across last 10 s of the 30-s pretask period, the post-task baseline was determined as the mean across last 5 s of the 50 and 5-s post-task periods for the cognitive and motor activations according to Suto et al. (2004) and Ito et al. (2005), respectively, and linear fitting was applied to the data between these two baselines. The moving average methods were used to exclude short-term motion artifacts in the analyzed data (moving average window: 1 s).

Data analysis

The waveforms of [oxy-Hb], [deoxy-Hb], and [total-Hb] changes were acquired from all the subjects in all 48 channels during the cognitive and motor activations. NIRS data that clearly contained motion artifacts determined by close observations of the subjects were excluded from further analyses.

The grand averaged waveforms of three types of hemoglobin concentration changes and superimposed individual waveforms of [oxy-Hb] changes were obtained for all the subjects on the basis of individual subjects' waveforms in all 48 channels. These grand averaged waveforms of [oxy-Hb] changes in all 48 channels were also imaged as topographs of [oxy-Hb] changes by the linear compensation method.

[oxy-Hb] data in the channels with low signal-to-noise (S/N) ratios were excluded from further statistical analyses when their standard deviations during the pretask period exceeded 0.040 in any subject group: seven frontal (F1–3, F5, F6, F9, and F11), eight left temporal (L1–5, L7, L9, and L10), and five right temporal (R1, R2, R5, R7, and R12) channels. The S/N ratios of [oxy-Hb] data in these channels positioned over hair-covered areas tended to be low due to the paucity of near-infrared light detected.

[oxy-Hb] data in the three groups were compared in two ways. First, as usually employed in block design experiments, the [oxy-Hb] data were averaged across three task segments (pretask, task, and post-task), analyzed using three-way repeated-measures analysis of variance (ANOVA) with "diagnosis" (healthy controls, patients with bipolar disorder, and patients with major depression), "task segments" (pretask, task, and post-task), and "channels" (28 channels) as independent variables, and compared using post hoc one-way ANOVA. Second, [oxy-Hb] changes were compared between two of the three groups by Welch's t test using the grand averaged waveforms every 0.1 s in each channel. This analysis enabled a more detailed comparison of [oxy-Hb] changes along the time course of the task. The differences were interpreted as meaningful if 30 and 20 consecutive comparison points reached a significance level of 5% among 600 and 400 points during the task periods in the verbal fluency and finger-tapping tasks, respectively, to avoid multiple comparison errors.

The relationships of the [oxy-Hb] changes with the clinical symptoms, medications, and task performances were investigated in the bipolar disorder and major depression groups. For the bipolar disorder group, correlations of the [oxy-Hb] changes during the task period with the HRSD scores, imipramine equivalents of antidepressant dosages (Inagaki et al., 1999, with some modification), lithium dosages, and number of words generated were examined. In addition, multiple regression analyses were performed with the [oxy-Hb] changes as a dependent variable, and the HRSD scores, antidepressant dosages, lithium dosages, number of words generated, and age of the subjects as independent variables for the verbal fluency task, and the HRSD score, antidepressant dosages, lithium dosages, age of the subjects as independent variables for the finger-tapping task. For the major depression group, the same analyses were performed, except that lithium dosages were eliminated from the correlation and multiple regression analyses because only one patient with major depression was treated with lithium. The [deoxy-Hb] data were also analyzed in the same way as the [oxy-Hb] data.

Results

Cognitive activation

The numbers of words generated during the verbal fluency task were not significantly different among the three groups (healthy control: mean, 16.5; SD, 3.6; bipolar disorder: mean, 14.7; SD, 4.4; major depression: mean, 14.2; SD, 5.6; one-way ANOVA $F = 1.15$, $P = 0.33$). The grand averaged waveforms of [oxy-Hb], [deoxy-Hb], and [total-Hb] during the verbal fluency task in the healthy control, bipolar disorder, and major depression groups are shown in Figs. 1–3, respectively. The three sets of waveforms of hemoglobin concentrations enlarged below the figures were from the representative channels, and the superimposed individual waveforms of the [oxy-Hb] changes are shown at the bottom of the figures. The [oxy-Hb] changes during the verbal fluency task can be displayed as the topographs for the three groups, as shown in Fig. 4.

As for [oxy-Hb], the three-way repeated-measures ANOVA revealed a significant main effect of "time segments" ($F = 493.3$, $P < 0.001$) and "channels" ($F = 5.2$, $P < 0.001$); the results of the post hoc one-way repeated-measures ANOVA, in which the pretask and task periods were compared, demonstrated significant [oxy-Hb] increases during the task period: in 16 frontal (F4, F7, F8, F10, and F13–24) channels and two left (L8 and L11) and four right temporal (R8–11) channels in the healthy control group; in 16 frontal (F4, F7, F8, F10, and F13–24) channels and four left (L6, L8, L11, and L12) and six right temporal (R3, R6, and R8–11) channels in the bipolar disorder group; and in six frontal (F14, F17, F18, F19, F21, and F22) channels and one left temporal (L8) channel in the major depression group. The main effect of "diagnosis" was also significant ($F = 46.4$, $P < 0.001$), and the post hoc one-way ANOVA clarified that the [oxy-Hb] increases during the task period in the major depression group were smaller than those in the bipolar disorder (F20 and F23) and healthy control (L8) groups. The interactions of "time segments" and "diagnosis" ($F = 34.3$, $P < 0.001$), and "time segments" and "channels" ($F = 4.2$, $P < 0.001$) were significant, but the interactions of "diagnosis" and "channels" ($F = 0.6$, $P = 0.987$), and "time segments", "diagnosis", and "channels" ($F = 0.6$, $P = 0.994$) were not significant. These significant effects were similarly observed when the [oxy-Hb] changes during the task period were divided into three time segments of 20 s (the early, middle, and late task segments).

