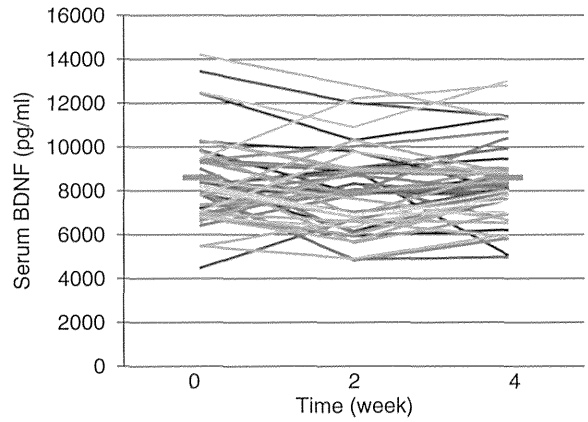

Figure 7 Serum BDNF change during treatment with fluvoxamine. Red line shows the mean of value.

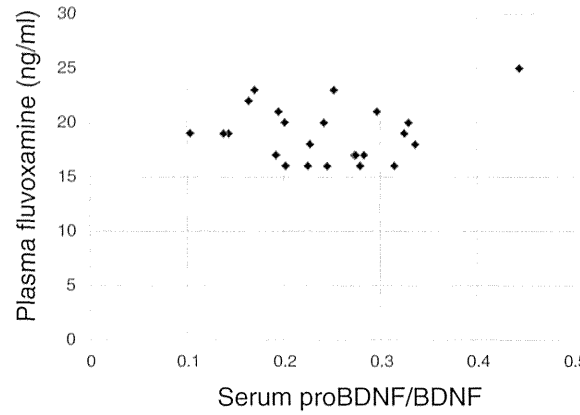

Figure 9 The proBDNF/BDNF ratio and plasma fluvoxamine concentration.

between plasma fluvoxamine levels at 4 weeks and the changes in serum BDNF levels (from 0 to 4 weeks) (r = 0.117, p = 0.691) (Figure 11).

Discussion

Recent meta-analyses demonstrated that mature BDNF levels in serum in patients with MDD were decreased compared to those in healthy controls. The result in the present study regarding serum BDNF confirms the recent meta-analyses [10,14,15]. Low serum mature BDNF levels increased over the course of antidepressant treatment [10,14,15]. We have previously reported that a significant correlation was found between the HAMD17 score and serum BDNF levels before pharmacotherapy [16]. In the present study, we reconfirmed our previous finding. A correlation was not however observed between serum proBDNF levels and the HAMD17 scores before starting fluvoxamine. In addition, there was no relationship between serum levels of proBDNF/BDNF and HAMD17. Taking these findings into account, the BDNF level, but not proBDNF and proBDNF/BDNF, reflects the severity of MDD. Moreover, no correlation was observed between serum fluvoxamine levels and serum levels of proBDNF/BDNF. The result in the present study suggests that the plasma fluvoxamine level was not independent of proBDNF/BDNF in MDD patients after fluvoxamine treatment. In addition, serum levels of BDNF and proBDNF/BDNF did not change at least 4 weeks after fluvoxamine administration. However, serum proBDNF increased during fluvoxamine treatment but did not reach the statistically significant level. Taking these findings into account, our hypothesis was not confirmed. In other words, the influence of fluvoxamine on serum levels of proBDNF, BDNF, and proBDNF/BDNF is complicated. Another interpretation is that 4 weeks is not enough to alter the dynamics of proBDNF and BDNF. Zhou et al. [17] reported that protein and serum levels of proBDNF were higher in MDD than in

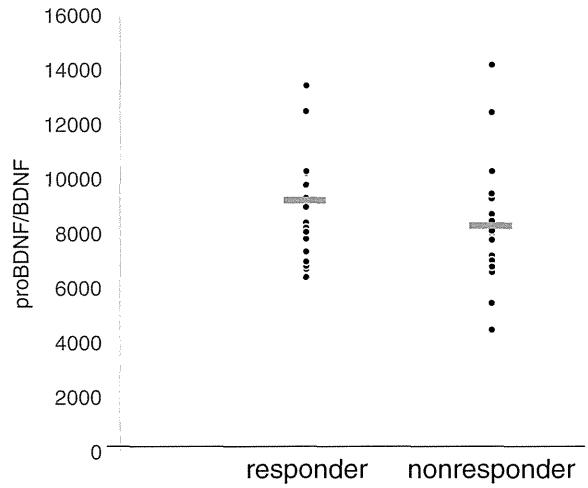

Figure 8 The proBDNF/BDNF ratio between the responders and the nonresponders. Red line shows the mean of value.

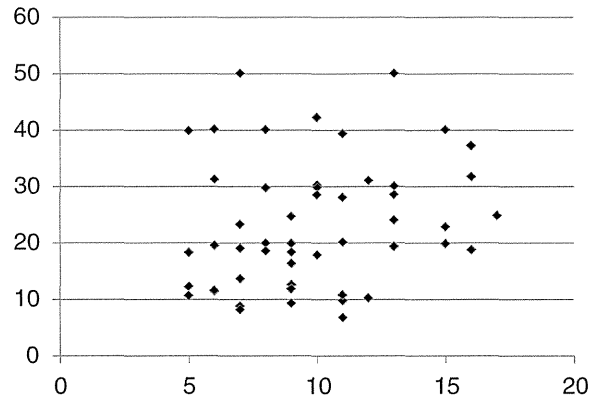

Figure 10 The changes in the HAMD17 (0–4 weeks) and plasma fluvoxamine concentration at week 4.

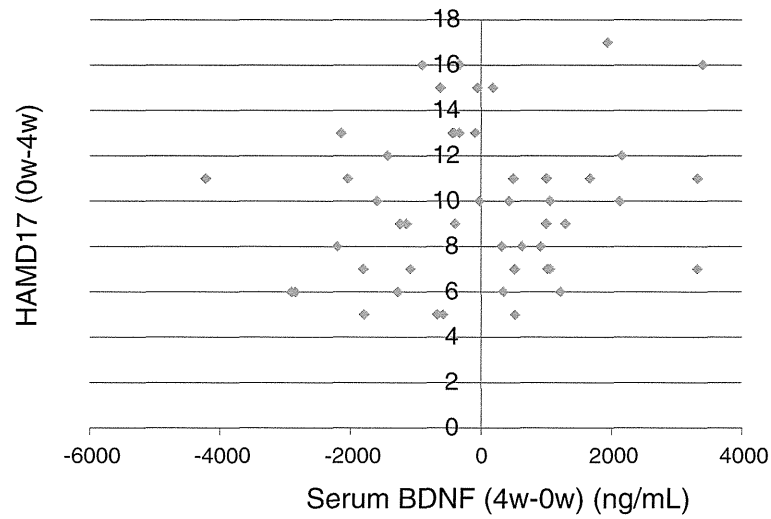

Figure 11 The changes in the HAMD17 (0–4 weeks) and plasma concentration at week 4.

healthy control subjects while BDNF levels were lower. The authors also demonstrated that the levels of BDNF and proBDNF negatively and positively correlated with major depression severity, respectively. These results suggest that the balance between proBDNF and BDNF is disturbed in MDD. Sodersten et al. [18] recently reported a very interesting finding using two independent cohort studies (Sahlgrenska cohort and Karolinska cohort). The authors found that serum MDNF, proBDNF, the ratio of BDNF/proBDNF, and interaction with MMP-9 were different between patients with bipolar disorders and healthy controls. The function of proBDNF however remains precisely elucidated.

Furthermore, there is little information about the role of serum proBDNF. We know that a controversy exists about the relationship between brain BDNF and peripheral BDNF. A recent study reported that circulating BDNF revealed a positive correlation with hippocampal BDNF, which reinforces the relevance to identify a potentially useful therapeutic biomarker [19].

No correlation was found between the changes in the HAMD17 scores and plasma fluvoxamine levels, which indicates that the effect of plasma fluvoxamine levels is independent of an individual's depressive clinical efficacy in fluvoxamine. In other words, the pharmacodynamic factors of each patient might also be involved in the effects of fluvoxamine. We should consider various factors for predicting the treatment response, and it could be more complicated in the fluvoxamine response. The present study had several limitations: (i) small samples, (ii) assaying serum proBDNF was tricky and the detection rate of serum proBDNF was very low using the ELISA kit, and (iii) we did not measure MMP-9 levels. Thus, we are undergoing reconfirmation of these preliminary results using another ELISA kit or Western blotting method.

Conclusions

We reconfirmed that serum levels of BDNF, but not proBDNF or proBDNF/BDNF ratio, in MDD were lower than those in healthy controls. Fluvoxamine however did not change serum levels of BDNF, proBDNF, and proBDNF/BDNF ratio at least within 4 weeks. Finally, no correlations exist between plasma levels of fluvoxamine and the changes in the HAMD17 scores or serum BDNF levels. In short, there is no association between serum levels of proBDNF, BDNF, or proBDNF/BDNF ratio and fluvoxamine response in MDD patients at least within 4 weeks of treatment. Using a different antidepressant medication on proBDNF/BDNF could be useful to determine the specificity of the effect of fluvoxamine.

Competing interests

Professor Nakamura has received grant support from Dainippon-Sumitomo Pharma Co., Tanabe-Mitsubishi Pharma Co., and Astellas Pharma Co., Ltd in 2013. The other authors declare that they have no competing interests.

Authors' contributions

RY designed the study, measured the serum BDNF, proBDNF, and plasma fluvoxamine, wrote the first draft, and managed the literature searches. TK performed the statistical analyses. HH, KA, AK, WU-N, and Al-S collected the clinical data. NI and JN wrote the final manuscript. All of the authors took part in either drafting the article or revising it critically for important intellectual content and approved the final manuscript.

