BRAIN RESEARCH 1252 (2009) 152–160

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

# Subjective feeling of psychological fatigue is related to decreased reactivity in ventrolateral prefrontal cortex

*Masashi Suda*[a], *Masato Fukuda*[a,*], *Toshimasa Sato*[a], *Shinya Iwata*[b], *Mingqiao Song*[a], *Masaki Kameyama*[a], *Masahiko Mikuni*[a]

[a]*Department of Psychiatry and Neuroscience, Gunma University Graduate School of Medicine, 3-39-22 Showa, Maebashi, Gunma 371-8511, Japan*

[b]*Gunma University Faculty of Medicine, 3-39-22 Showa, Maebashi, Gunma 371-8511, Japan*

ARTICLE INFO

*Article history:*
Accepted 18 November 2008
Available online 6 December 2008

*Keywords:*
Near-infrared spectroscopy (NIRS)
Subjective fatigue
Frontal function
Sleeping hour
Verbal fluency task

ABSTRACT

The purpose of this study is to examine the relationship between subjective fatigue and brain function. Twenty-three healthy young volunteers participated in this study. Relationships were investigated between subjective fatigue assessed using visual-analogue scale (VAS) score and sleep duration, and cerebral cortex reactivity during a verbal fluency task by 52-channel near-infrared spectroscopy (NIRS). The VAS score negatively correlated with oxygenated hemoglobin concentration ([oxy-Hb]) increases in the bilateral channels over the regions from the ventrolateral part of the frontal lobe to the upper part of the temporal lobe during the verbal fluency task. Sleep duration in the previous night positively correlated with [oxy-Hb] increases in the bilateral channels over the dorsolateral prefrontal lobe also during the verbal fluency task. No significant correlations between the VAS score and sleep duration in the previous night with [oxy-Hb] increases were found during a control task, the left-finger-tapping task. The subjective feeling of psychological fatigue is related to decreased reactivities in the lateral frontal and superior temporal cortices and is unrelated to sleep duration in the previous night, which is reflected in the reactivity in the dorsolateral prefrontal cortices. These results suggest that transient hypofunction and persistent dysfunction in the lateral prefrontal and temporal lobes are among the brain substrates of fatigue. These also demonstrate the advantage of NIRS for investigating brain function during subjective phenomena such as fatigue because it enables examination in a natural setting.



## 1. Introduction

Fatigue is a feeling frequently experienced in daily life by both healthy subjects and patients with various medical and psychiatric conditions, and is classified into physiological and psychological fatigue. It is generally considered as one of the most important feelings in daily life because it is closely related to the quality of life. However, fatigue is usually assessed only subjectively and no gold standard for its objective measurement has been established owing to its very complex nature and difficulty in providing a reliable definition.

* *Corresponding author.* Fax: +81 27 220 8187.
*E-mail address:* fkdpsy@med.gunma-u.ac.jp (M. Fukuda).

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.11.077

In the search for biological indices of fatigue, brain function correlates of fatigue have been studied using, for example, event-related potentials (Lorist et al., 2005; Boksem et al., 2006). Recently, more direct studies of brain substrates of fatigue have been carried out using functional neuroimaging, particularly for medical conditions such as chronic fatigue syndrome (CFS) (de Lange et al., 2004; Tanaka et al., 2006; Cook et al., 2007), multiple sclerosis (Filippi et al., 2002; Roelcke et al., 1997), and Parkinson disease (Abe et al., 2000). Most of these studies demonstrated decreased brain activities in patients with fatigue. Fatigue in healthy subjects is mainly studied with such subjects as the control group in the medical setting, and thus, studies focusing on fatigue in healthy subjects going about their daily lives are lacking. One exception is a study in which fatigue was induced by tryptophan ingestion in healthy subjects, which demonstrated smaller activation in the inferior frontal and the lateral orbital gyri during a Stroop task, using functional magnetic resonance imaging (fMRI) (Morgan et al., 2007).

One of the serious problems in functional neuroimaging studies of fatigue is their examination settings: fMRI data have to be obtained from subjects in the supine position in a very noisy room. Brain function examined under such an unusual environment does not necessarily reflect daily life situations. Near-infrared spectroscopy (NIRS) is one of the functional neuroimaging techniques that can enable the detection of regional cerebral blood volume (CBV) by measuring oxygenated ([oxy-Hb]) and deoxygenated

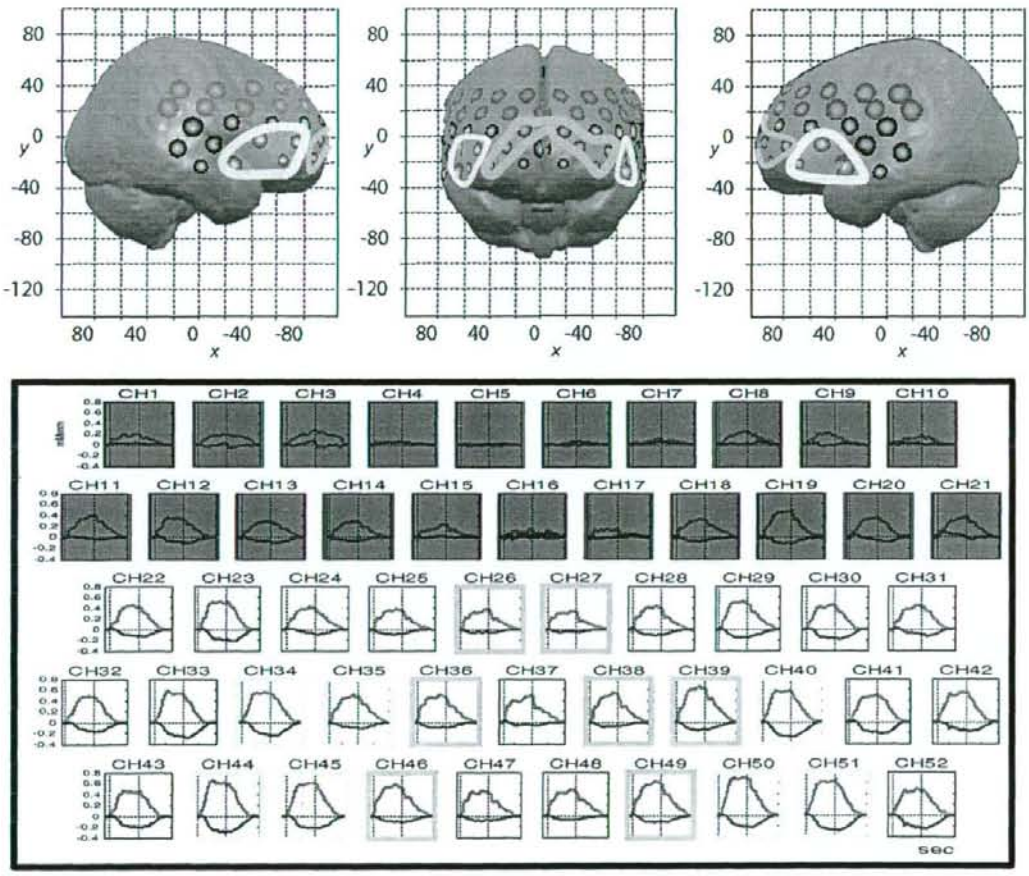


**Fig. 1 – Grand averaged waveforms of oxygenated and deoxygenated hemoglobin concentration ([oxy-Hb] (red line) and [deoxy-Hb] (blue line), respectively) changes during a 60-s verbal fluency task (between two dotted vertical lines) measured using a 52-channel NIRS machine over frontal and temporal regions. Channels excluded from the analyses because of motion artifacts are shaded in gray. Yellow and green frames indicate channels showing significant partial correlations with VAS score and sleep duration in multiple regression analyses, respectively. The location of NIRS channels was probabilistically estimated and anatomically labeled in standard brain space (Okamoto et al., 2004).**

hemoglobin concentration ([deoxy-Hb]) changes using near-infrared light (for reviews, see Hock et al., 1997; Strangman et al., 2002; Fallgatter et al., 2004; Boas et al., 2004). NIRS has higher time resolution such as 0.1 s than fMRI, and more direct correspondence to brain structures beneath than topographic EEG, and has been used to examine, for example, the time course of brain activation (Sato et al., 2007; Hanaoka et al., 2007), language dominance (Watanabe et al., 1998), brain substrates of personality (Ito et al., 2005) and sleepiness (Suda et al., 2008) in healthy subjects, brain activation in awake infants (Taga et al., 2003), and pathophysiology of psychiatric disorders (for example, see Suto et al., 2004; Kameyama et al., 2006; Uehara et al., 2007). In addition to its complete noninvasiveness and the portability of the apparatus, NIRS has another advantage for studies of fatigue, that is, its examination setting: NIRS can measure the brain activity of subjects in the sitting position in a quiet room. This advantage extends its applicability to normal children, healthy volunteers while writing and speaking, and psychiatric patients.

In this study, we examined the relationship between the subjective feeling of psychological fatigue and brain activation in healthy subjects going about their daily lives. The subjective feeling of psychological fatigue was assessed using the visual analogue scale (VAS) score, and brain activation was measured using NIRS during cognitive activation. In addition to subjective fatigue, sleepiness was included in the clinical assessment using sleep duration because fatigue and sleepiness are distinct but interrelated phenomena and are difficult to differentiate (Dawson and McCulloch, 2004; Shen et al., 2006). Brain activation was also measured during motor activation as the control condition for contrasts with cognitive activation. We hypothesized that the fatigue-related decrease in brain activity observed in medical conditions associated with fatigue is also observed in healthy subjects going about their daily lives.

## 2. Results

### 2.1. *Behavioral data*

Behavioral data were as follows: VAS score (mean, 37.2; SD, 17.4; range, 5–70), sleep duration in the previous night (mean, 6.1; SD, 1.8; range, 1–10), average sleep duration in usual nights (mean, 6.4; SD, 1.4; range, 4–10), and task performance score (mean, 17.0; SD, 4.8; range, 9–26). Sleep duration in the previous night was subjected to further correlational analyses because there was no significant difference between sleep duration in the previous night and average sleep duration in usual nights ($t=-0.722$, $P=0.474$). There were also no significant correlations among VAS score, sleep duration in the previous night, and task performance.

### 2.2. *NIRS data during verbal fluency task (Fig. 1)*

#### 2.2.1. *[oxy-Hb] data*

The two-way repeated-measures ANOVA revealed a significant main effect of "task" in all the 31 channels ($F=5.6$ to 60.7, $P<0.02$) and "segment" in 28 channels (ch 22–25, 28–36, 38–52; $F=6.5$ to 21.3, $P<0.002$): [oxy-Hb] increases were significantly larger during the VFT than during the finger tapping task, and during the task segment than the pretask and the posttask segments. Post hoc ANOVA demonstrated that the [oxy-Hb] increases during the verbal fluency task were significant in all the 31 channels ($F=9.48$ to 40.55, $P<0.0015$). The two-way interactions of "task" and "segment" were significant in 25 channels (ch 22, 24–29, 32–40, 42, 44–51; $F=3.4$ to 17.6,

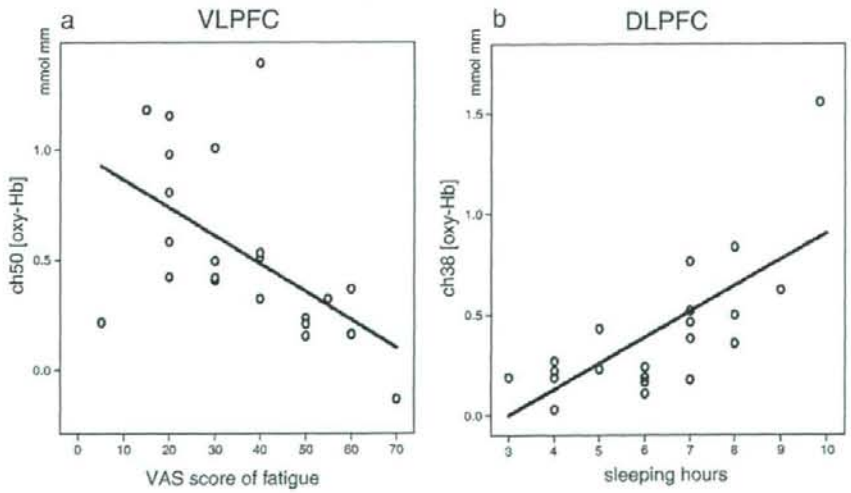


**Fig. 2 – (a) Correlation between [oxy-Hb] changes in ch 50 (VLPFC) during the verbal fluency task and VAS score of subjective feeling of psychological fatigue. (b) Correlation between [oxy-Hb] changes in ch 38 (DLPFC) during verbal fluency task and sleep duration in the previous night.**

$P<0.036$), indicating that the [oxy-Hb] increases during the task segment relative to those during the pretask and the posttask segments were more pronounced during the VFT than during the finger tapping task.

In the multiple regression analysis, the [oxy-Hb] increases during the verbal fluency task correlated with the VAS score in seven channels (ch 34–35, 40, 44–45, 50–51, yellow channels in Fig. 1; beta = −0.669 to −0.419, $P=0.002$ to 0.038; Fig. 2), with sleep duration in seven channels (ch 26–27, 36, 38–39, 46, 49, green channels in Fig. 1; beta = 0.676 to 0.435, $P=0.001$ to 0.026; Fig. 3), and with age in four channels (ch 29, 34, 40, 44; beta = −0.484 to −0.397, $P=0.019$ to 0.04). Task performance and sex did not correlate with [oxy-Hb] increases in any channel. The channels showing significant correlation with fatigue were found mainly in the ventral region and those with sleep duration in the dorsal region.