The results of the t test for the between-group comparison of the [oxy-Hb] changes during the cognitive activation are shown in Fig. 5, and some of these results are shown in Fig. 6 in the form of topographs. Compared with those in the healthy control group, the [oxy-Hb] increases in the bipolar disorder group were significantly smaller during the early task period in six lower frontal (F13, F16, F18, F20, F21, and F24) channels and two left lower anterior temporal (L8 and L12) channels, but were significantly larger during the late task period in four frontal (F14, F15, F20, and F23) channels. When compared with those in the major depression group, the [oxy-Hb] increases in the bipolar disorder group were

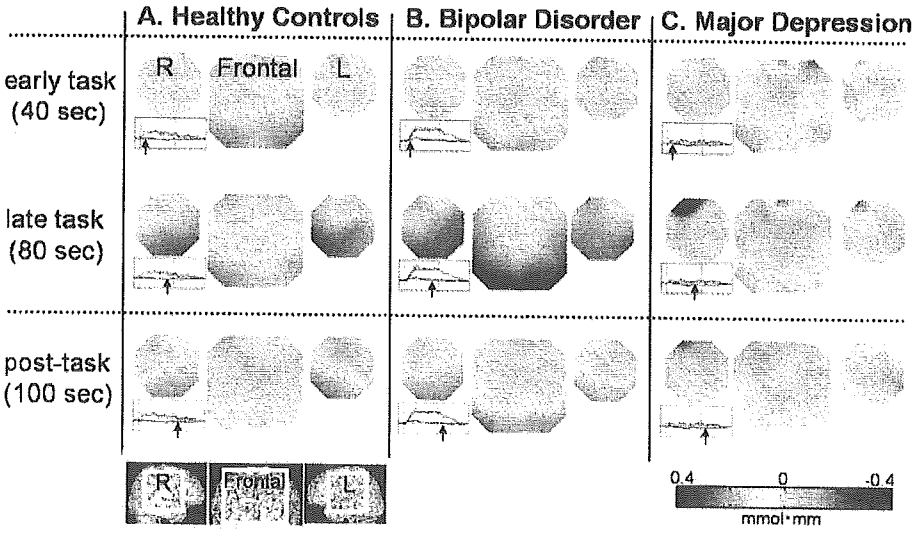

Fig. 4. Topographical presentation of [oxy-Hb] changes in the three groups during cognitive activation. The [oxy-Hb] changes in the healthy control (A, left), bipolar disorder (B, center), and major depression (C, right) groups are presented as topographical maps along the time course of the task (from top to bottom). Each set of topographical maps is composed of three maps corresponding to the frontal and bilateral temporal probes. The times from the task start are presented in seconds on the left side and are indicated by arrows on the representative grand averaged waveforms of hemoglobin concentration in each group, and the red, green, and blue areas in the topographs indicate an increase, no change, and a decrease in [oxy-Hb], respectively. Topographical maps do not exclude data of channels with low signal-to-noise ratios for general view of the activation.

significantly larger during the late task period in 14 frontal (F4, F7, F8, F10, F12, F14, and F16–23) channels and four left (L6, L8, L11, and L12) and four right anterior temporal (R3, R6, R9, and R11) channels, and were significantly smaller during the early task period in one frontal (F18) channel. Additionally, the [oxy-Hb] increases in the major depression group were significantly smaller than those in the healthy control group in eight frontal (F4, F10, F12, F13, F16, F20, F23, and F24) channels and four left (L6, L8, L11, and L12) and four right lower anterior temporal (R6, R8, R9, and R11) channels mainly during the early task period.

In the healthy control group (Fig. 1), [oxy-Hb] rapidly increased immediately after the start of the task period, remained activated during the task period, and then decreased gradually after the end of the task. Such [oxy-Hb] increases during the task period were clearly observed in the lower frontal channels. In the bipolar disorder group (Fig. 2), [oxy-Hb] gradually increased after the start of the task period, reached its peak with a comparable magnitude to but with a longer latency than that in the healthy control group, and then decreased gradually in the post-task period, mainly in the lower frontal and bilateral anterior temporal channels. In the major depression group (Fig. 3), rapid but small increases in [oxy-Hb] were observed after the start of the task period, but the subsequent [oxy-Hb] increases during the task period were smaller.

