score decreased from 87.05 (SD = 29.40) to 54.00 (SD = 29.99) over the course of the study. The PHQ-9 and GAD-7 scores reduced from 11.11 (SD = 6.88) to 6.84 (SD = 5.07) and 9.32 (SD = 5.86) to 5.74 (SD = 4.74), respectively. A within-group *t*-test revealed significantly different scores between the pre- and post-CBT scores on the assessed scales: $t(1, 18) = 5.627$, $p < .001$, for the LSAS; $t(1, 18) = 3.338$, $p = .003$ for the PHQ-9; and $t(1, 18) = 2.486$, $p = .002$ for the GAD-7. The effect sizes between pre- and post-CBT were 1.124 (large), .620 (medium), and 0.611 (medium) for the LSAS, PHQ-9, and GAD-7, respectively.

Result of feedback from trainees

Ten trainees took part in the post-hoc survey about the training course. Regarding satisfaction with the length (i.e., one day a week for two years) of the training course, eight selected "satisfied," and two selected "very satisfied." As for the workshops, six selected "satisfied," three selected "very satisfied," and one selected "slightly satisfied." With respect to the frequency and the duration of the supervision, five selected "satisfied," three selected "slightly satisfied," and two selected "satisfied."

In response the question about the distinctive aspects of our training course compared to previous CBT training, five mentioned the continuity and practicality of our course, in contrast to classroom lectures for a short period. Additionally, four trainees appreciated the colleagues they had made through the training, and noted that they still support each other. Moreover, two pointed out that they obtained a wider perspective of CBT because both psychologists and psychiatrists were instructors in the training course. Regarding the difficulties trainees had during the training, three referred to their reluctance to record the sessions, although their clients usually agreed to be recorded. Finally, two noted that it was difficult to write clinical case reports.

Discussion

The purpose of this study was to report the preliminary outcomes of individual CBT for OCD, BN, and SAD delivered by the trainees of the Chiba CBT training course. We included patients with comorbid mood disorders if OCD, SAD, **or** BN was the principal diagnosis to reflect routine clinical practice. The results demonstrated that individual CBT for OCD, BN, and SAD in Japan led to significant reductions in symptom severity for these primary diagnoses. The effect size for OCD was comparable with those obtained in past trials involving psychological treatment for OCD (Rosa-Alcázar et al., 2008), and those for BN and SAD were large. Our study was designed not only to recruit patients similar to those seen in routine clinical practice but also to train clinicians who **will be** engaged in routine clinical practice; they were not fully trained therapists **before** this study.

Although it is difficult to directly compare our effect sizes with other published data due to a variety of factors (e.g., patient demographic and type/intensity of CBT), the overall effect sizes of 0.63 for PHQ-9 (medium) and 0.66 for GAD-7 (medium) were less than were those in other IAPT and other studies (Clark et al. 2009; Radhakrishnan et al. 2013; Richards & Borglin, 2011; Richards & Suckling, 2009; Westbrook & Hill, 1998; Westbrook & Kirk, 2005). It is possible that severity of depression and anxiety among our recruited patients was lower than that observed in previous reports, and thus resulted in a lower effect size. However, it is noteworthy that our results showed the lowest scores of PHQ-9 and GAD-7 at post-treatment (Table 4).

Table 4 about here

Our training course was highly evaluated by the trainees regarding the satisfaction with the length of the training course, the content of the workshops, and the frequency and duration of the supervision. This was confirmed by their comments suggesting that our course offered more comprehensive training than other courses. The trainees valued colleagues, probably because most of them do not have someone to

consult (even to talk) about CBT at their workplace. In order to address the difficulties in writing case reports, the supervisors addressed this issue by providing a special seminar about academic writing.

Dissemination of CBT across Japan

As noted in the Introduction, CBT is only covered by national health insurance for the treatment of mood disorders, primarily because the quantity of outcome research in CBT, particularly **using randomized control trials**, is exceptionally low in Japan. As Gunter and Whittal (2010) proposed, we need to conduct studies and evaluate more research-based data to obtain required funding and organizational support. The other issue hindering the dissemination of CBT in Japan is the paucity of training opportunities. Opportunities are limited for both pre- and post-qualification training. More than 160 universities **and** colleges provide postgraduate master's programs in clinical psychology, but only a few courses incorporate CBT in their curricula because of the scarcity of CBT experts. Our hope is that post-qualification training courses will be established in other areas of Japan so that more health professionals **can** attend workshops and benefit from regularly supervised practice.

Through the development and administration of the training course, the supervisors gained a wealth of professional knowledge concerning the dissemination of CBT in Japan. For example, approximately two years before the commencement of the training course, a survey was conducted with a number of psychiatric hospitals and clinics in Chiba province to identify the type of therapies that patients desired and clinicians would like to learn (Haraguchi et al., submitted for publication). The result of this survey revealed the strong need for CBT and provided rationale for establishing our training course. Additionally, as they ran the training course, supervisors had to identify and solve problems and difficulties as these arose. For instance, some trainees had difficulties with academic writing because they had completed their

undergraduate or postgraduate course many years ago. A special workshop was organized for the improvement of writing skills. To modify bias in their assessments, supervisors occasionally watched a video of a session together and compared each other's scores on the CTS-R. Moreover, they asked their supervisees to rate the Process Evaluation of Training and Supervision scale (Wilson, 2007) to assess the duration, frequency, supportive, and formative factors of supervision.

The concept of CBT as a Western therapy requiring major adaptation for effective use in Japanese culture must be considered further in on-going research. Compared to Western cultures, more emphasis is placed on interpersonal relationships than on self-fulfillment or self-development in Asian cultures. However, we believe that similar factors support the efficacy and utilization of CBT in Japan. **For example**, in a randomized trial, Nakatani et al. (2005) demonstrated that behavioral therapy is highly effective for Japanese patients with OCD. Matsunaga et al. (2008) elucidated the transcultural stability of the symptom structure of OCD, which is consistent with the hypothesis that OCD is mediated by universal psychobiological mechanisms.

