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; Ehlis 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; Semkovska 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

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symptoms were assessed using the Drug-Induced Extrapyramidal 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 Extrapyramidal 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 place 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

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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

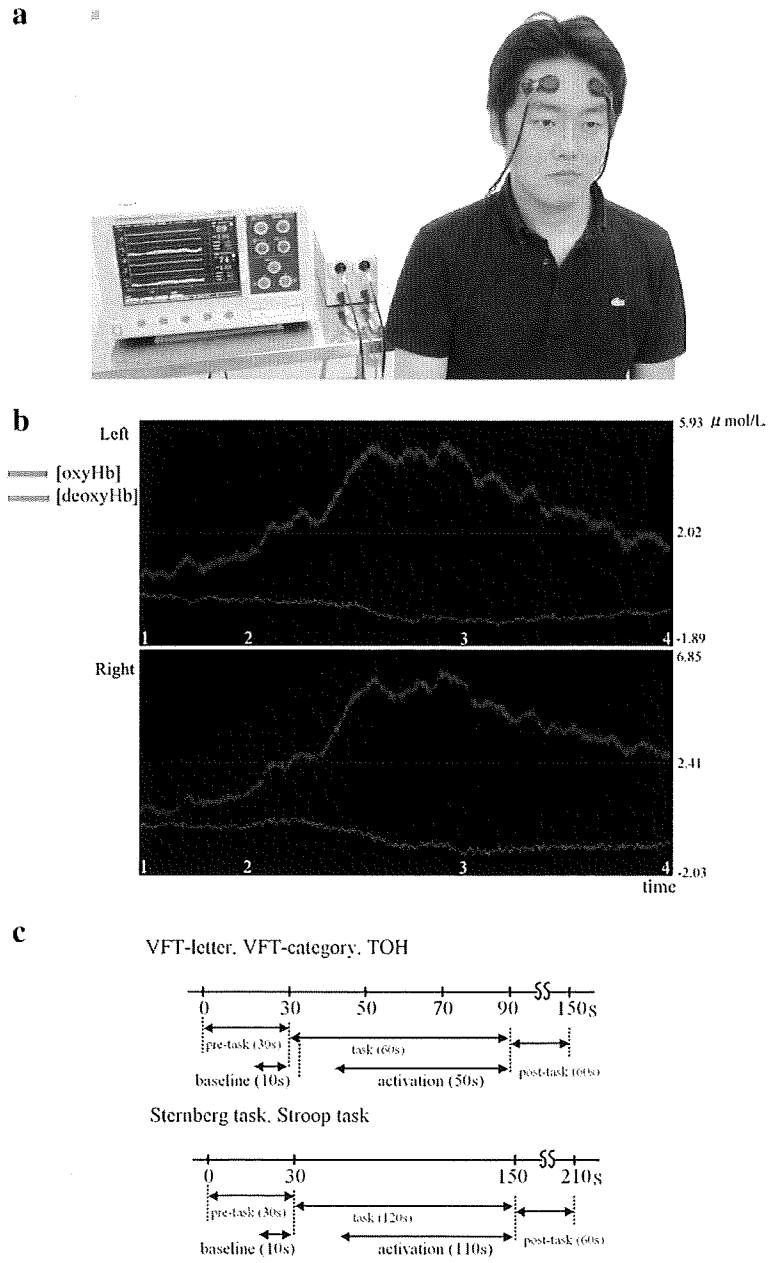

Fig. 1. a. The 2-channel NIRS measurements and the probe setting. b. Actual NIRS data along time course during TOH in one representative healthy control. Upper row shows measurement at left forehead, lower row shows measurement at right forehead. Red line means [oxyHb], blue line means [deoxyHb]. In 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 μmol/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 (Δ[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 Δ[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 Δ[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 (Δ[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 Δ[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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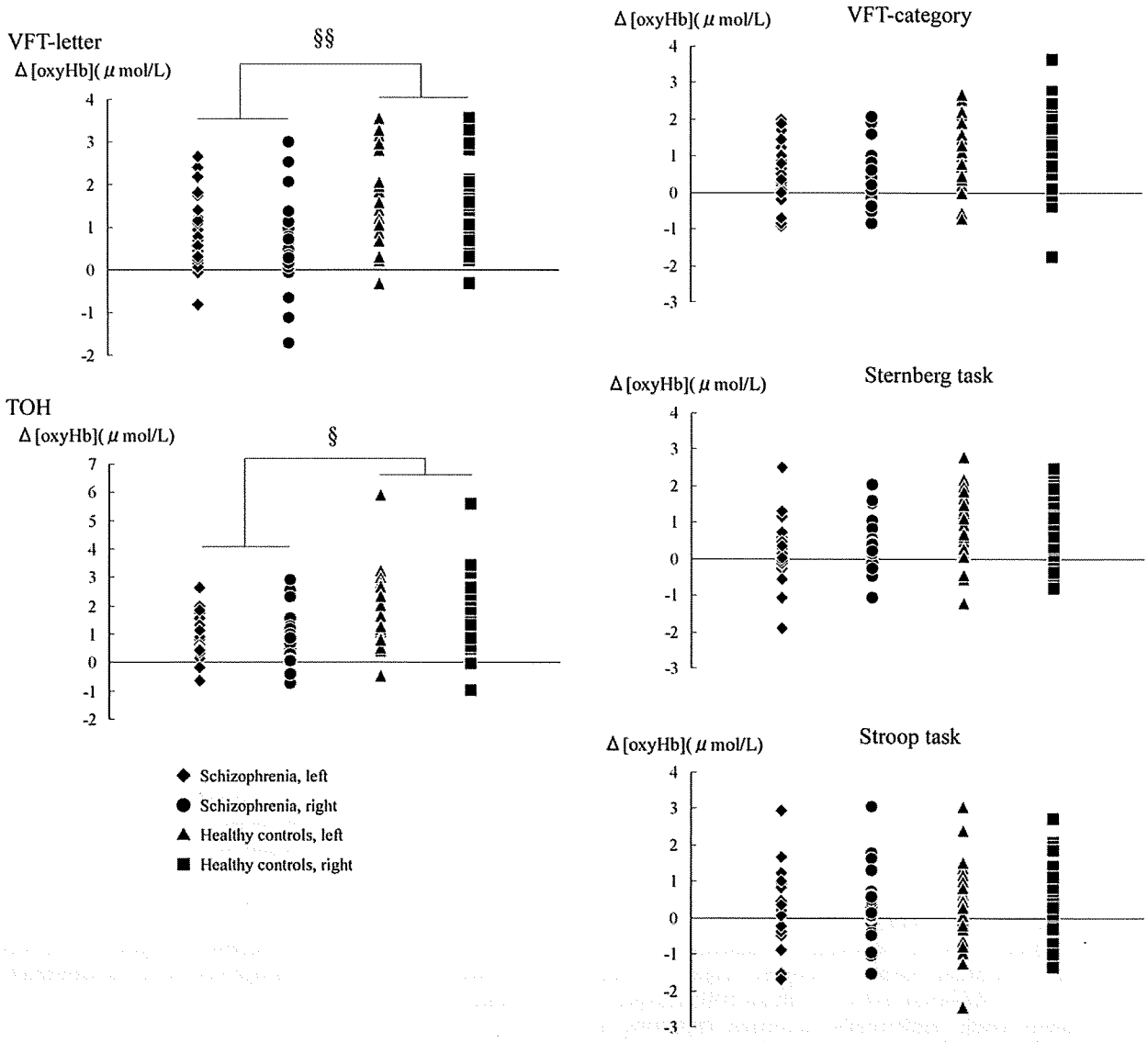