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Relationships between brain-derived neurotrophic factor, clinical symptoms, and decision-making in chronic schizophrenia: data from the Iowa Gambling Task

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The levels of brain-derived neurotrophic factor (BDNF) are significantly decreased in patients with schizophrenia and correlate with impairments in cognitive function. However, no study has investigated the relationship between the serum BDNF levels and decision-making. We compared patients with schizophrenia to healthy controls with respect to their decision-making ability and serum BDNF levels. Eighty-six chronic schizophrenia patients and 51 healthy controls participated in this study. We controlled for gender, age, and estimated intelligence quotient (IQ), and we investigated the differences in decision-making performance on the Iowa Gambling Task (IGT) between the schizophrenia patient and control groups. We also compared the IGT scores, the serum BDNF levels, and the clinical symptoms between the groups. The IGT scores of the schizophrenia patients were lower than those of the controls. A negative correlation was detected between the mean net scores on the trials in the final two blocks and the serum BDNF levels ($p < 0.05$). Multiple regression analysis revealed that depressive symptoms and the serum BDNF levels were significantly associated with the mean net scores on the trials in the final two blocks. Based on these results, impaired sensitivity to both reward and punishment is associated with depressive symptoms and reduced serum BDNF levels in chronic schizophrenia patients and may be related to their poor performance on the IGT.

Keywords: brain-derived neurotrophic factor, decision-making, schizophrenia, gambling task, cognition, depression

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), is a member of neurotrophins involved in growth, differentiation, maturation, and survival in immature neurons. In mature neurons, it plays an important role in synaptic plasticity, augmentation of neurotransmission and regulation of receptor sensitivity (Numakawa et al., 2010). BDNF and its high affinity receptor TrkB are widely expressed in developing and adult nervous system, and BDNF is the most abundantly expressed neurotrophic factor in the central nervous system (Balaratnasingam and Janca, 2012). Recent research has provided evidence for the contribution of BDNF to the pathophysiology of schizophrenia. Studies of the BDNF Val66Met (rs6265) showed that the Met allele is associated with lower levels of BDNF secretion and with abnormal hippocampal structure and function, providing evidence for the direct involvement of BDNF in schizophrenia (Egan et al., 2003).

Recent advances in clinical neuroscience indicate that the hippocampus and the orbitofrontal cortex (OFC) play a critical role in complex decision-making processes (Rolls, 1999; Krawczyk, 2002; Johnson et al., 2007; Yu and Fank, 2014). Patients experiencing damage to the hippocampus and the OFC exhibit striking deficits in real-life decision-making, especially social or emotional decision-making, in the context of generally well-preserved intellectual functioning. In addition, growing evidence demonstrates that schizophrenia patients exhibit emotional disturbances and social dysfunction (Mandal et al., 1999; Kohler et al., 2000; Chemerinski et al., 2002), which could be partially explained by impaired decision-making. This impairment in decision-making may occur during interpersonal interactions and social situations (Damasio, 1994). In general, decisions are made based on the assessment of reward and punishment outcomes using both cognitive and affective information (Solms and Turnbull, 2004).

The Iowa Gambling Task (IGT) was developed to assess the role of affective information in decision-making (Bechara et al., 1994). In this task, subjects are presented with four decks of cards and are asked to select any deck in any sequence, and then to take a card from it. The subjects win or lose money with each turn of a card. The participants do not appear to understand the contingencies of the game at the onset. Nevertheless, they can quite rapidly develop a "feeling," or "hunch" in the absence of conceptual awareness.

Because cognitive dysfunction is associated with schizophrenia, we hypothesized that the serum BDNF levels are associated with the IGT scores of the chronic schizophrenia patients.

MATERIALS AND METHODS

SUBJECTS

Eighty-six chronic schizophrenia outpatients recruited from the University of Occupational and Environmental Health participated in the present study and met the following inclusion

criteria: (1) aged 20–60 years; (2) chronic illness without acute exacerbation; and (3) continuously receiving a stable dose of antipsychotics for at least 3 months. The exclusion criteria were: (1) any comorbid central nervous system disorder; (2) severe psychotic symptoms; (3) meeting the DSM-IV criteria for alcohol or other substance dependence; (4) meeting the DSM-IV criteria for mental retardation; (5) receiving antidepressants; (6) treatment with electroconvulsive therapy in the 6 months preceding the study; (7) receiving clozapine; and (8) inability to understand the study protocol. The diagnosis of schizophrenia was established based on the Structured Clinical Interview for DMS-IV (SCID) (First et al., 1996) and a comprehensive review of the patients' medical records. All patients met the criteria for schizophrenia. None were comorbid with any other psychiatric disorders. Seventy-eight of the schizophrenia patients were receiving stable dose of one antipsychotic drug (risperidone, olanzapine, quetiapine, aripiprazole, blonanserin, or perospirone). The remaining schizophrenia patients were receiving at least two antipsychotic drugs. Regarding other medications, nine patients were taking stable dosages of mood stabilizer (lithium, valproic acid, or carbamazepine), five were taking stable dosages of anticholinergic drugs and two were taking antidepressant (paroxetine or mirtazapine). Schizophrenic symptoms were rated using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987).

Additionally, we recruited 51 healthy volunteers (26 females and 25 males) for the healthy control group. The healthy controls consisted of individuals matched to the patients with respect to age, gender, and estimated intelligence quotient (IQ). The healthy controls had not experienced a head injury and did not suffer from any neurologic, psychotic, mood, or substance use disorder as evaluated by the SCID.

INTELLIGENCE TEST

The IQ of the participants was estimated using the Japanese Adult Reading Test (Matsuoka et al., 2002; Hori et al., 2008), a Japanese version of the National Adult Reading Test (Nelson and Wilson, 1991), and those individuals exhibiting estimated IQ scores of less than 80 were excluded from this study.

IGT

Decision-making ability was assessed using the computerized version of the IGT in Japanese (Bechara et al., 1994, 1999, 2005). Decks of cards labeled "A," "B," "C," or "D" were placed in front of the subjects from left to right. Initially, 200,000 yen were given to each subject. The subjects were told that (1) they are to draw one playing card from one of the four decks on each turn, (2) this game involves betting across multiple turns, (3) they receive money every time that they draw a card but that a penalty is occasionally applied, and (4) the objective of this game is to maximize the amount of money that they have. When selecting a card, the subjects can draw a card from any of the decks and can change their selection any time as many times as they choose. The game ended at the 100th draw of a card by a subject, but the subjects were not informed about this rule beforehand. The subjects received a reward each time they drew a card; if they selected a card from the deck A or B, a reward of 10,000 yen was applied, and if they selected a card from the deck C or D, a reward of 5000 yen was applied. Simultaneously, a penalty is applied; decks A and B are referred to as "bad decks" because the immediate reward at the time of the draw is high but the penalty is also high and frequent; therefore, the player ultimately loses money as cards are drawn from these decks. Alternatively, decks C and D are referred to as the "good decks" because the immediate reward is low but the frequency and amount of the penalty is low; therefore, players who draw cards from these decks ultimately earn money. Additionally, decks A and C are categorized as "low magnitude decks" in which a low penalty is applied at a relatively high frequency, whereas decks B and D are categorized as "high magnitude decks" in which a high penalty is applied at a relatively low frequency. The task ended after 100 selections. Neither the risks of rewards or penalties for each deck nor the number of selections allowed was disclosed to the subjects. The composition of the final score and the total amount of money held by each subject at the end of the task was not disclosed to the subjects. This score represented the extent to which socially valuable resources had been increased and may also indicate the amount of risk that the subject was willing to accept given that they may have continued to lose. The frequency of shifting between advantageous (C and D) and disadvantageous (A and B) decks by the subject was computed for every 20 cards, for a total of 5 blocks.

This examination is an exercise in which the subjects are rewarded as well as occasionally penalized with each draw of a card. The subjects can learn the types of rewards and penalties that are applied and can evaluate and change their selections during the process of the game. In this examination, the subjects must use cognitive processing to predict outcomes associated with their selection of cards and generate future predictions using a complex set of results and repeated decisions.

BDNF MEASUREMENT

All blood samples were obtained between 7:00 and 10:00 a.m. in the morning fasting. Fifteen milliliters of venous blood was drawn with subjects in the supine position, after the subjects had been lying at rest overnight.

The serum BDNF levels were measured using a BDNF Emax Immunoassay Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. In short, 96-well microplates were coated with an anti-BDNF monoclonal antibody and incubated at 4°C for 18 h. The plates were incubated in a blocking buffer for 1 h at room temperature. The samples were diluted 100 times with assay buffer, and BDNF standards were maintained at room temperature under horizontal shaking for 2 h, followed by washing with the appropriate washing buffer. The plates were incubated with anti-human BDNF polyclonal antibody at room temperature for 2 h and washed with the washing buffer. Then, the plates were incubated in an anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature, followed by incubation in peroxidase substrate and a tetramethylbenzidine solution to induce a colorized reaction. This reaction was stopped using 1 mol/L hydrochloric acid. The absorbance at 450 nm was measured using an Emax automated microplate reader. The measurements were performed in duplicate. The standard curve was linear from 5 to 5000 pg/mL, and the detection limit was 10 pg/mL. The intra- and inter-assay coefficients of variation were 5 and 7%,

respectively. The recovery rate of exogenously added BDNF to the measured plasma samples was more than 95%.

Written informed consent was obtained from all of the subjects who participated in this study. The study protocols were approved by the Ethics Committee of the University of Occupational and Environmental Health and included standard procedures for clinical research involving vulnerable participants in Japan. This study was performed according to the ethical standards of the Declaration of Helsinki. If a participant exhibited a compromised ability to consent, we excluded this individual from this study. All participants who declined to participate or otherwise did not participate were eligible for treatment and were not disadvantaged in any other manner because of their lack of participation in this study.

STATISTICAL ANALYSIS

The differences in the clinical variables between the groups were assessed using the *t*-test and the χ^2 test for parametric data or using the Mann–Whitney *U*-test and the Fisher exact test for non-parametric variables. Data analysis of the IGT outcome variables was conducted using *t*-tests and repeated-measures analysis of variance. A multiple linear regression was employed to analyze the effect of the serum BDNF levels on the IGT scores while adjusting for confounding factors [depression score on the PANSS, age, estimated IQ, Chlorpromazine-equivalent (CPZ-eq), and PANSS-T score]. The correlations between the PANSS scores, the IGT scores, and the serum BDNF levels were evaluated using Pearson's correlation analysis. *P*-values of <0.05 were considered to be significant. The data were analyzed using stata13.1 software for Windows.

RESULTS

The demographic characteristics of the subjects are summarized in **Table 1**. No significant differences in age, gender, estimated IQ, or the serum BDNF levels were detected between the two groups.