The limitations of this study

Although the present study provided valuable information, it does have several limitations. This was a single-arm study without a concurrent control group. Moreover, our waiting period was not fixed, and scores were not obtained at a pre-treatment baseline point. Although these design factors reflect the real-world nature of mental health services, it remains unknown whether the observed improvements in symptom severity was related to the natural extinction of the disorders. More studies employing psychological placebo conditions to control for nonspecific factors, such as positive outcome expectancy and self-efficacy for problem management, are needed.

This study established the acute effectiveness of the treatment, but the lack of follow-

up data limits the generalizability of the study (e.g., long-term effects, relapse rates). Further, there was no control for the patients' use of medication, although our patient group had typically taken antidepressant medication for an extended period before referral to Chiba University Hospital. Again, this circumstance reflects the reality of the population of patients who access secondary mental health services in Japan. Further studies will need to include fixed waiting periods, control groups, and long-term follow-up to provide more insight into the implementation of CBT in routine practice in Japan. Currently, our research team is running a randomized control trial for SAD (Yoshinaga et al., in press: trial number: UMIN000007552) and single-arm trials for OCD and BN with fixed waiting periods. Changes in employment status, such as fewer days absent from work, should be examined after completion of the therapy. This would be a crucial test of whether increased access to psychological therapies would largely pay for itself by reducing other depression- and anxiety-related public costs (e.g., welfare benefits and medical costs) and increasing revenues (e.g., taxes, increased productivity).

This study focused on the effectiveness of CBT delivered by trainees to evaluate our training program. However, it remains unknown if the result of the CBT is due to the training or whether trainees had been already competent. Thus, other measures could also be employed to gain a better understanding of the ways training should be provided. Comparing scores on the cognitive therapy awareness scale (Sudak et al., 2003)— a multiple-choice questionnaire (Maunder et al., 2008; Myles & Milne, 2004)—between pre- and post-training would **reveal** how competent trainees felt as they progressed **through** training. A video assessment task (Myles & Milne, 2004) would provide a more objective perspective of the trainee competence.

References

Andrews G, & Titov N (2009). Hit and miss: innovation and the dissemination of evidence based psychological treatments. Behaviour Research and Therapy 47, 974–979.

Blackburn I, James IA, Milne DL, Baker C, Standart S, Garland A, & Reichert FK (2001). The revised cognitive therapy scale (CTS-R): psychometric properties. Behavioural and Cognitive Psychotherapy 29, 431–446.

Clark D, & Wells A (1995). A cognitive model of social phobia. In: Social Phobia: Diagnosis, Assessment, and Treatment (ed. R. G. Heimberg & M. R. Liebowitz), pp. 69-93. New York: Guilford Press.

Clark DM, Layard R, Smithies R, Richards DA, Suckling R, & Wright B. (2009). Improving access to psychological therapy: Initial evaluation of two UK demonstration sites. Behaviour Research and Therapy, 47, 910-920

Cohen J (1988). Statistical Power Analysis for the Behavioral Sciences, Hillsdale, NJ: Erlbaum.

First MB, & Gibbon M (1997). User's Guide for the Structured Clinical Interview for DSM-IV Axis I Disorders SCID-I: Clinician Version, Arlington: American Psychiatric Pub.

Foa EB, Kozak MJ, Salkovskis PM, Coles M, & Amir N (1998). The validation of a new obsessive-compulsive disorder scale: The Obsessive-Compulsive Inventory. Psychological Assessment 3, 206–214.

Gunter RW, & Whittal ML (2010). Dissemination of cognitive-behavioral treatments for anxiety disorders: overcoming barriers and improving patient access. Clinical Psychology Review 30, 194–202.

Haraguchi T, Shimizu, E, Nakazato M, Kobori O, & Iyo M. (submitted for publication). Current status of cognitive behavioural therapy in Chiba prefecture: a questionnaire survey.

Henderson M, & Freeman CP (1987). A self-rating scale for bulimia. The 'BITE'. British Journal

of Psychiatry 150, 18–24.

Houghton, S., Saxon, D., Bradburn, M., Ricketts, T., & Hardy, G. (2010), The effectiveness of routinely delivered cognitive behavioural therapy for obsessive-compulsive disorder: A benchmarking study. British Journal of Clinical Psychology, 49: 473–489

Kessler RC, Andrews G, Colpe LJ, Hiripi E, Mroczek DK, Normand SLT...Zaslavsky AM (2002). Short screening scales to monitor population prevalences and trends in non-specific psychological distress. Psychological Medicine 32(6), 959–976.

Liebowitz MR (1987). Social phobia. Modern Problems of Pharmacopsychiatry 22, 141–173.

Löwe B, Decker O, Müller S, Brähler E, Schellberg D, Herzog W, & Herzberg PY (2008). Validation and standardization of the Generalized Anxiety Disorder Screener (GAD-7) in the general population. Medical Care 46, 266–274.

Maunder L, Milne D, & Cameron L (2008). Pilot evaluation of brief training in CBT for primary care practitioners. Behavioural and Cognitive Psychotherapy 36, 341–353.

Matsunaga H, Maebayashi K, Hayashida K, Okino K, Matsui T, Iketani T,...Stein DJ (2008). Symptom structure in Japanese patients with obsessive-compulsive disorder. American Journal of Psychiatry 165, 251–253.

Myles PJ, & Milne DL (2004). Outcome evaluation of a brief shared learning programme in cognitive behavioural therapy. Behavioural and Cognitive Psychotherapy 32, 177–188.

Nakatani E, Nakagawa A, Nakao T, Yoshizato C, Nabeyama M, Kudo A,..Kawamoto M (2005). A randomized controlled trial of Japanese patients with obsessive-compulsive disorder: Effectiveness of behavior therapy and fluvoxamine. Psychotherapy and Psychosomatics 74, 269–276.

Radhakrishnan M, Hammond G, Jones PB, Watson A, McMillan-Shields F, & Lafortune L (2013). Cost of improving Access to Psychological Therapies (IAPT) programme: an analysis of cost of session, treatment and recovery in selected Primary Care Trusts in the East of England.

Richards, DA, & Borglin, G. (2011). Implementation of psychological therapies for anxiety and depression in routine practice: Two year prospective cohort study. Journal of Affective Disorders. 133, 51–60.