By dividing the 24 NIRS channels into four clusters of neighboring six channels, namely, right VLPFC (ch 23, 24, 33, 34, 44, and 45), left VLPFC (ch 29, 30, 40, 41, 50, and 51), right DLPFC (ch 25, 26, 35, 36, 46, and 47), and left DLPFC (ch 27, 28, 38, 39, 48, and 49) and averaging [oxy-Hb] changes in each cluster with six channels, significant correlations were obtained in the right VLPFC ($r=-0.551$, $P=0.006$) and left VLPFC ($r=-0.527$, $P=0.001$) for VAS scores and right DLPFC ($r=0.638$, $P=0.001$) and left DLPFC ($r=0.730$, $P<0.001$) for sleep duration.

The correlations between [oxy-Hb] changes during the verbal fluency task and VAS scores and sleeping hours at every 0.1 s point are shown in Fig. 3. [oxy-Hb] changes correlated with both the VAS scores and sleep duration in many channels when examined along the time courses of the task: significant correlations as high as 1% were obtained during the later part of the task segment in the channels corresponding to VLPFC for the VAS scores and during the earlier part of the task segment in the channels corresponding to DLPFC for sleep duration.

### 2.2.2. [deoxy-Hb] data

The two-way repeated-measures ANOVA revealed a significant main effect of "task" in 16 channels (ch 23, 25–29, 33, 39, 44, 46–52; $F=4.6$ to 31.6, $P<0.018$), and "segment" in 27 channels (ch 22–25, 28–35, 38–52; $F=3.4$ to 19.9, $P<0.039$): [deoxy-Hb] decreases were significantly larger during the VFT than during the finger tapping task, and during the task segment than the pretask and the posttask segments. Post hoc ANOVA showed that the [deoxy-Hb] decreases during the verbal fluency task were significant in 22 channels (ch 23, 25–27, 29–30, 33–36, 38–41, 44–51; $F=5.646$ to 53.024, $P<0.036$). The


**Fig. 3 – Pearson's r value graphs showing correlation at the point of 0.1 s between [oxy-Hb] changes during a 60-s verbal fluency task (between two dotted vertical lines) and VAS scores of subjective feeling of psychological fatigue (upper part) or sleep duration in the previous night (lower part) in the available 31 channels as presented in Fig. 1. Blue and red lines in each r graph correspond to 5% and 1% statistical significance levels, respectively.**

two-way interactions of "task" and "segment" were significant in ch 47 ($F=7.8$, $P=0.001$), indicating that the [deoxy-Hb] decreases during the task segment relative to those during the pretask and the posttask segments were more pronounced during the VFT than during the finger tapping task.

In the multiple regression analyses, there were no significant correlations except a negative correlation between [deoxy-Hb] decreases and sleep duration in the previous night in ch 47 (beta=−0.695, $P=0.0002$).

### 2.3. *NIRS data during finger tapping task*

[oxy-Hb] changes during the finger tapping task were significant in four channels (Ch 33, 44–46; $F=7.37–11.11$, $P<0.003$) and no channel exhibited significant [deoxy-Hb] changes (Fig. 4). The correlations between [oxy-Hb] changes during the finger tapping task and VAS scores and sleeping hours at every 0.1 s point are shown in Fig. 5. There were no correlations between [oxy-Hb] changes and VAS score or sleep duration in the previous night.

## 3. Discussion

In this study, we examined the correlations of subjective fatigue assessed in terms of the VAS score and sleep duration in the previous night with cerebral cortex reactivity in young healthy volunteers during a verbal fluency task and a left-finger-tapping task using a multichannel NIRS machine. The results obtained demonstrated that (1) subjective fatigue negatively correlated with [oxy-Hb] increases in the channels over the ventrolateral prefrontal cortex in both hemispheres during cognitive task, (2) sleep duration in the previous night positively correlated with [oxy-Hb] increases in the channels over the dorsolateral prefrontal cortex in both hemispheres also during cognitive task, and (3) no such significant correlations of VAS score and sleep duration in the previous night with [deoxy-Hb] decreases during the cognitive task or with either [oxy-Hb] or [deoxy-Hb] changes during the simple motor task were found. These findings suggest that the subjective feeling of psychological fatigue, i.e., *the subjectively perceived aspect of psychological fatigue*, is related to decreased reactivity in the ventrolateral prefrontal cortex and is unrelated to sleep duration in the previous night, which is reflected in the reactivity in the dorsolateral prefrontal cortex.

To the best of our knowledge, this is the first study in which the subjective feeling of psychological fatigue was examined using NIRS. The subjective fatigue related to decrease in ventrolateral prefrontal cortex reactivity in healthy subjects is in good agreement with a previous fMRI study that demonstrated smaller activations in the inferior frontal and lateral orbital gyri during a Stroop task in healthy subjects who ingested tryptophan, which induces fatigue (Morgan et al., 2007). Fatigue-related abnormalities in the frontal lobe have also been observed in patients with some medical conditions such as chronic fatigue syndrome (CFS) (de Lange et al., 2004, Tanaka et al., 2006, Okada et al., 2004, Kuratsune et al., 2002, Cook et al., 2007), multiple sclerosis (Filippi et al., 2002, Roelcke et al., 1997), and Parkinson disease (Abe et al., 2000).

NIRS methodology is assumed to be advantageous for obtaining such a finding because it enabled the measurement of brain activation in a natural setting, that is, in subjects in the sitting position in a quiet room, instead of in the supine

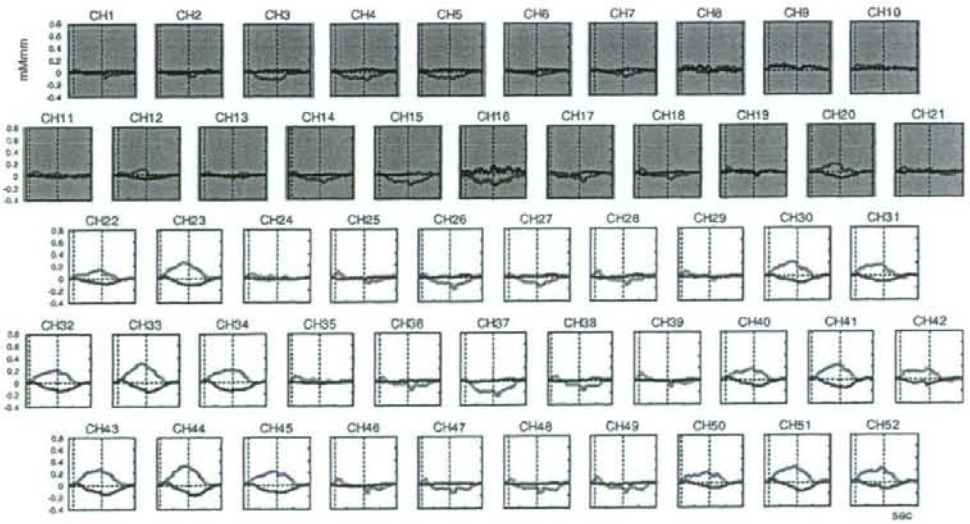


**Fig. 4 – Grand averaged waveforms of [oxy-Hb] (red line) and [deoxy-Hb] (blue line) changes during a 60-s left-finger-tapping task (between two dotted vertical lines) measured using a 52-channel NIRS machine over frontal and temporal regions as shown in Fig. 1.**

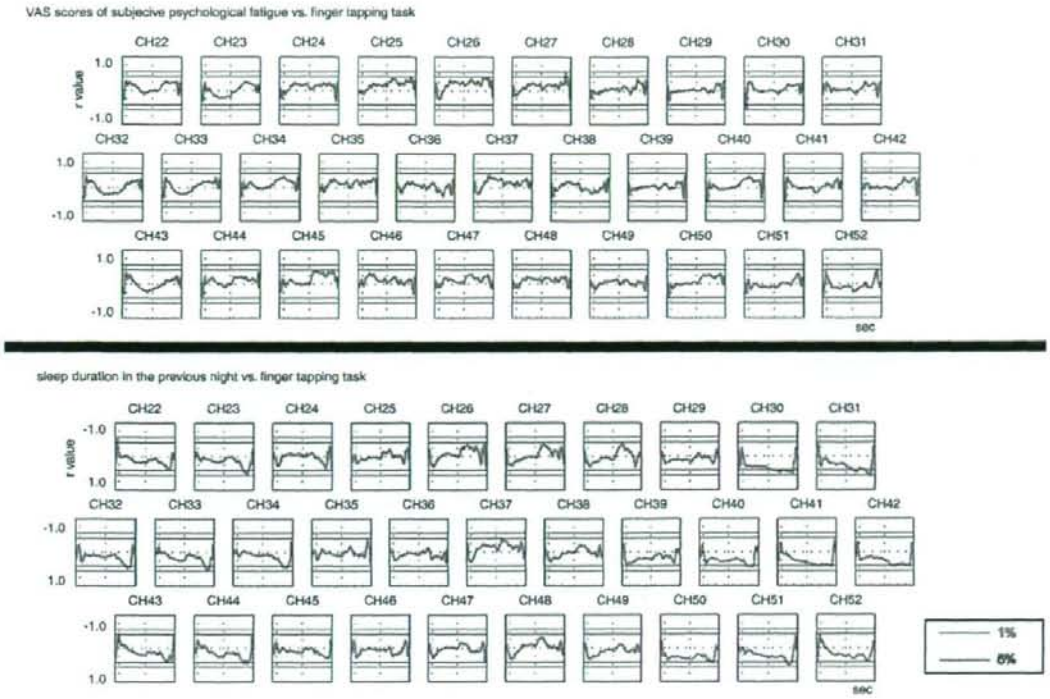


**Fig. 5 – Pearson's r value graphs showing correlation at the point of 0.1 s between [oxy-Hb] changes during a 60-s left finger tapping task (between two dotted vertical lines) and VAS scores of subjective feeling of psychological fatigue (upper part) or sleep duration in the previous night (lower part) in the available 31 channels as presented in Fig. 1. Blue and red lines in each r graph correspond to 5% and 1% statistical significance levels, respectively.**

position in a noisy gantry as in the case of fMRI studies. The obtained results cannot be attributed to task-nonspecific physiological factors possibly related to fatigue such as reduced neurovascular coupling due to altered autonomic function in fatigue, but are considered to reflect the function of the ventrolateral prefrontal cortex because significant correlations between NIRS data and the VAS score of subjective feeling of psychological fatigue were obtained only in the VFT task but not in the finger tapping task.

Measurements that enable the differentiation of subjective feeling of fatigue and sleepiness are often difficult, as mentioned in the Introduction. To differentiate them, we also examined the relationships of NIRS data with sleep duration in the previous night and the average in usual nights, which we used as alternative indices of the subjective feeling of sleepiness. A clear difference in their correlations suggests that the obtained findings are related not to sleepiness but to fatigue: the significant correlation of the NIRS data with the VAS score of the subjective feeling of psychological fatigue was negative in the bilateral channels over the ventrolateral prefrontal cortex, and that with the sleep duration was positive in the bilateral channels over the dorsolateral prefrontal cortex.

The latter finding is consistent with the studies showing decreased cerebral blood flow in sleepiness during arithmetic (Drummond et al., 1999) and verbal working memory tasks (Mu et al., 2005a,b) and reduced cerebral blood volume increases in the medial prefrontal cortex during a verbal fluency task (Suda et al., 2008), but is inconsistent with the studies showing increased cerebral blood flow in sleepiness using verbal learning (Drummond et al., 2000), short-term attention (Portas et al., 1998), divided attention (Drummond et al., 2001), and grammatical reasoning tasks after total sleep deprivation (Drummond et al., 2004), and is partly consistent with the studies showing mixed results during verbal (Chee and Choo, 2004; Habeck et al., 2004) and nonverbal (Bell McGinty et al., 2004) item recognition tasks. An increased brain activity in sleepiness is interpreted as reflecting compensatory efforts to overcome performance deficits after sleep deprivation (Drummond et al., 2005).

There are several methodological limitations of this study. First, fatigue was assessed only using the VAS score of the subjective feeling; no established assessment scale of fatigue was employed. Second, sleepiness was also assessed using only sleep duration without employing any assessment scale of subjective sleepiness or an objective method such as

electroencephalography. Third, the subjects included were going about their usual daily lives at the time of examination and their fatigue or sleepiness was not controlled. Diurnal physiological fluctuations might have had an effect on the results of this study. In a future study, the subjects should be controlled in terms of their condition or might be grouped according to the causes of fatigue and analyzed separately for each cause. Fourth, only the VFT task was employed for cognitive activation. The relationship between psychological fatigue and activations over the ventrolateral prefrontal lobe should be replicated using other cognitive tasks such as working memory and executive function tasks. Moreover, repeated-measure design would be preferable. To repeat the task such as stressful calculation to induce the psychological fatigue would make it possible to evaluate both objective and subjective fatigue. Fifth, activation was examined only in the frontal lobe but not in other cerebral cortices or deep brain structures. Although focusing on cortical function using NIRS favors the studies on *the subjectively perceived* aspect of psychological fatigue, future study is needed to assess the participation of deep brain structures such as the brainstem, thalamus, and limbic region in provoking the observed cortical change. Sixth, there was a statistical problem. We did not apply the false-positive correction in multiple regression analyses, although we applied it in two-way ANOVA and post hoc analyses. The main reason for this is that false-positive correction was already performed in the first step, that is, two-way ANOVA and post hoc analyses on a channel-by-channel basis. However, because the possible type I error cannot be completely excluded, further study with a large number of subjects is required to exclude.