GAMBLING TASK PERFORMANCE

Descriptive data for the performance on the IGT are presented in **Table 2**. The schizophrenia patients displayed significantly smaller difference scores on the advantageous minus disadvantageous deck selection index and earned significantly less money than the controls.

The learning curves of each group are shown in **Figure 1**. There were significant main effects of the group ($p < 0.001$) and the block ($p < 0.001$) and a significant group × block interaction ($p < 0.001$). A follow-up independent *t*-test indicated that the controls performed better than the schizophrenia patients during the final three blocks but not during the first two blocks. Even after correction for multiple comparisons, this between-group difference remained statistically significant for the final three blocks.

The between-group differences in selections from each deck were examined using a 2 (group) × 4 (deck) repeated-measures ANOVA. There was a significant main effect of the deck ($p < 0.001$) and a significant group × deck interaction ($p < 0.001$). Follow-up *t*-tests evaluated the between-group differences in the selection from each deck. As shown in **Figure 2**, the schizophrenia patients selected deck B more frequently and deck C less frequently than the controls, whereas the two groups did not differ in the selection of decks A and D ($p < 0.05$, even after the Bonferroni correction). Within-group comparisons were used to clarify the pattern of performance; the controls selected the advantageous decks more frequently [(C + D) − (A + B)], ($t = 3.95, p < 0.001$) than the patients with schizophrenia.

Table 1 | Demographic and clinical information of the schizophrenia patients and the healthy control group.

	Healthy control	Schizophrenia group	*p*-value
Age (year)	36.7 ± 9.9	35.1 ± 12.1	0.43
Gender (M/F)	25/26	43/43	0.91
Education (years)	13.4 ± 2.2	12.7 ± 2.7	0.15
Estimated IQ	101.8 ± 7.6	99.4 ± 8.2	0.09
Duration of the illness (years)		11.4 ± 13.2	
SCHIZOPHRENIA DIAGNOSIS			
Paranoid type		47	
Disorganized type		27	
Catatonic type		5	
Indifferenciated type		7	
PANSS-P		17.2 ± 4.7	
PANSS-N		20.4 ± 4.6	
PANSS-G		33.2 ± 7.0	
PANSS-T		70.7 ± 11.7	
CPZeq of total antipsychotic drugs(mg/day)		470.1 ± 293.0	
Serum BDNF(ng/ml)	14.1 ± 7.3	11.8 ± 7.0	0.06

PANSS-P, PANSS positive score; PANSS-N, PANSS negative score; PANSS-G, PANSS general psychopathological score; PANSS-T, PANSS total score; CPZ-eq, chlorpromazine-equivalent.

GAMBLING TASK PERFORMANCE, CLINICAL SYMPTOMS, AND SERUM BDNF LEVELS

No correlation was found between the total scores and mean net scores on the IGT and the PANSS-P, PANSS-N, or PANSS-T (**Table 3**). A significant negative correlation was detected between the mean net scores on trials 61–100 and the PANSS-G scores. A significant negative correlation was observed between the mean net scores on the trials in the final two blocks (61–80 and 81–100) and the serum BDNF levels (**Table 3**). **Table 4** shows the multiple regression analysis results for the mean net scores on each block of the trials. Depressive symptoms and the serum BDNF levels were significantly associated with the mean net scores on the trials in the final two blocks.

DISCUSSION

A significant finding in the present study was that the serum BDNF levels and depressive symptoms correlated with decision-making. Several previous studies reported that schizophrenia patients exhibited diminished decision-making abilities compared to healthy individuals (Beninger et al., 2003; Ritter et al., 2004; Lee et al., 2007; Kim et al., 2009; Struglia et al., 2011). To the best of our knowledge, this is the first report to provide

Table 2 | Iowa Gambling Task performance of the schizophrenia patients and the healthy control group.

	Healthy control	Schizophrenia	*t*	*df*	*p*
Mean amount of money earned (yen)	219902.0 ± 72770.1	162348.8 ± 72369.9	4.48	135	< 0.001
No. of cards chosen from deck A	17.7 ± 6.9	18.6 ± 6.0	−0.75	135	0.44
No. of cards chosen from deck B	26.0 ± 12.2	34.5 ± 11.1	−4.1	135	< 0.001
No. of cards chosen from deck C	30.7 ± 12.6	19.6 ± 6.1	5.89	135	< 0.001
No. of cards chosen from deck D	25.2 ± 8.9	27.2 ± 9.0	−1.21	135	0.23
Choice advantageous minus disadvantageous decks	12.2 ± 32.6	−6.3 ± 22.3	−3.6	135	< 0.001

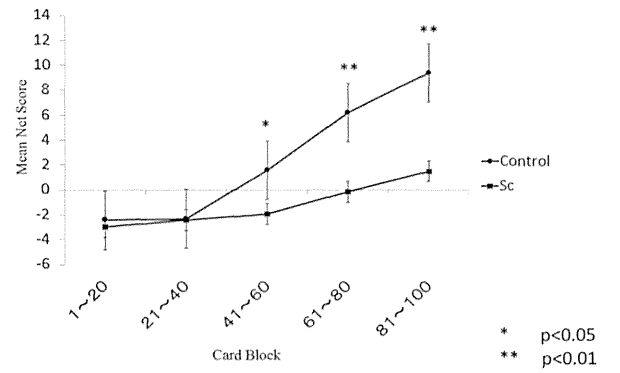

FIGURE 1 | Iowa Gambling Task in schizophrenia and healthy subjects.

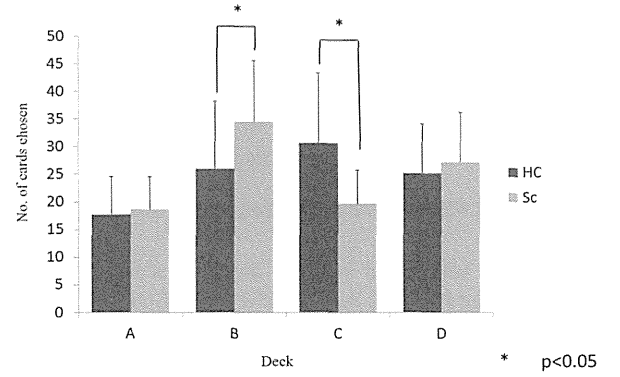

FIGURE 2 | Number of card choice selected from each deck during the 100-card task.

evidence suggesting that the serum BDNF levels reflect decision-making ability in patients with chronic schizophrenia. A recent meta-analysis revealed that the serum BDNF levels in schizophrenia patients are lower than those in healthy individuals (Green et al., 2011). In addition, recent studies have reported an association between poor performance on the IGT and the volume of several lesions in the hippocampus (Bonatti et al., 2009; Labudda et al., 2009; Yamano et al., 2011). The expression level of BDNF is normally high in the hippocampus. Taking these findings into account, diminished serum BDNF level in schizophrenia patients may reflect their poor decision-making performance. In short, serum BDNF may serve as a biomarker of decision-making ability in schizophrenia patients.

Wilder et al. reported that schizophrenia patients often selected the decks corresponding to infrequent and high-magnitude punishments (Wilder et al., 1998). Another study confirmed the finding that schizophrenia patients performed poorly compared to healthy individuals and often selected cards from the deck corresponding to high-magnitude punishments (Shurman et al., 2005). In the present study, the schizophrenia patients selected from good decks during the latter half of the task; however, optimizing the selection pattern appeared to be more difficult for the schizophrenia patients compared to the healthy individuals. Therefore, schizophrenia patients may tend to perform the same pattern of changes in the selection of the decks on the IGT as the control subjects. The magnitude of the occasional penalties has been reported to have little impact on the pattern of card selection.

The results in the present study are in accordance with those of previous publications (Beninger et al., 2003; Ritter et al., 2004; Shurman et al., 2005; Lee et al., 2007; Kim et al., 2009; Struglia et al., 2011). In addition, the present study revealed a significant correlation between decision-making performance and certain psychiatric symptoms. Previous studies have reported inconsistent results regarding the relationship between the IGT score and symptoms of schizophrenia, including a significant correlation to not only negative symptoms of schizophrenia (Shurman et al., 2005) but also positive symptoms of schizophrenia (Struglia et al., 2011). Several studies demonstrated an association between the serum BDNF levels and severity of major depressive disorder (Shimizu et al., 2003; Dell'Osso et al., 2010; Kurita et al., 2012; Yoshiumra et al., 2012). In contrast, no correlations existed between the serum BDNF levels and severity of depression (Karege et al., 2002b; Piccinni et al., 2008; Park et al., 2014). Taken together, it remains controversial that severity of a depressive state influences decision-making in schizophrenia patients. In short, it is not elucidated whether serum BDNF levels reflect or not depressive factors in schizophrenia. One study reported that depressive symptoms are associated with QOL in schizophrenia (Ueoka et al., 2011). Therefore, treatment targeting depressive symptoms may improve the QOL in schizophrenia patients.

The present study contained several limitations. First, the patients with schizophrenia were not classified into subtypes. Second, the schizophrenia patients were receiving various antipsychotic medications when the IGT was performed. Third, the number of subjects in the control group was small. Fourth, the schizophrenia patient group was receiving antipsychotics, a

Table 3 | Correlation between the Iowa Gambling Task performance and serum BDNF, estimated IQ, and clinical symptoms.

	Mean amount of money earned (yen)	1~20	21~40	41~60	61~80	81~100
Serum BDNF concentration (ng/ml)	0.06	0.02	0.03	0.09	0.24*	0.24*
CPZeq of total antipsychotic drugs(mg/day)	0.06	−0.05	−0.09	0.00	−0.1	−0.07
Estimate IQ	0.02	−0.06	−0.04	−0.01	−0.03	−0.06
PANSS-P	0.08	0.02	−0.08	0.00	0.02	0.02
PANSS-N	0.07	−0.03	−0.02	−0.1	−0.08	−0.02
PANSS-G	0.04	−0.03	−0.07	−0.01	−0.25*	−0.23*
PANSS-T	0.08	−0.01	−0.08	−0.04	−0.17	−0.13

CPZ-eq, chlorpromazine-equivalent.
*$p < 0.05$.

Table 4 | Multiple regression analysis results for the mean net scores on each block of the trials.

