehensive look at PFC activity is not available. To address this, we employed a 2-channel NIRS (2ch-NIRS) system and four kinds of cognitive tasks (VFT, Tower of Hanoi or TOH, the Sternberg, and the Stroop) to examine which tasks were suitable for finding significant differences in task-induced changes in [oxyHb], and for showing association between the changes in rCBR and demographic and clinical parameters in schizophrenia. VFT, TOH,

the Sternberg and the Stroop tasks measure fluency, executive function, working memory, and attention/inhibition, respectively (Ma et al., 2007; Johnson et al., 2006), and they are generally regarded as representative neuropsychological tasks to elicit PFC activation (Ragland et al., 2007; Alvarez and Emory, 2006; Fincham et al., 2002; Schlösser et al., 2008; Johnson et al., 2006). Using 2ch-NIRS to measure changes in rCBR during these tasks is relatively simple and readily adoptable for clinical purposes.

Unlike previous studies with multi-channel NIRS, in this study, changes in [oxyHb] were measured at the bilateral forehead overlying the PFC using a 2ch-NIRS system. Despite it having only two channels, there are several advantages to the 2ch-NIRS system. For instance, invalid NIRS data from a low signal/noise ratio common to multi-channel systems on haired scalp (Suto et al., 2004) is not a problem for NIRS measurements on the forehead. In addition, preparations for 2ch-NIRS measurements take only few seconds, while several minutes are needed for the placement of the probes with multi-channel systems. Furthermore, identical cross-subject anatomical positioning in 2ch-NIRS is supported by fixing the probes at Fp1–F7 and Fp2–F8 according to the 10/20 international electrode placement system for electroencephalography; similar anatomical channel positioning is difficult in multi-channel systems due to variations in head size. Subjects also feel considerable pain at the scalp with multi-channel systems, but not with 2ch-NIRS, which may improve the quality of the PFC recording. Finally, 2-channel data acquisition has no need for statistical corrections for multiple comparisons as does multi-channel data acquisition (Nakahachi et al., 2008).

The purpose of this study was to compare changes in rCBR in the PFC between schizophrenia and healthy controls, and to investigate potential association between changes in rCBR and demographic and clinical parameters in schizophrenia using four frontal lobe tasks and simple, non-invasive 2ch-NIRS measurements. Based on previous reports indicating an association between hypofrontality and negative symptoms in schizophrenia (Pratt et al., 2008; Semkowska et al., 2001), we hypothesized that PFC activation during these tasks in schizophrenia patients would be less than that of healthy controls. We also anticipated a significant correlation between the magnitude of PFC activation and clinical parameters in schizophrenia patients.

2. Methods

2.1. Subjects

This study was conducted with 30 schizophrenia patients and 30 age/gender-matched healthy controls. All patients with schizophrenia were diagnosed according to the Structured Clinical Interview of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV; American Psychiatric Association, 1994). They were treated as inpatients or outpatients at the Department of Psychiatry, Osaka University Hospital from November 2006 to April 2007. Psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987) and summarized using the five-factor model of PANSS (Lindenmayer et al., 1994). Antipsychotic medication-related extrapyramidal

symptoms were assessed using the Drug-Induced Extrapyr- amidal Symptom Scale (DIEPSS; Inada, 1996). Control subjects were not taking any medications at the time of recruitment and had no personal or family history of psychiatric and/or neurological disorders. All subjects were right-handed as indicated by the Edinburgh Handedness Inventory (Oldfield, 1971). Their premorbid IQs were assessed using the Japanese version of the National Adult Reading Test (JART; Matsuoka et al., 2006). This study was approved by the Ethics Committee of Osaka University, and written informed consent was obtained from all subjects prior to the experiments. All procedures were carried out in accordance with the policies and principles contained in the Declaration of Helsinki. The demographic and clinical characteristics of all subjects are shown in Table 1.

2.2. Tasks and procedure

All subjects sat on a comfortable chair in a silent room. The tasks comprised pre-task (30 s), task and post-task periods (60 s). The durations of the task period of VFT-letter, VFT-category and TOH was 60 s, while the Sternberg and the Stroop tasks had durations of 120 s. Because most previous NIRS studies which applied VFT used a 60-sec task period, we allocated the same period of time to VFT in this study. As for TOH, a 60-sec task period proved to elicit sufficient cortical activation in our preliminary measurements. However, the task period for the Sternberg and the Stroop tasks were set at 120 s based on preliminary findings indicating that 60 s was insufficient for significant activation.

Table 1
Demographic data and clinical characteristics

	Schizophrenia (30)	Healthy controls (30)
Age	38.7±11.7	37.3±8.7
Sex (M/F)	12/18	13/17
Years of education	13.3±2.4**	15.5±2.1
JART	101.2±10.8	105.9±8.4
Onset age	23.4±8.4	
Duration of illness (year)	14.7±13.0	
Duration of antipsychotic medication (year)	11.9±12.5	
Equivalents of CPZ (mg)	842.6±704.2	
Equivalents of BZD (mg)	17.1±11.1 ^a	
Admission (time)	3.0±3.7	
Duration of admission (month)	15.0±41.5	
PANSS		
Positive symptoms	18.8±5.5	
Negative symptoms	18.2±6.4	
General psychopathology	36.8±9.1	
Five-factor model of PANSS		
Positive	15.7±5.3	
Negative	16.7±6.5	
Cognitive	10.9±4.0	
Excitement	6.7±2.5	
Depression/anxiety	9.2±3.0	
DIEPSS	2.5±2.9	

Data are presented as mean±SD. JART, the Japanese version of the National Adult Reading Test; CPZ, chlorpromazine; BZD, benzodiazepines; PANSS, the Positive and Negative Syndrome Scale; DIEPSS, the Drug-Induced Extrapyr- amidal Symptom Scale.

** $p < 0.01$, Mann-Whitney U test was used.

^a Eleven patients were taking BZD medications.

The VFT-letter version asks the subjects to generate loudly as many nouns as possible, all of which start with the Japanese *hiragana* letters 'a', 'ka', and 'sa', with a duration of 20 s for each letter. The VFT-category version asks the subjects to generate loudly as many nouns as possible related to three different categories: animals, vegetables, and vehicles, with a 20-sec duration for each category. In both tasks, the subjects were asked to pronounce the vowels 'a', 'i', 'u', 'e', 'o' repeatedly during the pre- and post-task periods. These tasks evaluate the ease with which a person can produce words. The total number of generated words was deemed task performance. The generated words during the measurement were written down by testers immediately. This procedure is explained in detail elsewhere (Kubota et al., 2005).

The TOH consists of three pegs of equal size and a number of disks of different sizes which can slide onto any peg. The subjects were instructed to follow three rules to perform the task. First, only one disk may be moved at a time. Second, no disk may be placed on top of a smaller disk. Third, at no time may a disk be put in a place other than a peg. Upon following these rules, the subjects were asked to transfer the disks, which were neatly piled up in order of size on one peg, to another peg to form the original conical shape in as few moves as possible. To remove non-specific activation elicited by hand motion, they were instructed to move the smallest disk slowly and continuously during the pre- and post-task periods. Before the measurements, the subjects were asked to perform the task with three disks to confirm that they understood the instructions. In TOH with four disks, the fewest moves to achieve the goal is 15. The total number of effective moves was deemed task performance. If the subjects completed the task within 60 s, they were asked to repeat the task. In this case, the total number of effective moves was deemed task performance. The total number of moves and total number of effective moves during measurement were written down by testers immediately. For details on the procedure see Giménez et al. (2003).