In conclusion, the subjective feeling of psychological fatigue was reflected in the decreased reactivities of the ventrolateral prefrontal cortex during cognitive activation in healthy subjects going about their daily lives. On the basis of the findings, we assume that transient hypofunction in the ventrolateral prefrontal cortex is the brain substrate of psychological fatigue in healthy subjects, and a more persistent dysfunction of the lobes is observed under pathological conditions associated with fatigue. Further functional neuroimaging studies specifically focusing on fatigue are required because elucidating the brain mechanism underlying psychological fatigue is beneficial for promoting the quality of life of healthy subjects and patients suffering from fatigue. Among several functional neuroimaging techniques currently employed, NIRS is advantageous for studies of fatigue and sleepiness because it enables the measurement of brain activation in a natural setting.

## 4. Experimental procedures

### 4.1. Subjects

Twenty-three young healthy volunteers participated in this study (11 males and 12 females; average age 22.6 years, SD 2.8, range 19–27). All the subjects were determined to be right-handed using the Edinburgh Handedness Inventory scale (Oldfield, 1971). They had no history of any major psychiatric disorder, neurological disorder, substance abuse, head injury, or major physical illness, and they were not on any psychotropic medications at the time of the study. This study was approved by the Institutional Review Board of the Gunma University Graduate School of Medicine. Written informed consent was obtained from all the subjects prior to the study.

### 4.2. *Assessment of subjective feeling of psychological fatigue*

The subjective feeling of psychological fatigue was assessed using the VAS. The participants were instructed to evaluate their subjective fatigue at the time of the examination in terms of the VAS from 0 (no fatigue) to 100 (total exhaustion) immediately before the NIRS measurement. VAS was selected for subjective fatigue assessment because it has been shown to provide valid measures of subjective feelings such as pain (McCormack et al., 1988, Price et al., 1983) and has actually been employed in the studies of subjective fatigue (Schneider et al., 2004, Tanaka et al., 2006, Cook et al., 2007). Sleep duration in the previous night and the average in a usual night were also obtained.

### 4.3. *NIRS measurements*

#### 4.3.1. *Activation tasks*

Cognitive and motor activation tasks were employed for NIRS measurements. The cognitive activation task was a modified letter version of the verbal fluency task. Each subject sat on a comfortable chair in a daylight room with his or her eyes open throughout each measurement. The cognitive activation task consisted of a 30-s pretask baseline, a 60-s verbal fluency task, and a 70-s posttask baseline. During the verbal fluency task, the subjects were instructed to verbally generate as many words whose initial syllable was either /a/, /ki/, or /ha/ as they could. These three initial syllables were employed in the above-mentioned order and changed every 20 s during the 60-s task to decrease the time during which the subjects remained silent. The number of words generated during the verbal fluency task was determined as a measure of task performance. During the pretask and posttask baseline periods, the subjects were instructed to repeat the syllables /a/, /i/, /u/, /e/, and /o/ as the Japanese counterparts of A, B and C in English.

The motor activation task was employed as a control task unrelated to cognitive activation and consisted of a 30-s pretask rest, a 60-s left-finger-tapping task, and a 70-s posttask rest. The left-finger-tapping task was selected because skilled movement of the dominant hand, i.e., right-finger tapping, may result in exceptionally lower or higher brain activity than other unskilled movements. The subjects were instructed to tap their four fingers with their thumb alternately as quickly and accurately as they could. They practiced the left-finger tapping after receiving the instructions for the task, and it was confirmed that they were able to perform the task correctly.

#### 4.3.2. *NIRS machine*

In this study, changes in [oxy-Hb] and [deoxy-Hb] were measured using a 52-channel NIRS machine (Hitachi ETG-4000) at two wavelengths of near-infrared light (i.e., 780 and

830 nm) whose absorption was measured, and [oxy-Hb] and [deoxy-Hb] were calculated. The distance between the pair of emission and detector probes was 3.0 cm, and it was considered that the machine measured points at a depth of 2–3 cm from the scalp, that is, the surface of the cerebral cortex (Hock et al., 1997, Toronov et al., 2001).

The probes of the NIRS machine were placed on the subject's frontal region. The frontal probes measured [Hb] changes at 52 measurement points in a 6×30 cm area, with the lowest probes positioned along the Fp1–Fp2 line in accordance with the international 10/20 system used in electroencephalography. The measurement points were labeled Channel 1 to Channel 52 from top to bottom. The correspondence between the NIRS channels and the measurement points on the cerebral cortex was confirmed by a multisubject study of anatomical craniocerebral correlation (Okamoto et al., 2004) and was displayed on the basis of the results of the virtual registration method (Tsuzuki et al., 2007).

The absorption of near-infrared light was measured with a time resolution of 0.1 s. The obtained data were analyzed using the "integral mode". The pretask baseline was determined as the mean across the last 10 s of the 30-s pretask period, the posttask baseline was determined as the mean across the last 10 s of the 70-s posttask period as described previously (Suto et al., 2004, Kameyama et al., 2006), and linear fitting was applied to the data between these two baselines. The moving average method was used to exclude short-term motion artifacts in the analyzed data (moving average window: 5 s).

### 4.4. *Data analyses*

The waveforms of [oxy-Hb] and [deoxy-Hb] changes in all the 52 channels were acquired from all the subjects during the cognitive and motor activation tasks. NIRS data of channels that clearly contained motion artifacts, as determined by close observation of the subjects, namely, channels 1–21, were excluded from further analyses. Absence of clear artifacts in the remaining NIRS channels was confirmed by close observation of experimental situations and analysis of the data obtained by two researchers.

[oxy-Hb] and [deoxy-Hb] data during the cognitive and motor activation tasks were analyzed in two steps. In the first step, the individually averaged [Hb] waveforms were divided into the following three time segments: the 'pretask' segment for 10 s before the task period, the 'task segment' for 60 s during the task period, and the 'posttask' segment for 60 s after the task period. [Hb] data were averaged across three task segments (pretask, task, and posttask) and were analyzed using two-way repeated-measures analysis of variance (ANOVA) with "task" (VFT and finger tapping) and "segment" (pretask, task, and posttask) as independent variables, followed by post hoc one-way ANOVA comparison for the significant factor with significance levels corrected by the false discovery rate method (Singh and Dan, 2006).

In the second step, for the channels with significant [Hb] changes, as determined by a significant main effect of "segment" in each channel in the first step, the relationships of the [Hb] changes with subjective fatigue and sleep duration were analyzed using multiple regression analyses with the averages of [Hb] during the task period as dependent variables and the VAS score, sleep duration, task performance score, sex, and age of the subjects as independent variables, to exclude the effects of sex, age, and performance score in this task. The [deoxy-Hb] data were also analyzed in the same manner as the [oxy-Hb] data.

In addition, we performed two types of analyses. First, the relationships between subjective fatigue or sleep duration and response latencies in the time domain or phase offsets in the frequency domain during verbal fluency task were determined. We calculated Pearson's r value at the point of 0.1 s between [oxy-Hb] changes in each channel and VAS scores or sleep duration during the verbal fluency task and the finger tapping task (shown in Figs. 3 and 5, respectively). Second, the localization of the channel that correlated with VAS and sleep duration was carried out. We divided the NIRS channels into four clusters corresponding to brain regions, namely, right VLPFC (ch 23, 24, 33, 34, 44, and 45), left VLPFC (ch 29, 30, 40, 41, 50, and 51), right DLPFC (ch 25, 26, 35, 36, 46, and 47), and left DLPFC (ch 27, 28, 38, 39, 48, and 49). The averaged [oxy-Hb] changes in each cluster were calculated as the average of the six channels, [oxy-Hb] changes during the verbal fluency task segment. Then, simple correlation was conducted using the averaged [oxy-Hb] changes of each cluster and VAS scores or sleep duration, with significance levels corrected by the false discovery rate method (Singh and Dan, 2006).

## REFERENCES


Abe, K., Takanashi, M., Yanagihara, T., 2000. Fatigue in patients with Parkinson's disease. Behav. Neurol. 12, 103–106.

Bell McGinty, S., Habeck, C., Hilton, H.J., Rakitin, B., Scarmeas, N., Zarahn, E., 2004. Identification and differential vulnerability of a neural network in sleep deprivation. Cereb. Cortex 14, 496–502.

Boas, D.A., Dale, A.M., Franceschini, M.A., 2004. Diffuse optical imaging of brain activation: approaches to optimizing image sensitivity, resolution, and accuracy. Neuroimage 23 (Suppl. 1), S275–S288.

Boksem, M.A., Meijman, T.F., Lorist, M.M., 2006. Mental fatigue, motivation and action monitoring. Biol. Psychol. 72, 123–132.

Chee, M.W., Choo, W.C., 2004. Functional imaging of working memory after 24 hr of total sleep deprivation. J. Neurosci. 24, 4560–4567.

Cook, D.B., O'Connor, P.J., Lange, G., Steffener, J., 2007. Functional neuroimaging correlates of mental fatigue induced by cognition among chronic fatigue syndrome patients and controls. Neuroimage 36, 108–122.

Dawson, D., McCulloch, K., 2004. Managing fatigue: it's about sleep. Sleep Med. Rev. 9, 365–380.

de Lange, F.P., Kalkman, J.S., Bleijenberg, G., Hagoort, P., van der Werf, S.P., van der Meer, J.W., 2004. Neural correlates of the chronic fatigue syndrome—an fMRI study. Brain 127, 1948–1957.

Drummond, S.P., Brown, G.G., Stricker, J.L., Buxton, R.B., Wong, E.C., Gillin, J.C., 1999. Sleep deprivation-induced reduction in cortical functional response to serial subtraction. Neuroreport 10, 3745–3748.

Drummond, S.P., Brown, G.G., Gillin, J.C., Stricker, J.L., Wong, E.C., Buxton, R.B., 2000. Altered brain response to verbal learning following sleep deprivation. Nature 403, 655–657.

Drummond, S.P., Gillin, J.C., Brown, G.G., 2001. Increased cerebral response during a divided attention task following sleep deprivation. J. Sleep Res. 10, 85–92.

Drummond, S.P., Brown, G.G., Salamat, J.S., Gillin, J.C., 2004. Increasing task difficulty facilitates the cerebral compensatory response to total sleep deprivation. Sleep 27, 445–451.

Drummond, S.P., Meloy, M.J., Yanagi, M.A., Orff, H.J., Brown, G.G., 2005. Compensatory recruitment after sleep deprivation and the relationship with performance. Psychiatry Res. 140, 211–223.

Fallgatter, A.J., Ehlis, A.Ch., Wagener, A., Michel, T., Herrmann, M.J., 2004. Nah-Infrarot-Spektroskopie in der Psychiatrie. Nervenarzt 75, 911–916.

Filippi, M., Rocca, M.A., Colombo, B., Falini, A., Codella, M., Scotti, G., Comi, G., 2002. Functional magnetic resonance imaging correlates of fatigue in multiple sclerosis. Neuroimage 15, 559–567.

Habeck, C., Rakitin, B.C., Moeller, J., Scarmeas, N., Zarahn, E., Brown, T., 2004. An event-related fMRI study of the neurobehavioral impact of sleep deprivation on performance of a delayed-match-to-sample task. Cogn. Brain Res. 18, 306–321.

Hanaoka, N., Aoyama, Y., Kameyama, M., Fukuda, M., Mikuni, M., 2007. Deactivation and activation of left frontal lobe during and after low-frequency repetitive transcranial magnetic stimulation over right prefrontal cortex: a near-infrared spectroscopy study. Neurosci. Lett. 414, 99–104.

Hock, C., Villringer, K., Muller Spahn, F., Wenzel, R., Heekeren, H., Schuh Hofer, S., 1997. Decrease in parietal cerebral hemoglobin oxygenation during performance of a verbal fluency task in patients with Alzheimer's disease monitored by means of near-infrared spectroscopy (NIRS)-correlation with simultaneous rCBF-PET measurements. Brain Res. 755, 293–303.

Ito, M., Fukuda, M., Suto, T., Uehara, T., Mikuni, M., 2005. Increased and decreased cortical reactivities in novelty seeking and persistence: a multichannel near-infrared spectroscopy study in healthy subjects. Neuropsychobiology 52, 45–54.

Kameyama, M., Fukuda, M., Yamagishi, Y., Sato, T., Uehara, T., Ito, M., Suto, T., Mikuni, M., 2006. Frontal lobe function in bipolar disorder: a multichannel near-infrared spectroscopy study. Neuroimage 29, 172–184.

Kuratsune, H., Yamaguti, K., Lindh, G., Evengard, B., Hagberg, G., Matsumura, K., 2002. Brain regions involved in fatigue sensation: reduced acetylcarnitine uptake into the brain. Neuroimage 17, 1256–1265.

Lorist, M.M., Boksem, M.A., Ridderinkhof, K.R., 2005. Impaired cognitive control and reduced cingulate activity during mental fatigue. Cogn. Brain Res. 24, 199–205.

McCormack, H.M., Horne, D.J., Sheather, S., 1988. Clinical applications of visual analogue scales: a critical review. Psychol. Med. 18, 1007–1019.

Morgan, R.M., Parry, A.M., Arida, R.M., Matthews, P.M., Davies, B., Castell, L.M., 2007. Effects of elevated plasma tryptophan on brain activation associated with the Stroop task. Psychopharmacology 190, 383–389.

Mu, Q., Mishory, A., Johnson, K.A., Nahas, Z., Kozel, F.A., Yamanaka, K., 2005a. Decreased brain activation during a working memory task at rested baseline is associated with vulnerability to sleep deprivation. Sleep 28, 433–446.

Mu, Q., Nahas, Z., Johnson, K.A., Yamanaka, K., Mishory, A., Koola, J., 2005b. Decreased cortical response to verbal working memory following sleep deprivation. Sleep 28, 55–67.

Okada, T., Tanaka, M., Kuratsune, H., Watanabe, Y., Sadato, N., 2004. Mechanisms underlying fatigue: a voxel-based morphometric study of chronic fatigue syndrome. BMC Neurol. 4, 14.

Okamoto, M., Dan, H., Sakamoto, K., Takeo, K., Shimizu, K., Kohno, S., Dan, I., 2004. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. Neuroimage 21, 99–111.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.