Fig. 2. The results of changes in [oxyHb] (μmol/L; Δ[oxyHb]) in five frontal lobe tasks. During VFT-letter and TOH, the repeated-measures analysis of covariance (the design-1 ANCOVA) revealed significant main effect of diagnosis group. During VFT-category, the Sternberg task, and the Stroop task, the 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 Δ[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 Δ[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 Δ[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 Δ[oxyHb] than schizophrenia patients in males ($p=0.003$), and male subjects showed a significantly larger increase in Δ[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 Δ[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-

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Table 3
Spearman's rank correlation coefficients between Δ[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 Δ[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 Δ[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 decease in Δ[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 Δ[deoxyHb] in healthy controls compared to schizophrenia patients in males ($p=0.002$), and a significantly larger decrease in Δ[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 Δ[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 Δ[oxyHb] and demographic and clinical parameters was analyzed using Spearman's rank-correlation. These results are shown in Table 3. In schizophrenia patients, left Δ[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 Δ[oxyHb] during TOH correlated negatively with chlorpromazine (CPZ) equivalents (rho=−0.42). Left Δ[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 Δ[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 Δ[oxyHb] and any of the variables in the analyses. Further analysis of multiple comparisons showed that these correlations between Δ[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 Δ[oxyHb] by the repeated-measure ANCOVA (the design-1 ANCOVA). Further analysis of the correlation between clinical parameters and Δ[oxyHb] revealed that left Δ[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 Δ[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

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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; Ehlis 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 Δ[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 Ehlis 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 Δ[oxyHb] during the letter-version task was larger than that during the category version. In addition, pronounced phonological (letter fluency) deficits in schizophrenia patients (Ehlis et al., 2008; Curtis et al., 1999) may also account for the difference between schizophrenia patients and healthy controls regarding Δ[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 Δ[oxyHb] in this study was almost identical during TOH and VFT-letter, the means of Δ[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 Δ[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 Δ[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 Δ[oxyHb] in the left inferior-frontal area induced by the Stroop task (Ehlis 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 (Ehlis 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 Δ[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 Δ[oxyHb] during performance of those tasks showing significant diagnosis group difference (i.e., VFT-letter and TOH). For both schizophrenia and healthy control groups, Δ[oxyHb] did not correlate with

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either age or level of performance. In schizophrenia patients, left Δ[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 Δ[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 Δ[oxyHb] during VFT-letter and TOH in which significant diagnosis group effects were observed. During TOH, we found a significant negative correlation of left Δ[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 Δ[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 Δ[oxyHb] correlates with negative and cognitive symptoms scores, specific Δ[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 Δ[oxyHb] between schizophrenia and healthy controls, but no correlation between Δ[oxyHb] and clinical parameters, including negative and cognitive symptoms. Upon confirmation of this finding, Δ[oxyHb] induced by VFT-letter might constitute a potential physiological marker of the disease. In summary, multiple comparisons showed that correlations between Δ[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 [deoxyHb] has been considered mostly related to fMRI BOLD signal, some researchers emphasize that [deoxyHb] and [oxyHb] may index neural activation. While decrease in Δ[deoxyHb] during neural activation is typical, Δ[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 [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 [deoxyHb] in NIRS (Strangman et al., 2002). Furthermore, BOLD signal increase in the area where neural activity was anticipated is sometimes not accompanied by [deoxyHb] decrease (Yamamoto and Kato, 2002). The BOLD signal is therefore not a reliable predictor of [deoxyHb]. As an index of neural activation, Δ[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 [deoxyHb] as less important than those of [oxyHb], since the interpretation of [deoxyHb] in NIRS measurement has not been established yet.

The results of the analysis of Δ[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 Δ[deoxyHb] as well as increase in Δ[oxyHb] could be interpreted as significant group difference in the prefrontal activation. However, the results of Δ[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 Δ[oxyHb].

4.3. Limitations

Our study is subject to several limitations. Since this is a cross-sectional study we cannot be sure that Δ[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 Δ[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 [oxyHb] and [deoxyHb] were based on the modified Beer–Lambert law, where

$$\Delta OD = \varepsilon(\lambda) * \Delta c * d * B,$$

with ΔOD being the change in absorbance, $\varepsilon(\lambda)$ is the molar absorbance efficient, 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 ($d*B$) 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 Δ[oxyHb] according to the formula. This Δ[oxyHb] variability corresponds to 20–30% of its standard deviation. If assumed to be noise, the standard deviation of estimated Δ[oxyHb] increases thus lowering the effect sizes, although the effect sizes of Δ[oxyHb] during VFT-letter and TOH were sufficiently large (>0.8). The effect size of Δ[oxyHb] would hence be greater than 0.8 in absence of inter-individual variability of the optical path length when estimating Δ[oxyHb]. Furthermore, taking into account the significant differences in Δ[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 Δ[oxyHb] improves, the potential clinical application of Δ[oxyHb] may dramatically increase. A third limitation is the potential influence of

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antipsychotic medications and benzodiazepines (BZD) on Δ[oxyHb]. A negative correlation was observed between right Δ[oxyHb] and CPZ equivalents during TOH, while no correlation was observed between left Δ[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 Δ[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 [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, Δ[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.I., 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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References

Alvarez, J.A., Emory, E., 2006. Executive function and the frontal lobes: a meta-analytic review. Neuropsychol. Rev. 16, 17–42.