	Independent variables	Multiple regression statistic			
		β	*SE*	*t*-value	*p*-value
IGT-1	Age	0.019	0.045	0.43	0.667
	CPZ-eq	−0.001	0.001	−0.62	0.535
	Depression	1.252	0.869	1.44	0.153
	PANSS-T	−0.459	0.051	−0.91	0.367
	Estimated-IQ	−0.399	0.060	−0.67	0.506
	Serum BDNF levels	0.009	0.070	0.13	0.894
IGT-2	Age	−0.050	0.054	−0.93	0.354
	CPZ-eq	−0.000	0.002	−0.21	0.831
	Depression	0.518	1.041	0.50	0.620
	PANSS-T	−0.049	0.06	−0.80	0.424
	Estimated-IQ	−0.039	0.071	−0.55	0.584
	Serum BDNF levels	0.019	0.084	0.23	0.819
IGT-3	Age	−0.117	0.056	−2.07	0.042*
	CPZ-eq	0.002	0.002	1.02	0.313
	Depression	0.834	1.084	0.77	0.444
	PANSS-T	−0.040	0.063	−0.63	0.532
	Estimated-IQ	−0.028	0.075	−0.37	0.710
	Serum BDNF levels	0.078	0.088	0.89	0.378
IGT-4	Age	−0.054	0.071	−0.75	0.453
	CPZ-eq	−0.000	0.002	−0.10	0.919
	Depression	−3.502	1.376	−2.54	0.013*
	PANSS-T	0.034	0.080	0.42	0.673
	Estimated-IQ	−0.017	0.095	−0.18	0.857
	Serum BDNF levels	0.23	0.112	2.06	0.043*
IGT-5	Age	−0.674	0.084	−0.81	0.423
	CPZ-eq	0.000	0.003	0.09	0.929
	Depression	−4.686	1.619	−2.89	0.005*
	PANSS-T	0.077	0.094	0.82	0.414
	Estimated-IQ	−0.458	0.111	−0.41	0.682
	Serum BDNF levels	0.269	0.131	2.05	0.044*

PANSS-T, PANSS total score; CPZ-eq, chlorpromazine-equivalent; IGT-1: Card block 1-20; IGT-2: Card block 21-40; IGT-3: Card block 41-60; IGT-4: Card block 61-80; IGT-5: Card block 81-100.
*$p < 0.05$.

relevant problem because of the influence of the drugs on the BDNF levels. Fifth, since we evaluated the depressive symptoms in schizophrenia with only PANSS depressive items, we were poorly assessed and insufficient. Last, there is increasing evidence that sampling characteristics, several sociodemographic variables (such as age, sex, urbanicity, BMI), life-style factors (such as food, alcohol intake, and smoking status), somatic disease, and even self-reported depressive symptoms are relevant determinants of serum BDNF levels (Radka et al., 1996; Karege et al., 2002a; Bus et al., 2011). In conclusion, both depressive symptoms and the serum BDNF levels may be associated with the impairment of decision-making in schizophrenia patients.

AUTHOR CONTRIBUTIONS

Dr. Hikaru Hori designed the study, performed the cognitive battery, collected the clinical data, performed the statistical analyses, wrote the first draft of the manuscript, and performed literature searches. Dr. Reiji Yoshimura and Dr. Jun Nakamura developed the study protocol and wrote the final manuscript. Dr. Asuka Katsuki performed the cognitive battery. Dr. Kiyokazu Atake collected the clinical data. All authors contributed to and approved the final manuscript.

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Research report

Characteristic distributions of regional cerebral blood flow changes in major depressive disorder patients: A pseudo-continuous arterial spin labeling (pCASL) study

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ABSTRACT

Background: Most previous studies that examined regional cerebral blood flow (rCBF) abnormalities in major depressive disorder (MDD) required the injection of radioisotopes into subjects. Here by using magnetic resonance imaging (MRI) with the pseudo-continuous arterial spin labeling (pCASL) method which does not require radioisotopes, we examined rCBF in patients with MDD in comparison with that in patients with schizophrenia and healthy subjects, taking the regional cerebral gray matter volume into account.
Methods: Subjects were 27 patients with MDD, 42 with schizophrenia and 43 healthy volunteers who underwent 3-T MRI with pCASL. Obtained pCASL imaging data were subject to the voxel-by-voxel statistical analysis.
Results: There were significant reductions of rCBF in the right inferior prefrontal cortex and anterior cingulate cortices (ACCs) in the MDD patients compared with the healthy controls. When compared with the schizophrenic patients, the MDD patients showed lower rCBF in the subgenual ACC and higher rCBF in left occipital region.
Limitation: The abnormalities of rCBF in MDD were known to reverse during symptom remission. Further study with follow-up period would bring the perception about the treatment response.
Conclusion: The rCBF reduction in the subgenual region may be a specific functional abnormality to MDD patients, which may provide a biological marker for MDD. The MRI with pCASL method is a promising tool to detect rCBF abnormalities controlling for gray matter volume in psychiatric disorders.

1. Introduction

Major depressive disorder (MDD) is a common disorder, and the lifetime prevalence was reported to be 8–12% (Andrade et al., 2003). Structural brain abnormalities in areas involved in emotional processing including the dorsolateral prefrontal region, orbitofrontal region, cingulate cortex, temporal region, hippocampus and striatum have been reported in MDD (reviewed in Arnone et al., Bora et al., 2012; Murphy and Frodl, 2011; Sexton et al., 2009). Previous studies using nuclear medicine techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) showed significant reductions of regional cerebral blood flow (rCBF) and metabolism in the frontal, parietal, and temporal regions of patients with MDD (Drevets et al., 2002; Mayberg et al., 2000; Smith and Cavanagh, 2005).

Arterial spin labeling (ASL) magnetic resonance imaging (MRI) is a novel noninvasive (i.e., non-radioactive) technique that can measure rCBF by taking advantage of arterial water as a freely diffusible tracer. This technique has recently been applied to detect functional abnormalities of the brain in MDD patients (Duhameau et al., 2010; Ho et al., 2013; Järnum et al., 2011; Lui et al., 2009; Walther et al., 2012). Some of these studies showed rCBF reduction in the frontal regions (Ho et al., 2013; Lui et al., 2009) and anterior cingulate cortex (ACC) (Walther et al., 2012). However, the others found no significant reduction in MDD patients (Duhameau et al., 2010; Järnum et al., 2011). Importantly, the limited spatial resolution of ASL images precludes accurate rCBF measurements because

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the partial volume effects on ASL cause an underestimation of activity in small brain structures. Since many MDD patients have altered brain structures as described above, their rCBF images are likely to be influenced by the partial volume effect. To our knowledge, however, no ASL study in MDD has thus far taken account of thise partial volume effect.

In this study, we examined differences in rCBF between patients with MDD and healthy controls by a recently developed method, pseudo-continuous ASL (pCASL), taking the regional cerebral gray matter volume into account (Dai et al., 2008; Wu et al., 2007). In addition, depressive features or syndromes are often manifested in patients with schizophrenia. It has been estimated that depression is manifested in 21 to 74% of acute patients with recent onset schizophrenia and in 13 to 50% of patients with chronic schizophrenia, while depressive features have been found in even higher rates, up to 80%, in patients with schizophrenia (Kollias et al., 2008). So it would be useful if there is a method to differentiate between MDD and schizophrenia by using a neuroimaging method. We previously reported MRI-pCASL study on schizophrenia that showed a significant reduction of rCBF in the left inferior frontal cortex (Ota et al., 2014). So we evaluated rCBF of MDD patients in comparison with that of schizophrenia patients as well.

2. Methods

2.1. Participants

Subjects were 27 patients with MDD, 42 patients with schizophrenia and 43 age- and gender-matched healthy subjects. The subjects partially overlapped with those in the previous report (Ota et al., 2014). A consensus diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) criteria (American Psychiatric Association, 1994), by a research psychiatrist (MO, HH, or TT). The MDD patients were rated with Hamilton Depression Rating scale (HAM-D) for their depressive symptoms (Hamilton, 1960), and the schizophrenic patients were done with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). MDD patients who were in remission, as defined by the total score on the HAM-D of less than 8, were not enrolled in the study (Mayberg et al., 2005). Daily doses of antidepressants were converted to imipramine equivalents, and daily doses of antipsychotics including depot antipsychotics were converted to chlorpromazine equivalents using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999).

Controls were recruited from the community through local magazine advertisements and our website announcement. These participants were interviewed for enrollment by a research psychiatrist using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998). Participants were excluded if they had a prior medical history of central nervous system disease or severe head injury, or if they met the criteria for substance abuse or dependence. Those individuals who demonstrated a history of psychiatric illness or contact with psychiatric services were excluded from the control group.

After the study was explained to each participant, his or her written informed consent was obtained for participation in the study. This study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. MRI data acquisition and processing

Imaging was performed on a 3-T MR system (Philips Medical Systems, Best, the Netherlands). 3D T1-weighted images and pCASL images were acquired as the same parameter as described previously (Ota et al., 2014). For measurement of the magnetization of arterial blood and also for segmentation purposes, an EPI M0 image was obtained separately with the same geometry and the same imaging parameters as the pCASL without labeling.

2.3. Postprocessing of the ASL data

Because the pCASL and M0 images were acquired separately, the image signal intensities of both were corrected for data scaling. Corrected data were transferred to a workstation and analyzed using ASLtbx software (Wang et al., 2008) running on statistical parametric mapping 5 (SPM5). For the rCBF calculations, we added the attenuation correction for the transversal relaxation rate of gray matter to the original equation. Details of this process are described elsewhere (Ota et al., 2013a).

The mean rCBF image derived using the ASLtbx software contained some patchy noise, and thus we used a median filter (a nonlinear digital filtering technique). In median filtering, the neighboring pixels are ranked according to their intensity, and the median value becomes the new value for the central pixel. Since the slice gap that we used was somewhat large, simple 2D median filtering (3 voxels $\times$ 3 voxels) was used. To evaluate rCBF voxel-basically, we normalized the mean rCBF images to the standard space. First, each individual 3D-T1 image was coregistered and resliced to its own M0 image. Next, the coregistered 3D-T1 image was normalized to the "avg152T1" image regarded as the anatomically standard image using with the DARTEL (diffeomorphic anatomical registration using exponentiated lie) registration method (Ashburner, 2007). Finally, the transformation matrix was applied to the mean rCBF images treated with the median filter. The spatially normalized images were resliced with a final voxel size of approx. $4 \times 4 \times 8$ mm. Each map was then spatially smoothed with a 4-mm full-width at half-maximum Gaussian kernel in order to decrease spatial noise and compensate for the inexactitude of normalization.