Price, D.D., McGrath, P.A., Rafii, A., Buckingham, B., 1983. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 17, 45–56.

Portas, C.M., Rees, G., Howseman, A.M., Josephs, O., Turner, R., Frith, C.D., 1998. A specific role for the thalamus in mediating the interaction of attention and arousal in humans. J. Neurosci. 21, 8979–8989.

Roelcke, U., Kappos, L., Lechner Scott, J., Brunnschweiler, H., Huber, S., Ammann, W., 1997. Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. Neurology 48, 1566–1571.

Sato, T., Ito, M., Suto, T., Kameyama, M., Suda, M., Yamagishi, Y., Fukuda, M., Mikuni, M., 2007. Time courses of brain activation and their implications for function: a multichannel near-infrared spectroscopy study during finger tapping. Neurosci. Res. 58, 297–304.

Schneider, C., Fulda, S., Schulz, H., 2004. Daytime variation in performance and tiredness/sleepiness ratings in patients with insomnia, narcolepsy, sleep apnea and normal controls. J. Sleep Res. 13, 373–383.

Shen, J., Barbera, J., Shapiro, C.M., 2006. Distinguishing sleepiness and fatigue: focus on definition and measurement. Sleep Med. Rev. 10, 63–76.

Singh, A.K., Dan, I., 2006. Exploring the false discovery rate in multichannel NIRS. Neuroimage 33, 542–549.

Strangman, G., Culver, J.P., Thompson, J.H., Boas, D.A., 2002. A quantitative comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation. Neuroimage 17, 719–731.

Suda, M., Sato, T., Kameyama, M., Ito, M., Suto, T., Yamagishi, Y., Fukuda, M., Mikuni, M., 2008. Decreased cortical reactivity underlies subjective daytime light sleepiness in healthy subjects: a multichannel near-infrared spectroscopy study. Neurosci. Res. 60, 319–326.

Suto, T., Fukuda, M., Ito, M., Uehara, T., Mikuni, M., 2004. Multichannel near-infrared spectroscopy in depression and schizophrenia: cognitive brain activation study. Biol. Psychiatry 55, 501–511.

Taga, G., Asakawa, K., Maki, A., Konishi, Y., Koizumi, H., 2003. Brain imaging in awake infants by near-infrared optical topography. Proc. Natl. Acad. Sci. U. S. A. 100, 10722–10727.

Tanaka, M., Sadato, N., Okada, T., Mizuno, K., Sasabe, T., Tanabe, H.C., 2006. Reduced responsiveness is an essential feature of chronic fatigue syndrome: a fMRI study. BMC Neurol. 6, 9.

Toronov, V., Webb, A., Choi, J.H., Wolf, M., Michalos, A., Gratton, E., Hueber, D., 2001. Investigation of human brain hemodynamics by simultaneous near-infrared spectroscopy and functional magnetic resonance imaging. Med. Phys. 28, 521–527.

Tsuzuki, D., Jurcak, V., Singh, A.K., Okamoto, M., Watanabe, E., Dan, I., 2007. Virtual spatial registration of stand-alone fNIRS data to MNI space. Neuroimage 34, 1506–1518.

Uehara, T., Fukuda, M., Suda, M., Ito, M., Suto, T., Kameyama, M., Mikuni, M., 2007. Cerebral blood volume changes in patients with eating disorders during word fluency: a preliminary study using multi-channel near infrared spectroscopy. Eat Weight Disord. 12, 183–190.

Watanabe, E., Maki, A., Kawaguchi, F., Takashiro, K., Yamashita, Y., Koizumi, H., 1998. Non-invasive assessment of language dominance with near-infrared spectroscopic mapping. Neurosci. Lett. 256, 49–52.

Neuroscience Research 63 (2009) 47–51

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

# Stimulus intensity dependence of cerebral blood volume changes in left frontal lobe by low-frequency rTMS to right frontal lobe: A near-infrared spectroscopy study

Yoshiyuki Aoyama [a], Naoki Hanaoka [b], Masaki Kameyama [a], Masashi Suda [a], Toshimasa Sato [a], Mingqiao Song [a], Masato Fukuda [a,*], Masahiko Mikuni [a]

[a] Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi 371-8511, Gunma, Japan
[b] Department of Psychiatry, Sanmaibashi Hospital, 1744 Nagate-machi, Ota 373-0054, Gunma, Japan

ARTICLE INFO

*Article history:*
Received 6 August 2008
Received in revised form 6 October 2008
Accepted 8 October 2008
Available online 15 October 2008

*Keywords:*
rTMS
Near-infrared spectroscopy
Hemoglobin concentration
Depression
Magnetic stimulation
Therapy

ABSTRACT

Repetitive transcranial magnetic stimulation (rTMS) has recently been widely employed for the investigation of brain function and treatment of psychiatric and neurological disorders. Although high and low stimulation frequencies are assumed to activate and deactivate brain function, respectively, the optimal parameters of rTMS for treatment of depression have been determined only on the basis of their clinical efficacy. In this study, we administered a 60-s low-frequency rTMS of three grades low intensities over the right dorsolateral prefrontal cortex (DLPFC) in 10 healthy volunteers, and monitored functional changes of the contralateral DLPFC by near-infrared spectroscopy (NIRS) during and immediately after rTMS. Obtained results demonstrated significant [oxy-Hb] decreases during rTMS, and significant differences in the time courses of [oxy-Hb] changes among three stimulus intensities, that is, [oxy-Hb] decreases were most prominent during the latter half of the stimulation and the first 30 s of poststimulation only at 15 mm condition (58% intensity). These results suggest that monitoring of brain functional changes due to rTMS using NIRS is useful for elucidating the brain mechanisms underlying the clinical effects of rTMS, and the effects of rTMS over contralateral DLPFC are obtained if the stimulus intensities are more than one-half of the motor thresholds.



## 1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is one of the brain stimulation techniques using electromagnetic induction, and minimal invasiveness is one of its most beneficial features. Recently, rTMS has been employed for the treatment of various medical conditions, particularly depression (Pascual-Leone et al., 1996; George et al., 1995; Klein et al., 1999). Its efficacy for depression treatment has been demonstrated in several randomized trials (Fitzgerald et al., 2003, 2006; Hausmann et al., 2004; Januel et al., 2006; Loo et al., 1999). Two modes of stimulation have been employed for depression treatment, low-frequency rTMS over the right prefrontal cortex to deactivate the right prefrontal cortex and high-frequency rTMS over the left prefrontal cortex to activate the left prefrontal cortex. A conclusion has not been reached regarding the optimal rTMS parameters for depression treatment. For treatment of depression, parameters of rTMS such as frequency, intensity, and duration of stimuli have generally been determined empirically on the basis of their follow-up clinical efficacy and the possible risk of rTMS side effects such as convulsion.

There were several functional neuroimaging studies in which regional cerebral blood flow (rCBF) changes due to rTMS were examined by position emission tomography (PET) (Knoch et al., 2006; Speer et al., 2000, 2003; Fox et al., 1997; Kimbrell et al., 2002), single photon emission computed tomography (SPECT) (Loo et al., 2003; Shajahan et al., 2002), and functional magnetic resonance imaging (fMRI) (Li et al., 2004; Bohning et al., 1999, 2003; Bestmann et al., 2005; Nahas et al., 2001). These studies demonstrated that rTMS induced different responses in rCBF depending on parameter settings such as frequency or intensity, and rCBF changes may occur ipsilateral as well as contralateral to the stimulated cortex. Ideally, rTMS parametors should be determined on the basis of these data on rTMS-induced changes in brain function. A near-infrared spectroscopy (NIRS), which enables the measurement of regional cerebral blood volume (rCBV) in terms of oxygenated hemoglobin concentration ([oxy-Hb]) and deoxygenated hemoglobin concentration ([deoxy-Hb]) changes

* Corresponding author. Tel.: +81 27 220 8185; fax: +81 27 220 8187.
*E-mail address:* fkdpsy@med.gunma-u.ac.jp (M. Fukuda).

0168-0102/$ – see front matter 
doi:10.1016/j.neures.2008.10.003

with high temporal resolution (Suto et al., 2004; Kameyama et al., 2006), has also been used recently in rTMS studies (Hanaoka et al., 2007; Hada et al., 2006; Iwata and Ugawa, 2002; Speer et al., 2003). For example, we demonstrated [oxy-Hb] decreases and increases during and after rTMS, respectively, in the left prefrontal cortex by low-frequency rTMS over the right prefrontal cortex (Hanaoka et al., 2007). Owing to its complete noninvasiveness and the use of a small device, NIRS can be employed for determining rTMS parameters in clinical settings in the future.

Here, as the first step for such a NIRS application in rTMS treatment, we investigated the effects of stimulus intensity on functional changes in the left prefrontal cortex during and immediately after low-frequency rTMS over the right prefrontal cortex using NIRS. We hypothesized that a strong but not weak intensity in low-frequency rTMS over the right prefrontal cortex which can deactivate the left prefrontal cortex.

## 2. Materials and methods

This study was approved by the Institutional Review Board of the Gunma University Graduate School of Medicine. All the experimental procedures regarding rTMS strictly followed the Report and Suggested Guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation by Wassermann (1998), and the recommendation by the Japanese Society of Clinical Neurophysiology (2003).

### 2.1. Subjects

Ten healthy volunteers participated in this study (3 females and 7 males; age: mean, 37.5; S.D., 10.9 years) after giving their written informed consent (Table 1). All the participants were right-handed as determined by the Edinburgh handedness inventory (Oldfield, 1971). The position of the motor cortex (M1) in the right hemisphere and stimulus intensities for the resting motor threshold (RMT) were determined individually by recording an electromyogram (Neuropac8; Nihon Koden, Tokyo, Japan) from the left first dorsal interosseous (FDI) muscle with single-pulse TMS. RMT was defined as the minimum intensity of stimulation that is required to produce a motor evoked potential (MEP) of at least 50 μV in 5 out of 10 trials. The mean average RMT was 53.8% (S.D. 12.9).

### 2.2. rTMS procedure

A Dantec Magpro stimulator connected to a figure-of-eight coil was used for the experiment (Dantec Medical A/S, Skovlunde, Denmark). The rTMS parameters were set as follows: a frequency of 1 Hz, a duration of 60 s, and an intensity of 100% RMT. While during measurement, the subjects used earplugs to reduce TMS click noises and were required to copy a landscape picture on an LCD monitor to reduce sleepiness, in accordance with the method of Watanabe et al. (2000). All the subjects underwent active and sham rTMS three times, and the order of two stimulations was counterbalanced between subjects.

**Table 1**
Characteristics of subjects.

| ID | Sex | Age | Handedness | RMT (%) |
|---|---|---|---|---|
| 1 | M | 44 | R(100) | 64 |
| 2 | F | 36 | R(100) | 34 |
| 3 | M | 30 | R(100) | 60 |
| 4 | F | 64 | R(100) | 50 |
| 5 | M | 32 | R(100) | 50 |
| 6 | M | 30 | R(100) | 35 |
| 7 | M | 31 | R(100) | 65 |
| 8 | F | 33 | R(100) | 72 |
| 9 | M | 45 | R(100) | 60 |
| 10 | M | 30 | R(100) | 48 |

The summary of the characteristics of study subjects. RMT means the Restimg Motor Threshold in table.

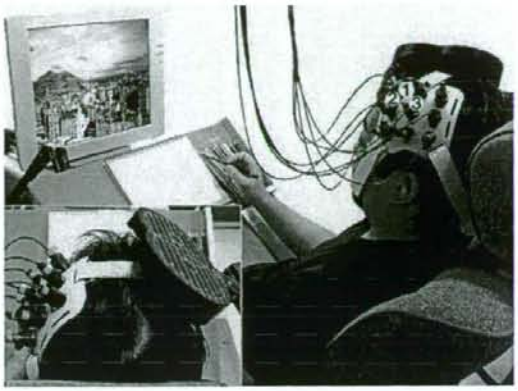

**Fig. 1.** Left and rear views of experimental setting. rTMS coil was positioned over the right prefrontal cortex and NIRS probes were set over the left frontal cortex. NIRS measured [oxy-Hb] and [deoxy-Hb] at the midpoints between emission and detector probes.

In the active stimulation, the subjects were administered rTMS at 5 cm anterior to the right RMT defined position, and the long axis of the figure-of-eight coil was set in parallel to the Fz–Cz line (Fig. 1). As the method of fixing the rTMS coil, we manually marked the MEP point on the scalp surface using a felt-tipped marker pen. We then measured and marked a position 5 cm anterior to the MEP point along the Fz–Cz line as the DLPFC equivalent site and the rTMS stimulation target lesion. Three stimulus intensities were employed by setting the distance between the undersurface of the coil and the scalp at 15, 25, and 35 mm, instead of changing instrument intensity itself. Stimulus intensities under each distance condition were assumed to be 58% RMT under the 15 mm condition, 41% RMT under the 25 mm condition, and 28% RMT under the 35 mm condition, on the basis of the equation showing the relationship between magnetic field intensity ($B$ in tesla) and distance from the coil ($d$ in mm), $B = e^{-0.36d}$ (Bohning et al., 1997).

Under the sham condition, a disconnected figure-of-eight coil was placed at the same setting as the 15 mm condition, and the stimulus was delivered by another coil that was positioned 50 cm behind the subjects so that no meaningful stimulation was applied. In this experiment, the difference in skin sensation between the real and sham stimulations was not disturbing because even the 15 mm condition in the active stimulation evoked no sensation on the subjects.

As the method of maintaining the distance between the undersurface of the rTMS coil and the scalp surface, urethane-made spacers of 15, 25, and 35 mm thicknesses were interposed in appropriate spaces for each stimulus setting. The participants sat on the bucket seats for cars with high-holding features, and the rTMS coil was fixed in the appropriate position with a fixing stand that was attached to the rTMS device. We confirmed the appropriate conformation by close observations during the entire measurement.