American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. American Psychiatric Association, Washington, DC.

Bell, M.D., Lysaker, P.H., Milstein, R.M., Beam-Goulet, J.L., 1994. Concurrent validity of the cognitive component of schizophrenia: relationship of PANSS scores to neuropsychological assessments. Psychiatry Res. 54, 51–58.

Boucart, M., Mobarek, N., Cuervo, C., Danion, J.M., 1999. What is the nature of increased Stroop interference in schizophrenia? Acta Psychol. 101, 3–25.

Buchsbaum, M.S., Hazlett, E.A., 1997. Update on PET glucose neuroimaging. Int. Rev. Psychiatry 20, 396–404.

Casement, M.D., Broussard, J.L., Mullington, J.M., Press, D.Z., 2006. The contribution of sleep to improvements in working memory scanning speed: a study of prolonged sleep restriction. Biol. Psychol. 72, 208–212.

Curtin, F., Schulz, P., 1998. Multiple correlations and Bonferroni's correction. Biol. Psychiatry 44, 775–777.

Curtis, V.A., Bullmore, E.T., Morris, R.G., Brammer, M.J., Williams, S.C., Simmons, A., Sharma, T., Murray, R.M., McGuire, P.K., 1999. Attenuated frontal activation in schizophrenia may be task dependent. Schizophr. Res. 37, 35–44.

Ehlis, A.C., Herrmann, M.J., Wagener, A., Fallgatter, A.J., 2005. Multi-channel near-infrared spectroscopy detects specific inferior-frontal activation during incongruent Stroop trials. Biol. Psychol. 69, 315–331.

Ehlis, A.C., Bähne, C.G., Jacob, C.P., Herrmann, M.J., Fallgatter, A.J., 2008. Reduced lateral prefrontal activation in adult patients with attention-deficit/hyperactivity disorder (ADHD) during a working memory task: a functional near-infrared spectroscopy (fNIRS) study. J. Psychiatr. Res. 42, 1060–1067.

Fallgatter, A.J., Strik, W.K., 2000. Reduced frontal functional asymmetry in schizophrenia during a cued continuous performance test assessed with near-infrared spectroscopy. Schizophr. Bull. 26, 913–919.

Fallgatter, A.J., Roesler, M., Sitzmann, L., Heidrich, A., Msueller, T.J., Strik, W.K., 1997. Loss of functional hemispheric asymmetry in Alzheimer's dementia assessed with near-infrared spectroscopy. Brain Res. Cogn. Brain Res. 6, 67–72.

Fincham, J.M., Carter, C.S., van Veen, V., Stenger, V.A., Anderson, J.R., 2002. Neural mechanisms of planning: a computational analysis using event-related fMRI. Proc. Natl. Acad. Sci. 99, 3346–3351.

Folley, B.S., Park, S., 2005. Verbal creativity and schizotypal personality in relation to prefrontal hemispheric laterality: a behavioral and near-infrared optical imaging study. Schizophr. Res. 15, 271–282.

Fox, P.T., Raichle, M.E., 1986. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc. Natl. Acad. Sci. 83, 1140–1144.

Giménez, M., Junqué, C., Pérez, M., Vendrell, P., Baeza, I., Salamero, M., Mercader, J.M., Bernardo, M., 2003. Basal ganglia N-acetylaspartate correlates with the performance in the procedural task 'Tower of Hanoi' of neuroleptic-naive schizophrenic patients. Neurosci. Lett. 347, 97–100.

Goel, V., Grafman, J., 1995. Are the frontal lobes implicated in "planning" functions? Interpreting data from the Tower of Hanoi. Neuropsychologia 33, 623–642.

Gonul, A.S., Kula, M., Eşel, E., Tutuş, A., Sofuoglu, S., 2003. A Tc-99m HMPAO SPECT study of regional cerebral blood flow in drug-free schizophrenic patients with deficit and non-deficit syndrome. Psychiatry Res. 30, 199–205.

Harvey, P.D., Howanitz, E., Parrella, M., White, L., Davidson, M., Mohs, R.C., Hoblyn, J., Davis, K.L., 1998. Symptoms, cognitive functioning, and adaptive skills in geriatric patients with lifelong schizophrenia: a comparison across treatment sites. Am. J. Psychiatry 155, 1080–1086.

Heinrichs, R.W., Zakzanis, K.K., 1998. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12, 426–445.

Heydebrand, G., Weiser, M., Rabinowitz, J., Hoff, A.L., DeLisi, L.E., Csernansky, J.G., 2004. Correlates of cognitive deficits in first episode schizophrenia. Schizophr. Res. 68, 1–9.

Hoshi, Y., 2003. Functional near-infrared optical imaging: utility and limitation in human brain mapping. Psychophysiology 40, 511–520.

Hoshi, Y., Kobayashi, N., Tamura, M., 2001. Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. J. Appl. Physiol. 90, 1657–1662.

Hoshi, Y., Shinba, T., Sato, C., Doi, N., 2006. Resting hypofrontality in schizophrenia: a study using near-infrared time-resolved spectroscopy. Schizophr. Res. 84, 411–420.

Inada, T., 1996. Evaluation and Diagnosis of Drug-induced Extrapyramidal Symptoms: Commentary on the DIEPSS and Guide to its Usage. Seiwa Shoten, Tokyo.

Jobsis, F.F., 1977. Noninvasive infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. Science 198, 1264–1267.

Johnson, M.R., Morris, N.A., Astur, R.S., Calhoun, V.D., Mathalon, D.H., Kiehl, K.A., Pearlson, G.D., 2006. A functional magnetic resonance imaging study of working memory abnormalities in schizophrenia. Biol. Psychiatry 60, 11–21.