2.4. Statistical analysis

Statistical analyses were performed using SPM5 software. Differences in rCBF among 3 diagnostic groups were assessed using the age and gender as non-imaging nuisance variables and the individual normalized gray matter volume image as an imaging nuisance covariate using Biological Parametric Mapping (BPM) (Casanova et al., 2007). Only differences that met the following criteria were deemed significant: a seed level of $p < 0.05$ (false discovery rate [FDR] correction for multiple comparisons) and a cluster level of $p < 0.05$ (uncorrected).

3. Results

Demographic and clinical characteristics of the participants are shown in Table 1. There was no significant difference in age or gender among the 3 diagnostic groups.

There were significant rCBF reductions in the ACCs and right inferior prefrontal cortex in the patients with MDD compared with the controls (Fig. 1). We found significant rCBF reductions in the ACC, bilateral prefrontal cortex, left superior temporal cortex, and bilateral occipital cortex in the patients with schizophrenia compared with the controls, which is consistent with the results of our previous study (Fig. 2) (Ota et al., 2014). When the 2 patient groups were compared, the MDD patients showed significantly lower rCBF in subgenual ACC (Fig. 3A) and higher rCBF in left occipital cortex (Fig. 3B) compared with the schizophrenic patients.

Table 1
Demographic and clinical characteristics of subjects.

Variable	MDD $n=27$			Schizophrenia $n=42$			Healthy subjects $n=43$			p Value
	Mean	±	SD	Mean	±	SD	Mean	±	SD	
Gender (M:F)	13	:	14	22	:	20	21	:	22	0.93
Age (years)	38.9	±	9.9	39.2	±	11.5	37.4	±	12.7	0.75
Antidepressant medication (mg/day)[a]	####	±	75.7							
Antipsychotic medication (mg/day)[b]				627.1	±	503.0				
HAM-D	16.6	±	7.3							
PANSS total				63.4	±	18.9				

MDD; major depressive disorder.
HAM-D; Hamilton's rating scale for depression.
PANSS; positive and negative synptom scale.
[a] Impramine equivalent.
[b] Chlorpromazine equivalent.

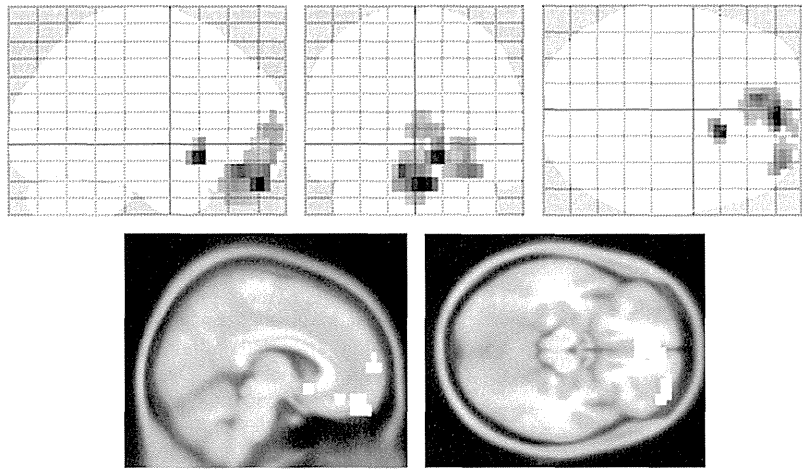

Fig. 1. Regional cerebral blood flow (rCBF) changes in major depressive disorder (MDD). There were significant reductions of rCBF in the right inferior prefrontal and anterior cingulate cortices (ACCs) compared with healthy subjects ($p < 0.05$, (false discovery rate [FDR])).

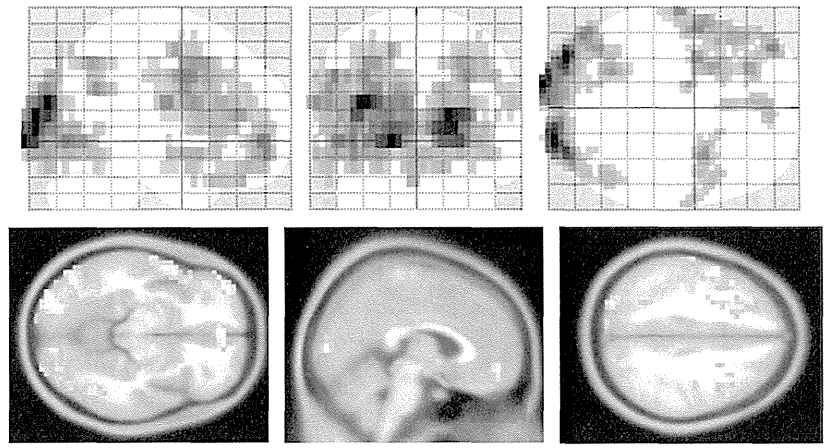

Fig. 2. Regional CBF changes in schizophrenia. There were significant reductions of rCBF in the bilateral prefrontal and occipital cortices, left temporal cortex, and ACC compared with healthy subjects ($p < 0.05$, FDR).

4. Discussion

We examined rCBF changes inMDD patients compared with healthy subjects and schizophrenia patients. By using the pCASL method and the regional gray matter volume correction, we found significant changes of rCBF in cingulate and frontal regions in MDD patients. To our knowledge, this is the first study of ASL-based rCBF changes in MDD patients that took the regional gray matter volume into account. In addition, we found differences in rCBF between MDD and schizophrenia patients by using this method.

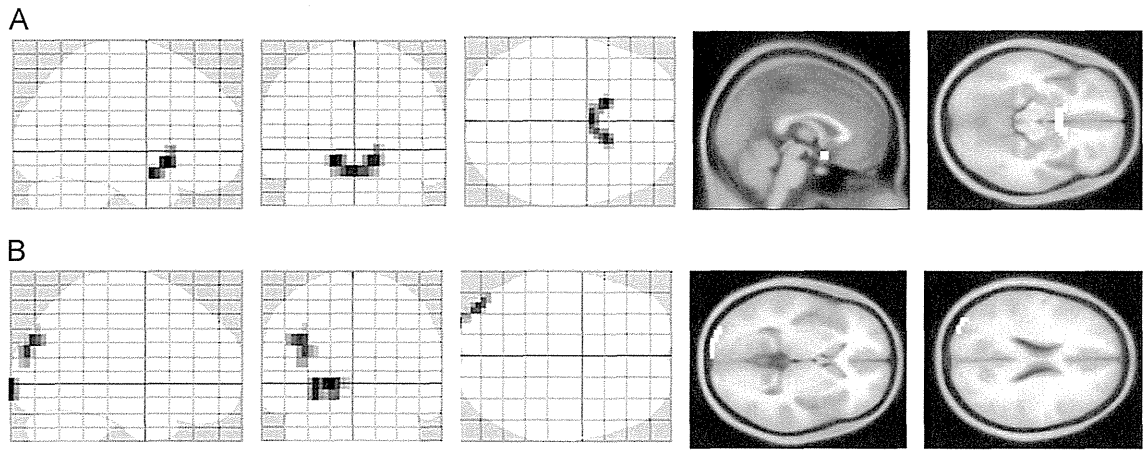

Fig. 3. Comparison of rCBF between the patients with MDD and schizophrenia. The MDD patients showed the lower rCBF in the subgenual ACC (A) and the higher rCBF in left occipital region (B) than schizophrenic patients. ($p < 0.05$, FDR).

Many MRI studies have focused on structural brain volume changes in MDD (reviewed in Arnone et al., 2012; Bora et al., 2012), and some studies have reported altered functions by using ASL (Ho et al., 2013; Lui et al., 2009; Walther et al., 2012). In addition, studies using PET and SPECT found that patients with MDD have decreased rCBF and metabolism (see review; Fitzgerald et al., 2008). We observed reduction of rCBF in the inferior prefrontal cortex in the MDD relative to healthy subjects. These findings mirror previous ASL studies showing lower baseline frontal rCBF (Ho et al., 2013; Lui et al., 2009; Walther et al., 2012). Hypoactivity in frontal areas has been strongly linked to psychomotor retardation (Galynker et al., 1998; Bench et al., 1992), and impaired executive functioning (e.g., attention, working memory, and decision making) (Paelecke-Habermann et al., 2005; Fossati et al., 2002). Together with findings from these studies, our results suggest that reduced perfusion in these regions may be associated with some of the cognitive and motor symptoms found in MDD. Our finding of hypoperfusion in the ACC of MDD patients is consistent with several perfusion and metabolism studies of MDD (Fitzgerald et al., 2008; Ho et al., 2013; Walther et al., 2012). The ACC has been shown to be involved in the processing of emotion and motivation (Carter et al., 1999). Thus, dysfunction in the ACC may underlie some of the core affective symptoms seen in MDD. Specifically, previous studies have shown that there were structural (Coryell et al., 2005; Costafreda et al., 2009; Lee et al., 2011; Wagner et al., 2011) and functional (Drevets et al., 1997; Liotti et al., 2002) dysfunctions in the subgenual ACC of MDD. And some studies showed that such change in the subgenual ACC was noticeable not in schizophrenia but in MDD, compared with healthy subjects (Coryell et al., 2005; Ota et al., 2013b). In line, we detected the lower rCBF in the subgenual ACC of MDD patients than schizophrenic patients, which is consistent with the preceding results.

ASL studies of schizophrenia revealed several rCBF changes (Horn et al., 2009; Pinkham et al., 2011; Scheef et al., 2010; Walther et al., 2011). However, the results of these studies differ substantially. For the frontal and temporal cortices, three and two out of these four studies consistently reported reduced rCBF, which is compatible with our results. We found rCBF reduction in bilateral occipital cortices of the individuals with schizophrenia, which is consistent with the study by Pinkham et al. (2011) in medicated schizophrenic patients and the study by Scheef et al. (2010) in drug-free subjects. Several studies obtained evidence of deficits of schizophrenia in visual processing, using electroencephalography (EEG) (Butler et al., 2001, 2005; Doniger et al., 2002), and other studies documented the abnormal EEG activities in the occipital lobe of patients with schizophrenia (Spencer et al., 2003, 2004). Thus, it seems likely that the occipital lobe is involved in some aspects of the pathophysiology of schizophrenia.