### 2.3. NIRS system

Brain functional changes due to rTMS were monitored using a multichannel NIRS system (Hitachi ETG-100; Hitachi Medical

Corporation, Tokyo, Japan). This system measures [oxy-Hb] and [deoxy-Hb] changes 2–3 cm beneath the scalp every 100 ms by detecting absorption of near-infrared light with wavelengths of 780 and 840 nm emitted. The following two features of this system are advantageous for monitoring rTMS effects on brain function: it enables measurements while the subjects are in the sitting position and simultaneous application of rTMS. In this study, we measured NIRS signals during 60 s of rTMS and 120 s immediately after rTMS.

Three channels were located in the left prefrontal area, and channel 1 of these was located contralateral to the stimulation point. The data obtained from the channels positioned over hair-covered areas showed a low signal to noise ratio because of the paucity of near-infrared light detected. Because the contralateral position of rTMS was located around and near the upper forehead, near-infrared light was clearly detected, and it was thought that the reliability of the NIRS [Hb] data obtained was high in this position. Therefore, we used three channels as the measurement points in this study.

The obtained [oxy-Hb] and [deoxy-Hb] data in three channels were averaged individually and then were grand-averaged for all the subjects. Differences between the active and sham stimulations were compared by calculating mean [oxy-Hb] and [deoxy-Hb] values from their grand-averaged waveforms across every 30 s interval, two 30 s intervals during rTMS, and four 30 s intervals after rTMS. The calculated mean [oxy-Hb] and [deoxy-Hb] values were analyzed by three-way repeated analysis of variance (ANOVA) with [oxy-Hb] and [deoxy-Hb] as the dependent variables, 'stimulation' (15, 25, and 35 mm, sham) and 'channel' (1, 2, and 3) as the between-subject factors, and 'time' (six 30 s intervals) as the within-subject factor, followed by post hoc comparisons.

## 3. Results

None of the subjects reported any side effect due to rTMS or NIRS measurement in this study.

The grand averaged [Hb] waveforms showed [oxy-Hb] decrease and a small [deoxy-Hb] increase during the stimulation period followed by an increase in [oxy-Hb] that returned to the baseline level or became higher after stimulation under the 25 and 35 mm conditions (Fig. 2). Three-way repeated ANOVA revealed a significant main effect of time both on [oxy-Hb] (d.f. = 5, 540; $F = 31.9$, $P < 0.001$) and [deoxy-Hb] (d.f. = 5, 540; $F = 36.5$, $P < 0.001$), a significant main effect of stimulation on [oxy-Hb] (d.f. = 3, 108; $F = 6.06$, $P = 0.001$), and a significant interaction between stimulation and time for [oxy-Hb] (d.f. = 15, 540; $F = 2.96$, $P = 0.003$) (Table 1). Post hoc comparison with Bonferroni correction revealed significant differences in [oxy-Hb] ($P < 0.015$) between the 15 mm condition and all the other conditions during two stimulation intervals and the first 30 s interval after rTMS. Because the main effect of 'channel' was not significant, [oxy-Hb] data averaged across three channels can be presented in relation to stimulation and time (Fig. 3): [oxy-Hb] decreases were most prominent during the latter half of the stimulation and the first 30 s of poststimulation only under the 15 mm condition (58% RMT).

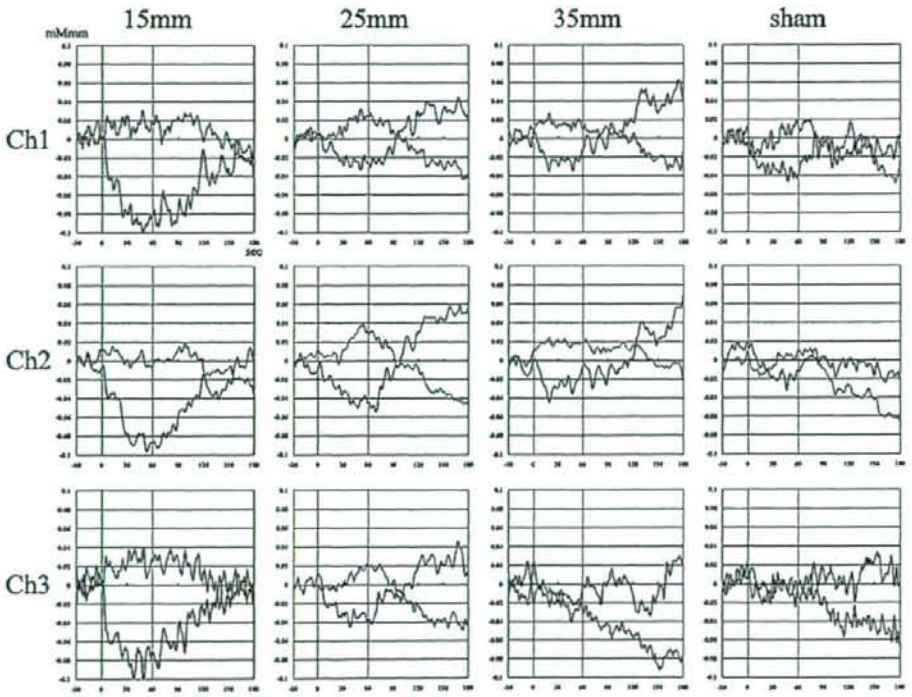


**Fig. 2.** Grand averaged waveforms at channels 1, 2, and 3. Grand averaged waveforms of [oxy-Hb] (thick line) and [deoxy-Hb] (thin line) before (−30 to 0 in the horizontal axis), during (0–60, between dotted lines), and after rTMS (60–120) measured at channels 1 (upper), 2 (middle), and 3 (lower) under four conditions (15, 25, and 35 mm under active conditions and sham stimulation, from left to right).

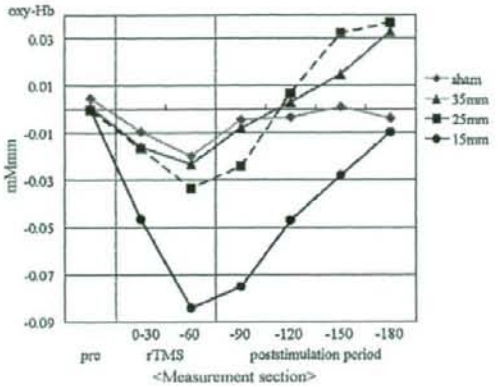


**Fig. 3.** [oxy-Hb] changes under four stimulation conditions. [oxy-Hb] changes averaged across three channels under four stimulation conditions (circle, 15 mm condition; square, 25 mm condition; triangle, 35 mm condition; diamond, sham stimulation) along stimulation time course.

## 4. Discussion

We measured brain functional changes using NIRS over the left prefrontal cortex during and immediately after the low-frequency rTMS at three low intensities administered over the right prefrontal cortex in 10 healthy volunteers. The obtained data demonstrated: (1) significant [oxy-Hb] decreases during rTMS, and (2) significant differences in the time courses of [oxy-Hb] changes among three stimulus intensities, that is, [oxy-Hb] decreases were most prominent during the latter half of the stimulation and the first 30 s of poststimulation only under the 15 mm condition. These results suggest that for contralateral hemisphere functional changes induced by low-frequency rTMS over the prefrontal cortex were successfully detected using NIRS and that a TMS intensity of more than one-half of the motor threshold is required to evoke such brain functional changes.

The deactivation of brain function during TMS is in agreement with the results of some previous studies. [oxy-Hb] decreases have been demonstrated in the stimulated primary motor cortex during and after high- (2 Hz) and low- (0.5 Hz) frequency rTMS over the motor cortex (Hada et al., 2006) and during and immediately after low- (0.25 Hz) frequency rTMS at an intensity of the motor threshold over the motor cortex in the corresponding contralateral site (Iwata and Ugawa, 2002). The [oxy-Hb] decreases also agreed with our preliminary report for the contralateral stimulated prefrontal site (Hanaoka et al., 2007). However, in contrast to the results in this study, the activation of brain function is also observed in other studies, for example, Oliviero et al., 1999. The contralateral inhibitory or dysfacilitatory effect of rTMS might generalize to other areas, such as primary motor areas and the visual cortex (Kosslyn et al., 1999). For example, low-frequency rTMS over the right prefrontal cortex increased rCBF in the ipsilateral anterior cingulate cortex, contralateral ventrolateral prefrontal cortex, and contralateral ventral striatum (Ohnishi et al., 2004), and low-frequency rTMS over the left prefrontal cortex increased relative rCBF in the right anterior cingulate, bilateral parietal cortices, insula, and left cerebellum in depressive patients in a SPECT study (Loo et al., 2003).

Conflicting results of rTMS-induced deactivation and activation can be explained by different time resolutions employed in functional brain imaging techniques. Brain activation can be monitored simultaneously with rTMS using NIRS, whereas it has to be averaged across a longer period in PET and SPECT measurements. [oxy-Hb] decreases followed by [oxy-Hb] increases observed in some NIRS studies (Hanaoka et al., 2007; Iwata and Ugawa, 2002) suggest that brain function may vary with time after rTMS: deactivation during and immediately after rTMS followed by a sustained activation for a long period. Such deactivation and activation might have been detected in NIRS studies and PET and SPECT studies, respectively. [oxy-Hb] increases after rTMS were not significant in this study, in contrast to our previous study (Hanaoka et al., 2007). This inconsistency may arise from the difference in stimulators employed (a Dantec magpro with a Medtronic MC-B70 double coil in this study and a Magstim rapid stimulator with a 70-mm double coil (Magstim Company, UK) in the previous study). The diameter of each coil was 70 mm. In a study in which these coils were compared (Thielscher and Kammer, 2004), they found the following: (i) the size of the electric field distribution does not largely differ between the two coils, (ii) the amount of induced field for a given stimulator intensity is slightly higher for the Medtronic coil than for the Magstim coil. The electric field induced by the Medtronic coil is approximately 1.19 times stronger than that induced by the Magstim coil for superficial cortical areas. They assumed the difference in the electric field intensity as arising from the geometrical design of the Medtronic MC-B70 coil; its two coils are fixed a little bent along the curvature of the head. Such difference in the electric field intensity between two stimulators may underlie the inconsistency in the contralateral deactivating effects between the present and the previous studies.

There are some limitations in this study. First, rTMS stimulus intensities were set as low as 58% RMT. The effects of rTMS at higher intensities should be examined. Second, NIRS signals were monitored only over the regions contralateral to the rTMS site. Ipsilateral measurement would show a more detailed information about brain functional changes due to rTMS. Third, NIRS measurement was stopped only after 120 s of rTMS. In the procedures of this study, as measurement time became long, the motion artifacts occurred more frequently and the irregularities in [Hb] data owing to sleepiness occurred occasionally during long-term measurement. Therefore it was difficult to decide whether the [Hb] changes were due to rTMS in the procedures of this study during long-term measurement. In order to exclude NIRS data contaminated with such artifacts, we used data within 120 s after stimulation for analysis as the brain functional changes induced by rTMS with high dependability. A further study of brain function for a longer period may reveal long-term effects of rTMS with the establishment of a procedure that can exclude these disturbances. Fourth, only healthy subjects were recruited in this study. Patients with psychiatric disorders that respond to rTMS, e.g., major depressive disorder, may show different patterns of brain activation. If these limitations are resolved, NIRS measurement can be employed for individual titration of rTMS parameters for successful treatment by rTMS.

In conclusion, the stimulus intensity dependence of functional changes in the left prefrontal cortex induced by low-frequency rTMS over the right prefrontal cortex was successfully monitored using NIRS. A direct monitoring of rTMS-induced brain functional changes using NIRS may be useful for elucidating the brain mechanisms underlying the clinical efficacy of rTMS and for establishing the appropriate rTMS parameters for individual patients.

## References

Bestmann, S., Baudewig, J., Siebner, H.R., Rothwell, J.C., Frahm, J., 2005. BOLD MRI responses to repetitive TMS over human dorsal premotor cortex. Neuroimage 28 (October (1)), 22–29 Epub 2005 Jul 5.

Bohning, D.E., Pecheny, A.P., Epstein, C.M., Speer, A.M., Vincent, D.J., Dannels, W., George, M.S., 1997. Mapping transcranial magnetic stimulation (TMS) fields in vivo with MRI. Neuroreport 8 (July (11)), 2535–2538.

Bohning, D.E., Shastri, A., McConnell, K.A., Nahas, Z., Lorberbaum, J.P., Roberts, D.R., Teneback, C., Vincent, D.J., George, M.S., 1999. A combined TMS/fMRI study of intensity-dependent TMS over motor cortex. Biol. Psychiatry 45 (February (4)), 385–394.

Bohning, D.E., Shastri, A., Lomarev, M.P., Lorberbaum, J.P., Nahas, Z., George, M.S., 2003. BOLD-fMRI response vs. transcranial magnetic stimulation (TMS) pulse-train length: testing for linearity. J. Magn. Reson. Imaging 17 (March (3)), 279–290.

Fitzgerald, P.B., Brown, T.L., Marston, N.A., Daskalakis, Z.J., De Castella, A., Kulkarni, J., 2003. Transcranial magnetic stimulation in the treatment of depression: a double-blind, placebo-controlled trial. Arch. Gen. Psychiatry 60 (October (10)), 1002–1008.

Fitzgerald, P.B., Benitez, J., de Castella, A., Daskalakis, Z.J., Brown, T.L., Kulkarni, J., 2006. A randomized, controlled trial of sequential bilateral repetitive transcranial magnetic stimulation for treatment-resistant depression. Am. J. Psychiatry 163 (January (1)), 88–94.