In the Sternberg and the Stroop tasks, digits and characters, respectively, were displayed on a 15-inch monitor connected to a desktop PC placed approximately 1 meter away from the subjects. These tasks were performed using the Multi Trigger System version 2.10 (MedicalTrySystem, Japan). The Sternberg task consisted of a modified version of the Sternberg Item Recognition Paradigm (Sternberg, 1966), which has two phases, namely the encoding phase and the probe phase. During the encoding phase, five random digits were displayed one by one for 2 s. Following an eye fixation mark (cross mark) for 3 s, a probe digit was displayed for 1 s with an inter-stimulus interval (ISI) of 2 s and the probe presentation was repeated three times during the probe phase. For each probe, subjects were asked to recognize whether a probe digit was included or not in the random digits displayed during the encoding phase. For each question, they answered quickly "yes" or "no", as appropriate. The eye fixation mark was displayed for 3 s between each set of digits. The total number of sets was eight. Each random five digits and each combination with a probe digit were different among the eight sets. The presentation was the same for all subjects. The subjects were asked to watch the eye fixation mark during the pre- and post-task periods. They performed practice trials prior to the measurements to allow them to

understand the task's components. The total number of correct answers was deemed task performance. The full performance was 24. The answers during the measurement were written down by testers immediately. For details on the procedure see Casement et al. (2006).

During the Stroop task, each name of four colors (blue, yellow, red, green), written in Japanese *kanji* character, was presented in congruent (e.g., the word BLUE displayed in blue color) or incongruent combinations (e.g., the word YELLOW displayed in red color). This task consisted of twelve

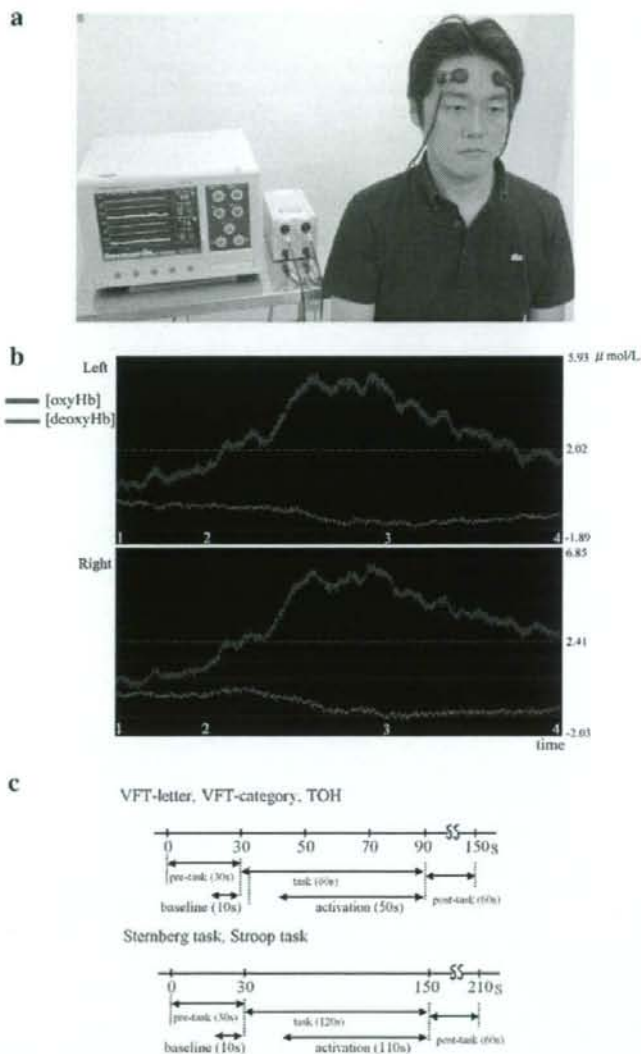


Fig. 1. a. The 2-channel NIRS measurements and the probe setting. b. Actual NIRS data along time course during TOH in one representative healthy control. Upper row shows measurement at left forehead, lower row shows measurement at right forehead. Red line means [oxyHb], blue line means [deoxyHb]. In figure, "1" means the beginning of pre-task, "2" means the beginning of task, "3" means the end of task (also the beginning of post-task), "4" means the end of post task. c. Protocols and procedures of data analysis in each task. Pre-task and post-task periods for all tasks had durations of 30 s and 60 s, respectively. The durations of the task periods of VFT-letter, VFT-category and TOH were 60 s, and those of the Sternberg and the Stroop tasks were 120 s. In data analysis, the mean levels of [oxyHb] ([deoxyHb]) during the last 10 s of the pre-task period were defined as baseline. The mean levels of [oxyHb] ([deoxyHb]) during the last 50 s task period of VFT-letter, VFT-category, and TOH were defined as activation levels. The mean levels of [oxyHb] ([deoxyHb]) during the last 110 s task period of the Sternberg task and the Stroop task were defined as activation levels. The difference between activation and baseline levels was deemed size of activation (Δ [oxyHb], Δ [deoxyHb]). VFT-letter, Verbal Fluency Test-letter; VFT-category, Verbal Fluency Test-category; TOH, Tower of Hanoi. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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congruent and twelve incongruent trials. The colored characters were presented for 1.25 s with an ISI of 1.75 s. Each trial was presented consecutively in a random order to avoid habituation. For each trial, the subjects were asked to orally identify the color of each printed color name, but not the color name displayed on the screen, as quickly as possible. An eye fixation mark was also used during the pre- and post-task periods of this task, and all subjects performed practice trials as well. The total number of correct answers was deemed task performance. The full performance was 40. The answers during the measurement were written down by testers immediately. This procedure is explained in detail elsewhere (Kawaguchi et al., 2005). To address the potential order effect, the order of these tasks was divided into the two following patterns, which were applied to each subject alternately: order A) VFT-letter → VFT-category → TOH → Sternberg task → Stroop task, order B) Stroop task → Sternberg task → TOH → VFT-category → VFT-letter.

2.3. NIRS measurements

NIRS measurements were carried out with a 2-channel system (NIRO-200, Hamamatsu Photonics, Japan). The NIRO-200 utilizes near-infrared light emitted at three different wavelengths (775, 810, and 850 nm) to detect primal changes in [oxyHb] and [deoxyHb] of venous blood in brain cortical regions. Two pairs of emission (light source: laser-diode) and detection probes (light detector: photo-diode) were attached to the bilateral forehead of the subjects. One detection probe was located at Fp1 and the corresponding emission probe at F7, while the other pair of probes was located at Fp2-F8, according to the international 10/20 electrode placement system for electroencephalography (see Fig. 1-a). The distance between the detection probe and the corresponding emission probe was 3 cm. These probe settings enabled us to detect hemodynamic changes in two separate cortical areas. The anatomical location of these areas likely corresponded to part of the superior and inferior frontal gyri (Okamoto et al., 2004). The two sets of probes do not interfere with each other for simultaneous recording of [oxyHb] or [deoxyHb] changes. Recordings were acquired at a sampling rate of 6 Hz. The estimated path length factor was 24 cm. All hemoglobin oxygen concentration values are expressed in $\mu\text{mol/L}$. Actual NIRS data along time course is shown in Fig. 1-b.