As for [deoxy-Hb], the three-way repeated-measures ANOVA revealed a significant main effect of "time segments" ($F = 85.1$, $P < 0.001$) and "channels" ($F = 2.8$, $P < 0.001$); the results of the post hoc one-way repeated-measures ANOVA, which the pretask and task periods were compared, demonstrated significant [deoxy-Hb] changes during the task period: decreases in four frontal (F14, F17, F22, and F24) channels and three left (L8, L11, and L12) and two right temporal (R8 and R11) channels in the healthy control group; decreases in one frontal (F14) channel and one left temporal (L8) channel and increases in one right temporal (R4) channel in the bipolar disorder group; and decreases in three frontal (F15, F17, and F20) channels in the major depression group. The main effect of "diagnosis" was also significant ($F = 6.7$, $P = 0.001$), and the post hoc one-way ANOVA clarified that the degrees of [deoxy-Hb] decreases during the task period in the bipolar disorder group were smaller than those in the healthy control group (F17 and F20). The interactions of "time segments" and "diagnosis" ($F = 9.3$, $P < 0.001$), and "time segments" and "channels" ($F = 2.2$, $P < 0.001$) were significant, but the interactions of "diagnosis" and "channels" ($F = 0.9$, $P = 0.669$), and "time segments", "diagnosis", and "channels" ($F = 0.9$, $P = 0.710$) were not significant.

Motor activation

The grand averaged waveforms of [oxy-Hb], [deoxy-Hb], and [total-Hb] changes during the finger-tapping task are shown in Fig. 7. As for [oxy-Hb], the three-way repeated-measures ANOVA revealed a significant main effect of "time segments" ($F = 493.3$, $P < 0.001$) and "channels" ($F = 5.2$, $P < 0.001$); the results of the post hoc one-way repeated-measures ANOVA, in which the pretask and task periods were compared, demonstrated significant [oxy-Hb] increases during the task period: in 13 frontal (F8, F12–16, and F18–24) channels and seven right temporal (R3, R4, R6, and R8–11) channels in the healthy control group; in seven frontal (F13, F15, F16, F20, and F22–24) channels and one left (L6) and three right temporal (R3, R4, and R6) channels in the bipolar disorder group; and in 13 frontal (F4, F7, F12–14, F16–20, and F22–24) channels and one left (L6) and three right temporal (R3, R6, and R9) channels in the major depression group. The main effect of "diagnosis" was significant ($F = 46.4$, $P < 0.001$), and the post hoc one-way ANOVA clarified that the [oxy-Hb] increases during the task period in the bipolar disorder group were smaller than those in the healthy control (R11) and major depression (R9) groups. The interaction of "task segments" and "diagnosis" was significant in one right temporal (R11) channel. The t test results were consistent with the results of ANOVA mentioned above.

As for [deoxy-Hb], the three-way repeated-measures ANOVA revealed a significant main effect of "time segments" ($F = 19.7$, $P < 0.001$) and "channels" ($F = 1.5$, $P = 0.048$). The results of