Richards DA, & Suckling R (2009). Improving access to psychological therapies: phase IV prospective cohort study. *British Journal of Clinical Psychology 48,* 377-96.

Rosa-Alcázar AI, Sánchez-Meca J, Gómez-Conesa A, & Marín-Martínez F (2008). Psychological treatment of obsessive-compulsive disorder: a metaanalysis. Clinical Psychology Review 28(8), 1310–1325.

Schmidt U, & Treasure J (1983). Getting Better Bit(e) by Bit(e): A Survival Kit for Sufferers of Bulimia Nervosa and Binge Eating Disorders, Hove, UK: Psychology Press, Ltd.

Shafran R, Clark DM, Fairburn CG, Arntz A, Barlow DH, Ehlers A...Wilson GT (2009). Mind the gap: Improving the dissemination of CBT. Behaviour Research and Therapy 47(11), 902–909.

Sudak DM, Beck JS, & Wright J (2003). Cognitive behavioral therapy: A blueprint for attaining and assessing psychiatry resident competency. Academic Psychiatry 27, 154–159.

Westbrook, D. & Hill, L. (1998). The long-term outcome of cognitive-behaviour therapy for adults in routine clinical practice. Behaviour Research & Therapy, 36, 635-643.

Westbrook, D. & Kirk, J. (2005). The clinical effectiveness of cognitive behaviour therapy: outcome for a large sample of adults treated in routine practice. Behaviour Research & Therapy, 43, 1243-1261.

Wilson, M. (2007). Can experiences of supervision be qualified? My PETS: A new tool for measuring supervisees' satisfaction with clinical supervision. Unpublished undergraduate thesis, available from the Psychology Department, Newcastle University, NE1 7RU.

Yoshinaga N, Niitsu T, Hanaoka H, Sato Y, Ohshima F, Matsuki S,...Shimizu E (2013). Strategy for treating selective serotonin reuptake inhibitor-resistant social anxiety disorder in the

clinical setting: A randomised controlled trial protocol of cognitive behavioural therapy in combination with conventional treatment. BMJ Open 3, e002242 doi:10.1136/bmjopen-2012-002242.

Tables

Table 1.

Demographic and clinical characteristics for OCD[a] (N = 14)

Variable		Value
Gender, female, n (%)		11 (79)
Age, years, mean (SD)		36.79 (9.88)
Comorbid Axis I diagnosis, N (%)	No comorbid condition (OCD only)	8 (57)
	With comorbidity	6 (43)
Age of onset, years, mean (SD)		31.57 (9.18)
Duration of OCD, years, mean (SD)		5.21 (4.67)
Employment status, N (%)	Employed full-time	2 (14)
	Part-time/homemaker	7 (50)
	Unemployed	5 (36)
Marital status, N (%)	Single	6 (43)
	Married	7 (50)
	Dating	1 (7)
Educational background, N (%)	High school	3 (22)
	Diploma	4 (28)
	Degree	7 (50)
Currently on medication, N (%)	AD and/or BZ	12 (86)

[a] Abbreviations: OCD = Obsessive-compulsive Disorder; BZ = Benzodiazepines; AD = Antipsychotics.

Table 2.

Demographic and clinical characteristics for BN[a] (N = 8)

Variable		Value
Gender, female, N (%)		8 (100)
Age, years, mean (SD)		31.3 (10.4)
BMI (kg/m^2)		23.5(5.7)
Comorbid Axis I diagnosis, N (%)	Without comorbidity (BN only)	5 (62.5)
	With comorbidity	3 (37.5)
Age of onset, years, mean (SD)		20.8 (7.1)
Duration of BN, years, mean (SD)		10.4 (8.9)
Employment status, N (%)	Employed full-time	1 (12.5)
	Full-time student	3 (37.5)
	Homemaker	2 (25.0)
	Unemployed	2 (25.0)
Marital status, N (%)	Single	5 (62.5)
	Married	3 (37.5)
	Divorced	2 (25.0)
Educational background, N (%)	Junior high school	0 (0)
	High school	1 (12.5)
	Diploma	3 (37.5)
	Degree	4 (50.0)
Currently on medication, N (%)	BZ and/or AD and/or MS	5 (62.5)

[a] Abbreviations: BN = Bulimia Nervosa; BZ = Benzodiazepines; AD = Antipsychotics; MS = Mood Stabilizers

Table 3.

Demographic and clinical characteristics for SAD[a] (N = 19)

Variable		Value
Gender, female, N (%)		14 (74)
Age, years, mean (SD)		32.3 (9.7)
Subtype, generalized, N (%)		16 (84)
Comorbid Axis I diagnosis, N (%)	Without comorbidity (SAD only)	11 (58)
	With comorbidity	8 (42)
Age of onset, years, mean (SD)		17.9 (8.8)
Duration of SAD, years, mean (SD)		14.3 (10.5)
Employment status, N (%)	Employed full-time	6 (32)
	Full-time student	5 (26)
	Part-time/homemaker	4 (21)
	Unemployed	4 (21)
Marital status, N (%)	Single	12 (63)
	Married	6 (32)
	Divorced	1 (5)
Educational background, N (%)	Junior high school	2 (13)
	High school	7 (37)
	Diploma	6 (32)
	Degree	4 (21)
Currently on medication, N (%)	AD and/or BZ	17 (87)

[a] Abbreviations: SAD = Social Anxiety Disorder; BZ = Benzodiazepines; AD = Antidepressants

Table 4.