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Kameyama, M., Fukuda, M., Uehara, T., Mikuni, M., 2004. Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study. Neuroimage 22, 1715–1721.
Kameyama, M., Fukuda, M., Yamagishi, Y., Sato, T., Uehara, T., Ito, M., Suto, T., Mikuni, M., 2006. Frontal lobe function in bipolar disorder: a multichannel near-infrared spectroscopy study. Neuroimage 29, 172–184.
Kawaguchi, S., Ukai, S., Shinosaki, K., Ishii, R., Yamamoto, M., Ogawa, A., Mizuno-Matsumoto, Y., Fujita, N., Yoshimine, T., Takeda, M., 2005. Information processing flow and neural activations in the dorsolateral prefrontal cortex in the Stroop task in schizophrenic patients. A spatially filtered MEG analysis with high temporal and spatial resolution. Neuropsychobiology 51, 191–203.
Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13, 261–276.
Keefe, R.S., Silva, S.G., Perkins, D.O., Lieberman, J.A., 1999. The effects of atypical antipsychotic drugs on neurocognitive impairment in schizophrenia: a review and meta-analysis. Schizophr. Bull. 25, 201–222.
Kerns, J.G., Cohen, J.D., MacDonald III, A.W., Johnson, M.K., Stenger, V.A., Aizenstein, H., Carter, C.S., 2005. Decreased conflict- and error-related activity in the anterior cingulate cortex in subjects with schizophrenia. Am. J. Psychiatry 162, 1833–1839.
Kleinschmidt, A., Obrig, H., Requardt, M., Merboldt, K.D., Dirnagl, U., Villringer, A., Frahm, J., 1996. Simultaneous recording of cerebral blood oxygenation changes during human brain activation by magnetic resonance imaging and near-infrared spectroscopy. J. Cereb. Blood Flow Metab. 16, 817–826.
Kubota, Y., Toichi, M., Shimizu, M., Mason, R.A., Coconcea, C.M., Findling, R.L., Yamamoto, K., Calabrese, J.R., 2005. Prefrontal activation during verbal fluency tests in schizophrenia–a near-infrared spectroscopy (NIRS) study. Schizophr. Res. 77, 65–73.
Lindenmayer, J.P., Bernstein-Hyman, R., Grochowski, S., 1994. A new five factor model of schizophrenia. Psychiatr. Q. 65, 299–322.
Ma, X., Wang, Q., Sham, P.C., Liu, X., Rabe-Hesketh, S., Sun, X., Hu, J., Meng, H., Chen, W., Chen, E.Y., Deng, W., Chan, R.C., Murray, R.M., Collier, D.A., Li, T., 2007. Neurocognitive deficits in first-episode schizophrenic patients and their first-degree relatives. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144, 407–416.
Manoach, D.S., 2003. Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. Schizophr. Res. 60, 285–298.
Martin, A., Wiggs, C.L., Lalonde, F., Mack, C., 1994. Word retrieval to letter and semantic cues: a double dissociation in normal subjects using interference tasks. Neuropsychologia 32, 1487–1494.
Matsuoka, K., Uno, M., Kasai, K., Koyama, K., Kim, Y., 2006. Estimation of premorbid IQ in individuals with Alzheimer's disease using Japanese ideographic script (Kanji) compound words: Japanese version of National Adult Reading Test. Psychiatry Clin. Neurosci. 60, 332–339.
Mehagnoul-Schipper, D.J., van der Kallen, B.F., Colier, W.N., van der Sluijs, M.C., van Erning, L.J., Thijssen, H.O., Oeseburg, B., Hoefnagels, W.H., Jansen, R.W., 2002. Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. Hum. Brain Mapp. 16, 14–23.
Morris, R.G., Miotto, E.C., Feigenbaum, J.D., 1997. Planning ability after frontal and temporal lobe lesions in humans: the effects of selection equivocation and working memory load. Cognitiveneuropsychology 14, 1007–1027.
Nakahachi, T., Ishii, R., Iwase, M., Canuet, L., Takahashi, H., Kurimoto, R., Ikezawa, K., Azechi, M., Sekiyama, R., Honaga, E., Uchiumi, C., Iwakiri, M., Motomura, N., Takeda, M., 2008. Frontal activity during the digit symbol substitution test determined by multichannel near-infrared spectroscopy. Neuropsychobiology 57, 151–158.
Nasrallah, H.A., Schwarzkopf, S.B., Olson, S.C., Coffman, J.A., 1990. Gender differences in schizophrenia on MRI brain scans. Schizophr. Bull. 16, 205–210.
Okada, F., Tokumitsu, Y., Hoshi, Y., Tamura, M., 1994. Impaired interhemispheric integration in brain oxygenation and hemodynamics in schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci. 244, 17–25.
Okamoto, M., Dan, H., Sakamoto, K., Takeo, K., Shimizu, K., Kohno, S., Oda, I., Isobe, S., Suzuki, T., Kohyama, K., Dan, I., 2004. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. Neuroimage 21, 99–111.
Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.
Pratt, J.A., Winchester, C., Egerton, A., Cochran, S.M., Morris, B.J., 2008. Modelling prefrontal cortex deficits in schizophrenia: implications for treatment. Br. J. Pharmacol. 153 (Suppl 1), S465–S470.
Ragland, J.D., Yoon, J., Minzenberg, M.J., Carter, C.S., 2007. Neuroimaging of cognitive disability in schizophrenia: search for a pathophysiological mechanism. Int. Rev. Psychiatry 19, 417–427.
Reinsel, R.A., Veselis, R.A., Dnistrian, A.M., Feshchenko, V.A., Beattie, B.J., Duff, M.R., 2000. Midazolam decreases cerebral blood flow in the left prefrontal cortex in a dose-dependent fashion. Int. J. Neuropsychopharmacol. 3, 117–127.
Richter, M.M., Herrmann, M.J., Ehlis, A.C., Plichta, M.M., Fallgatter, A.J., 2007. Brain activation in elderly people with and without dementia: influences of gender and medication. World J. Biol. Psychiatry 8, 23–29.
Schlösser, R.G., Koch, K., Wagner, G., Nenadic, I., Roebel, M., Schachtzabel, C., Axer, M., Schultz, C., Reichenbach, J.R., Sauer, H., 2008. Inefficient executive cognitive control in schizophrenia is preceded by altered functional activation during information encoding: an fMRI study. Neuropsychologia 46, 336–347.
Semkovska, M., Bédard, M.A., Stip, E., 2001. Hypofrontality and negative symptoms in schizophrenia: synthesis of anatomic and neuropsychological knowledge and ecological perspectives. Encephale 27, 405–415.
Shinba, T., Nagano, M., Kariya, N., Ogawa, K., Shinozaki, T., Shimosato, S., Hoshi, Y., 2004. Near-infrared spectroscopy analysis of frontal lobe dysfunction in schizophrenia. Biol. Psychiatry 55, 154–164.
Soul, J.S., du Plessis, A.J., 1999. New technologies in pediatric neurology. Near-infrared spectroscopy. Semin. Pediatr. Neurol. 6, 101–110.
Sternberg, S., 1966. High-speed scanning in human memory. Science 153, 652–654.
Strangman, G., Culver, J.P., Thompso, J.H., Boas, D.A., 2002. A quantitative comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation. Neuroimage 17, 719–731.
Suto, T., Fukuda, M., Ito, M., Uehara, T., Mikuni, M., 2004. Multichannel near-infrared spectroscopy in depression and schizophrenia: cognitive brain activation study. Biol. Psychiatry 55, 501–511.
Takizawa, R., Kasai, K., Kawakubo, Y., Marumo, K., Kawasaki, S., Yamasue, H., Fukuda, M., 2008. Reduced frontopolar activation during verbal fluency task in schizophrenia: a multi-channel near-infrared spectroscopy study. Schizophr. Res. 99, 250–262.
Toronov, V., Webb, A., Choi, J.H., Wolf, M., Michalos, A., Gratton, E., Hueber, D., 2001. Investigation of human brain hemodynamics by simultaneous near-infrared spectroscopy and functional magnetic resonance imaging. Med. Phys. 28, 521–527.
Watanabe, A., Kato, T., 2004. Cerebrovascular response to cognitive tasks in patients with schizophrenia measured by near-infrared spectroscopy. Schizophr. Bull. 30, 435–444.
Yamamoto, T., Kato, T., 2002. Paradoxical correlation between signal in functional magnetic resonance imaging and deoxygenated haemoglobin content in capillaries: a new theoretical explanation. Phys. Med. Biol. 47, 1121–1141.