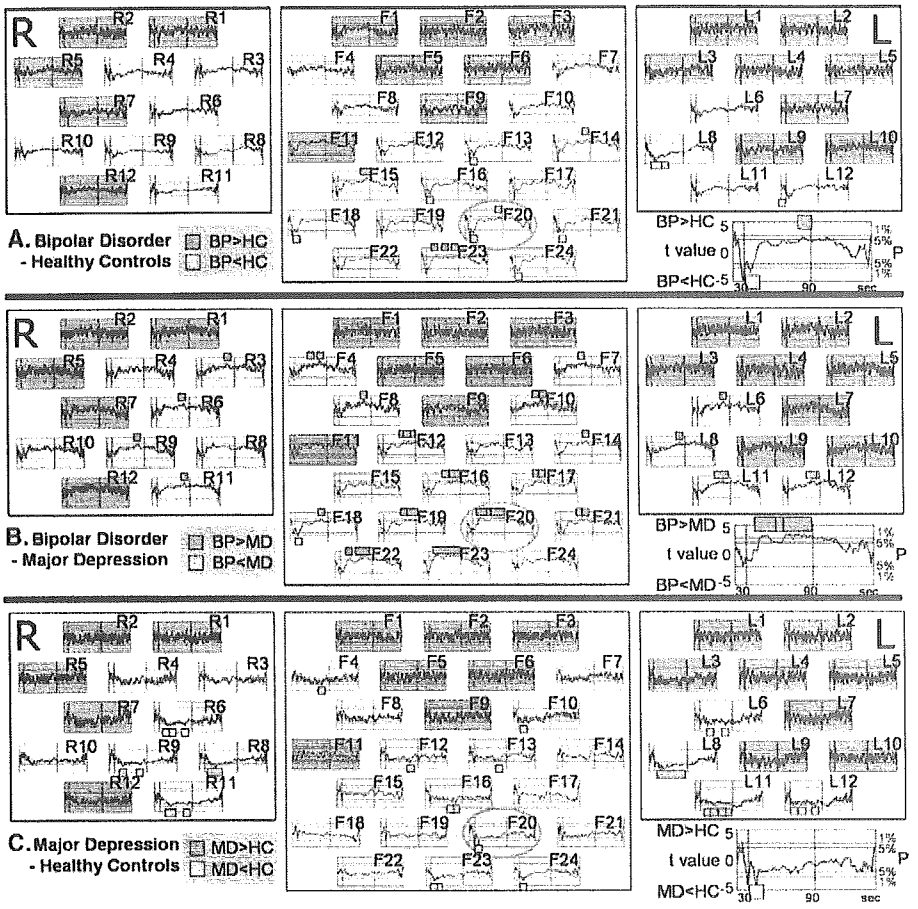

Fig. 5. t value graphs of [oxy-Hb] comparison between the groups during cognitive activation. BP, bipolar disorder group; MD, major depression group; HC, healthy control group. The t values of [oxy Hb] comparison between the bipolar disorder and healthy control groups (A, upper), between the bipolar disorder and major depression groups (B, middle), and between the major depression and healthy control groups (C, bottom) in 48 channels are presented, as shown in Fig. 1. The channels with low signal-to-noise ratios were presented with gray meshing. Three graphs (circled in orange) are enlarged below as typical representatives. The blue and red lines in each t graph correspond to the statistical significance levels of 5% and 1%, respectively, and the times with significant differences in each graph are marked orange (yellow) when the [oxy-Hb] changes in the bipolar disorder group were larger (smaller) than those in the healthy control groups in panel A, when the [oxy-Hb] changes in the bipolar disorder group were larger (smaller) than those in the major depression group in panel B, and when the [oxy-Hb] changes in the major depression group were larger (smaller) than those in the healthy control group in panel C.

the post hoc one-way repeated-measures ANOVA, which the pretask and the task periods were compared, demonstrated significant [deoxy-Hb] changes during the task period: increases in one frontal (F23) channel and one right temporal (R8) channel in the bipolar disorder group, and increases in one left (L12) and one right temporal (R11) channels and decreases in one right temporal (R3) channel in the major depression group. The interaction of "time segments" and "channels" (F = 1.6, P = 0.009) was also significant. The main effect of "diagnosis" (F = 2.0, P = 0.130) and the interactions of "time segments" and "diagnosis" (F = 1.4, P = 0.233), "diagnosis" and "channels" (F = 0.8, P = 0.774), and "time segments", "diagnosis", and "channels" (F = 0.9, P = 0.768) were not significant.

Correlation with clinical symptoms and medications

As for the verbal fluency task, the [oxy-Hb] changes were not significantly correlated with the HRSD scores and antidepressant dosages in all the channels in both the bipolar disorder and major depression groups, and were not significantly correlated with the task performance in all the channels in all the three groups even when significance level corrections for multiple correlations were not employed. The [oxy-Hb] changes were significantly correlated with the lithium dosages in two right temporal channels (R9, Spearman's rho = 0.50, P = 0.043; R11, *rho* = 0.56, P = 0.019) in the bipolar disorder group when significance level corrections were not employed. However, no significant correlations were obtained in the multiple regression analyses including the HRSD scores, antidepressant dosages, lithium dosages, number of words generated, and age of the subjects in both the bipolar disorder and major depression groups.

The [deoxy-Hb] changes were significantly correlated with the HRSD scores in one frontal channel (F20, rho = −0.50, P = 0.041) and one right temporal channel (R11, rho = −0.52, P = 0.031), and with the lithium dosages in one right temporal channel (R3, rho = 0.49, P = 0.045), but were not significantly correlated with the antidepressant dosages and task performance in the bipolar disorder group when significance level corrections were not employed. However, no significant correlations were obtained in the multiple regression analyses in the bipolar disorder group. The [deoxy-Hb] changes were significantly correlated with the antidepressant dosages in four frontal channels (F7, rho = 0.64, P =

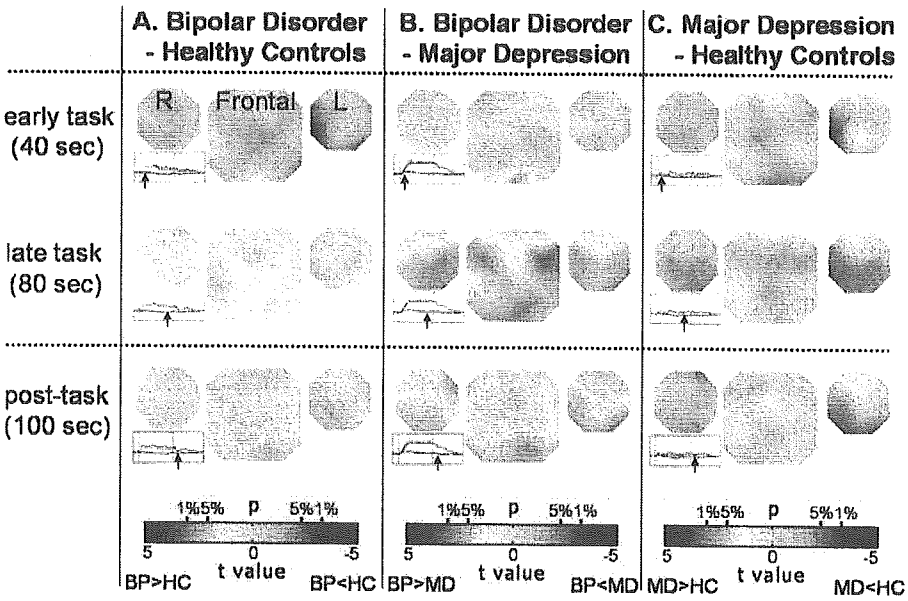

Fig. 6. Topographical presentation of t value of [oxy-Hb] comparison between the groups during cognitive activation. BP, bipolar disorder group; MD, major depression group; HC, healthy control group. The t values of [oxy-Hb] for the bipolar disorder and healthy control groups (A, left), the bipolar disorder and major depression groups (B, center), and the major depression and healthy control groups (C, right) are presented as topographical maps along the time course of the task (from top to bottom). Each set of topographical maps is composed of three maps corresponding to the frontal and bilateral temporal probes. The times from the task start are presented in seconds on the left side and are indicated by arrows on the representative grand averaged waveforms of hemoglobin concentration in each group. The red, green, and blue areas in the topographs indicate positive, zero, and negative t values, with 2.0 and 2.7 for the statistical significance levels of 5% and 1%, respectively, in panel A, and with 2.1 and 2.8 for the statistical significance levels of 5% and 1%, respectively, in panels B and C. Topographical maps do not exclude data of channels with low signal-to-noise ratios for general view of the activation.