Comparison of effect sizes among various studies

Symptom	Data source	Intensity of CBT[a]	N (Dep , Anx)	Outcome	Pre Mean (SD)	Post Mean (SD)	ES[b]
Depression	Current data	High	45 (0%, 82%)	PHQ-9	10.6 (6.3)	6.6 (5.5)	0.63
	Westbrook (1988)	N/A	36 (27%, 36%)	BDI	18.2 (9.9)	10.9 (10.4)	0.79
	Westbrook (2005)	N/A	776 (19%, 56%)	BDI	16.9 (10.5)	9.8 (9.0)	0.68
	Clark (2009): Doncaster	High and low	1648 (95%, 5%)	PHQ-9	15.8 (6.2)	7.5 (6.9)	1.34
	Clark (2009): Newham	High and low	221 (46%, 43%)	PHQ-9	15.3 (6.2)	8.2 (7.2)	1.15
	Richards (2009)	High and low	1274 (N/A)	PHQ-9	16.0 (6.15)	8.1 (7.2)	1.28
	Richards (2011)	High and low	4183 (77%, 8%)	PHQ-9	16.2 (6.2)	9.0 (7.3)	1.17
	Radhakrishnan (2013)	High	2230 (N/A)	PHQ-9	14.4 (6.7)	9.2 (9.0)	0.79
		Low	4854 (N/A)	PHQ-9	12.5 (6.3)	8.0 (9.4)	0.72
Anxiety	Current data	High	45 (0%, 82%)	GAD-7	9.1 (5.8)	5.2 (4.6)	0.66
	Westbrook (1988)	N/A	36 (27%, 36%)	BAI	15.2 (10.4)	11.4 (11.1)	0.37
	Westbrook (2005)	N/A	473 (25%, 48%)	BAI	17.0 (11.8)	10.6 (8.9)	0.54
	Clark (2009): Doncaster	High and low	1648 (95%, 5%)	GAD-7	13.9 (5.2)	6.8 (6.2)	1.37
	Clark (2009): Newham	High and low	221 (46%, 43%)	GAD-7	13.7 (5.1)	6.8 (5.8)	1.35
	Richards (2009)	High and low	1274 (N/A)	GAD-7	14.0 (5.2)	7.2 (6.3)	1.07
	Richards (2011)	High and low	4183 (77%, 8%)	GAD-7	14.1 (5.1)	8.1 (6.4)	1.17
	Radhakrishnan (2013)	High	2230 (N/A)	GAD-7	12.9 (5.3)	8.2 (8.2)	0.89
		Low	4854 (N/A)	GAD-7	11/7 (5.4)	7.3 (9.0)	0.82

Abbreviations: CBT = Cognitive Behavioural Therapy; PHQ-9 = Patients Health Questionnaire-9 items; GAD-7 = Generalized Anxiety Disorder-7 items; BDI = Beck Depression Inventory;

BAI = Beck Anxiety Inventory; Dep = Depressive disorder; Anx = Anxiety disorder; ES = Effect Size.

[a] High = one-to-one, face-to-face psychological therapy; Low = guided self-help (e.g., using books, leaflets or computer support) and goup psychoeducation.

[b] Effect sizes (cohen's *d*) for each study were recalculated using same formula.

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Correlation of prefrontal activity measured by near-infrared spectroscopy (NIRS) with mood, BDNF genotype and serum BDNF level in healthy individuals

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ABSTRACT

Depression has been known to reduce the prefrontal activity associated with the execution of certain cognitive tasks, although whether a temporarily depressed or anxious mood in healthy individuals affects the prefrontal blood oxygen level during cognitive tasks is unknown. Combining the measurement of prefrontal activity with near-infrared spectroscopy (NIRS) and the two cognitive tasks, namely the letter version of the verbal fluency test (VFT-l) and the Stroop test, we measured the effect of a depressed or anxious mood and gender on the changes in the prefrontal oxygenated hemoglobin (Oxy-Hb) levels during those cognitive tests in healthy individuals. Depressed mood or anxious mood was assessed by the Hospital Anxiety and Depression Scale (HADS). Thereby we aimed to explore the possibility of NIRS measurement for detecting the early subclinical manifestation of major depression. Moreover, we examined the possible relationships between prefrontal activation and the functional Val66Met polymorphisms of the brain derived neurotropic factor (BDNF) gene and serum BDNF level. As a result, the increased prefrontal Oxy-Hb levels during cognitive tasks were significantly correlated with the severity of depressed mood in males. The course of the prefrontal Oxy-Hb increase was different depending on the cognitive tasks, *i.e.*, the VFT-l or the Stroop test, in both genders. Correlations of BDNF genotype and serum BDNF level with the prefrontal Oxy-Hb levels during those cognitive tasks were negative. Our results suggest that the early subclinical manifestation of depressed mood in males might be detected by the NIRS measurement, which is not correlated with the individual properties of BDNF.

Keywords: Near-Infrared Spectroscopy (NIRS); Depression; Anxiety; Brain Derived Neurotropic Factor (BDNF)

1. INTRODUCTION

Depression is a common psychiatric disease and results in considerable social-economic burdens due to loss of productivity [1-3]. Thus, the prevention of depression with early detection and intervention is critically important for those exposed to chronic stress because of its potential to induce depression in some individuals [4,5].

Near-infrared spectroscopy (NIRS) is a non-invasive method of measuring the relative change in the concentrations of oxygenated and deoxygenated hemoglobin (Δ[Oxy-Hb]) and Δ[DeOxy-Hb], respectively), which are closely correlated to the neural activity of the brain [6]. Using this technique, altered prefrontal activation has been reported in various psychiatric diseases such as schizophrenia, major depression, bipolar disorder and anxiety disorder [7]. In the case of major depression, a reduced increase of the dorsolateral prefrontal Oxy-Hb level compared to healthy controls during the letter version of the Verbal Fluency Test (VFT-l-l) as determined by the measurement of NIRS has been reported repeatedly [8-10]. However, it has not been clarified whether a temporarily depressed or anxious mood in healthy individuals affects the frontal blood oxygen level during cognitive tasks. Given that one of the main symptoms of major depression is depressive mood, it is a matter of concern whether prefrontal activations could be affected by such a mood. In addition, several lines of evidence

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have shown that there exist gender differences in the prevalence, comorbidity, and symptom presentation pattern in major depression [11], thus, early manifestations of the disease might be different between the genders, and these differences might be observed in the altered prefrontal activation measured by NIRS.