2.4. Statistical analysis

Since [oxyHb] is a more sensitive indicator of changes in rCBR compared to [deoxyHb] (Hoshi et al., 2001; Hoshi, 2003), changes in [oxyHb] laid the foundation for the analyses. The mean levels of [oxyHb] during the last 10 s of the pre-task period was deemed baseline. The mean levels of [oxyHb] during the last 50 s task period of VFT-letter, VFT-category, and TOH, and the last 110-sec task period of the Sternberg task and the Stroop task were deemed activation levels, since stable elevation of [oxyHb] by task execution was observed 10 s after task initiation. The difference between activation and baseline levels was deemed size of activation ($\Delta[\text{oxyHb}]$). Task protocols and procedures of data analysis in each task are shown in Fig. 1-c. Statistical analyses were carried out using the SPSS version 10 (SPSS Inc., Chicago, IL). Chi-square test for

independence of group and gender, Mann-Whitney test for age, years of education, JART, task performance, were performed. Two designs of repeated-measures ANCOVA were performed. One design of ANCOVA (the design-1 ANCOVA) was performed to test $\Delta[\text{oxyHb}]$ for each task, using diagnosis group, gender, hemisphere, and task order as the factors, and age, education, and behavioral performance as the covariates. In the design-1 ANCOVA, diagnosis group was set as a factor since our main interest was group difference. Gender was included as a factor based on Kameyama et al. (2004) report of sex differences in cortical activation, as indicated by NIRS. As for the hemisphere factor, potential difference in NIRS activation has been reported in association with linguistic tasks and hemisphere dominance (Kubota et al., 2005). Since the total time of tasks could influence the results of cortical activation, task order was included as a factor. Moreover, age, education, and behavioral performance were set as the covariates since these variables could potentially affect neural activation. Another design of ANCOVA (the design-2 ANCOVA) was performed, using tasks, diagnosis group, gender, hemisphere, and task order as the factors, and age and education as the covariates. In the design-1 ANCOVA, Spearman's rank-correlation and multiple comparisons with Bonferroni's correction (Curtin and Schulz, 1998) were performed between $\Delta[\text{oxyHb}]$ and demographics and major clinical parameters for the tasks when significant group effects were found. For changes in [deoxyHb], repeated-measures ANCOVA was performed to test for differences between activation and baseline levels ($\Delta[\text{deoxyHb}]$) for each task, using diagnosis group, gender, hemisphere, and task order as the factors, and age, education, and behavioral performance as the covariates. The level of significance was set at $p < 0.05$.

3. Results

3.1. Performance data

The results for task performance for each task in both schizophrenia patients and healthy control groups are shown in Table 2. For all tasks, task performance was lower in schizophrenia than in healthy controls (Mann-Whitney *U* test).

3.2. NIRS data

The results of $\Delta[\text{oxyHb}]$ for each task (the design-1 ANCOVA) are shown in Fig. 2. For VFT-letter, the repeated-measures ANCOVA revealed significant difference for

Table 2
Performance data

	Schizophrenia (30)	Healthy controls (30)
VFT-letter	13.0 ± 3.9**	17.4 ± 3.8
VFT-category	23.7 ± 5.0**	28.9 ± 4.6
TOH	6.1 ± 3.1**	11.0 ± 3.6
(Schizophrenia 2/Healthy controls 11) ^a		
Sternberg task	20.8 ± 2.8**	22.6 ± 1.7
Stroop task	37.8 ± 4.0*	39.6 ± 0.7

Performance data are presented as mean ± SD. VFT-letter, Verbal Fluency Test-letter; VFT-category, Verbal Fluency Test-category; TOH, Tower of Hanoi. ** $p < 0.01$, * $p < 0.05$; Mann-Whitney *U* test was used.

^a Subjects to complete the task.

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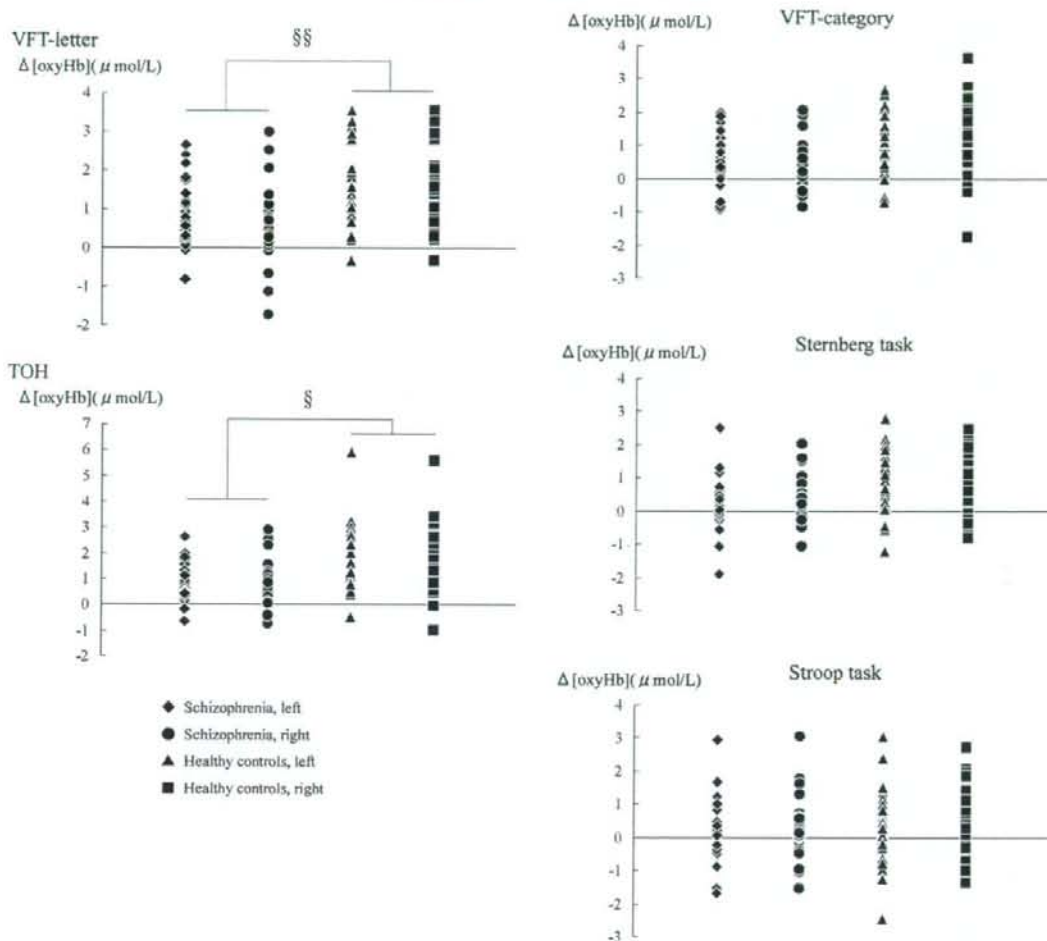


Fig. 2. The results of the changes in [oxyHb] ($\mu\text{mol/L}$; $\Delta[\text{oxyHb}]$) in five frontal lobe tasks. During VFT-letter and TOH, the repeated-measures analysis of covariance (the design-1 ANCOVA) revealed significant main effect of diagnosis group. During VFT-category, the Sternberg task, and the Stroop task, the design-1 ANCOVA revealed no significant main effect of diagnosis group. The effect size of each task was as follows: VFT-letter (left: 0.82, right: 0.88), VFT-category (left: 0.61, right: 0.77), TOH (left: 0.90, right: 0.86), the Sternberg task (left: 0.54, right: 0.61), the Stroop task (left: 0.04, right: 0.21). §§ $p < 0.01$, § $p < 0.05$; significant group effect VFT-letter, Verbal Fluency Test-letter; VFT-category, Verbal Fluency Test-category; TOH, Tower of Hanoi.