0.033; F18, rho = 0.62, $P = 0.043$; F21, rho = 0.60, $P = 0.049$; F24, rho = 0.83, $P = 0.002$) and with the task performance in one frontal channel (F22, rho = −0.61, $P = 0.047$), but were not significantly correlated with the HRSD scores in the major depression group when significance level corrections were not employed. The significant correlations were obtained in the multiple regression analyses in one frontal channel (F24, $R = 0.87$, $P = 0.043$): the significant variable was the antidepressant dosages (beta = −0.83, $P = 0.036$) in the major depression group.

As for the finger-tapping task, the significant correlations in [oxy-Hb] were obtained in the multiple regression analyses in one right temporal channel (R10, $R = 0.73$, $P = 0.041$): the significant variables were the lithium dosages (beta = −0.48, $P = 0.046$) and age (beta = 0.61, $P = 0.012$) in the bipolar disorder group. The significant correlations in [deoxy-Hb] were obtained in the multiple regression analyses in one right temporal channel (R11, $R = 0.78$, $P = 0.016$): the significant variables were the HRSD scores (beta = 0.49, $P = 0.031$), antidepressant dosages (beta = −0.47, $P = 0.028$), and age (beta = −0.57, $P = 0.010$) in the bipolar disorder group. However, no significant correlations were obtained in both [oxy-] and [deoxy-Hb] in the multiple regression analyses in the major depression group.

Discussion

Summary of obtained results

In the present study, the rCBV changes during the cognitive and motor tasks in the bipolar disorder group were compared with those in the healthy control and major depression groups. rCBV increases were successfully demonstrated by NIRS during both the cognitive and motor activations in all the three groups. Their time courses were different among the three groups: the [oxy-Hb] increases in the bipolar disorder group were smaller in the early task period but larger in the late task period than those in the healthy control group, and were comparable to those in the early task period but larger than those in the late task period in the major depression group. The differences in the time courses of the [oxy-Hb] changes were found specifically during the verbal fluency task but not during the finger-tapping task. These [oxy-Hb] changes during the verbal fluency task reflected the cognitive activation in the frontal lobe and the finger-tapping task cognitively undemanding activation. On the other hand, the number of channels with significant changes of [deoxy-Hb] during the task period was smaller than that with significant changes of [oxy-Hb] in any group, probably due to the lower S/N ratios in [deoxy-Hb] than in [oxy-Hb]. Cognitive activation was accompanied consistently by increases in [oxy-Hb] but inconsistently by decreases or increases in [deoxy-Hb]. The discrepancy in the changes in [oxy-Hb] and [deoxy-Hb] corresponds to the findings of Ehlis et al. (2005) that decreases in [deoxy-Hb] during cerebral activation are not as consistently observed increases in [oxy-Hb], and that the changes in [deoxy-Hb] are to be interpreted carefully.

Three points should be noted regarding the task parameters used in this study. First, the task period was relatively long in the present study. Most previous NIRS studies used a short task period for the finger-tapping and verbal fluency tasks (i.e., 10–30 s Colier et al., 1999; Maki et al., 1995; Obrig et al., 1996; Watanabe et al., 1998); on the other hand, some studies used a 60-s task period (Hirth et al., 1997; Hock et al., 1997; Kameyama et al., 2004; Matsuo et al., 2000, 2002, 2004, 2005; Suto et al., 2004). We adopted longer task periods (40 s for the finger-tapping task and 60 s for the verbal fluency task) to examine in detail the time course of