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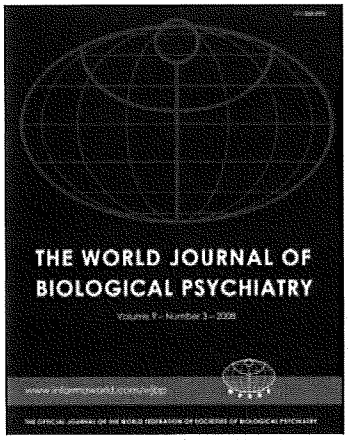

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Abnormal microstructures of the basal ganglia in schizophrenia revealed by diffusion tensor imaging

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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-naive 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-naive 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/mm^2, 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: 6×6×6 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

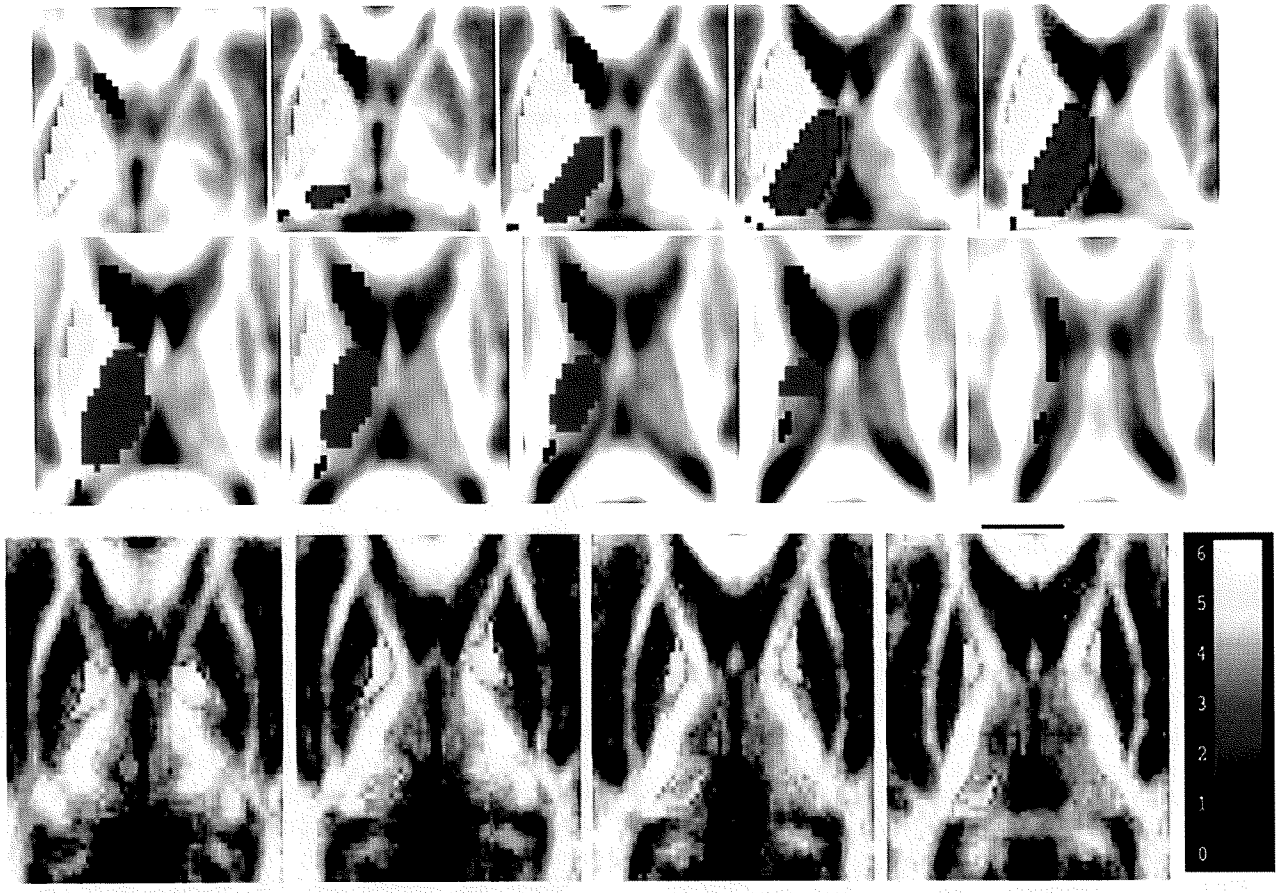

Figure 1. Top: A half of the explicit mask is displayed onto mean FA images of warped FA images obtained from 42 controls (dark blue: caudate nucleus; yellow: putamen; light blue: globus pallidus; red: thalamus). Even after averaging, the mean images are not blurred. Since globus pallidus is traversed 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-naive 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.

References

Andreasen NC. 1999. A unitary model of schizophrenia: Bleüler's 'fragmented phrene' as schizencephaly. Arch Gen Psychiatry 56:781–787.

Ashburner J, Andersson JL, Friston KJ. 1999. High-dimensional image registration using symmetric priors. Neuroimage 9:619–628.