diagnosis group ($F(1, 58) = 13.138, p < 0.001$). Post-hoc analysis revealed that healthy controls exhibited a significantly larger increase in $\Delta[\text{oxyHb}]$ than schizophrenia patients. There was a significant main effect of task order ($F(1, 58) = 9.148, p = 0.004$). Post-hoc analysis revealed that an increase in $\Delta[\text{oxyHb}]$ in order A was significantly larger than in order B. Regarding TOH, the design-1 ANCOVA revealed a significant main effect for diagnosis group ($F(1, 58) = 5.824, p = 0.02$). Post-hoc analysis revealed that healthy controls exhibited a significantly larger increase in $\Delta[\text{oxyHb}]$ than schizophrenia patients. There was a significant interaction between diagnosis group and gender ($F(1, 58) = 4.432, p = 0.04$). The post-hoc analysis using Bonferroni's correction revealed that healthy controls showed a significantly larger increase in $\Delta[\text{oxyHb}]$ than schizophrenia patients in males ($p = 0.003$),

and male subjects showed a significantly larger increase in $\Delta[\text{oxyHb}]$ than female subjects in healthy controls ($p = 0.016$). In VFT-category and the Sternberg task, the design-1 ANCOVA revealed no significant main effect of diagnosis group ($F(1, 58) = 3.905, p = 0.054, F(1, 58) = 2.771, p = 0.102$ respectively). For the Stroop task, the design-1 ANCOVA revealed no significant main effect of diagnosis group ($F(1, 58) = 0.379, p = 0.541$), but there was a significant main effect of task order ($F(1, 58) = 8.810, p = 0.005$). Post-hoc analysis revealed that an increase in $\Delta[\text{oxyHb}]$ in order B were significantly larger than in order A. Except for TOH, there was no interaction between factors for the tasks. The design-2 ANCOVA revealed a significant main effect of diagnosis group ($F(1, 58) = 9.511, p = 0.003$). The post-hoc analysis using Bonferroni's correction revealed a significant main effect of tasks (VFT-letter – Sternberg task; $p < 0.001$, VFT-

Table 3
Spearman's rank correlation coefficients between $\Delta[\text{oxyHb}]$ and demographic, clinical data

	Schizophrenia(30)				Healthy controls(30)			
	VFL-l	VFL-r	TOH-l	TOH-r	VFL-l	VFL-r	TOH-l	TOH-r
Age	-0.14	-0.17	-0.27	-0.20	-0.11	-0.17	-0.30	-0.29
Years of education	-0.25	-0.15	0.07	0.01	0.05	0.01	0.35	0.39 ^a
JART	0.23	0.20	0.45 ^a	0.16	-0.09	-0.06	-0.04	0.02
Onset age	-0.19	-0.19	0.27	0.07				
Duration of illness	-0.11	-0.01	-0.48 ^a	-0.25				
Equivalents of CPZ	0.00	-0.14	-0.11	-0.42 ^a				
PANSS								
Positive symptoms	0.19	0.18	-0.34	-0.12				
Negative symptoms	0.07	0.02	-0.43 ^a	-0.29				
General psychopathology	0.26	0.21	-0.27	-0.15				
Five-factor model of PANSS								
Positive	0.20	0.12	-0.33	-0.21				
Negative	0.01	-0.03	-0.39 ^a	-0.21				
Cognitive	0.18	0.07	-0.52 ^a	-0.53 ^a				
Excitement	0.25	0.36	-0.17	0.19				
Depression/Anxiety	0.01	0.13	-0.10	0.08				

Correlation coefficients are presented using Spearman's rank correlation in both groups. JART, the Japanese version of the National Adult Reading Test; CPZ, chlorpromazine; PANSS, the Positive and Negative Syndrome Scale; DIEPSS, the Drug-Induced Extrapyramidal Symptom Scale. VFL-l, Verbal Fluency test Letter-left; VFL-r, Verbal Fluency test Letter-right; TOH, Tower of Hanoi.

^a $p < 0.05$.

letter - Stroop task; $p < 0.001$, VFT-category - TOH; $p = 0.011$, VFT-category - Stroop task; $p = 0.014$, TOH - Sternberg task; $p < 0.001$, TOH - Stroop task; $p < 0.001$). The design-2 ANCOVA showed no interaction between any factors.

The results of $\Delta[\text{deoxyHb}]$ for each task are as follows. In the VFT-category, the repeated-measures ANCOVA revealed a significant main effect of diagnosis group ($F(1, 58) = 7.545$, $p = 0.008$). Post-hoc analysis revealed that healthy controls exhibited a significantly larger decrease in $\Delta[\text{deoxyHb}]$ than schizophrenia patients. For TOH and the Sternberg task, the repeated-measures ANCOVA revealed a significant main effect of diagnosis group (TOH: $F(1, 58) = 5.957$, $p = 0.018$; Sternberg: $F(1, 58) = 6.297$, $p = 0.015$), and post-hoc analysis revealed that healthy controls exhibited a significantly larger decrease in $\Delta[\text{deoxyHb}]$ than schizophrenia patients. However, there was a significant interaction between diagnosis group and gender ($F(1, 58) = 4.951$, $p = 0.031$) only for TOH, with post-hoc analysis using Bonferroni's correction indicating a significantly larger decrease in $\Delta[\text{deoxyHb}]$ in healthy controls compared to schizophrenia patients in males ($p = 0.002$), and a significantly larger decrease in $\Delta[\text{deoxyHb}]$ in male subjects compared to female subjects in healthy controls ($p = 0.039$). Regarding VFT-letter and the Stroop task, the repeated-measures ANCOVA revealed no significant main effect of diagnosis group ($F(1, 58) < 0.001$, $p = 0.989$, $F(1, 58) = 2.328$, $p = 0.133$ respectively). In the Stroop task, we found a significant effect of task order via the repeated-measures ANCOVA ($F(1, 58) = 8.972$, $p = 0.004$). Post-hoc analysis revealed that a decrease in $\Delta[\text{deoxyHb}]$ in order B was significantly larger than in order A. There was no interaction between factors, except for TOH.

3.3. Correlation

For VFT-letter and TOH, in which a significant main effect of diagnosis group was revealed by the repeated-measures ANCOVA (the design-1 ANCOVA), the correlation between $\Delta[\text{oxyHb}]$ and demographic and clinical parameters was analyzed using Spearman's rank-correlation. These results are

shown in Table 3. In schizophrenia patients, left $\Delta[\text{oxyHb}]$ during TOH showed a significant positive correlation with JART ($\rho = 0.45$) and a significant negative correlation with illness duration ($\rho = -0.48$). Right $\Delta[\text{oxyHb}]$ during TOH correlated negatively with chlorpromazine (CPZ) equivalents ($\rho = -0.42$). Left $\Delta[\text{oxyHb}]$ during TOH also showed a significant negative correlation with negative symptoms scores on PANSS ($\rho = -0.43$) and with negative ($\rho = -0.39$) and cognitive symptoms scores on the five-factor model of PANSS ($\rho = -0.52$). Similarly, right $\Delta[\text{oxyHb}]$ during TOH showed a significant negative correlation with cognitive symptoms scores on the five-factor model of PANSS ($\rho = -0.53$). During VFT-letter, no significant correlation was found between $\Delta[\text{oxyHb}]$ and any of the variables in the analyses. Further analysis of multiple comparisons showed that these correlations between $\Delta[\text{oxyHb}]$ during TOH and clinical parameters in schizophrenia were not statistically significant.