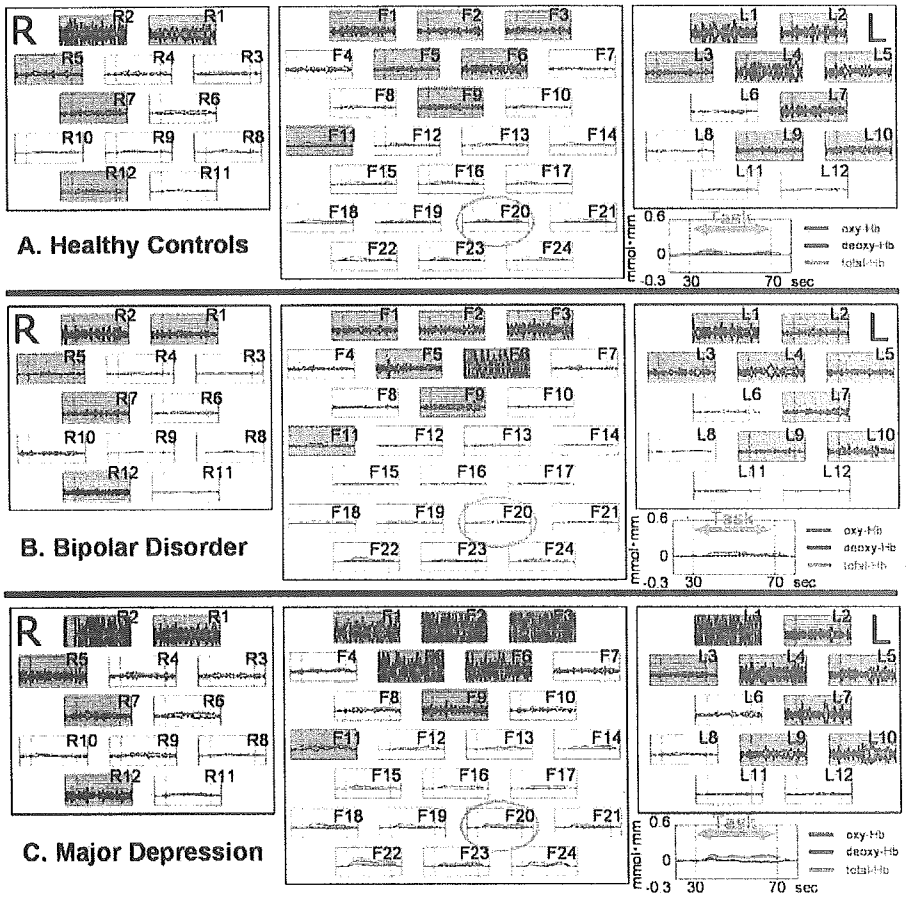

Fig. 7. Grand averaged waveforms of hemoglobin concentration changes during motor activation in the three groups. Grand averaged waveforms of [oxy-Hb] (red line), [deoxy-Hb] (blue line), and [total-Hb] (green line) during motor activation (between two vertical dotted lines) measured by the frontal (center) probe and the left (right) and right temporal (left) probes in the healthy control group (A, upper), bipolar disorder (B, middle), and major depression (C, bottom) groups. The channels with low signal-to-noise ratios were presented with gray meshing. The grand averaged waveform hemoglobin concentration changes in the representative channels (circled in orange) are enlarged below.

cerebral activation. Second, a modified version of the verbal fluency task was adopted: the syllables assigned were changed every 20-s during the 60-s task period in the verbal fluency task to decrease the time during which the subjects were silent. This task procedure not only contributed to the minimization of poor cerebral activation caused by the subjects' silence, but also made the task easier, particularly for clinical patients. The modification could result in no significant differences in task performance among the groups. Third, word repetition was performed in the pretask baseline period instead of keeping silent as in many other studies. This procedure enabled the exclusion of the effect of simple utterance from cerebral activation in word generation.

[oxy-Hb] changes

The discrepancies in the results of previous studies of the bipolar disorder described in the Introduction could be due to the differences in their task designs employed: the duration of the task (30–60 s), number of task repetitions (1–10 times), and baseline conditions before and after the task period (rest vs. word repetition). Among these three factors, the baseline condition could be the most critical because frontal lobe hyperactivity was observed when the subjects were required to perform some simple tasks during the baseline periods (Curtis et al., 2001; Chang et al., 2004), while frontal lobe hypoactivity was observed when the subjects were resting without performing any task during the baseline periods (Matsuo et al., 2002, 2004; Yurgelun-Todd et al., 2000). The larger [oxy-Hb] increases observed in the bipolar disorder group in the present study are consistent with this baseline condition hypothesis. The mechanisms underlying frontal lobe hyperactivity due to the simple task during the baseline periods could be that a simple task during the pretask period could (1) accelerate the following activation or (2) reduce the frontal activity level by relaxing the patients with bipolar disorder much more than the healthy controls.

Another factor of the task design that can influence the frontal activation in bipolar disorder is the initial syllable designation in the verbal fluency task. In the present study, the initial syllables were changed every 20 s during the 60-s task to decrease the time during which the subjects remained silent, while in Matsuo's (2002) study, the initial syllables were unchanged throughout the 60-s task period, resulting in a smaller number of generated words (means: 11.2, 9.2, and 12.4 for the healthy control, bipolar disorder, and major depression groups, respectively) than that in the present study (means: 16.5, 14.7, and 14.2, respectively). Better performances in the present study, particularly in the later task period, may result in a larger activation in the bipolar disorder group. This interpretation is supported by the differences in the

time course of [oxy-Hb] changes between the bipolar disorder and healthy control groups: the results of t test, in which the two groups were compared every 0.1 seconds, revealed significant differences in [oxy-Hb] increases mainly in the later task period.

The baseline condition and initial syllable designation mentioned above can explain the results in the bipolar disorder group but not those in the major depression group. The frontal lobe hypoactivity in the major depression group has been consistently demonstrated irrespective of the baseline condition of the task employed and was also demonstrated in the present study even with task performances comparable to those in the healthy control and bipolar disorder groups. The differences in [oxy-Hb] increases between the bipolar disorder and major depression groups, therefore, could be interpreted to reflect the differences in frontal lobe function between these two groups: the frontal lobe function is potentially preserved in terms of its reactivity, but it is difficult to be activated in the bipolar disorder group and shows markedly reduced reactivity in the major depression group. The decreased reactivity in the major depression group was consistent with the results of previous studies (reviewed by Drevets, 2000; Malhi et al., 2004b; Rogers et al., 2004). The difference in frontal lobe reactivity between the bipolar disorder and major depression groups can be employed for differential diagnosis between bipolar disorder and major depression in clinical settings in the future.