There was limitation in this study. The abnormalities of rCBF in MDD were known to reverse during symptom remission (see review; Drevets, 2000). Our MDD patients showed the HAM-D score of > 8, depressed non-remitters, and they showed the lower rCBF than healthy subjects. Further study with follow-up period that provides the information about the response to therapy would bring the prediction about the treatment response.

In conclusion, our pCASL study with partial volume effect correction demonstrated hypoactivity in the right inferior prefrontal area and cingulate cortex in MDD patients. The rCBF reduction in the subgenual region was specific in MDD patients compared with not only the healthy subjects, but also with schizophrenic patients. This point may provide objective biological information pertaining to the clinical diagnosis of schizophrenia and MDD. Finally, the present study demonstrate that the MRI with pCASL method is a promising tool to detect rCBF abnormalities controlling for gray matter volume in psychiatric disorders.

Role of funding source

The founding source had no involvement.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Neuroimaging-aided differential diagnosis of the depressive state

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ABSTRACT

A serious problem in psychiatric practice is the lack of specific, objective biomarker-based assessments to guide diagnosis and treatment. The use of such biomarkers could assist clinicians in establishing differential diagnosis, which may improve specific individualised treatment. This multi-site study sought to develop a clinically suitable neuroimaging-guided diagnostic support system for differential diagnosis at the single-subject level among multiple psychiatric disorders with depressive symptoms using near-infrared spectroscopy, which is a compact and portable neuroimaging method. We conducted a multi-site, case–control replication study using two cohorts, which included seven hospitals in Japan. The study included 673 patients (women/men: 315/358) with psychiatric disorders (major depressive disorder, bipolar disorder, or schizophrenia) who manifested depressive symptoms, and 1007 healthy volunteers (530/477). We measured the accuracy of the single-subject classification in differential diagnosis among major psychiatric disorders, based on spatiotemporal characteristics of fronto-temporal cortical haemodynamic response patterns induced by a brief (<3 min) verbal fluency task. Data from the initial site were used to determine an optimal threshold, based on receiver-operator characteristics analysis, and to generate the simplest and most significant algorithm, which was validated using data from the remaining six sites. The frontal haemodynamic patterns detected by the near-infrared spectroscopy method accurately distinguished between patients with major depressive disorder (74.6%) and those with the two other disorders (85.5%; bipolar disorder or schizophrenia) that presented with depressive symptoms. These results suggest that neuroimaging-guided differential diagnosis of major psychiatric disorders developed using the near-infrared spectroscopy method can be a promising biomarker that should aid in personalised care in real clinical settings. Potential confounding effects of clinical (e.g., age, sex) and systemic (e.g., autonomic nervous system indices) variables on brain signals will need to be clarified to improve classification accuracy.

Introduction

Among non-communicable diseases, neuropsychiatric conditions, including depression, contribute most significantly to overall disability-adjusted life years (DALYs), surpassing both cardiovascular disease and cancer (Mathers and Loncar, 2006; Prince et al., 2007). Therefore, early and accurate diagnosis and treatment are critical in psychiatric disorders, for which the development of specific biomarkers is of special importance. Currently, however, the diagnostic process in psychiatry is mainly based on patients' reports of symptoms, observed behaviours and disease course. Overcoming the limitations of relying on clinical interviews alone for the diagnosis of psychiatric disorders has been a great challenge.

To complicate this issue further, the manifestation of only a major depressive episode hampers the reliable differentiation of major depressive disorder (MDD) from bipolar disorder (BP) or

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schizophrenia (SZ) based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria alone (Zimmermann et al., 2009). Although many clinical symptoms are common to various psychiatric disorders, depressive symptoms are particularly ubiquitous in the disease process or clinical staging of various psychiatric disorders (Hafner et al., 2005). For instance, differentiation between BP presenting with depressive symptoms and unipolar MDD is a topical issue (Akiskal et al., 1995). Indeed, most patients with BP with depressive symptoms are initially diagnosed with and treated for MDD (Akiskal et al., 1995; Goldberg et al., 2001). Therefore, biomarkers that can facilitate early and accurate differentiation of BP with depressive symptoms from MDD are necessary.

In addition, depressive symptoms that fulfil the operational diagnostic criteria for a depressive episode/major depression can also occur at any stage of SZ and can contribute substantially to its associated morbidity and even mortality (an der Heiden et al., 2005). The differentiation of SZ from MDD, especially in the early stages, is also important because patients with SZ also exhibit non-psychotic and non-specific prodromal symptoms (e.g., depressive or negative symptoms and cognitive deficits) for several years before the onset of full-blown psychosis (McGorry et al., 2008). Therefore, the availability of clinically useful and cost-effective biomarkers for the differential diagnosis of major psychiatric disorders would likely enhance patient management, improve treatment/therapeutic response and lead to targeted therapies tailored to the individual (Holsboer, 2008). Despite their potential, to date, no such biomarkers have been established.

Functional imaging studies are one source of potential biomarkers (Gur et al., 2007; Phillips and Vieta, 2007); these studies have previously elucidated subtle brain abnormalities in patients with major psychiatric disorders relative to healthy control (HC) individuals and have been applied to the differential diagnosis of psychiatric disorders (e.g., to differentiate MDD from SZ, Barch et al., 2003 or BP, Almeida et al., 2009). However, some functional neuroimaging techniques are limited by the fact that, during the procedure, the individuals need to be placed in an uncomfortable or unnatural setting (e.g., lying in a supine position in a narrow gantry with the head fixed during the entire examination), for accurate measurement.

In contrast, multi-channel near-infrared spectroscopy (NIRS) using near-infrared light provides a completely non-invasive measurement of the spatiotemporal characteristics of brain function in ordinary clinical settings and allows patients to be comfortably seated in a well-lit room; therefore, it is considered a method for 'real-world neuroimaging'. Additionally, NIRS has relatively low maintenance costs and does not involve ionising radiation or objectionable noise; thus, it can be repeated as needed even for patients with psychiatric disorders. The utility and limitations of NIRS have been discussed extensively in previous reports (Ferrari and Quaresima, 2012; Obrig and Villringer, 2003; Strangman et al., 2002a). NIRS allows the measurement of haemoglobin concentration changes (1) only in the cortical surface area located immediately beneath the probes, but not in deeper brain structures, and (2) with limited spatial resolution, although it has a high temporal resolution. In NIRS, typical cortical activation represents not only decreased concentration of deoxy-haemoglobin ([deoxy-Hb]), which is considered the main source of blood oxygenation level-dependent (BOLD) contrast increase in functional magnetic resonance imaging (fMRI), but also a relatively larger increase in oxy-haemoglobin concentration ([oxy-Hb]) (Fig. 1).

The verbal fluency task (VFT) is a cognitive task that is used as a neuropsychological test or a neuroimaging task. The VFT elicits different abnormalities relevant to each diagnostic group of major psychiatric disorders (Curtis et al., 2001; Zanelli et al., 2010). We previously developed a very brief (<3 min) VFT and used it to investigate the differential fronto-temporal haemodynamic pattern between MDD and SZ (Suto et al., 2004) or MDD and BP (Kameyama et al., 2006), as well as the relationship between NIRS signals and functional impairment in SZ (Takizawa et al., 2008). We also found functional NIRS abnormalities in individuals at ultra-high risk for SZ and patients with first-episode psychosis (Koike et al., 2011). However, the clinical applicability of NIRS to the differential diagnosis of individuals remains uncertain. In this study, we extended our translational approach to replicate our previous findings (Kameyama et al., 2006; Suto et al., 2004) in a seven-site collaborative study using a large, fully independent sample set, and to evaluate the application of NIRS to psychiatric differential diagnosis in natural clinical settings.

Specifically, we used NIRS with wide coverage of the prefrontal and temporal cortices to investigate whether the frontal and temporal brain haemodynamic responses induced by cognitive activation could serve as biomarkers of underlying major psychiatric disorders with depression. To validate the reproducibility and generalisability of the results, we applied an algorithm developed using the data generated at the initial site to the test data derived from the remaining 6 sites. We hypothesised that the spatiotemporal characteristics of the haemodynamic responses detected by NIRS would not only differentiate patients with psychiatric disorders from HCs with acceptable sensitivity and specificity, but would also differentiate correctly and with a high concordance rate patients with MDD from patients with bipolar disorder and schizophrenia who present with depressive symptoms.

Material and methods

Participants

This multi-site study was performed in 7 hospitals: 6 were affiliated with universities (Fukushima, Gunma, Mie, Tokyo, Showa, and Tottori) and one was affiliated with the National Centre of Neurology and Psychiatry of Japan. The sites were situated in the Tokyo metropolitan area and in moderate-scale prefectural capital cities (Fukushima, Maebashi, Tsu and Yonago). The participants were recruited from June 2004 to June 2009, with the exception of recruitment at the initial site (Gunma University Hospital in Maebashi City), which was conducted over 6 years (March 2003 to March 2009). The ethics committees of the participating hospitals approved this collaborative study. In accordance with the Declaration of Helsinki, all participants gave written informed consent after receiving a complete explanation of the study.

Six hundred and seventy-three in-patients and out-patients with psychiatric disorders (MDD, BP and SZ), in addition to 1007 HC volunteers (Flow diagram (1)), were initially enrolled. Of note, these individuals were not the same as those included in our previous studies (Kameyama et al., 2006; Suto et al., 2004). The patients were diagnosed by experienced psychiatrists based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First et al., 1997). The HC volunteers were hospital staff members, university students and members of the general population who responded to website or newspaper advertisements in each city. The SCID non-patient edition was used to screen HC individuals. The exclusion criteria of the initial enrolment were neurological illness, traumatic brain injury with any known cognitive consequences and alcohol/substance abuse or addiction. All participants were native Japanese speakers who were capable of performing a Japanese version of the VFT easily.