Fox, P., Ingham, R., George, M.S., Mayberg, H., Ingham, J., Roby, J., Martin, C., Jerabek, P., 1997. Imaging human intra-cerebral connectivity by PET during TMS. Neuroreport 8 (August (12)), 2787–2791.

George, M.S., Wassermann, E.M., Williams, W.A., Callahan, A., Ketter, T.A., Basser, P., Hallett, M., Post, R.M., 1995. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. Neuroreport 6 (October (14)), 1853–1856.

Hada, Y., Abo, M., Kaminaga, T., Mikami, M., 2006. Detection of cerebral blood flow changes during repetitive transcranial magnetic stimulation by recording hemoglobin in the brain cortex, just beneath the stimulation coil, with near-infrared spectroscopy. Neuroimage 32 (September (3)), 1226–1230 Epub 2006 Jun 9.

Hanaoka, N., Aoyama, Y., Kameyama, M., Fukuda, M., Mikuni, M., 2007. Deactivation and activation of left frontal lobe during and after low-frequency repetitive transcranial magnetic stimulation over right prefrontal cortex: a near-infrared spectroscopy study. Neurosci. Lett. 414 (March (2)), 99–104 Epub 2007 Feb 9.

Hausmann, A., Kemmler, G., Walpoth, M., Mechtcheriakov, S., Kramer-Reinstadler, K., Lechner, T., Walch, T., Deisenhammer, E.A., Kofler, M., Rupp, C.I., Hinterhuber, H., Conca, A., 2004. No benefit derived from repetitive transcranial magnetic stimulation in depression: a prospective, single centre, randomised, double blind, sham controlled "add on" trial. J. Neurol. Neurosurg. Psychiatry 75 (February (2)), 320–322.

Iwata, N., Ugawa, Y., 2002. Imaging functional connectivity by combining transcranial magnetic stimulation (TMS) and functional neuroimaging techniques. Clin. Electroencephal. 44, 80–85 (in Japanese).

Januel, D., Dumortier, G., Verdon, C.M., Stamatiadis, L., Saba, G., Cabaret, W., Benadhira, R., Rocamora, J.F., Braha, S., Kalalou, K., Vicaut, P.E., Fermanian, J., 2006. A double-blind sham controlled study of right prefrontal repetitive transcranial magnetic stimulation (rTMS): therapeutic and cognitive effect in medication free unipolar depression during 4 weeks. Prog. Neuropsychopharmacol. Biol. Psychiatry 30 (January (1)), 126–130 Epub 2005 Oct 20.

The committee on the guidelines for transcranial magnetic stimulation in the Japanese Society of Clinical Neurophysiology, Recommendation on the safety of transcranial magnetic stimulation. Jpn. J. Clin. Neurophysiol. 31 (2003) 69 (in Japanese).

Kameyama, M., Fukuda, M., Yamagishi, Y., Sato, T., Uehara, T., Ito, M., Suto, T., Mikuni, M., 2006. Frontal lobe function in bipolar disorder: a multichannel near-infrared spectroscopy study. Neuroimage 29 (January (1)), 172–184 Epub 2005 Aug 25.

Kimbrell, T.A., Dunn, R.T., George, M.S., Danielson, A.L., Willis, M.W., Repella, J.D., Benson, B.E., Herscovitch, P., Post, R.M., Wassermann, E.M., 2002. Left prefrontal-repetitive transcranial magnetic stimulation (rTMS) and regional cerebral glucose metabolism in normal volunteers. Psychiatry Res. 115 (October (3)), 101–113.

Klein, E., Kreinin, I., Chistyakov, A., Koren, D., Mecz, L., Marmur, S., Ben-Shachar, D., Feinsod, M., 1999. Therapeutic efficacy of right prefrontal slow repetitive transcranial magnetic stimulation in major depression: a double-blind controlled study. Arch. Gen. Psychiatry 56 (April (4)), 315–320.

Knoch, D., Treyer, V., Regard, M., Muri, R.M., Buck, A., Weber, B., 2006. Lateralized and frequency-dependent effects of prefrontal rTMS on regional cerebral blood flow. Neuroimage 31 (June (2)), 641–648 Epub 2006 Feb 23.

Kosslyn, S.M., Pascual-Leone, A., Felician, O., Camposano, S., Keenan, J.P., Thompson, W.L., Ganis, G., Sukel, K.E., Alpert, N.M., 1999. The role of area 17 in visual imagery: convergent evidence from PET and rTMS. Science 284, 167–170.

Li, X., Nahas, Z., Kozel, F.A., Anderson, B., Bohning, D.E., George, M.S., 2004. Acute left prefrontal transcranial magnetic stimulation in depressed patients is associated with immediately increased activity in prefrontal cortical as well as subcortical regions. Biol. Psychiatry 55, 882–890.

Loo, C., Mitchell, P., Sachdev, P., McDarmont, B., Parker, G., Gandevia, S., 1999. Double-blind controlled investigation of transcranial magnetic stimulation for the treatment of resistant major depression. Am. J. Psychiatry 156 (June (6)), 946–948.

Loo, C.K., Sachdev, P.S., Haindl, W., Wen, W., Mitchell, P.B., Croker, V.M., Malhi, G.S., 2003. High (15 Hz) and low (1 Hz) frequency transcranial magnetic stimulation have different acute effects on regional cerebral blood flow in depressed patients. Psychol. Med. 33 (August (6)), 997–1006.

Nahas, Z., Lomarev, M., Roberts, D.R., Shastri, A., Lorberbaum, J.P., Teneback, C., McConnell, K., Vincent, D.J., Li, X., George, M.S., Bohning, D.E., 2001. Unilateral left prefrontal transcranial magnetic stimulation (TMS) produces intensity-dependent bilateral effects as measured by interleaved BOLD fMRI. Biol. Psychiatry 50 (November (9)), 712–720.

Ohnishi, T., Matsuda, H., Imabayashi, E., Okabe, S., Takano, H., Arai, N., Ugawa, Y., 2004. rCBF changes elicited by rTMS over DLPFC in humans. Suppl. Clin. Neurophysiol. 57, 715–720.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9 (March (1)), 97–113.

Oliviero, A., Di Lazzaro, V., Piazza, O., Profice, P., Pennisi, M.A., Della Corte, F., Tonali, P., 1999. Cerebral blood flow and metabolic changes produced by repetitive magnetic brain stimulation. J. Neurol. 246 (December (12)), 1164–1168.

Pascual-Leone, A., Rubio, B., Pallardo, F., Catala, M.D., 1996. Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. Lancet 348 (July (9022)), 233–237.

Shajahan, P.M., Glabus, M.F., Steele, J.D., Doris, A.B., Anderson, K., Jenkins, J.A., Gooding, P.A., Ebmeier, K.P., 2002. Left dorso-lateral repetitive transcranial magnetic stimulation affects cortical excitability and functional connectivity, but does not impair cognition in major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 26 (June (5)), 945–954.

Speer, A.M., Kimbrell, T.A., Wassermann, E.M., D Repella, J., Willis, M.W., Herscovitch, P., Post, R.M., 2000. Opposite effects of high and low frequency rTMS on regional brain activity in depressed patients. Biol. Psychiatry 48 (December (12)), 1133–1141.

Speer, A.M., Willis, M.W., Herscovitch, P., Daube-Witherspoon, M., Shelton, J.R., Benson, B.E., Post, R.M., Wassermann, E.M., 2003. Intensity-dependent regional cerebral blood flow during 1-Hz repetitive transcranial magnetic stimulation (rTMS) in healthy volunteers studied with H215O positron emission tomography. I. Effects of primary motor cortex rTMS. Biol. Psychiatry 54 (October (8)), 818–825 (Review).

Suto, T., Fukuda, M., Ito, M., Uehara, T., Mikuni, M., 2004. Multichannel near-infrared spectroscopy in depression and schizophrenia: cognitive brain activation study. Biol. Psychiatry 55 (March (5)), 501–511.

Thielscher, A., Kammer, T., 2004. Electric field properties of two commercial figure-8 coils in TMS: calculation of focality and efficiency. Clin. Neurophysiol. 115 (July (7)), 1697–1708.

Wassermann, E.M., 1998. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the safety of repetitive transcranial magnetic stimulation, June 5–7 1996. Electroencephalogr. Clin. Neurophysiol. 108 (January (1)), 1–16.

Watanabe, E., Maki, A., Kawaguchi, F., Yamashita, Y., Koizumi, H., Mayanagi, Y., 2000. Noninvasive cerebral blood volume measurement during seizures using multichannel near infrared spectroscopic topography. J. Biomed. Opt. 5 (July (3)), 287–290.

*Psychophysiology*, *46* (2009), 52–61. Wiley Periodicals, Inc. Printed in the USA.

DOI: 10.1111/j.1469-8986.2008.00748.x

# Preattentive dysfunction in major depression: A magnetoencephalography study using auditory mismatch negativity

YUICHI TAKEI,[a] SUMIE KUMANO,[a] SUGURU HATTORI,[a] TORU UEHARA,[a] YUKI KAWAKUBO,[b] KIYOTO KASAI,[b] MASATO FUKUDA,[a] AND MASAHIKO MIKUNI[a]

[a]Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine, Gunma, Japan
[b]Department of Neuropsychiatry, Tokyo University Graduate School of Medicine, Tokyo, Japan

**Abstract**

Information processing deficits in major depressive disorder have been infrequently examined electrophysiologically. Its preattentive and sensory information processing was examined using mismatch field (MMNm) and P1m components, respectively, by magnetoencephalography. Fourteen major depressive disorder patients and 19 healthy volunteers participated in the study. MMNm was elicited in response to duration and frequency changes of pure-tone stimuli and in response to a vowel across-category change. The magnetic global field power (mGFP) of MMNm was significantly smaller in the major depressive disorder patients than in the healthy volunteers, although that of P1m did not differ between the two groups. Information processing at the preattentive level is impaired functionally in major depressive disorder, and this dysfunction is not due to the dysfunction at the lower level of information processing.

**Descriptors:** MEG, Mismatch negativity, Major depressive disorder, MMN, Magnetoencephalography, Attention

Cognitive dysfunctions are assumed to underlie clinical symptoms in major depressive disorder patients and have been included in the criteria for establishing diagnosis: For example, a diminished ability to think or concentrate during a major depressive episode is included as an item in the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (*DSM-IV*). Cognitive dysfunctions in major depressive disorder patients have been experimentally demonstrated in many neuropsychological studies. During a depressive episode of major depressive disorder patients, neuropsychological deficits have been demonstrated in memory, learning, attention, alertness, and executive functions (Austin et al., 1992; Veiel, 1997; Zakzanis, Leach, & Kaplan, 1998). In addition, a number of recent studies have shown that some of these cognitive deficits persist during the euthymic state and symptomatological remission (Austin et al., 1992; Tham et al., 1997).

Information processing deficits underlying these cognitive dysfunctions in major depressive disorder patients can be examined electrophysiologically using event-related potentials (ERPs). For example, among late ERPs, the P300 component has often been reported to be reduced in amplitude and delayed in peak latency in major depressive disorder patients (Karaaslan, Gonul, Oguz, Erdinc, & Esel, 2003; Kawasaki, Tanaka, Wang, Hokama, & Hiramatsu, 2004; Papageorgiou et al., 2004; Roschke & Wagner, 2003; Urretavizcaya et al., 2003), and the results are interpreted as reflecting abnormalities in the capacity of attentional operation resource (Kok, 2001). However, because late ERPs such as P300 are often vulnerable to motivational factors and task involvement of the participants, the interpretation of these findings in major depressive disorder patients is difficult.

Among the earlier components of ERPs, mismatch negativity (MMN) has been the most extensively investigated to elucidate preattentive cognitive function. MMN and its magnetic counterpart (MMNm) are considered to reflect preattentive information processing and are elicited approximately 150–200 ms after the onset of physically deviant auditory stimuli in identical and repeated stimulus sequences (Hari et al., 1984; Näätänen, Gaillard, & Mäntysalo, 1978). Although there is some evidence for attentional modulation of MMN (Trejo, Ryan-Jones, & Kramer, 1995; Woldorff, Hillyard, Gallen, Hampson, & Bloom, 1998), MMN is elicited even when attention is directed away from the auditory input. Thus, the significance of MMN results can be more easily interpreted than that of P300 results if the motivational factor of the subjects cannot be controlled, as in the case of psychiatric patients. This automatic mismatch process might have an important role in initiating an involuntary switching of attention to an auditory stimulus change occurring outside the focus of attention (Giard, Perrin, Pernier, & Bouchet, 1990; Lyytinen, Blomberg, & Näätänen, 1992; Näätänen, 1992).

Among various psychiatric disorders, MMN has been studied mainly in schizophrenia. In a meta-analysis of MMN (Umbricht & Krljes, 2005), it was concluded that MMN deficits are a robust

We thank Dr. Masato Yumoto of the Tokyo University Graduate School of Medicine for his collaboration in this study.

Address reprint requests to: Masato Fukuda, 3-39-22 Showa, Maebashi, Gunma 371-8511, Japan. E-mail address: fkdpsy@med.gunma-u.ac.jp

feature of chronic schizophrenia and represent the underlying mechanism of attention dysfunction in schizophrenia. Studies of MMNm in schizophrenia also indicated an MMNm amplitude decrease in schizophrenia and changes in the equivalent current dipole (ECD) location (Kasai et al., 2003; Kreitschmann-Andermahr et al., 1999; Oades et al., 2006; Pekkonen et al., 2002).