Correlations with clinical symptoms and medications

Clinical symptoms, antidepressant medications, and age were generally not related to the altered frontal lobe activation in both the bipolar disorder and major depression groups in the present study. Lithium medication was correlated only in a temporal channel in the bipolar disorder group. The findings regarding clinical symptoms are consistent with the results of some previous activation studies that found no correlation between frontal lobe activation and clinical symptoms (major depression, Okada et al., 1996; bipolar disorder, Yurgelun-Todd et al., 2000), but they are inconsistent with the results of a study that found significant correlations (major depression, Fu et al., 2004). Future studies are warranted to clarify the influences of clinical symptoms and medications on frontal lobe activation by examining the same patient population longitudinally, because these correlations in the present study were examined across many subjects.

Limitation of the study

There are three points that should be improved in the present study. The first point is the limited cerebral regions that could be measured using NIRS probes. A considerable nonmeasured area existed between the areas covered by the frontal and temporal probes due to their arrangement on the skull, which prevented the examination of the lower posterior frontal cortex. In addition, 20 of the 48 channels in the upper frontal and upper posterior temporal probes had to be excluded from detailed analyses because of their low S/N ratios, which prevented the examination of some parts of the upper frontal, parietal, and temporal cortices. The second point is the subjects' characteristics. The sample sizes were small, the sex ratios were somewhat skewed, and the patients' symptoms were rather mild. The mild symptomatology may contribute to the absence of significant correlations between clinical symptoms and frontal lobe activation. The third point is that all the patients were on medications at the time of the examination. Although there were no significant correlations between the antidepressant or lithium dosages and frontal lobe activation in the present study, the possibility remains: a part of the observed findings could result from the effects of the psychotropic drugs the patients were taking, for example, the anticholinergic effects of the antidepressant drugs or the influences of different lithium dosages between the bipolar disorder and major depression groups. Further studies are required to improve these three points.

In conclusion, bipolar disorder and major depression were characterized by preserved but delayed and reduced frontal lobe activation patterns, respectively, in the present study by multichannel NIRS with a high time resolution. NIRS with its noninvasiveness and high time resolution could be a useful research tool for examining in detail the time courses of brain activation in mood disorders, as well as a clinically useful tool for the differential diagnosis of patients with bipolar disorder and those with major depression in the near future.

Acknowledgments

This research was supported in part by a Grant-in-Aid for Scientific Research (C) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MF), a Health and Labor Sciences Research Grant for Research on Psychiatric and Neurological Diseases and Mental Health (MF), and a Health and Labor Sciences Research Grant for Special Research from the Japanese Ministry of Health, Labor and Welfare (MM).

We thank Dr. Itsuro Ida, Dr. Akihiko Oshima, and Mr. Suguru Hattori of the Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine, for collaboration.

The preliminary results of this study were presented in the 59th Annual Meeting of the Society of Biological Psychiatry on April 29–May 1, 2004 in New York, U.S.A.

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Neuropsychopharmacology (2006) 31, 212–220

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Assessment of the Dexamethasone/CRH Test as a State-Dependent Marker for Hypothalamic-Pituitary-Adrenal (HPA) Axis Abnormalities in Major Depressive Episode: A Multicenter Study

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There is compelling evidence for the involvement of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in depression. Growing evidence has suggested that the combined dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test is highly sensitive to detect HPA axis abnormalities. We organized a multicenter study to assess the DEX/CRH test as a state-dependent marker for major depressive episode in the Japanese population. We conducted the DEX/CRH test in 61 inpatients with major depressive episode (Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV)) and 57 healthy subjects. In all, 35 patients were repeatedly assessed with the DEX/CRH test on admission and before discharge. The possible relationships between clinical variables and the DEX/CRH test were also examined. Significantly enhanced pituitary–adrenocortical responses to the DEX/CRH test were observed in patients on admission compared with controls. Such abnormalities in patients were significantly reduced after treatment, particularly in those who underwent electroconvulsive therapy (ECT) in addition to pharmacotherapy. Age and female gender were associated with enhanced hormonal responses to the DEX/CRH test. Severity of depression correlated with DEX/CRH test results, although this was explained, at least in part, by a positive correlation between age and severity in our patients. Medication *per se* was unrelated to DEX/CRH test results. These results suggest that the DEX/CRH test is a sensitive state-dependent marker to monitor HPA axis abnormalities in major depressive episode during treatment. Restoration from HPA axis abnormalities occurred with clinical responses to treatment, particularly in depressed patients who underwent ECT.
Neuropsychopharmacology (2006) **31,** 212–220. doi:10.1038/sj.npp.1300868; published online 17 August 2005

Keywords: hypothalamic-pituitary-adrenal axis; dexamethasone/CRH test; depressive disorder; electroconvulsive therapy; cortisol; ACTH

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Received 29 March 2005; revised 9 June 2005; accepted 10 June 2005
Online publication: 14 July 2005 at http://www.acnp.org/citations/Npp071405050209/default.pdf