American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders. 4th ed. (DSM-IV). Washington, DC: American Psychiatric Association.

Bogerts B, Meertz E, Schonfeldt-Bausch R. 1985. Basal ganglia and limbic system pathology in schizophrenia. A morphometric study of brain volume and shrinkage. Arch Gen Psychiatry 42:784–791.

Davis KL, Stewart DG, Friedman JI, et al. 2003. White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry 60:443–456.

Falkai P, Wobrock T, Lieberman J, Glenthoj B, Gattaz WF, Moller HJ. 2005. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of schizophrenia, Part 1: acute treatment of schizophrenia. World J Biol Psychiatry 6:132–191.

Falkai P, Wobrock T, Lieberman J, Glenthoj B, Gattaz WF, Moller HJ. 2006. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of schizophrenia, part 2: long-term treatment of schizophrenia. World J Biol Psychiatry 7:5–40.

Gur RE, Maany V, Mozley PD, Swanson C, Bilker W, Gur RC. 1998. Subcortical MRI volumes in neuroleptic-naive and

treated patients with schizophrenia. Am J Psychiatry 155:1711–1717.

Lieberman JA, Tollefson GD, Charles C, et al. 2005. Antipsychotic drug effects on brain morphology in first-episode psychosis. Arch Gen Psychiatry 62:361–370.

Menon V, Anagnoson RT, Glover GH, Pfefferbaum A. 2001. Functional magnetic resonance imaging evidence for disrupted basal ganglia function in schizophrenia. Am J Psychiatry 158:646–649.

Mori T, Ohnishi T, Hashimoto R, et al. 2007. Progressive changes of white matter integrity in schizophrenia revealed by diffusion tensor imaging. Psychiatry Res 154:133–145.

Ohnishi T, Hashimoto R, Mori T, et al. 2006. The association between the Val158Met polymorphism of the catechol-O-methyl transferase gene and morphological abnormalities of the brain in chronic schizophrenia. Brain 129:399–410.

Spinks R, Nopoulos P, Ward J, Fuller R, Magnotta VA, Andreasen NC. 2005. Globus pallidus volume is related to symptom severity in neuroleptic naive patients with schizophrenia. Schizophr Res 73:229–233.

Uranova N, Orlovskaya D, Vikhreva O, et al. 2001. Electron microscopy of oligodendroglia in severe mental illness. Brain Res Bull 55:597–610.

Yoshikawa K, Nakata Y, Yamada K, Nakagawa M. 2004. Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI. J Neurol Neurosurg Psychiatry 75:481–484.

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Impaired long-term memory retention and working memory in sdy mutant mice with a deletion in Dtnbp1, a susceptibility gene for schizophrenia

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Abstract

Background: Schizophrenia is a complex genetic disorder caused by multiple genetic and environmental factors. The dystrobrevin-binding protein 1 (DTNBP1: dysbindin-1) gene is a major susceptibility gene for schizophrenia. Genetic variations in DTNBP1 are associated with cognitive functions, general cognitive ability and memory function, and clinical features of patients with schizophrenia including negative symptoms and cognitive decline. Since reduced expression of dysbindin-1 has been observed in postmortem brains of patients with schizophrenia, the sandy (sdy) mouse, which has a deletion in the Dtnbp1 gene and expresses no dysbindin-1 protein, could be an animal model of schizophrenia. To address this issue, we have carried out a comprehensive behavioral analysis of the sdy mouse in this study.

Results: In a rotarod test, sdy mice did not exhibit motor learning whilst the wild type mice did. In a Barnes circular maze test both sdy mice and wild type mice learned to selectively locate the escape hole during the course of the training period and in the probe trial conducted 24 hours after last training. However, sdy mice did not locate the correct hole in the retention probe tests 7 days after the last training trial, whereas wild type mice did, indicating impaired long-term memory retention. A T-maze forced alternation task, a task of working memory, revealed no effect of training in sdy mice despite the obvious effect of training in wild type mice, suggesting a working memory deficit.

Conclusion: Sdy mouse showed impaired long-term memory retention and working memory. Since genetic variation in DTNBP1 is associated with both schizophrenia and memory function, and memory function is compromised in patients with schizophrenia, the sdy mouse may represent a useful animal model to investigate the mechanisms of memory dysfunction in the disorder.

Background

Schizophrenia is a complex genetic disorder characterized by profound disturbances of cognition, emotion and social functioning. DTNBP1 (dystrobrevin binding protein 1; dysbindin-1) has been one of the most studied and promising schizophrenia susceptibility genes [1-3]. Postmortem brain studies have demonstrated reduced expression of dysbindin-1 protein and mRNA in the schizophrenic brain [4-6]. DTNBP1 risk haplotypes for schizophrenia have been associated with decreased gene expression, whereas DTNBP1 protective haplotypes for the disorder have been associated with increased gene expression [7]. Furthermore, chronic treatment of mice with antipsychotics was not found to affect the expression levels of dysbindin-1 protein and mRNA in their brains [6,8], suggesting that prior evidence of lower dysbindin-1 protein and mRNA levels in the postmortem brains of schizophrenics is not likely to be an artifact of antemortem drug treatment. Together, these data indicate that the dysbindin-1 gene may confer susceptibility to schizophrenia through reduced expression.

Dysbindin-1 is expressed relatively ubiquitously in the brain, localized to neuronal cell bodies. It is expressed in regions implicated in schizophrenia, including the frontal cortex, temporal cortex, hippocampus, caudate, putamen, nucleus accumbens, amygdala, thalamus, and midbrain [5]. It may be involved in glutamatergic and dopaminergic function related to the pathophysiology of schizophrenia [9-13]. As the behavioral level, a genetic variation of DTNBP1 was reported to influence general cognitive ability and to be associated with cognitive decline in schizophrenia [14,15]. Memory function, one of the representative neurobiological traits related to the risk for developing schizophrenia, was also associated with genetic variations in DTNBP1 [16,17]. Moreover, the association between some clinical features of schizophrenia, such as its negative symptoms, and a risk haplotype of DTNBP1 has been demonstrated [18,19]. Risk genetic variations in DTNBP1, therefore, might be related to the cognitive functions affected in schizophrenia.