There have been few studies of MMN in major depressive disorder patients. Umbricht et al. (2003) examined MMN generated in response to a pure-tone duration and a frequency change in major depressive disorder patients, bipolar disorder patients, schizophrenia patients, and in healthy volunteers and found no significant differences in MMN amplitude between major depressive disorder patients and healthy volunteers. Ogura, Nageishi, and Omura (1995) investigated major depressive disorder patients and bipolar disorder patients in the depressive state using the oddball paradigm of a tone-burst frequency change. They reported that the mean amplitude of the early N200 component (N2a), which corresponds to the MMN component, of the patients in the depressive state was smaller than that of the healthy volunteers. Lepistö et al. (2004) investigated MMN using a consonant sound change and a novel sound and found a short MMN latency and an unchanged MMN amplitude in children with major depressive disorder patients compared with those in healthy children. Taken together, these previous studies of MMN in major depressive disorder patients have not yielded sufficiently consistent results.

Another earlier ERP component, P1, often called P50, and its magnetic counterpart P1m are considered to reflect earlier stages of auditory information processing and, in accordance with the assumption, its dipole is located in the primary auditory cortex. This component is employed in studies of auditory information processing in psychiatric disorders. For example, Ahveninen et al. (2006) reported a significant P50 amplitude reduction and a marked deviation of P50m dipole sources in a twin study of schizophrenia, and assumed that a P50 amplitude reduction and a P50m dipole deviation might be a marker of brain function changes related to the genetic predisposition to schizophrenia and that these changes might be inherited as morphological changes in auditory cortex neurons. In fact, from these findings, the left superior temporal gyrus and left medial temporal lobe are considered to be key regions of structural difference in patients with schizophrenia (Honea, Crow, Passingham, & Mackay, 2005).

Whole-head magnetoencephalography (MEG) has advantages over scalp electroencephalography (EEG) in terms of its higher spatial resolution with many recording channels and its more accurate estimation of MMNm dipole locations. These advantages are due to the fact that magnetic fields are less affected by intervening tissues of different conductivities than electrical fields. To the best of our knowledge, there has been only one study of MMNm and P1m in major depressive disorder patients using MEG (Kähkönen et al., 2007). They used a pure-tone frequency deviant to elicit MMNm and found no significant differences in MMN amplitude or latency between major depressive disorder patients and healthy volunteers. In this study, we recorded MMNm and P1m by MEG in major depressive disorder patients in the passive oddball paradigms of vowel sounds as well as pure tone and examined their power and latency in relation to clinical symptoms and psychotropic medications. We also estimated the current dipoles of MMNm and P1m. Our hypotheses are as follows: (1) Preattentive information processing deficits are revealed as reduced MMNm power and/or delayed MMNm latency in major depressive disorder patients and (2) the locations of estimated current dipoles do not differ between major depressive disorder patients and healthy volunteers because most voxel-based morphometry studies showed no anatomical differences in temporal structures between major depressive disorder patients and healthy volunteers (Beyer & Krishnan, 2002).

## Methods

### *Participants*

Fourteen major depressive disorder patients and 19 healthy volunteers participated in this study (Table 1). The major depressive disorder patients were recruited from the Department of Psychiatry, Gunma University Hospital, Japan. Each patient was diagnosed as having major depressive disorder in accordance with *DSM-IV* (American Psychiatric Association, 1994).

The major depressive disorder patients included 9 men and 5 women (age: mean, 41.4 years; *SD*, 10.2; range, 25–60). Major depressive disorder patients over 60 years old were not included in this study to eliminate the effects of additional pathophysiological factors on major depressive disorder such as aging and vascular changes.

During this study, all the subjects were euthymic or depressive, as indicated by their 17-item Hamilton Rating Scale for Depression (HRSD) scores (mean, 10.7; *SD*, 4.1; range, 5–19; Hamilton, 1960). According to the minimental state examination (MMSE) scores, there were no patients with dementia in the major depressive disorder group. However, 13 of the 14 patients were on medication with antidepressants, mood stabilizers, antipsychotics, anxiolytics, or hypnotics during this study. The imipramine equivalent dose of antidepressants, the diazepam equivalent dose of anxiolytics, and the flunitrazepam equivalent dose of hypnotics were calculated for each patient (Inagaki & Inada, 2006).

The healthy volunteers included 13 men and 6 women (age: mean, 37.7 years; *SD*, 10.0; range, 26–56). They had no history of any major psychiatric disorders or major physical illnesses and were not on any major psychiatric medications during this study. The mean age and sex ratio did not significantly differ between the two groups, $F(1,31) = 1.0$, $p = .32$; $\chi^2(1) = 0.62$, $p = .80$. All the subjects were right-handed as indicated by their Edinburgh inventory scores (mean, 96.8; *SD*, 6.0; range, 80–100; Oldfield, 1971). Their sleepiness scores at the time of MEG examination were assessed using the Stanford sleepiness scale. The sleepiness scores before, $F(1,31) = 0.29$, $p = .59$, and during the task performance, $F(1,31) = 1.52$, $p = .23$, did not differ significantly between the two groups.

For both the patients and healthy volunteers, Japanese was the first language. The exclusion criteria for both groups included clear abnormality of MRI results, neurological illness, traumatic brain injury with any of the known cognitive consequences or loss of consciousness for more than 5 min, substance use or addiction, and presence of hearing or vision impairment. This study was approved by the Institutional Review Board of Gunma University Hospital, and written informed consent was obtained from all the participants prior to the study.

### *Task Procedures*

P1m and MMNm were recorded during an auditory task while the subjects were instructed to perform another visual task and to ignore the auditory stimuli.

**Table 1.** *Characteristics of Participants*

| Case | Age (years) | Sex | Age of onset (years) | MMSE score | HRSD score | Total imipramine equivalent dose (mg/day) | Antidepressant (imipramine equivalent dose) | Mood stabilizer (mg/day) | Others (mg/day) |
|---|---|---|---|---|---|---|---|---|---|
| Major depressive disorder ($n = 14$) | | | | | | | | | |
| 1 | 41 | M | 39 | 29 | 10 | 25 | Sulpiride 50(25) | | Zopiclone 7.5, Loflazepate 1 |
| 2 | 37 | M | 34 | 30 | 10 | 72.9 | Milnacipran 100(66.7), Trazodone 25(12.5) | | Nitrazepam 5 |
| 3 | 31 | F | 28 | 29 | 8 | 0 | | | Loflazepate 1 |
| 4 | 37 | M | 24 | 29 | 6 | 75 | Amitriptyline 75(75) | Lithium 600 | Lorazepam 1, Zolpidem 5 |
| 5 | 37 | M | 36 | 30 | 13 | 60 | Maprotiline 60(60) | Lithium 600 | Lorazepam 1 |
| 6 | 54 | F | 24 | 30 | 9 | 112.5 | Milnacipran 75(50), Sulpiride 100(50), Trazodone 25(12.5) | | Clonazepam 1, Etizolam 1.5 |
| 7 | 58 | M | 57 | 29 | 7 | 25 | Sulpiride 50(25) | | Loflazepate 1 |
| 8 | 34 | M | 33 | 30 | 9 | 37.5 | Paroxetine 10(37.5) | | Levothyroxine 25 |
| 9 | 48 | F | 45 | 29 | 19 | 150 | Paroxetine 40(150) | | Quetiapine 100, Clonazepam 1.5, Nitrazepam 20, Quazepam 15, Flunitrazepam 2, Levothyroxine 25 |
| 10 | 42 | F | 20 | 30 | 12 | 125 | Milnacipran 75(50), Amoxapine 75(75) | | Etizolam 1.5 |
| 11 | 25 | F | 24 | 28 | 13 | 82.5 | Milnacipran 30(20), Trazodone 125(62.5) | Valproate 200 | Levomepromazine 5 |
| 12 | 40 | M | 34 | 30 | 11 | 300 | Imipramine 150(150), Setiptiline 6(150) | | Methylphenidate 10, Brotizolam 0.25, Triazolam 0.5, Flunitrazepam 2 |
| 13 | 60 | M | 34 | 30 | 18 | 141.7 | Sulpiride 150(75), Maprotiline 25(25), Trazodone 25(12.5), Paroxetine 20(75) | | |
| 14 | 35 | M | | | | | Sulpiride 150(75), Milnacipran 100(66.7) | | Etizolam 1.5 |
| Mean | 41.4 | M9/F5 | 34.6 | 29.5 | 10.7 | 99.6 | | | |
| *SD* | 10.2 | | 10.9 | 0.7 | 4.1 | 79 | | | |
| Healthy control (n = 19) | | | | | | | | | |
| Mean | 37.7 | M13/F6 | | | | | | | |
| *SD* | 10.0 | | | | | | | | |

*Note.* M: male; F: female; HRSD: 17-item Hamilton Rating Scale for Depression.

*Auditory mismatch negativity task.* In the auditory task, the subjects were presented with sequences of auditory stimuli consisting of standard and deviant stimuli delivered randomly. The interstimulus interval was 445 ± 15 ms. The stimuli were delivered binaurally through plastic tubes. The experiment consisted of two conditions. The first condition was designed to elicit MMNm in response to duration and frequency changes of pure-tone stimuli (standard: 50-ms duration, 1000-Hz frequency, probability = 83%; duration deviant: 100-ms duration, 1000-Hz frequency, probability = 8.5%; frequency deviant: 50-ms duration, 1200-Hz frequency, probability = 8.5%). The second condition was designed to elicit MMNm in response to a vowel across-category change condition (standard, Japanese vowel sound/a/, probability = 90%; deviant, Japanese vowel sound/o/, probability = 10%). These vowel stimuli were spoken by a native Japanese, digitized using the NeuroStim system (NeuroScan, USA), and edited to have a loudness of 80 dBSPL and a rise/fall time of 10 ms. The frequency spectra for the vowels were as follows./a/: formant (F) 0 = 140, F1 = 760, F2 = 1250, F3 = 2750, and F4 = 3600 Hz;/o/: F0 = 140, F1 = 480, F2 = 770, F3 = 2820, and F4 = 3600 Hz. The frequency of the pure-tone stimuli was 1000 Hz, nearly equal to the central frequency of the formants of the vowel stimuli. The order of the two conditions was counterbalanced across the subjects.

*Visual task.* The subjects performed a visual task while ignoring the auditory stimuli during MMNm measurement. The visual stimuli consisted of three sequences. Sequence 1 consisted of pictures of animals, flowers, buildings, and fruits. Sequence 2 consisted of pictures of sweets, flowers, animals, insects, castles, festivals, stone lanterns, and fruits. Sequence 3 consisted of pictures of birds, landscapes, flowers, and roads. The target pictures of Sequence 1 were animals, those of Sequence 2 were sweets, and those of Sequence 3 were birds. In each sequence, the target pictures were randomly presented with a probability of 30%. The pictures were sequentially presented at 2000-ms duration on a screen placed 1.7 m from the subjects. The subjects were instructed to press a button immediately after a target

picture was presented. The order of the three sequences was counterbalanced across the subjects.

### *Data Acquisition*

*MEG.* Magnetic fields were recorded in a magnetically shielded room (JFE Mechanical Co., Japan) with a 306-channel magnetometer (Knuutila et al., 1993). This whole-head magnetometer consisted of 102 triple-sensor units, each with two orthogonal planar gradiometers and one magnetometer that records maximal signals directly above the source (Hämäläinen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993). We used a 204-channel gradiometer for data analysis except for a 102-channel magnetometer, because we could not record the data obtained with the 102-channel magnetometer for all subjects. A subject was instructed to sit on a chair with his/her head inside the helmet-shaped magnetometer. The position of the magnetometer with respect to the head was determined at the beginning of the task under each condition according to the magnetic fields produced by currents fed into three indicator coils at predetermined locations on the scalp. The locations of these coils in relation to the pre-auricular points and nasion were determined with an Isotrak 3D digitizer (Polhemus TM, USA) before the start of the experiment.

MEG epochs were averaged separately for standard and deviant stimuli. The duration of the averaging period was 420 ms, including a 100-ms prestimulus baseline. The recording bandpass range was 0.1–100 Hz, with a sampling rate of 512 Hz. The first 10 stimuli were automatically excluded from averaging. MEG epochs exceeding 3000 fT/cm were also excluded from averaging. Data collection under each condition lasted until 100 deviant stimuli that did not generate artifacts were presented. This number for averaging was adopted according to those adopted in previous studies (Alho et al., 1998; Kasai et al., 2003) and considering the balance between the signal-to-noise ratio and a possible habituation effect of MMN in response to speech sound (McGee et al., 2001). Average responses were digitally filtered in the bandpass range of 1–20 Hz.

*Magnetic resonance imaging (MRI) of brain.* In all the major depressive disorder patients and volunteers, a set of 2-mm-thick, sagittal MRI slices were acquired with 1.5-T equipment (MAGNETOM Symphony Maestro Class, Siemens Medical Solutions, Erlangen, Germany) using a three-dimensional (3D) fast spoiled gradient recalled acquisition in the steady state (FSPGR). The MEG coordinate system was aligned with the MRI-based coordinates by identifying the left and right preauricular points, as well as the nasion, from the MRI slices.

### *Data Analysis*

*Magnetic counterpart of global field powers (mGFPs) of P1m and MMNm.* The mismatch reaction is defined as the difference between the magnetic field of the standard tone and the evoked field of the deviant tone. The magnetic fields of standard tones were subtracted from those of deviant tones for each channel, and the root mean squares of the differences over the 54 channels positioned over the temporal region (Figure 1) were calculated for each subject as the magnetic counterpart of the global field power (mGFP) of the mismatch reaction, separately for each condition and hemisphere, using the formula shown in Figure 2 (Kreitschmann-Andermahr et al., 1999; Lehmann & Skrandies, 1980). The peak latency of P1m was determined as the maximum amplitude of the individual mGFP curve of standard stimuli for each condition between 40 and 100 ms (Tervaniemi et al., 1999). The individual curves of MMNm were calculated by subtracting the wave at each channel elicited in response to the standard tone from that elicited in response to deviant tones under the same experimental condition (Alho et al., 1998). The peak latency of MMNm was determined as the maximum amplitude of the individual mGFP curves between 100 and 250 ms (Kasai et al., 2003; Tervaniemi et al., 1999). The individual curve whose peak amplitude could not be found in the designated periods of those of P1m and MMNm was excluded from analysis.

$$\text{mGFP (fT/cm)} = \sqrt{\frac{1}{n} \cdot \sum_{i=1}^{n} \left( Ui - \frac{\sum_{j=1}^{n} Uj}{n} \right)^2}$$

**Figure 1.** Grand mean waveform of magnetic fields for MMNm response to duration changes of pure-tone stimuli in major depressive disorder patients and healthy volunteers. Thick circles indicate selected channels. Thin circle indicates an enlarged picture of part of the channels. Left side: focused parts of the left hemisphere. Solid lines, major depressive disorder patients; dashed lines, healthy volunteers.