4. Discussion

In this study, we measured PFC activity during four well-established frontal lobe tasks, namely VFT, TOH, the Sternberg, and the Stroop task, using a 2ch-NIRS system. Our findings indicate a significant main effect of diagnosis group for VFT-letter and TOH as analyzed $\Delta[\text{oxyHb}]$ by the repeated-measure ANCOVA (the design-1 ANCOVA). Further analysis of the correlation between clinical parameters and $\Delta[\text{oxyHb}]$ revealed that left $\Delta[\text{oxyHb}]$ during TOH showed a significant negative correlation with negative symptoms scores on PANSS and with negative and cognitive symptoms scores on the five-factor model of PANSS for schizophrenia patients. However, no significant correlation was found between $\Delta[\text{oxyHb}]$ and any clinical parameters during VFT-letter. In the design-2 ANCOVA, a significant main effect of diagnosis group was recognized.

4.1. OxyHb data

The use of a 2ch-NIRS system to evaluate PFC activation during VFT was considered appropriate based on findings

from previous studies using multi-channel systems, which indicated that maximum activation in the PFC corresponded to Fp1-Fp7 and Fp2-Fp8 locations, where the probes were placed for our measurements (Takizawa et al., 2008; Ehliis et al., 2008; Kubota et al., 2005; Watanabe and Kato, 2004; Suto et al., 2004). In both schizophrenia and healthy control groups, task performance was found to be better for VFT-category than for VFT-letter, whereas task-induced $\Delta[\text{oxyHb}]$ were found to be larger during VFT-letter than during VFT-category. In particular, a group difference was more apparent for VFT-letter than VFT-category, which is in line with recent findings by Ehliis et al. (2008). With regard to the retrieval of words during VFT-letter, the task may require larger cognitive demands, as the letter version has been reported unfamiliar to subjects compared to the category version (Martin et al., 1994). This may explain in part why performance of the category-version was better than performance of the letter-version, and $\Delta[\text{oxyHb}]$ during the letter-version task was larger than that during the category version. In addition, pronounced phonological (letter fluency) deficits in schizophrenia patients (Ehliis et al., 2008; Curtis et al., 1999) may also account for the difference between schizophrenia patients and healthy controls regarding $\Delta[\text{oxyHb}]$ during VFT-letter. Thus, VFT-letter appears to be more appropriate than VFT-category when screening for prefrontal dysfunction in schizophrenia.

As for TOH, several studies have reported PFC activation. For instance, activation in the right dorsolateral PFC, bilateral parietal, bilateral premotor area, and the left inferior frontal gyrus was reported in normal subjects using fMRI (Fincham et al., 2002). Findings from studies of patients with PFC lesions have proposed that this cortical area plays a crucial role in the execution of TOH (Morris et al., 1997; Goel and Grafman, 1995). Since no neuroimaging studies using TOH have been reported in schizophrenia, an accurate location of the peak of activation remains to be determined. However, the behavior of $\Delta[\text{oxyHb}]$ in this study was almost identical during TOH and VFT-letter, the means of $\Delta[\text{oxyHb}]$ in healthy controls were larger for TOH (left: 1.78 ± 1.16 , right: 1.77 ± 1.22) than for VFT-letter (left: 1.58 ± 1.01 , right: 1.58 ± 1.01), and the effect size of $\Delta[\text{oxyHb}]$ between healthy controls and schizophrenia patients was larger for TOH (left: 0.90, right: 0.86) than for VFT-letter (left: 0.82, right: 0.88). These reflect the validity of measurements with a 2ch-NIRS system in limited cortical areas like those corresponding to Fp1-Fp7 and Fp2-Fp8. In the present study, there was significant interaction between diagnosis group and gender only for TOH, with male healthy controls showing larger activation than schizophrenia patients. Despite evidence of clinical and biological differences in schizophrenia, particularly in structural brain abnormalities (Nasrallah et al., 1990), specific functional changes in the brain of schizophrenia patients during cognitive tasks in relation to gender have not been reported (Buchsbaum and Hazlett, 1997). Thus, gender difference in cortical activation in schizophrenia needs further investigation.

With regard to the Sternberg task, the measurement areas in this study corresponded well with locations exhibiting significant task-induced activation and significant difference by diagnosis in a previous fMRI study (Johnson et al., 2006). However, no significant difference in $\Delta[\text{oxyHb}]$ was observed between schizophrenia patients and healthy controls in this study. A number of methodological differences between our

study and previous investigations may explain partly the discrepancy in the findings. Firstly, the presence of variability in activation size due to working memory load and diagnosis group effect may account for the difference in the findings. In this regard, previous fMRI studies reported that the relation between activation size and working memory load of the Sternberg task exhibited an inverse U-curve, and that the difference of activation between schizophrenia patients and healthy controls was the smallest when working memory load consisted of a 5-digit paradigm (Johnson et al., 2006; Manoach, 2003), which we used in this study. Secondly, we should consider the differences in data analyses: in previous fMRI studies, the encoding phase and the delay phase were analyzed separately, resulting in significant difference in activation for each phase (Schlösser et al., 2008; Johnson et al., 2006). Since our analyses do not allow phases discrimination, significant findings for each phase cannot be determined in the present study. Thirdly, in the Sternberg task in this study, the encoding and delay phases were comparatively shorter than those used in other studies (Schlösser et al., 2008; Johnson et al., 2006). This might also obscure significant differences in activation. The Stroop task has also been employed in previous PET and fMRI studies where it indicated strong anterior cingulate cortex activation (Alvarez and Emory, 2006). A NIRS study conducted with healthy subjects found a significant increase in $\Delta[\text{oxyHb}]$ in the left inferior-frontal area induced by the Stroop task (Ehliis et al., 2005). This location was somewhat posterior to our measurement position. Thus, although the main purpose of this study was comprehensive assessment of multiple frontal lobe functions using a 2ch-NIRS system, our measurement position might be outside of the major significant activations induced by the Stroop task. Moreover, recent reports suggest that evident activation is associated with the presentation of incongruent stimuli only (Ehliis et al., 2005; Kerns et al., 2005). However, the analyses used in our study do not allow us to distinguish incongruent from congruent stimuli. Furthermore, Boucart et al. (1999) reported that the main alteration evident during the Stroop task for schizophrenia patients was recognized when the words were surrounded by others. In this study, single words were presented on-screen, one at a time. Thus, we cannot rule out the possibility that group difference in $\Delta[\text{oxyHb}]$ could have been found, if we employed the paradigm by Boucart et al. (1999). Limitations in analysis methods may hence account for the lack of Stroop task-induced activation in this study.

As for the effect of the task order, we found that task order was relevant in VFT-letter and the Stroop task. As a result of the design-1 ANCOVA, mean activation values were larger during order A than during order B in VFT letter for both groups. However, mean activation values were larger during order B than during order A in the Stroop task for both groups. Because activation values collected in the latter line of experiments decreased, we concluded that a lower number of tasks and a short time of tasks were most appropriate in clinical examinations.