On the day of NIRS measurement, the depressive symptoms of participants were evaluated using the 17-item Hamilton Rating Scale for Depression (HAMD) (Hamilton, 1960) and their psychotic and manic symptoms were evaluated using both the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1991) and the Young Mania Rating Scale (YMRS) (Young et al., 1978), respectively, by well-trained psychiatrists with no knowledge of the NIRS data. During the study, all patients with psychiatric disorders were medicated with one or more agents (anti-psychotics, anti-depressants, anxiolytics and/or anti-parkinsonian agents), with the exception of 10 drug-free patients with MDD and 5 drug-free patients with SZ.

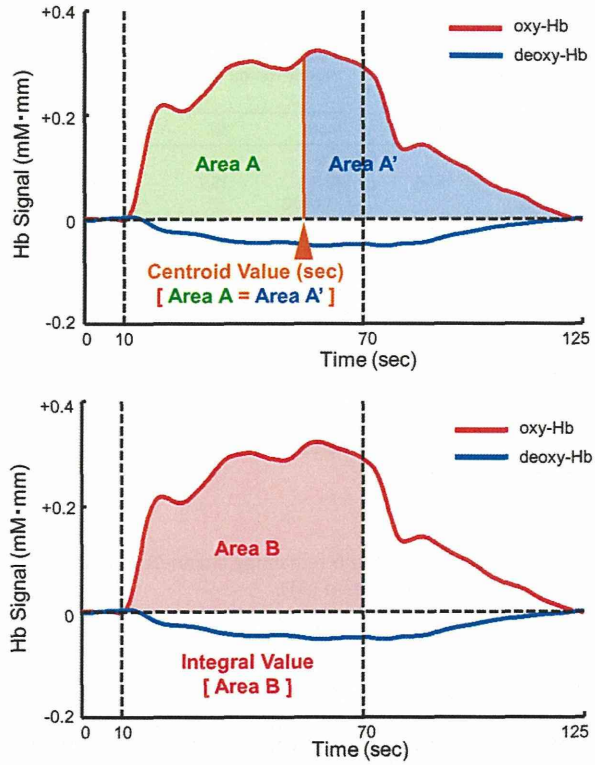

Fig. 1. Typical time-course pattern of near-infrared spectroscopy (NIRS) signals coupled with the verbal fluency task. The 'centroid value' is indicated by the time [s], which is indicated by a perpendicular line from the centroid of an NIRS signal-change area (calculated with positive change) throughout all the task periods. Oxy-Hb: oxygenated haemoglobin signal; deoxy-Hb: deoxygenated haemoglobin signal.

To minimise the influence of confounding factors, we performed group matching for age and gender among the 4 diagnostic groups using one-way analysis of variance (ANOVA) and a chi-squared test, which excluded randomly selected individuals and brought the mean age of the HC individuals and patients with MDD or SZ in closer alignment with that of the patients with BP (44.0 ± 14.9 years old (y.o.)), which was the group with the fewest individuals (Table 1 and Flow diagram (2)). For confirmation, we also analysed demographically non-matched samples that are identified in the Supplementary Material (I). The overall results were the same as those described in the main manuscript and the reduction in the total number of study participants after demographical matching did not appear to have an influence on the development of the algorithm (see Supplementary Material (I)).

Because our clinically valuable target were help-seeking unremitted patients, subsequently we excluded study participants with extremely mild symptoms (HAMD score ≤ 5, PANSS depression item score ≤ 1, PANSS negative symptom score ≤ 11, PANSS general psychopathology score ≤ 21, or PANSS positive symptom score − negative symptom score ≤ 11; the latter 3 criteria were based on the criteria from the PANSS manual for the 5th percentile of patients with mild SZ, Kay et al., 1991). We also excluded patients in a manic phase (YMRS score > 10) from the NIRS measurement; rather, we focused on patients with BP who were in the depressive phase because the different phases may produce different brain dysfunctions in patients with BP (Phillips and Vieta, 2007), and manic patients with BP were diagnosed without apparent difficulty (Flow diagram (3)).

Activation task

The activation task used in this study was similar to that used in our previous studies (Kameyama et al., 2006; Suto et al., 2004; Takizawa et al., 2008). Briefly, a VFT (letter version) was administered and NIRS signal ([oxy-Hb] and [deoxy-Hb]) changes were measured during a 10 s pre-task baseline period, a 60 s activation period and a 55 s post-task baseline period. During the activation period, the participants were instructed to utter as many Japanese words beginning with a designated syllable as possible. For the pre- and post-task baseline periods, the individuals were instructed to simply repeat Japanese vowels out loud. The total number of correct words generated during the 60 s activation period was used as the measure of task performance (Table 1).

Among the many neuropsychological tasks used for detecting neurocognitive deficits in patients with major psychiatric disorders (Barrett et al., 2009; Zanelli et al., 2010), we selected the VFT because it is an executive task that exhibits distinct differences in performance and neuroimaging data among each diagnostic group of major psychiatric disorders (Costafreda et al., 2006; Curtis et al., 2001; Zanelli et al., 2010). In addition, the VFT is easy to understand and execute; in fact, all participants generated more than one word during the VFT. Therefore, this task is suitable for translational research aimed at identifying practical biomarkers.

NIRS measurement

The NIRS apparatus and measurement procedure were described in full previously (Takizawa et al., 2008). Briefly, we used a 52-channel NIRS system (ETG-4000; Hitachi Medical Co., Tokyo, Japan). The preparation of the apparatus, including the audiovisual on-screen instructions, usually took less than 7 min and our brief version of the VFT took less than 3 min, which is less demanding for participants (10–15 min is necessary for the entire procedure).

NIRS is based on the principle that near-infrared light is preferentially absorbed by haemoglobin and less so by other tissues. Near-infrared light emitted from the skin travels into the body, is reflected and absorbed by the internal tissues and reappears on the skin. Thus, the absorption of near-infrared light reflects haemoglobin concentration ([Hb]) in the tissue placed beneath emission and detection probe pairs. Measurements taken using 2 or more wavelengths of near-infrared light enable the determination of [oxy-Hb] and [deoxy-Hb] changes because their absorptions are different at different wavelengths. The ETG-4000 system measures relative changes in [oxy-Hb] and [deoxy-Hb] using 2 wavelengths (695 and 830 nm) of infrared light, based on the modified Beer–Lambert law.(Yamashita et al., 1996) In this continuous-wave NIRS system, these [Hb] values include a differential pathlength factor (DPF); therefore, the unit of this form of NIRS measurement is mM·mm. The distance between pairs of source-detector probes was set to 3.0 cm and each measurement area located between pairs of source-detector probes was defined as one 'channel'. It is assumed that a machine in which the source-detector spacing is 3.0 cm measures points at a depth of 2–3 cm from the scalp (i.e., the surface of the cerebral cortex) (Okada and Delpy, 2003). The temporal resolution of NIRS was set to 0.1 s.

The arrangement of the probes measured relative [oxy-Hb] and [deoxy-Hb] signal changes in the bilateral prefrontal cortical area (i.e., dorso-lateral [Brodmann areas (BAs) 9 and 46], ventro-lateral [BAs 44, 45, and 47] and fronto-polar [BA 10] regions) and in the superior and middle temporal cortical surface regions, which was corroborated by a multi-individual study of anatomical cranio-cerebral correction via the international 10–20 system (Fig. 2 and Table S1) (Tsuzuki et al., 2007). However, in the 10–20 system, the anterior parts of the probes (e.g., Fpz) can be positioned precisely, whereas the position errors of more lateral probes might be increased due to inter-individual head size variability. In addition, although we initially aimed to analyse single-individual and single-channel levels in this study, studies of repeated NIRS measures have demonstrated acceptable reliability of the NIRS signal at the group and cluster levels, whereas retest reliability was unsatisfactory at the single-individual and single-channel levels (Schecklmann et al., 2008).

Table 1
Demographic and clinical characteristics of the 4 age- and gender-matched diagnostic groups at all 7 study sites.

	Major depressive disorder		Schizophrenia		Bipolar disorder		Healthy control		Group difference
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*p*-Value
n	153		136		134		590		
Age years	43.8	12.7	43.7	12.1	44.0	14.9	43.9	15.7	0.99
Gender, women/men	77/76		67/69		69/65		314/276		0.81[a]
Education, years	14.0	1.9	15.2	2.0	15.6	2.0	16.1	2.4	<0.01
Estimated premorbid IQ	106.0	10.1	103.7	11.2	106.9	8.6	107.2	10.1	0.23
Task performance	13.0	3.8	13.6	4.4	12.0	3.6	15.3	4.8	<0.01
Age at onset, years	39.2	11.3	23.4	7.4	32.9	12.4	–		
PANSS									
Positive	–		16.3	5.0	–		–		
Negative	–		21.0	6.0	–		–		
General psychopathology	–		37.0	7.6	–		–		
HAM-D	14.1	6.7	–		8.4	7.0	–		
YMRS	–		–		4.7	5.9	–		
GAF	53.9	9.7	47.3	11.4	55.5	13.3	–		

Abbreviations: IQ, intelligence quotient; PANSS, Positive and Negative Syndrome Scale; GAF, global assessment of functioning.
[a] Chi-square test was used for testing group difference. Otherwise, one-way ANOVA was used.

Therefore, instead of undertaking a full analysis at the single-individual and single-channel levels, here we performed an analysis of NIRS signals at the single-individual and cluster levels. A principal component analysis (PCA) of NIRS [oxy-Hb] signal changes in targeted fronto-temporal channels was performed at the initial study site as a preliminary analysis to capture a channel cluster of the analogous time-course pattern in HC individuals. Subsequently, the weight maps of the first and second principal component graphs were used to identify 2 cluster components.

These analyses suggested that 2 cluster components were identified and that the 2 clusters included the frontal region (11 channels) and the bilateral temporal region (10 channels each) (see Supplementary Material (II) and eFig. S1). The channels in these 2 respective regions of interest were averaged and transformed into representative 'Region 1 (R1)' and 'Region 2 (R2)' NIRS signals for each individual (Fig. 3). According to registration into the LONI Probabilistic Brain Atlas 40 (LBPA40) (Fig. 2) (see Supplementary Table S1 for LBPA40 anatomical labels) (Shattuck et al., 2008), the R1 NIRS signal consisted of signals from channels located approximately in the fronto-polar and dorsolateral prefrontal cortical regions (i.e., superior and middle frontal gyri), whereas the R2 NIRS signal consisted of signals from channels located approximately in the ventro-lateral prefrontal cortex and the superior and middle temporal cortical regions (i.e., inferior frontal gyrus and superior and middle temporal gyri).