Brain derived neurotrophic factor (BDNF), the most abundant neurotrophic factor in the human brain, plays important roles in the survival, development, and proliferation of the central and peripheral neurons [12,13]. A functional polymorphism of the BDNF gene, an amino acid substitution of a valine for a methionine at codon 66 (Val66Met) as a single nucleotide polymorphism (SNP), has been suggested to be associated with the performance of episodic memory in schizophrenia patients [14] as well as healthy control subjects [14,15], and with the performance of prefrontal cognitive tasks in bipolar disorder [16]. In addition, accumulating evidence suggests that serum BDNF level, which likely reflects brain BDNF level, is altered in mood disorders such as depression. Serum BDNF level has been reported to be associated with depression severity [17] and has been observed to be increased after antidepressant treatment in depressed patients [18-20]. One of the personally traits, neuroticism in the NEO-five factor inventory (NEO-FFI), which is considered to be a marker of depression, appeared to have a negative correlation with serum BDNF level in healthy humans [21]. Taken together, these findings suggest that prefrontal activation is potentially correlated with each individual's BDNF profile, but to date no NIRS study has been conducted to assess this potential correlation.

In the present study, we examined the correlation of changes in the prefrontal Oxy-Hb levels measured by NIRS during cognitive tasks with depressed or anxious mood, gender, Val66Met polymorphisms of the BDNF gene and serum BDNF level in basically healthy individuals. Thereby we aimed to explore the possibility of using NIRS measurement to detect early subclinical manifestations of major depression or anxiety disorder.

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2. MATERIALS AND METHODS

2.1. Subjects and Blood Samples

The research was performed after the study was approved by the ethics committee of Chiba University Graduate School of Medicine and Chiba Prefectural College of Allied Medical Science. All of the subjects were the students of Chiba Prefectural College of Allied Medical Science. Subjects were well explained about the experiments, and written informed consent was obtained. Thirty eight healthy volunteers (female = 17, male = 20, mean age = 22.6 ± SD 3.7, range = 19 - 31) were enrolled in the NIRS study. None of them had past history of psychotic disorders of the DSM-IV axis I or II, neurological disorders, drug dependence, and any major physical illness. One female subject was excluded from the following analyses because of developing depression which needed medical service three months after the NIRS measurements. All except one male subject were right-handed. Whole blood samples for extracting DNA and serum samples for BDNF measurement were collected from 30 of those 37 subjects (female = 13, male = 17) between 11:00-12:00 AM and stored at −80 C.

2.2. Assessment of Metal Status with HADS

Hospital Anxiety and Depression Scale (HADS) was used to assess subjects' mental status. HADS is a self-report screening scale that was developed to indicate the possible presence of anxiety and depression states in hospital and medical outpatient clinic settings. HADS consists of a seven-item anxiety subscale and a seven-item depression subscale. Each item is scored on a four-point scale, giving maximum subscale scores of 21 for depression and anxiety, respectively. Cut-off scores were recommended as ≥8 for possible anxiety or depression in the original study [22]. Self-assessment of HADS was conducted blind to the experimenter of NIRS (H.O. and J.T.), BDNF measurement and genotyping (D.M.). In addition, analysis of HADS score was performed after all the other results were obtained in order to keep subjects' mental status blind to those measurements. Some of them reached ≥8 in the scores of depression and anxiety subscales of HADS, but it was because the study was done right before the semester final examination, and none of them met the criteria of any mental illnesses in DSM-IV [23] when the NIRS measurement and blood collection were conducted. Thus, their mental statuses were assumed as healthy overall, even though temporal depressed or anxiety moods were existed.

2.3. NIRS Study

2.3.1. NIRS Recordings

NIRS recordings were performed using a multichannel optical topography system (FOIRE-3000, Shimadzu Co., Japan). Eleven light-emission and ten light-detection probes were arranged in a 3 × 7 rectangular lattice. Each recording channel consisted of one light-emission probe and one light-detection probe located 3 cm away from each other. A head shell that mounted the 21 probes for 32 recording channels was placed securely on the scalp overlying mainly the prefrontal area. The light-detection probe between channels No.3 and No.4 was located on Fz of the international 10 - 20 system (**Figure 1(a)**).

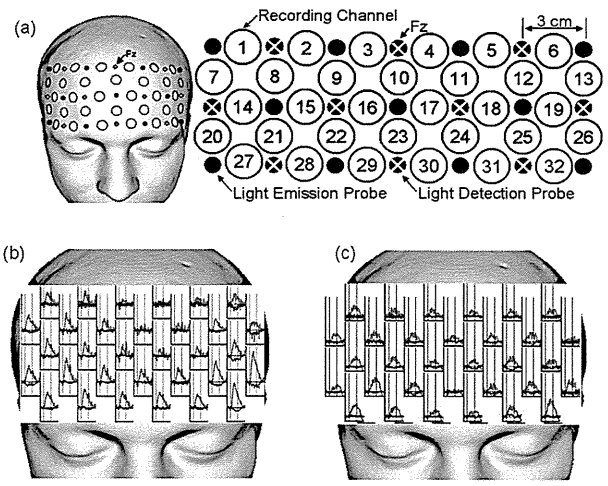

Figure 1. Probe setting and typical courses of the Oxy- and DeOxy-Hb change during the tasks. (a) 21 probes for 32 recording channels were placed securely on the scalp overlying mainly the prefrontal area. The light-detection probe between channels No.3 and No.4 was located on Fz of the international 10 - 20 system. Eleven light-emission and ten light-detection probes were arranged in a 3 × 7 rectangular lattice. Each recording channel consisted of one light-emission probe and one light-detection probe located 3 cm away from each other; (b), (c) Typical courses of the Oxy-Hb (red line) and DeOxy-Hb (blue line) change during the two tasks, VFT-l (b) and Stroop test (c).

2.3.2. Cognitive Tasks for NIRS Recording

Prefrontal activities were recorded during the letter version of verbal fluency test (VFT-l) and Stroop tasks. For NIRS recording, each cognitive task was consisted of a 30 s pre-task baseline period, a 60 s task period, and a 60 s post-task baseline period. Instruction cues for each task were displayed on a 22-inch monitor connected to a laptop personal computer placed approximately 1 meter in front from the subject who was sitting on a comfortable chair.

The VFT-l was carried out according to the previous studies [10,24]. In briefly, during the task period, subjects were instructed to produce orally as many words that began with the cue as possible. During the task period, Three Japanese letters (/a/, /ka/, and /sa/) were presented on the display as cues in turn every 20 s. During the pre- and post-task baseline periods, the subjects were instructed to repeat the syllables /a/, /i/, /u/, /e/, and /o/.