In the present study, we attempted to relate demographic and major clinical parameters to $\Delta[\text{oxyHb}]$ during performance of those tasks showing significant diagnosis group difference (i.e., VFT-letter and TOH). For both schizophrenia and healthy control groups, $\Delta[\text{oxyHb}]$ did not correlate with

either age or level of performance. In schizophrenia patients, left $\Delta[\text{oxyHb}]$ during TOH correlated positively with JART, and inversely with illness duration. These findings support that a higher premorbid IQ correlates with larger activation in the left PFC, while longer illness predicts smaller activation in the same area. In healthy controls, right $\Delta[\text{oxyHb}]$ during TOH correlated positively with years of education. These data associate higher education with larger activation in the right PFC, which does not hold true in schizophrenia patients. We also tested for a correlation between clinical parameters and $\Delta[\text{oxyHb}]$ during VFT-letter and TOH in which significant diagnosis group effects were observed. During TOH, we found a significant negative correlation of left $\Delta[\text{oxyHb}]$ with negative symptoms scores on PANSS as well as negative and cognitive symptoms scores on the five-factor model of PANSS for schizophrenia patients. However, no significant correlation was found during VFT-letter. A negative correlation between executive function and negative symptoms scores on PANSS or cognitive symptoms scores on the five-factor model of PANSS has been previously reported in schizophrenia (Heydebrand et al., 2004; Bell et al., 1994). Our finding of a significant negative correlation between $\Delta[\text{oxyHb}]$ and negative symptoms scores is consistent with that of these earlier studies and with a report of decreased regional cerebral blood volume in PFC in schizophrenia patients with severe negative symptoms (Gonul et al., 2003). If $\Delta[\text{oxyHb}]$ correlates with negative and cognitive symptoms scores, specific $\Delta[\text{oxyHb}]$ induced by TOH, as demonstrated by 2ch-NIRS, may be used as a physiological state marker of schizophrenia. VFT-letter, however, showed significant differences in $\Delta[\text{oxyHb}]$ between schizophrenia and healthy controls, but no correlation between $\Delta[\text{oxyHb}]$ and clinical parameters, including negative and cognitive symptoms. Upon confirmation of this finding, $\Delta[\text{oxyHb}]$ induced by VFT-letter might constitute a potential physiological marker of the disease. In summary, multiple comparisons showed that correlations between $\Delta[\text{oxyHb}]$ and demographic and clinical parameters in schizophrenia during TOH cannot be deemed significant; albeit, these correlations may hold some meaning.

4.2. DeoxyHb data

Although $[\text{deoxyHb}]$ has been considered mostly related to fMRI BOLD signal, some researchers emphasize that $[\text{deoxyHb}]$ and $[\text{oxyHb}]$ may index neural activation. While decrease in $\Delta[\text{deoxyHb}]$ during neural activation is typical, $\Delta[\text{deoxyHb}]$ behavior is in fact more complex. In simultaneous measurements of fMRI and NIRS, some studies report an increase in the BOLD signal, positively related to an increase in $[\text{oxyHb}]$ in NIRS (Mehagnoul-Schipper et al., 2002; Toronov et al., 2001; Kleinschmidt et al., 1996). Others found an increase in the BOLD signal, positively related to a decrease in $[\text{deoxyHb}]$ in NIRS (Strangman et al., 2002). Furthermore, BOLD signal increase in the area where neural activity was anticipated is sometimes not accompanied by $[\text{deoxyHb}]$ decrease (Yamamoto and Kato, 2002). The BOLD signal is therefore not a reliable predictor of $[\text{deoxyHb}]$. As an index of neural activation, $\Delta[\text{oxyHb}]$ may be suitable, owing to the highest sensitivity amongst the NIRS parameters (Hoshi et al., 2001; Hoshi, 2003). We therefore weighted the results of $[\text{deoxyHb}]$ as less important than those of $[\text{oxyHb}]$, since the

interpretation of $[\text{deoxyHb}]$ in NIRS measurement has not been established yet.

The results of the analysis of $\Delta[\text{deoxyHb}]$ in this study indicated a significant main effect of the diagnosis group was in VFT-category, TOH and the Sternberg task. A significant interaction between diagnosis group and gender was recognized in TOH. A significant effect of task order was recognized in the Stroop task. In TOH, a significant decrease in $\Delta[\text{deoxyHb}]$ as well as increase in $\Delta[\text{oxyHb}]$ could be interpreted as significant group difference in the prefrontal activation. However, the results of $\Delta[\text{deoxyHb}]$ in VFT-category, the Sternberg task, and the Stroop task are difficult to interpret because these three tasks did not show significant differences in $\Delta[\text{oxyHb}]$.

4.3. Limitations

Our study is subject to several limitations. Since this is a cross-sectional study we cannot be sure that $\Delta[\text{oxyHb}]$ during TOH and VFT-letter are reliable physiological markers of schizophrenia. Longitudinal studies are necessary. A second limitation lies in the effect of optical path length factor on estimated $\Delta[\text{oxyHb}]$. Although the optical path length may vary across individuals, we set the optical path length factor at 24 cm in the present study. Calculations of $[\text{oxyHb}]$ and $[\text{deoxyHb}]$ were based on the modified Beer-Lambert law, where

$$\Delta OD = \epsilon(\lambda) \cdot \Delta c \cdot d \cdot B,$$

with ΔOD being the change in absorbance, $\epsilon(\lambda)$ is the molar absorbance coefficient, Δc is the change in the concentration of absorbed materials, d is the distance between optical probes, and B is the differential path length factor. The optical path length ($d \cdot B$) of each subject is needed for quantitative estimation. This requires the assumption that optical path length factors are constant among positions and individuals. Nevertheless, studies using time-resolved spectroscopy methods of NIRS have reported no difference in the path length due to diagnosis (schizophrenia and healthy controls) and laterality (at Fp1-F7 and Fp2-F8), as well as at most 20% path length variability among positions and individuals (Shinba et al., 2004), which could produce about 20% variability in estimated $\Delta[\text{oxyHb}]$ according to the formula. This $\Delta[\text{oxyHb}]$ variability corresponds to 20–30% of its standard deviation. If assumed to be noise, the standard deviation of estimated $\Delta[\text{oxyHb}]$ increases thus lowering the effect sizes, although the effect sizes of $\Delta[\text{oxyHb}]$ during VFT-letter and TOH were sufficiently large (>0.8). The effect size of $\Delta[\text{oxyHb}]$ would hence be greater than 0.8 in absence of inter-individual variability of the optical path length when estimating $\Delta[\text{oxyHb}]$. Furthermore, taking into account the significant differences in $\Delta[\text{oxyHb}]$ during VFT-letter and TOH, and the lack of difference during the Stroop task in the present study, we consider that the group difference between schizophrenia patients and healthy controls in our study could not be affected by the path length factor. As measures of the optical path length factor become easier with technological advances, and quantitative accuracy of $\Delta[\text{oxyHb}]$ improves, the potential clinical application of $\Delta[\text{oxyHb}]$ may dramatically increase. A third limitation is the potential influence of

antipsychotic medications and benzodiazepines (BZD) on $\Delta[\text{oxyHb}]$. A negative correlation was observed between right $\Delta[\text{oxyHb}]$ and CPZ equivalents during TOH, while no correlation was observed between left $\Delta[\text{oxyHb}]$ and CPZ equivalents during either TOH or VFT-letter in this study. We do not have a clear explanation for this finding at the present time. Cognitive improvement in schizophrenia has been reported in association with atypical rather than typical antipsychotics (Keefe et al., 1999). The interpretation of the influence of antipsychotics in this study is complicated by the concomitant use of typical and atypical antipsychotics in some patients. Since BZD affects rCBF (Reinsel et al., 2000), we performed further analyses to divide the schizophrenia group into two subgroups, BZD-on group and BZD-off group, to test for possible differences in $\Delta[\text{oxyHb}]$ between the two subgroups, but none were found (data were not shown). Therefore, we conclude our present results were not influenced by BZD.

4.4. Conclusions

In summary, we have examined hemodynamic changes in $[\text{oxyHb}]$ in the bilateral PFC in schizophrenia patients and healthy controls using a 2ch-NIRS system during several cognitive tasks. VFT-letter and TOH appear to offer a better discriminative power than other neuropsychological tests to recognize PFC dysfunction in schizophrenia patients. In addition, $\Delta[\text{oxyHb}]$ in the left PFC correlated with negative and cognitive symptoms. This finding proposes that TOH and VFT-letter elicit PFC hemodynamic changes which might represent candidate physiological markers of schizophrenia. Despite the limitations of our study, we conclude that the 2ch-NIRS has potential for PFC activity measurements not only in schizophrenia patients, but also in other psychiatric disorders owing to several advantages such as simplicity and low running cost.