*Dipole analysis.* Under each condition and for each subject, equivalent current dipoles for P1m and MMNm were calculated separately for each hemisphere, utilizing a spherical head model constructed on the basis of an individual MRI and a subset of 54 channels over the temporal brain areas (Figure 3). ECD of P1m was calculated from standard stimuli for each condition. ECD of MMNm was calculated from the deviant-stimulus response wave minus the standard-stimulus response wave. ECDs of MMNm and P1m were calculated at the same latency determined by mGFP analysis. The ECDs with a goodness of fit (GOF) greater than 70% were included in the analysis. In this procedure, we reduced the number of channels to 32–49 when the dipole was not calculated or a certain channel had a considerable number of artifacts. The mean GOFs for P1m under the two conditions and in the two hemispheres ranged from 85.7% to 90.6% for the major depressive disorder patients and from 85.5% to 90.4% for the healthy volunteers. The mean GOFs for MMNm under the three conditions and in the two hemispheres ranged from 84.4% to 88.1% for the major depressive disorder patients and from 82.4% to 85.8% for the healthy volunteers. Whenever available, the ECD locations for each subject were superimposed on his/her 3D-reconstructed MRI. The *x*-axis defined the right and left directions, the *y*-axis defined the anterior and posterior directions, and the *z*-axis defined the superior and inferior directions.

### *Statistical Analyses*

The reaction time and correct answer rate during the visual task performance, age, and sleepiness scores before and during the task performance were compared between groups by one-way analysis of variance (ANOVA). We excluded the data of the visual task performance (reaction time and correct answer rate) over 3 *SD*s lower or higher than the average. The mGFP peak latencies and powers of P1m and MMNm were analyzed using three-way ANOVA with group (major depressive disorder patients and healthy volunteer), condition (pure-tone frequency change condition, pure-tone duration change condition, and vowel across-category change condition) and hemisphere (left and right) as independent variables, followed by Scheffe's post hoc test where appropriate. Spearman's rho was calculated in exploratory analyses of the relationships among the visual task

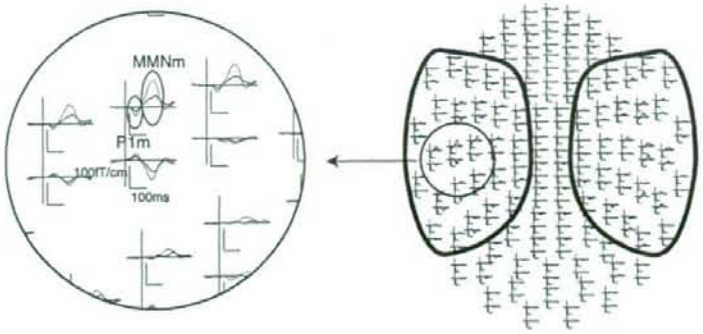


**Figure 2.** Formula for calculating mGFP. In this formula, $n$ is the number of channels and $u$ is the amplitude.

performance, clinical measures, and medications (reaction time, correct answer rate, age, HRSD score, age of onset, illness duration, and doses of antidepressants, anxiolytics, and hypnotics) and the mGFP power/peak latency or dipole location of P1m and MMNm.

The ECD locations of P1m and MMNm were compared between the two groups separately for each hemisphere using three-way ANOVA with group (major depressive disorder patients and healthy volunteer) and condition (pure-tone frequency change condition, pure-tone duration change condition, and vowel across-category change condition) as independent variables, followed by Scheffe's post hoc test where appropriate. The ECD location between P1m and MMNm was also compared separately for each hemisphere using one-way ANOVA. The statistical results were considered significant if $p < .05$ for the ANOVA of mGFP and the dipole analyses and $p < .01$ for the correlational analyses to avoid false positive findings in multiple correlation calculations.

## Results

### *Behavioral Data*

The reaction time during the visual task did not differ between the groups (major depressive disorder patients: mean, 588.3 ms; *SD*, 111.5; healthy volunteers: mean, 546.0 ms; *SD*, 70.6; $F[1,28] = 1.61$, $p = .22$). The correct answer rates in the visual task did not differ between the two groups (major depressive disorder patients: mean, 99.4%; *SD*, 0.5%; healthy volunteers: mean, 98.8%; *SD*, 1.3%). The Stanford sleepiness scores before and during the task did not significantly differ between the two groups.

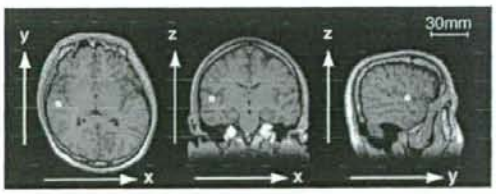


**Figure 3.** ECD location of MMNm of healthy volunteers for the pure-tone duration change condition in the left hemisphere. The arrows in this figure indicate each axis. The *x*-axis defines the right and left directions, the *y*-axis defines the anterior and posterior directions, and the *z*-axis defines the superior and inferior directions.

### *Magnetic Counterpart of Global Field Power (Table 2, Figures 4 and 5)*

*P1m.* A three-way ANOVA of the mGFP power of P1m with group, condition, and hemisphere as independent variables revealed a significant effect of condition, $F(1,121) = 20.67$, $p < .01$, but not of group, hemisphere, or any interaction. A three-way ANOVA of the mGFP latency of P1m also revealed a significant effect of condition, $F(1,121) = 126.40$, $p < .01$, but not of group, hemisphere, or any interaction. Larger power and prolonged latency of mGFP in the vowel across-category change condition than in the pure tone condition were revealed.

*MMNm.* A three-way ANOVA of the mGFP power of MMNm with group, condition, and hemisphere as independent variables revealed significant effects of group, $F(1,186) = 7.01$, $p < .01$, and condition, $F(2,186) = 16.52$, $p < .01$, but not of hemisphere or any interaction. The mGFP powers of MMNm in the major depressive disorder patients were significantly smaller than those in the healthy volunteers. The Scheffe's post hoc test clarified that the mGFP powers of MMNm for pure-tone frequency change condition were significantly smaller than those for pure-tone duration change condition, $t(130) = 11.12$, $p < .01$, and vowel across-category change condition, $t(130) = 7.68$, $p < .01$. A post hoc two-way ANOVA of the mGFP power of MMNm with group and hemisphere as independent variables for each condition revealed trend-level significant effects of group in the vowel across-category condition, $F(1,62) = 3.04$, $p = .09$, and in the pure-tone duration change condition, $F(1,62) = 3.52$, $p = .07$, but not in the pure-tone frequency change condition, $F(1,62) = 0.61$, $p = .44$.

A three-way ANOVA of the mGFP latency of MMNm with group, condition, and hemisphere as the independent variables revealed a significant main effect of condition, $F(2,186) = 19.75$, $p < .01$, but not of group, hemisphere, or any interaction. The Scheffe's post hoc test clarified that the MMNm latencies were smaller in the pure-tone frequency change condition than in the pure-tone duration change condition, $t(135) = 30.46$, $p < .01$, and the vowel across-category change condition, $t(135) = 21.35$, $p < .01$.

### *Dipole Analysis*

*P1m.* Reliable ECDs of P1m were successfully estimated in both the left and right hemispheres in 16 out of the 19 healthy

**Table 2.** *Three-Way Factorial ANOVA of mGFP Amplitudes of MMNm and P1m with Group, Condition, and Hemisphere*

| | | Group | Task | Hemisphere | Group × Task | Group × Hemisphere | Task × Hemisphere |
|---|---|---|---|---|---|---|---|
| MMNm | mGFP (fT/cm) | 7.0** | 16.5** | 0.5 | 0.8 | 0.0 | 0.2 |
| | mGFP latency (ms) | 0.1 | 19.7** | 0.3 | 1.8 | 1.2 | 0.2 |
| P1m | mGFP (fT/cm) | 2.2 | 20.7** | 0.2 | 1.2 | 0.9 | 0.1 |
| | mGFP latency (ms) | 0.9 | 126.4** | 3.5 | 0.1 | 1.0 | 0.0 |

*Note:* Group (major depressive disorder patients and healthy volunteers); Condition (vowel across-category change, pure-tone duration change, and frequency change); Hemisphere (right and left):
**$p < .01$.

subjects and in 12 out of 14 major depressive disorder patients and were estimated either in the left or the right hemisphere in 3 out of the 19 healthy subjects and in 3 out of the 14 major depressive disorder patients. A three-way ANOVA of x/mm, y/mm, and z/mm of estimated ECD locations with group, condition, and hemisphere as independent variables demonstrated a significant effect of hemisphere in y/mm, $F(1,118) = 31.93$, $p < .01$, and in z/mm, $F(1,118) = 13.13$, $p < .01$, but not of group, condition, or any interaction. These results indicate that the ECDs were located more anteriorly and superiorly in the left hemisphere than in the right hemisphere.

*MMNm.* Reliable ECDs of MMN1m were successfully estimated both in the left and right hemispheres in 15 out of the 19 healthy subjects and in 8 out of the 14 major depressive disorder patients, and were estimated either in the left or the right hemisphere in only 4 out of the 19 healthy subjects and in 6 out of the 14 major depressive disorder patients. A three-way ANOVA of x/mm, y/mm, and z/mm of estimated ECD locations with group, condition, and hemisphere demonstrated a main effect of condition in y/mm, $F(2,174) = 4.59$, $p = .01$, and hemisphere in y/mm, $F(1,174) = 26.69$, $p < .01$, but not of group or any interaction. The Scheffe's post hoc test clarified that the ECDs were located more anteriorly in the vowel across-category change condition than in the pure-tone duration change condition, $t(126) = 4.55$, $p = .02$, and also were located more anteriorly in the right hemisphere than in the left hemisphere.

*Comparison between P1m and MMNm.* A one-way ANOVA of x/mm, y/mm, and z/mm of estimated ECD locations with component (P1m and MMNm) in each hemisphere revealed a significant main effect of component in z/mm, $F(1,154) = 4.61$, $p = .03$: The MMNm dipole is located inferiorly to the P1m dipole in the left hemisphere.

### *Correlational Analysis*

In the healthy volunteers, the MMNm power in the right hemisphere in the pure-tone duration change condition significantly correlated with age ($\rho = -0.64$, $p < .01$) and the reaction time ($\rho = 0.61$, $p < .01$). The MMNm duration in the left hemisphere in the pure-tone duration change condition significantly correlated with the reaction time ($\rho = -0.71$, $p < .01$). In the major depressive disorder patients, there was no significant correlation of P1m or MMNm power/latency with age, HRSD score, age of onset, reaction time, correct answer rate, or doses of antidepressants, anxiolytics, and hypnotics. The P1m latency in the left hemisphere under the pure-tone condition significantly correlated with illness duration ($\rho = 0.73$, $p < .01$).

## Discussion

### *Summary of Results*

In this study, we investigated MMNm and P1m responses in major depressive disorder patients and healthy volunteers. The results are summarized as follows: (1) mGFP of MMNm was significantly smaller in the major depressive disorder patients than in the healthy volunteers; (2) mGFP of P1m did not differ between the two groups; (3) mGFP of MMNm did not correlate with depressive symptoms, psychotropic medication, age of onset, or illness duration; and (4) the locations of the estimated MMNm and P1m dipoles did not significantly differ between the two groups. These results suggest impaired preattentive information processing in major depressive disorder patients irrespective of the depressive state and psychotropic medication.

### *Comparison with Previous Studies*

As described in the introduction, there has been only one study of MMNm and P1m in major depressive disorder patients using MEG (Kähkönen et al., 2007), and there has been only one study that directly examined MMN in major depressive disorder patients using EEG (Umbricht et al., 2003). Kähkönen et al. found no significant differences in MMN amplitude or latency between major depressive disorder patients and healthy volunteers using pure-tone frequency deviant to elicit MMNm. Umbricht et al. (2003) found no significant differences in MMN amplitude or latency between major depressive disorder patients and healthy volunteers using pure-tone frequency deviant and pure-tone duration deviant to elicit MMNm. In our results, a three-way ANOVA of the mGFP power of MMNm with group, condition, and hemisphere as independent variables revealed significant effects of group, and a post hoc two-way ANOVA of the mGFP power of MMNm with group and hemisphere as independent variables for each condition revealed trend-level significant effects of group in the vowel across-category condition and in the pure-tone duration change condition but not in the pure-tone frequency change condition. This result is partly replicated in previous studies in the sense that the mGFP power and latency of MMNm were not significantly different between the major depressive disorder patients and the healthy volunteers in the pure-tone frequency change condition. However, our results were in disagreement with those of Umbricht et al. (2003) as well in the sense that the mGFP power of MMNm in the pure-tone duration change showed the trend-level significant effects in our study. There was a study showing that attention affects MMN power (Yucel, Petty, McCarthy, & Belger, 2005); thus, the difference in the methods of distracting the subjects' attention from the auditory stimuli may also be responsible for the difference between our results and Umbricht's results, because of the difference in the visual task procedure. However, an obvious difference between our study and their studies was in the physical