To assess the Stroop effect [25], a list of color names (blue, yellow, red, and green, written in Japanese Kanji characters), which were printed in a color not denoted by the name, was presented during the task period, and a list of color dots (blue, yellow, red, and green) was presented during the pre- and post-task baseline periods. A set of 24 dots/words was presented consecutively on the display. The subjects were instructed to name the ink color in which the dots/words were presented as quickly as possible and explicitly not to read the words. The investigator recorded the number of correct and incorrect verbal responses.

2.3.3. NIRS Data Analyses

Three near-infrared laser beams (wavelengths at 780, 805, and 830 nm) were emitted, and reflectance beams sampled at a 175-ms sampling interval were used to calculate Δ[Oxy-Hb] and Δ[DeOxy-Hb]. The linear trends of the continuous Δ[Oxy-Hb] and Δ[DeOxy-Hb] fluctuations were removed and smoothed with a five-point moving average. In the present study, we mainly report the Δ [Oxy-Hb] results because it may be the most sensitive parameter among hemodynamic responses [26]. Many previous NIRS studies calculated a z score in each recording channel for comparison among subjects or among recording channels [27-29], therefore, we also used the z score for comparison. In the present study, the z score was calculated in each recording channel as follows: the mean Δ[Oxy-Hb] value during the task period (30 - 60 s after task onset) versus that during a 30-s pre-task baseline period was divided by the standard deviation (SD) of Δ[Oxy-Hb] during the pre-task period. The signal processing was performed using MATLAB 7.5.0 (MathWorks, Natick, MA, USA). The significance level of Δ[Oxy-Hb] was set at $z > 3.163$, which reached the two-sided Bonferroni corrected significance level ($p < -0.05$). This calculation method for the significance level is similar to that used in previous NIRS studies [28, 29]. The mean z score of Δ[Oxy-Hb] over all channels was analyzed to measure the signal intensity, and the number of channels with a significant Δ[Oxy-Hb] was analyzed to measure the extent of cerebral activation. Typical courses of the change in Oxy- and DeOxy-Hb during the two tasks, namely the VFT-l and the Stroop test, are shown in **Figures 1(b)** and **(c)**, respectively.

2.4. Genotyping

The genomic DNA was extracted from peripheral leukocytes by standard procedures. Polymerase chain reaction (PCR) and the PCR-based restriction fragment length polymorphism (RFLP) assay were performed to genotype the DNA sequence variants of the BDNF gene as reported previously [30]. The primer sequences were forward: 5-GGTGAGAAGAGTGATGACCA-3 and reverse: 5-GCCAGCCAATTCTCTCTTTTT-3. The PCR products were digested at 37 C with restriction enzyme PmaC I (Takara Shuzo Ltd., Kyoto, Japan), followed by 2% agarose gel-electrophoresis with ethidium bromide staining. After testing the Hardy-Weinberg equilibrium for genotype frequencies (Val/Val, Val/Met and Met/Met), subjects were divided by two groups, Val/Val and Met carrier (Val/Met and Met/Met) group.

2.5. Serum BDNF Measurements

Serum BDNF levels were measured using the BDNF Emax Immunoassay System kit (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, 96-well plates were coated with anti-BDNF monoclonal antibody and incubated at 4 C for 18 hours. The plates were incubated in a blocking buffer for 1 hour at room temperature. The samples and BDNF standards were maintained at room temperature under conditions of shaking for 2 hours, followed by washing with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 hours, washed, and incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 hour at room temperature. The plates were incubated in peroxidase substrate and tetramethylbenzidine solution to produce a color reaction. The reaction was stopped with 1 mol/L hydrochloric acid, the absorbance at 450 nm was measured with an Emax automated microplate reader (Molecular Devices, Tokyo, Japan).

2.6. Statistical Analysis

All the calculations were performed with SPSS software (SPSS version 17.0J, Tokyo, Japan). Student's t-test (two-tailed) was employed for each comparison of NIRS measurements and BDNF levels between the genders, and BDNF Val66Met genotypes. Spearman's correlation coefficients were examined to identify the correlation of the intensity of the Oxy-Hb increase during the tasks with the genders, and with the serum BDNF level. The Hardy-Weinberg equilibrium for genotype frequencies was calculated using chi-square test. A value of $p < 0.05$ was used as the standard for statistical significance in all the analyses.

3. RESULTS

3.1. Scores of HADS and Cognitive Tasks between the Gender

The mean HADS scores and the cognitive task performance of males and females were summarized in **Table 1**. As is shown, none of the valuables was significantly different between the genders..

3.2. Gender Difference in the Prefrontal Activity

Table 2 shows the gender difference in the prefrontal activity. As shown, the mean z scores of Δ[Oxy-Hb] over all channels during the VFT-l were 6.52 ± 4.8 in males and 2.85 ± 2.1 in females. These results were significantly different between the genders ($t = 2.95$, $p < 0.007$). In addition, the mean number of channels with a significant increase in Δ[Oxy-Hb] during the VFT-l was significantly different between the genders, 19.1 ± 8.1 in males and 10.2 ± 7.5 in females ($t = 3.24$, $p = 0.003$).

Table 1. HADS and the cognitive test performances.

	Males	Females	p*
HADS score (mean score ± SD)			
depression	3.0 ± 2.6	3.1 ± 3.0	n.s.
anxiety	5.0 ± 3.6	4.3 ± 2.9	n.s.
Cognitive test performance (mean score ± SD)			
words production in the VFT-l	14.1 ± 4.1	16.3 ± 4.4	n.s.
correct words in the Stroop	73.8 ± 16.8	75.2 ± 11.5	n.s.
incorrect words in the Stroop	2.1 ± 2.0	2.6 ± 1.7	n.s.

*t-test. HADS: Hospital Anxiety and Depression Scale; VFT-l: Letter version of the verbal fluency test; Stroop: Stroop test.

Table 2. Effect of gender on the prefrontal activity.