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Contributors

K.I. and M.I. designed the study and wrote the protocol and undertook the statistical analysis. K.I., M.A., K.O., Y.Y., N.L., H.T., R.S., and T.Y. conducted data acquisition. K.I. and M.I. analyzed the data. K.I. wrote the first draft of the manuscript. L.C., R.K., T.N., and R.I. contributed to the editing of the final manuscript. All authors revised it critically for important intellectual content and have approved the final manuscript. R.H., H.K., and M.T. supervised the entire project.

Conflict of interest

None.

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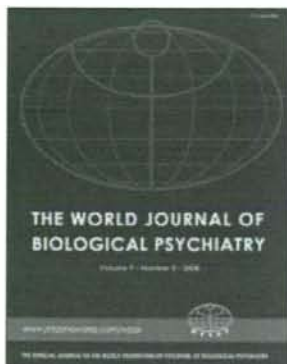
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BRIEF REPORT

Abnormal microstructures of the basal ganglia in schizophrenia revealed by diffusion tensor imaging

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Abstract

There has been a hypothesis that deficits in the basal ganglia–thalamic system may play an important role in the dysfunctional goal-directed behaviour in schizophrenia. By using diffusion tensor imaging, we measured fractional anisotropy (FA) values in the basal ganglia–thalamic system in 42 schizophrenics and 42 matched controls to investigate microstructural tissue alterations in the basal ganglia–thalamic system in schizophrenia. Schizophrenics had significantly lower FA values in the bilateral globus pallidus and left thalamus compared to controls, suggesting that schizophrenics might have microstructural abnormalities in globus pallidus and thalamus. These data support the notion that myelination abnormalities in basal ganglia–thalamic system are related to the pathophysiology of schizophrenia.

Key words: Schizophrenia, diffusion tensor imaging, basal ganglia, globus pallidus, MRI

Introduction

Schizophrenia often demonstrated movement abnormalities, such as catatonia, pacing and other stereotyped behaviours considered to be associated with basal ganglia dysfunction. The basal ganglia regulates not only motor behaviours but also aspects of cognitive and limbic behaviours. There has been a hypothesis that deficits in the basal ganglia–thalamic system may play an important role in the dysfunctional goal-directed behaviour in schizophrenia (Andreasen 1999). In fact, several studies demonstrated abnormalities in the basal ganglia in schizophrenic brains, including the volume reductions of the pallidum internum of postmortem brains of patients with schizophrenia (Bogerts et al. 1985), higher volumes in the globus pallidus of previously

treated patients with schizophrenia than the healthy comparison subjects and the neuroleptic-naïve patients (Gur et al. 1998), fMRI evidence for basal ganglia dysfunction in subjects with schizophrenia (Menon et al. 2001), abnormality of oligodendroglial cells in caudate nucleus in schizophrenia (Uranova et al. 2001), and positive correlation between globus pallidus and the severity of global symptoms in neuroleptic-naïve patients (Spinks et al. 2005).

Diffusion tensor imaging (DTI) is a relatively new technique, and it is useful for evaluating white matter abnormalities in schizophrenia. We have reported progressive changes of white matter integrity in schizophrenia using DTI (Mori et al. 2007). Recently, this technique was applied to investigate

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abnormalities of the subcortical regions in neurodegenerative diseases. Patients with Parkinson's disease had significantly decreased fractional anisotropy (FA) in the region of interest along a line between the substantia nigra and the lower part of the putamen/caudate complex, in which the nigrostriatal dopaminergic neurons are lost in Parkinson's disease, demonstrating its possibility to detect microstructural tissue alterations (Yoshikawa et al. 2004). To investigate possible microstructural abnormalities in the basal ganglia-thalamic system in schizophrenia, we measured FA values in the basal ganglia and the thalami in schizophrenics and in normal controls for comparison, as a sub-analysis of our previous study (Mori et al. 2007).

Material and methods

Subjects and clinical assessments

Forty-two patients with DSM-IV schizophrenia (26 male and 16 female, one left hander, mean age: 40.0 ± 9.3 years old, education: 13.0 ± 2.9 years, mean duration of illness; 16.8 ± 9.0 years, mean daily dose of antipsychotics (chlorpromazine equivalent): 1005.1 ± 735.3 mg/day) (Association 1994) and 42 controls (26 male and 16 female, one left hander, mean age: 39.2 ± 9.0 years old, education: 17.1 ± 3.5 years) were participated in our study. Written informed consent was obtained from all the subjects. This study has been approved by the local ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All the normal subjects were screened by a questionnaire on medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the central nervous system. We employed the Japanese version of National Adult Reading Test (JART) as a convenient tool to measure IQ for participants (premorbid IQ for schizophrenics). Patients had fewer years of education (two-sample *t*-test, $P < 0.0001$), lower scores of JART (controls: 78.8 ± 11.5 , schizophrenics: 58.7 ± 25.3 , two-sample *t*-test $P < 0.001$).

Neuroimaging analysis

MR studies were performed on a 1.5-Tesla Siemens Magnetom Vision Plus system. Axial DTI scans aligned to the plane containing anterior and posterior commissures were acquired with a pulsed-gradient, spin-echo, single-shot echo planar imaging (EPI) sequence (TR/TE = 4000/100 ms, 256×256 matrix, FOV 240 mm, $b = 1000$ s/mm², NEX = 4, 20 slices, 5 mm slice thickness, 1.5 mm gap). Diffusion was measured along six non-collinear directions,

because six directions were maximum number of this Vision Plus system. For each of six gradient directions, four acquisitions were averaged. Four acquisitions without diffusion weighting ($b = 0$) were also averaged. Additionally, a three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence with a gapless series of thin sagittal sections using an MPRage sequence (TR/TE = 11.4/4.4 ms; flip angle, 15°; acquisition matrix, 256×256 ; NEX = 1, FOV 315 mm; slice thickness 1.23 mm) was acquired for evaluating the volume of grey matter (GM), WM and cerebrospinal fluid (CSF) space. Seven diffusion images acquired as above by an in-house script described previously (Mori et al. 2007) on Matlab 6.5 software (Mathworks, Inc., MA, USA). Then, the FA images were spatially normalized using high-dimensional-warping algorithm (Ashburner et al. 1999) and were matched to the FA template image (Figure 1, top). To make the FA template image, we warped FA images of four normal subjects (other than 42 control subjects) to the single-subject T1 template (skull stripped image) using spatial normalization function of SPM2 and averaged the four warped FA images. The transformed FA images were smoothed with a Gaussian kernel (the filter size, full-width half-maximum: $6 \times 6 \times 6$ mm).

Since our interest was FA changes in the basal ganglia and thalamus, we excluded other brain areas by using an explicit mask (Figure 1, top). The resultant FA maps were analyzed using Statistical parametric mapping 2 (SPM2), which implements a 'general linear model'. To test hypotheses about regional population effects, data were analyzed by a two-sample *t*-test without global normalization. JART scores were treated as nuisance variables. Furthermore, we performed correlational analyses between duration of illness, age of onset, total daily dose of antipsychotic drugs (chlorpromazine equivalent) and FA value in the schizophrenics. Our a priori hypothesis is limited to the basal ganglia; however, investigation of the FA changes within this ROI is null hypothesis. Thus, we used $P < 0.05$, corrected for multiple comparisons with Family-Wise Error rate (FWE) within basal ganglia as a statistical threshold.