Obtaining an animal model of schizophrenia is extremely important in investigating the pathogenesis and treatment of the disease [20,21]. If a specific gene is suggested to be involved in schizophrenia by human genetic studies, the role of the gene should be examined in detail by using animals that carry abnormal expression and/or function of the genes [22]. Several mice with mutations in putative schizophrenia susceptibility genes have been shown to exhibit behavioral abnormalities reminiscent of schizophrenia [23-28]. Improved animal models of schizophrenia will provide valuable advances in the treatment of patients with the disorder.

Recently, we provided the first report of a behavioral analysis of the sandy (sdy) mutant mouse, which expresses no dysbindin-1 protein owing to a deletion in the dysbindin-1 gene [9]. Sdy was reported as a mutant mouse with diluted pigmentation that arose spontaneously in the DBA/2J inbred mouse strain and has simultaneous defects in melanosomes, lysosomes and platelet dense granules [29]. The sdy mice showed less activity and spent less time in the center of an open field apparatus [9]. Consistent with the latter observation, sdy mice also displayed evidence of heightened anxiety-like responses and deficits in social interaction [9]. However, cognitive ability has not been examined in sdy mice, although human genetic studies have consistently shown the effects of DTNBP1 genotypes on human cognitive function. Therefore, we performed a battery of behavioral analyses including memory performance in sdy mice.

Results

General behavioral characteristics of sdy mice

To address the behavioral effects of Dtnbp1 deficiency, we subjected sdy mutant mice to a comprehensive behavioral test battery that covers many distinct behavioral domains, from simple sensorimotor functions to higher brain functions, including learning and memory. We present here results showing significant impact of Dtnbp1 deficiency. The raw data of behavioral tests, which are not described in this paper, are disclosed in the gene-brain-phenotyping database https://behav.hmro.med.kyoto-u.ac.jp/. The results of social interaction, hot plate test, acoustic startle response and its prepulse inhibition and the passive avoidance test are open to the public in the database. Sdy mice did not differ significantly from wild type mice in overall health and appearance, body weight (wild type, 25.09 ± 0.386 g; sdy, 24.985 ± 0.623 g, $F(1, 38) = 0.021$, $p = 0.8868$; genotype effect), or core body temperature (wild type, 36.8 ± 0.146°C; sdy, 36.445 ± 0.121°C, $F(1, 38) = 3.509$, $p = 0.0688$; genotype effect). In addition, there was no significant difference between sdy mice and wild type mice in sensory-motor reflex (eye blink, ear touch, whisker twitch, righting reflex; data not shown) or muscular strength assessed in grip strength test (wild type, 0.623 ± 0.023 N; sdy, 0.675 ± 0.02 N, $F(1, 38) = 3.037$, $p = 0.0895$) and wire hang test (wild type, 42.05 ± 3.774 sec; sdy, 38.65 ± 4.234 sec, $F(1, 38) = 0.359$, $p = 0.5524$).

Locomotor activity and motor coordination of sdy mice

To examine spontaneous locomotor activity and response to a novel environment, sdy mice and wild type mice were assayed in an open field test. Sdy mice showed decreased locomotor activity and exploratory behavior (distance traveled in 120 min: wild type, 5829.850 ± 665.814 cm; sdy, 4208.250 ± 432.967 cm, $F(1, 38) = 4.220$, $p = 0.0469$) (Additional figure 1A). There was no significant difference in the vertical activity, stereotypic behavior or

time spent in the center area in the open field test (Additional figure 1B, C, and 1D).

Decreased locomotor activity and exploratory behavior were also detected in the light/dark transition test (Additional figure 2A, B). There was a significant genotype difference in distance traveled in the dark box ($F(1, 38) = 21.437$, $p < 0.0001$) and time course for the decrease in distance traveled in the dark box was significantly different between genotypes ($F(9, 342) = 1.958$, $p = 0.0434$) (Additional figure 2B). There was no significant difference in time spent in the light box, often used as an index of anxiety-like behavior. We also conducted an elevated plus maze test to assess anxiety-like behaviors and no significant difference between genotypes was observed (Additional figure 3).

In a rotarod test, wild type mice demonstrated significant improvement in latencies to fall ($F(5, 95) = 5.024$, $p = 0.0004$; trial effect), which was not evident in the sdy mice ($F(5, 95) = 1.290$, $p = 0.2749$; trial effect) (Figure 1). Since the effect of motor learning reached a plateau in wild type mice after the 5th trial, we compared the performance of each genotype in the 5th and 6th trials. In these trials, there was a significant difference in latency to fall between sdy and wild type mice ($F(1,38) = 5.720$, $p = 0.0218$; genotype effect). In addition, sdy mice showed a swimming deficit in a Porsolt forced swim test, where more sdy mice drawn to death than wild type mice (wild type, 0 out of 8 mouse was died; sdy, 3 out of 8 mice were died, Fisher's exact test, $p = 0.20$). While this difference did not reach statistical significance, an effect may have been seen if we had stopped the experiment prematurely due to the drastic consequences. We suspect the reason for the sdy mice drowning in the experiment may have been due to deficits in swimming ability and/or exercise performance.

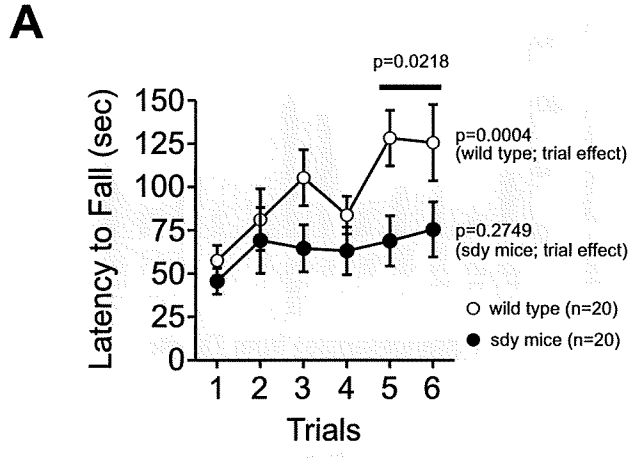

Figure 1
Motor coordination deficit of sdy mice. Latency to fall (second) from the rotating drum was counted in wild type mice and sdy mice in a rotarod test. First 3 trials were conducted on the first day, and later 3 trials were conducted on the second day. The trial effects of each genotype were analyzed by one-way repeated measures ANOVA and the genotype effect in 5th and 6th trial was analyzed by two-way repeated measures ANOVA.