	Gender (n)		
[Oxy-Hb] during VFT-l	Males (9)	Females (21)	p[3]
z scores over all channels[1]	6.52 ± 4.8	2.85 ± 2.1	0.007
the number of channels, $z > 3.163$[2]	19.1 ± 8.1	10.2 ± 7.5	0.003
Δ[Oxy-Hb] during Stroop			
z scores over all channels[1]	1.73 ± 1.8	1.67 ± 2.2	n.s.
the number of channels, $z > 3.163$[2]	8.75 ± 7.1	9.18 ± 8.0	n.s.

[1]Mean z score ± SD over all channels; [2]Mean number of channels ± SD with significant increase ($z > 3.163$) during the task [3]t-test.

Thus, prefrontal activation during the VFT-l was significantly more intense and broader in males than females.

In contrast, the mean z scores of Δ[Oxy-Hb] over all channels during the Stroop test, 1.73 ± 1.8 in males and 1.67 ± 2.2 in females ($t = 0.092$, $p = 0.93$), were not significantly different between the genders, and the mean number of channels with a significant increase in Δ [Oxy-Hb] during the Stroop test, 8.75 ± 7.1 in males and 9.18 ± 8.0 ($t = -0.17$, $p = 0.87$), was not significantly different between the genders.

3.3. Correlation of Prefrontal Oxy-Hb Increase with HADS Scores

Spearman's correlation coefficients revealed a significant correlation between the depression scores of HADS and the mean z score of Δ[Oxy-Hb] over all channels during both the VFT-l ($\rho = 0.57$, $p = 0.009$) and the Stroop test ($\rho = 0.55$, $p = 0.012$) in males (**Figures 2(a)** and **(b)**). In contrast, in females (**Figures 2(a)** and **(b)**), the depression scores of HADS were not correlated with the mean z score of Δ[Oxy-Hb] over all channels during both the VFT-l ($\rho = -0.29$, $p = 0.247$) and the Stroop test (r =

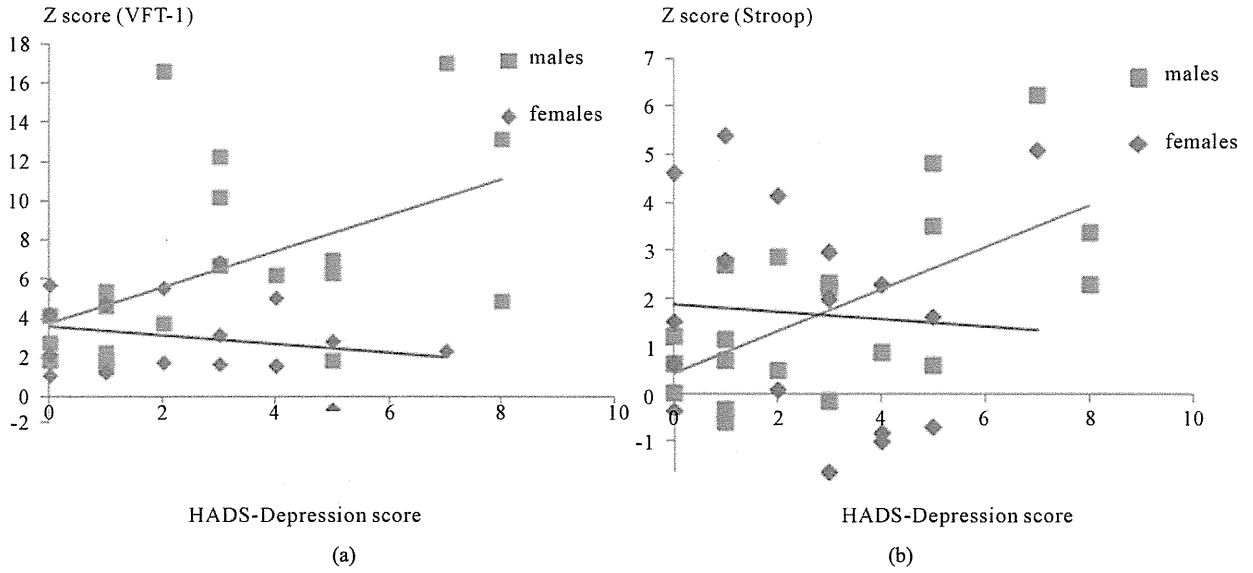

Figure 2. Correlation of the intensity of the prefrontal Oxy-Hb increase with HADS depression scores in both of the genders. (a) A significant correlation between the depression scores of HADS and the mean z score of Δ[Oxy-Hb] over all channels during the VFT-l was observed in the males (ρ = 0.57, p = 0.009) but not in the females (ρ = −0.29, p = 0.247); (b) A significant correlation between the depression scores of HADS and the mean z score of Δ[Oxy-Hb] over all channels during the Stroop test was observed in the males (ρ = 0.55, p = 0.012) but not in the females (ρ = −0.24, p = 0.340).

−0.24, p = 0.340). In both genders, the anxiety scores of HADS were not significantly correlated with the mean z score of Δ[Oxy-Hb] over all channels during both the VFT-l (males, ρ = 0.20, p = 0.402, females, ρ = −0.167, p = 0.507) and the Stroop test (males, ρ = 0.169, p = 0.477, females, ρ = 0.018, p = 0.945).

3.4. Effect of BDNF Val66Met Polymorphism on the Prefrontal Activity

Genotype distribution was in Hardy-Weinberg equilibrium (χ^2 = 1.15, df = 1, p = 0.564). As shown in **Table 2**, 9 subjects were Val/Val type and 21 subjects were Met carriers (17 Val/Met subjects and 4 Met/Met subjects). There was no significant difference between them in the mean z score of Δ[Oxy-Hb] over all channels or in the mean number of channels with a significant increase in Δ[Oxy-Hb] during both of the tasks (**Table 3**).The word "data" is plural, not singular.

3.5. Correlation between the Serum BDNF Level and the Prefrontal Activity

Spearman's correlation coefficients revealed no correlation between the serum BDNF level and the mean z score of Δ[Oxy-Hb] over all channels during both the VFT-l (ρ = −0.101, p = 0.595) and the Stroop test (ρ = −0.194, p = 0.305). In addition, there was no difference in the comparisons of mean serum BDNF level (μg/ml) between the genders (Male = 15.24 ± 3.9, Female = 14.31 ± 3.6, mean ± SD) and between the genotypes (Val/Val = 14.81 ± 3.3, Met carriers = 14.85 ± 4.0, mean ± SD).