An automatic artefact-rejection procedure (see Supplementary Material (III)) was followed and individual data were excluded when there were fewer than 6 remaining channels from each of the 2 cluster regions (Flow diagram (4)).

Statistical analyses

Taking into consideration the potential application of the technique in ordinary clinical settings and personalised care, a conservative receiver operating characteristic (ROC) analysis was performed and used to generate simple indices of NIRS signal patterns, to aid individual diagnoses.

The spatiotemporal characteristics of the frontal and temporal haemodynamic responses induced by VFT were assessed and subsequently applied to an algorithm using the simplest and fewest variables for differential diagnosis. Because previous studies have shown that the best way to differentiate patients with MDD from those with BP or SZ is to describe the time-course of changes in the NIRS signal associated with the VFT (Kameyama et al., 2006; Suto et al., 2004), we chose to create 2 simple visual indices, referred to here as 'integral value' and 'centroid value', to capture these time-course changes.

The integral value describes the size of the haemodynamic response during the 60 s activation task period, whereas the centroid value serves as an index of time-course changes throughout the task, with periods representing the timing of the haemodynamic response. The centroid value is indicated by a time shown with a perpendicular line from the centroid of the NIRS signal change area (calculated as a positive change) throughout the task periods (from 0 [s] to 125 [s]

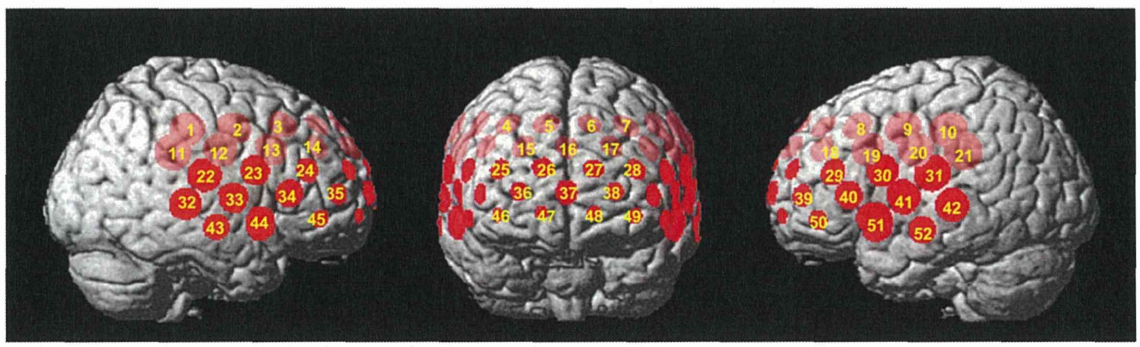

Fig. 2. Regions of interest (Regions 1 and 2) of the near-infrared spectroscopy (NIRS) signals. The locations of near-infrared spectroscopy (NIRS) measurements were probabilistically estimated and anatomically labelled in the standard brain space (LBPA40) according to Tsuzuki et al. (2007). Region 1: (ch 25–28, ch 36–38 and ch 46–49); Region 2, Right: (ch 22–24, ch 32–35 and ch 43–45); Left: (ch 29–31, ch 39–42 and ch 50–52).

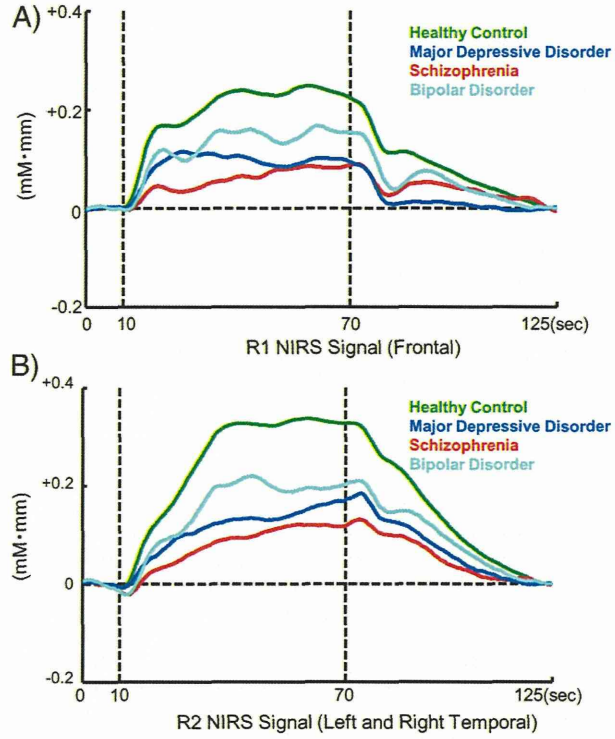

Fig. 3. Time courses of the haemodynamic responses in Region 1 (R1) and Region 2 (R2) in the 4 diagnostic groups. Panels A and B show the time courses of the haemodynamic responses in R1 and R2, respectively.

(= 10 [s] + 60 [s] + 55 [s])) (see Fig. 1). To confirm the reproducibility of each single index between the 2 measurements, a test–retest analysis (single-measure intra-class correlation (ICC) analysis using a one-way random effect model) revealed the presence of significant intra-class correlation coefficients for both the R1 and R2 integral values [$r = 0.47$, $p = 0.01$; $r = 0.59$, $p < 0.01$, respectively] and the R1 centroid value [$r = 0.65$, $p < 0.01$], but not for the R2 centroid value [$r = 0.20$, $p = 0.19$] (see Supplementary material (IV)). PCA and ICC analyses revealed that the 2 indices of NIRS analysis during VFT were acceptable at the single-individual and cluster levels. Thus, the 3 significant variables were used for further analysis.

The 2 representative R1 and R2 NIRS signals obtained from each individual were averaged separately for each type of [Hb] and the integral and centroid values were calculated using parametric statistical tests. Further analyses focused on the increases in [oxy-Hb], because these appear to reflect task-related cortical activation more directly than do decreases in [deoxy-Hb], as evidenced by the stronger correlation between the former and the blood oxygenation level-dependent signal measured by fMRI (Strangman et al., 2002b) and by the results of animal studies (Hoshi et al., 2001). As the typical [oxy-Hb] activation pattern had a positive direction (Fig. 1), data with positive [oxy-Hb] changes (i.e., data with an integral value ≥ 0) in R1 and R2 were used to create an algorithm (Flow diagram (4)). Data exhibiting negative [oxy-Hb] changes were added to the analysis and were described in the results as being appropriate. The analysis of [deoxy-Hb] changes was reported in Supplementary Material (V); however, no significant variable was found regarding [deoxy-Hb] changes.

First, as a preliminary analysis to identify the variable that differentiates patients with psychiatric diseases from HCs most robustly, the 3 variables, including both integral and centroid values of the R1 NIRS signal and the integral value of the R2 NIRS signal, were compared among all of the patients and the age- and gender-matched controls at the initial study site using ANOVA. The resulting significant variables were applied to ROC analyses at the remaining 6 sites.

Because mental health professionals in real clinical settings must differentiate patients with MDD from those with BP or SZ presenting with depression as accurately as possible, the second main analysis performed here aimed to determine the most informative variable and the optimal threshold to discriminate patients with MDD from those with non-MDD disorders. In the present study, the 3 variables, including both integral and centroid indices of R1 and the integral R2 index of the NIRS signal, were compared among patients with MDD and those with either of the other 2 disorders using ANOVA; the variables that were deemed to be significant were applied to the ROC analysis. The preliminary data from the initial site were used to determine an optimal threshold, which was then validated using the test data from the remaining 6 sites.

Third, Pearson's correlation analysis was performed between the significant variables and demographic confounding factors. Data were tested for a normal distribution using the Kolmogorov–Smirnov test. Data that were not normally distributed were analysed using Spearman's correlation analysis.

In particular, regarding clinical confounding factors, such as symptoms (HAMD, YMRS and PANSS scores) and medication doses (antidepressants: imipramine (IMP) equivalent dose; antipsychotics: chlorpromazine (CPZ) equivalent dose; anxiolytics: diazepam equivalent dose; and anti-parkinsonian drugs: biperiden equivalent dose, lithium dose, sodium valproate dose and carbamazepine dose), a stepwise multiple linear regression analysis was performed with a probability of F for conservative entry and removal criteria of 0.01 and 0.05, respectively, to elucidate the complicated relationships among these clinical confounding factors in each diagnostic group.

All data are expressed as mean and standard deviation (SD). The significance level was set to alpha = 0.05. When a difference was considered significant, we presented both the effect size (Cohen's d) and the 95% confidence interval (CI). Statistical analyses were performed using the SPSS 16.0.1J software (SPSS Inc., Tokyo, Japan).

Results

Demographic characteristics

Table 1 shows the demographic and clinical characteristics of the 4 age- and gender-matched diagnostic groups used in this study. One-way ANOVA revealed an absence of significant age differences among the groups ($p = 0.99$) and a chi-squared test showed an absence of gender differences among the groups ($p = 0.81$). In addition, the age and gender distributions among the 4 diagnostic groups were not significantly different at the initial site (Gunma University, MDD: 39.9 (11.7) y.o., 12/15; BP: 41.1 (13.2) y.o., 22/15; SZ: 40.1 (14.9) y.o., 11/20; and HC: 40.0 (4.2) y.o., 7/10) (age, $p = 0.98$; gender, $p = 0.24$) and at the other 6 sites (MDD: 44.6 (12.7) y.o., 65/61; BP: 45.1 (15.4) y.o., 47/50; SZ: 44.8 ± 11.0 y.o., 56/49; and HC: 44.0 ± 15.9 y.o., 307/266) (age, $p = 0.89$; gender, $p = 0.81$).

Preliminary test of the difference between HCs and patients

Although it was not the main theme of this study, to compare our results with those of studies of biomarkers performed only to detect functional abnormalities in patients against a control group, we also analysed the differences between HC individuals and patients to confirm the significance of the 3 variables chosen for analysis. Full analyses are described in Supplementary Material (VI).

From the analyses performed using data from the initial site, we adopted both R1 and R2 integral values as statistically significant variables for the algorithm. Thresholds were dependent on the