Performance in the Barnes circular maze test

Long term spatial memory, which is dependent on the functioning of the hippocampus, was assessed in sdy mice and wild type mice using a Barnes circular maze [30-32]. The task is similar to the Morris water maze as both tests require an escape response. The Barnes maze test was chosen for this study since it does not involve swimming like the Morris water maze [30-32]. Given the possible motor deficits in sdy mice, swimming ability might have given an advantage to wild type mice over sdy mice in the Morris water maze.

Both sdy mice and wild type mice learned to locate the escape hole during the course of the training period as indicated by a progressive reduction in latencies and numbers of errors to escape (wild type, $F(32, 512) = 2.896$, $p < 0.0001$, sdy, $F(32, 448) = 2.806$, $p < 0.0001$; trial effect was analyzed by one-way repeated measures analysis of variance (ANOVA)). Through the training trials, there were no statistical differences between sdy mice and wild type mice in latencies ($F(1, 30) = 0.001$, $p = 0.9707$; genotype effect), errors ($F(1, 30) = 0.429$, $p = 0.5176$; genotype effect), and distances ($F(1, 30) = 0.058$, $p = 0.8108$; genotype effect) to escape through the target hole (Figure 2A).

The probe trial was conducted 24 hours after the last training session. Both sdy mice and wild type mice selectively located the correct target hole where the escape box had been and both sdy mice and wild type mice spent significantly more time around the target hole compared to the holes adjacent to the target (paired t-test, wild type: $t(17) = 4.645$, $p = 0.0002$; sdy mice: $t(13) = 6.538$, $p < 0.0001$) (Figure 2B). To assess the long-term retention of spatial memory in sdy mice, we also conducted probe tests 7 days after the last training trial. During the retention probe test, wild type mice selectively located the correct target hole where the escape box had been and spent significantly more time around the target hole compared to the adjacent holes (paired-test, $t(17) = 3.239$, $p = 0.0048$), but sdy mice did not (paired-test, $t(13) = 0.983$, $p = 0.3437$)(Figure 2C). These results indicate that sdy mice are impaired in memory retention rather than memory recall.

Performance in the T-maze forced alternation task

We next examined a T-maze forced alternation task in sdy mice and wild type mice, a task of working memory [33-

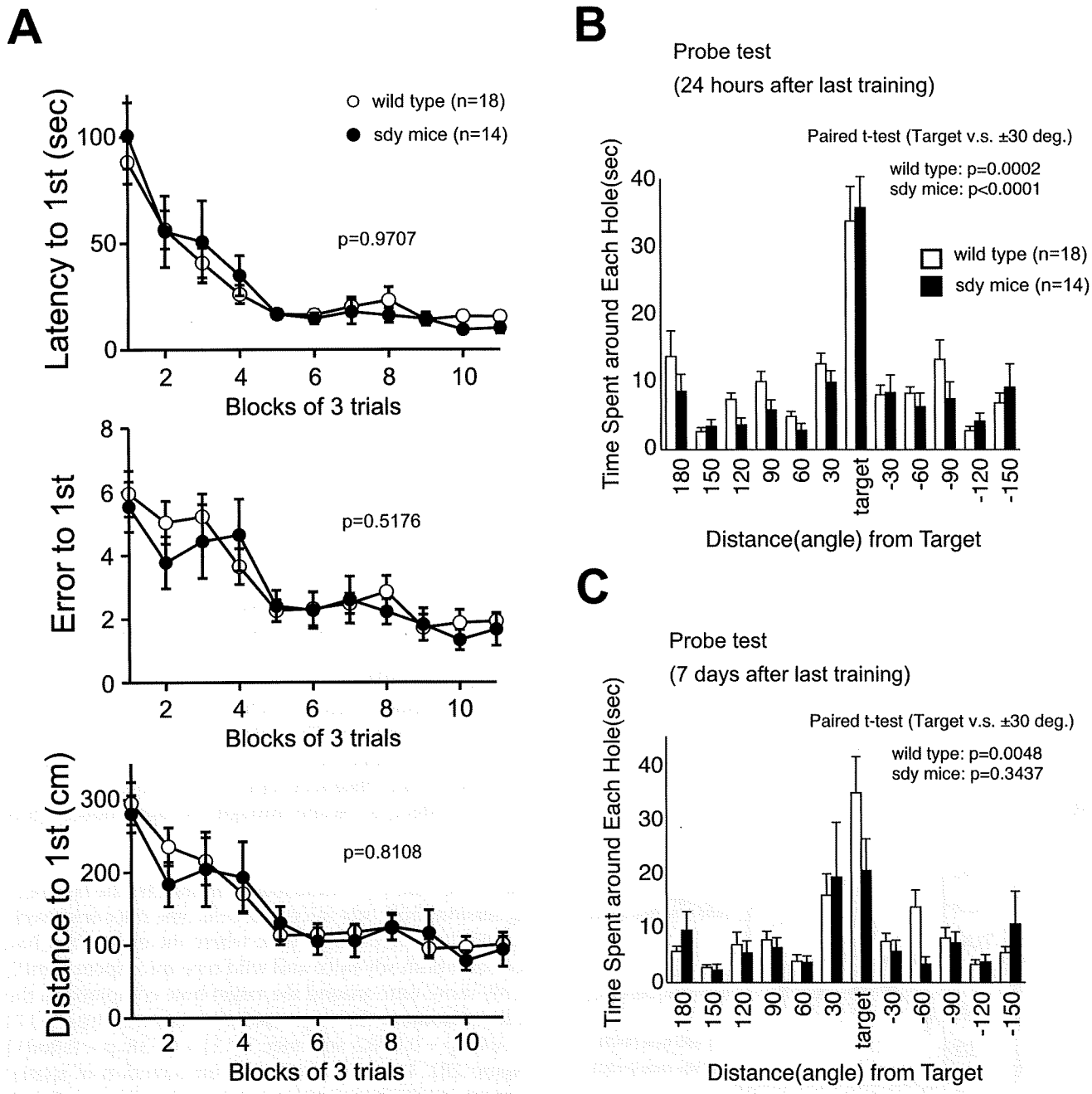

Figure 2

Deficit of long-term memory retention in sdy mice. (A) Latency to reach the target hole (up), numbers of errors (middle) and distance to reach the target hole (bottom) across training were recorded. Data were analyzed by two-way repeated measures ANOVA. Data are presented as averages of 3 trials. (B) Time spent around each hole in the probe trial conducted 24 hours after last training. (C) Time spent around each hole in the probe trial conducted 7 days after last training. Time spent around target hole and holes adjacent to the target were compared by paired t-test.