4. DISCUSSION

The major findings of this study were as follows. First, the increased Oxy-Hb levels in the prefrontal lobe during the cognitive tasks (VFT-l and Stroop test) were significantly correlated with the severity of depressed mood only in males. Second, the course of the Oxy-Hb increase in the frontal lobe was different depending on the cognitive task, *i.e.* VFT-l or Stroop task, in both genders. Finally, the correlations of the BDNF genotype and serum BDNF level with the Oxy-Hb levels in the prefrontal lobe during cognitive tasks were negative. To the best of

Table 3. Effect of BDNF Val66Met polymorphism on the pre frontal activity.

	Genotype (n)		
[Oxy-Hb] during FT-l	Val/Val (9)	Met carrier (21)	p[3)]
z scores over all channels[1)]	4.86 ± 3.3	5.00 ± 4.7	n.s
the number of channels, z > 3.163[2)]	15.7 ± 9.0	15.0 ± 9.3	n.s.
[Oxy-Hb] during Stroop			
z scores over all channels[1)]	1.98 ± 1.8	1.41 ± 2.2	n.s.
the number of channels, z > 3.163[2)]	10.3 ± 6.7	7.81 ± 7.9	n.s.

[1)]Mean z score ± SD over all channels; [2)]Mean number of channels ± SD with significant increase (z > 3.163) during the task [3)]t-test.

our knowledge, this is the first study to report the correlation of mood, BDNF genotype and BDNF level with the prefrontal activity as measured by NIRS in healthy individuals.

4.1. Correlation of the Increased Prefrontal Oxy-Hb Levels with Depressed Mood in Males

Even a healthy person sometimes falls into a temporary mood of depression or anxiety due to the stress or events of daily life, and if such a mood is long-lasting it can lead to disability. **Figure 2** shows that the depression scores of HADS were significantly correlated with the mean z-scores of Oxy-Hb level during the VFT-l (ρ = 0.57, p = 0.009) and Stroop (ρ = 0.55, p = 0.012) tasks in males. The results suggest that, in males, the heavier the depressed mood, the greater the blood oxygen level required in the prefrontal lobe for the completion of certain cognitive tasks. These were rather unexpected results considering the results of the previous NIRS studies on major depression, in which the increase of the Oxy-Hb levels of the prefrontal lobe during the VFT-l were reduced [8-10]. The major difference between our study and those previous studies was that the subjects of our study were basically healthy individuals and did not meet any criteria of DSM-IV. In healthy individuals, intra-subject reproducibility of the Oxy-Hb increase during the VFT-l over time as measured by NIRS has been reported [31,32]. However, the mental statuses of the healthy individuals in those studies were "within the healthy range", thus, it has not been clarified whether a temporary depressed or anxious mood in healthy individuals would affect the prefrontal Oxy-Hb increase during cognitive tasks. Two possibilities should be considered to explain the inconsistency. First, baseline blood perfusion does not change along with a temporarily depressed mood, but the prefrontal lobe of men with depressed mood does not work efficiently during cognitive tasks, and that leads to the high Oxy-Hb levels as seen in **Figure 2**. It is well known that cortical activation is affected by the complexity and difficulty of the experimental task, as difficult tasks demand more cortical activation than easier tasks [33,34]. On the other hand, the scores of the VFT-l and the Stroop task were not correlated with the HADS scores in either gender (data not shown). Taken together, these findings suggest that even in a depressed mood, males can do the tasks at the expected level by supplying the required brain area with a high Oxy-Hb level. However, when they fall into a serious depressive disorder that meets clinical criteria such as DSM-IV (American Psychiatric Association, 1994), they might no longer be able to supply sufficient Oxy-Hb to the required brain area, and this might cause their performance level to drop. Second, depressed mood might cause the baseline Oxy-Hb levels to decrease in males, but the Oxy-Hb level might rise substantially higher than in males in a normal mood status when cognitive tasks are undertaken. It is important to note that reduced global cerebral blood flow (CBF) or metabolism has been reported in major depression, but the regional reduction and its correlation with mood remain to be determined [35-37]. Currently, we cannot strictly differentiate those two possibilities since the changes in Oxy- or Deoxy-Hb level measured by NIRS are not absolute but relative changes.

4.2. Gender Difference in the Increase of Prefrontal Oxy-Hb Level during the Tasks

There was marked gender difference in the Oxy-Hb level change during the VFT-l. The mean relative increase of the Oxy-Hb level and the number of probes showing a highly increased Oxy-Hb level ($z > 3.163$) were both significantly greater in males than in females (**Table 2**). To date, a gender difference in prefrontal Oxy-Hb change during the VFT-l in healthy subjects has been studied in two studies. In one study, female subjects showed less increased Oxy-Hb levels during the VFT-l than male subjects [38], and in another they showed no change in Oxy-Hb levels compared to males during the VFT-l [39]. One of the possible reasons for this difference is the difference in experimental design. As indicated by Herrmann *et al.* [39], they used a passive resting baseline condition before and after the task, whereas Kameyama *et al.* [38] used an active baseline condition in which the subjects repeated a train of syllables. The procedure of the VFT-l in our study was almost the same as that in Kameyama *et al.* [38], and our results seemed to confirm their results. In contrast, this gender difference was not found during the Stroop test. As in our results, no clear gender difference was observed in the previous NIRS studies involving the Stroop test in healthy individuals [40-42]. One possibility is that, during the Stroop test, the medial part of the brain region such as the anterior cingulate cortex might be more involved than the dorsolateral prefrontal region [43,44]. Also, Kameyama *et al.* [38] discussed the gender difference by citing previous reports in which resting global CBF was observed to be higher in female subjects than in male subjects [45,46], thus, increased baseline perfusion could result in decreased activation due to the ceiling effect [38]. One of the limitations of our study was that we did not exchange the order of the two tasks (the VFT-l was always first), and so we cannot exclude the possibility that the order effect influenced our results. If a transient increase of prefrontal perfusion during the VFT-l pushed up the baseline perfusion in males, it is possible that the following increase of Oxy-Hb level during the Stroop test