Results

In comparison with controls, schizophrenics had significantly lower FA values in the bilateral globus pallidus (GP) (Figure 1, bottom). Increased FA values in schizophrenics were not found in any regions (data not shown).

A correlational analysis in the schizophrenics demonstrated a significantly negative correlation

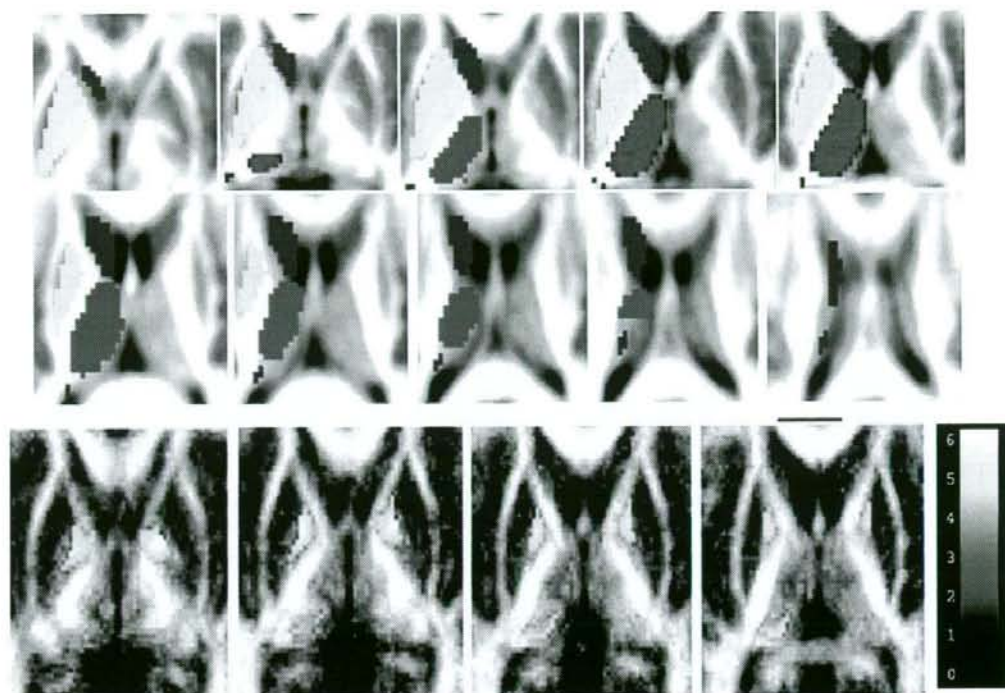


Figure 1. Top: A half of the explicit mask is displayed onto mean FA images of warped FA images obtained from 42 controls (dark blue: caudate nucleus; yellow: putamen; light blue: globus pallidus; red: thalamus). Even after averaging, the mean images are not blurred. Since globus pallidus is traversed by numerous myelinated nerve fibres, it shows higher FA value than other parts of basal ganglia. Bottom: The SPM t is displayed onto mean axial FA images of 42 schizophrenics. A significant reduction of FA value in schizophrenia was noted in the bilateral globus pallidus (right GP: t value = 6.52, Talairach coordinate x, y, z : 18, -2, -2, left GP: t value = 6.37, Talairach coordinate x, y, z : -18, -3, -2) and left thalamus (t value = 4.96, Talairach coordinate x, y, z : -18, -33, 10).

between duration of illness and FA in the left head of the caudate nucleus (t value = 4.77, Talairach coordinate x, y, z : -11, -17, -6). However, there is no significant correlation between duration of illness and FA values in the GP and the thalamus. There was no significant correlation between FA values in the basal ganglia-thalamic system with age of onset or total daily dose of antipsychotic drugs.

Discussion

In this study, we found a significantly reduced FA value in the bilateral GP and left thalamus in schizophrenics compared to controls. We consider that reduced FA may reflect microstructural abnormalities in the basal ganglia-thalamic system in schizophrenia. A previous fMRI study suggested that GP itself may be the primary locus of the functional deficits in the basal ganglia and may be dysfunctional in schizophrenia (Menon et al. 2001). A postmortem study of basal ganglia morphology reported that only

the GP were smaller in schizophrenics than in controls (Bogerts et al. 1985). These studies indicated functional and structural abnormalities in GP in schizophrenia. Our data, reduced FA in GP in schizophrenia, were obtained using a size-adjusted high-dimensional warping method (Ohnishi et al. 2006). Our results, microstructural abnormalities in the GP in schizophrenia, are consistent with previous reports.

Although the underlying mechanisms remain to be clarified, previous DTI studies in parkinsonism have well demonstrated ongoing pathological changes in neurodegenerative diseases, suggesting that this technique has the potential to detect microstructural alterations in the basal ganglia (Yoshikawa et al. 2004). Since pathological findings of schizophrenia are still ambiguous, the underlying pathological changes of reduced FA values in schizophrenia are unclear. However, multiple lines of evidence now converge to implicate oligodendroglia and myelin in schizophrenia (Davis et al.

2003). We assume that damage of myelinated nerve fibres may contribute to FA reduction in the basal ganglia-thalamic system. The GP is traversed by numerous myelinated nerve fibres that give it the pale appearance for which it is named, and has rich connections to the putamen and the thalamus. These histological characteristics of the GP may contribute to its higher FA values. A qualitative electron microscopic study reported the density of concentric lamellar bodies (an indicator of damage of myelinated fibres) was dramatically increased in the caudate nucleus in schizophrenia, as compared to controls (Uranova et al. 2001). Such pathological changes seem to explain decreased FA values in the schizophrenic brain. However, there have been no data on whether GP also have alterations of myelinated fibres. Further pathological studies need to be conducted to draw a firm conclusion on this matter.

Although some studies demonstrated abnormalities of GP in neuroleptic-naïve schizophrenics (Spinks et al. 2005), abnormalities in the basal ganglia have been considered to relate to antipsychotic medication (Gur et al. 1998). In this study, FA changes in the GP and thalamus were not associated with the duration of illness or the daily dose of antipsychotic drugs, suggesting that FA changes in these regions might be independent of medication with neuroleptics. Guidelines for the biological treatment of schizophrenia developed by an international Task Force of the World Federation of Societies of Biological Psychiatry recommended atypical antipsychotics as first line drugs (Falkai et al. 2005, 2006). The differential treatment effects on brain morphology could be due to typical antipsychotics-associated toxicity or greater therapeutic effects of atypical antipsychotics (Lieberman et al. 2005). It would be interesting to compare patients treated with atypical antipsychotics to those with a history of typical antipsychotics treatment; however, the subgroup of patients that were only treated with atypical antipsychotics or the subgroup of patients that were only treated with typical antipsychotics were too small to investigate a possible difference between two groups in FA in our sample. To conclude whether observed change of FA value is a result of medication or relates to the pathophysiology of schizophrenia itself, longitudinal studies on treated schizophrenics, and studies on neuroleptic-naïve schizophrenics should be conducted.

There is a limitation to our study: we used a 1.5-Tesla Siemens Magnetom Vision Plus system, which is a relatively old system. We chose six gradient directions, which is quite low, as this number is the maximum number of directions in this system. Slice thickness of 5 mm and 1.5-mm slice gaps are

methodological drawbacks to this study. The reason why we used a slice thickness of 5 mm and 1.5-mm slice gaps is to cover the whole brain as in our previous study (Mori et al. 2007). There may be a partial volume effect in our mapping parameters, although we minimized the problem by using the high-dimensional warping algorithm.

Our data suggest that patients with schizophrenia might have microstructural abnormalities in globus pallidus and thalamus. The DTI study may be a promising method to investigate microstructural abnormalities in schizophrenia.

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Declaration of Interest

None.

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