INTRODUCTION

There is compelling evidence for an important role of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in the pathophysiology of mood disorders (Holsboer, 1995; Plotsky *et al*, 1998). To quantify the dysregulation of HPA axis, the dexamethasone (DEX) suppression test (DST) has

been studied most extensively since Carroll *et al* (1981) standardized it as a biological marker for the diagnosis of melancholia. A major drawback of the DST, however, is its modest sensitivity (rate of nonsuppression of cortisol) of 40–50% (Carroll, 1982; American Psychiatric Association, 1987). Subsequently, a refined laboratory test that combines the DST and corticotropin-releasing hormone (CRH) challenge, the DEX/CRH test, has been introduced (Holsboer *et al*, 1987; von Bardeleben and Holsboer, 1989). In normal subjects, pretreatment with DEX suppresses pituitary–adrenal responses to CRH. In depressed patients, however, the same procedure enhances hormonal responses to CRH, resulting in higher sensitivity of the DEX/CRH test to major depression by up to 80% (Heuser *et al*, 1994b). Furthermore, the DEX/CRH test was shown to be more closely associated with the activity of HPA system (24-h cortisol profiles) than the standard DST in healthy and depressed subjects (Deuschle *et al*, 1998). We have confirmed the relatively high sensitivity of the DEX/CRH test in Japanese patients with depressive disorder (Oshima *et al*, 2000; Kunugi *et al*, 2004). These findings indicate that the DEX/CRH test is a useful laboratory tool to monitor HPA axis abnormalities in depressed patients in clinical practice. However, previous studies have not always provided consistent results as to whether clinical variables, such as age, severity, and diagnosis (unipolar *vs* bipolar), are associated with DEX/CRH test results.

In serial DST studies, conversion from nonsuppressor to suppressor status is temporally associated with clinical responses to antidepressants (eg Holsboer *et al*, 1982; Greden *et al*, 1983). In line with this, hormonal responses to the DEX/CRH test also restored after successful treatment with antidepressants (Holsboer-Trachsler *et al*, 1991; Heuser *et al*, 1996; Baghai *et al*, 2002; Hatzinger *et al*, 2002), suggesting that the DEX/CRH test is a useful state-dependent marker. However, it has been argued that DEX/CRH test results may be trait dependent, particularly in bipolar patients (Holsboer *et al*, 1995; Schmider *et al*, 1995; Watson *et al*, 2004; Ising *et al*, 2005).

Changes in repeated DEX/CRH tests may depend on treatment modality. In responders to repetitive transcranial magnetic stimulation therapy, post-DEX cortisol levels prior to CRH challenge decreased, while no change of CRH-induced ACTH and cortisol release was observed (Zwanzger *et al*, 2003). Similarly, partial changes in DEX/CRH tests were observed in responders to partial sleep deprivation therapy (Schule *et al*, 2002). Concerning electroconvulsive therapy (ECT), there is little information on changes in the DEX/CRH test. Previous studies employing conventional DST have provided inconsistent results: some authors reported a positive correlation between response to ECT and normalization of the HPA axis (Albala *et al*, 1981; Papakostas *et al*, 1981; Grunhaus *et al*, 1987; Devanand *et al*, 1991); an author reported an equivocal result (Devanand *et al*, 1987); and others failed to find such a relationship (Coryell, 1986; Fink *et al*, 1987). These inconsistent results require further investigations.

The present study aimed to assess the DEX/CRH test as a state-dependent marker to monitor HPA axis abnormalities in patients with major depressive episode, including patients receiving ECT. Furthermore, we examined DEX/CRH test results for a possible association with clinical variables, such as age, gender, severity, and medication.

METHODS

Subjects

Subjects were 57 healthy controls (37 men and 20 women; mean age: 31 years, range: 22–48, standard deviation (SD): 7) and 61 inpatients (24 men and 37 women; mean age: 54 years, range 30–82, SD: 13) with major depressive episode according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) (American Psychiatric Association, 1994), who were recruited from eight institutions in Japan. All the patients and controls were biologically unrelated Japanese. In all, 54 patients were diagnosed with unipolar major depression (29 with single episode and 25 with recurrent episodes), and the remaining seven with bipolar I disorder. The following were excluded: patients who had somatic disorders, such as inflammation, endocrine disorders, and neoplasm; patients who were treated with liver-enzyme-inducing drugs, lithium carbonate, or carbamazepine; and patients who were withdrawn from abused illicit drugs and other substances, such as benzodiazepines and alcohol. After full description of the study, written informed consent was obtained from every subject for his/her participation in the study. The study protocol was approved by the ethics committee of each institution.

Among the 61 patients, 35 (13 men and 22 women; 13 unipolar depression with single episode, 18 unipolar depression with recurrent episodes, and four bipolar disorder; mean age: 55 years, SD: 15) were repeatedly assessed with the DEX/CRH test on admission and before discharge, while the remaining 26 were assessed only once on admission. Among the 35 patients, 12 underwent ECT in addition to pharmacotherapy. Based on the effect size reported by Holsboer-Trachsler *et al* (1991), this sample size ($N=35$) had a power of 96% at the 0.05 level of significance (two-tailed) to detect effects of treatment on DEX/CRH test results.

The severity of index depressive episode was assessed with the 21-item version of the Hamilton depression rating scale (HDRS; Hamilton, 1967) when the DEX/CRH test was conducted. Patients who were scored less than 15 by the HDRS on admission were not enrolled in the study. The majority of the patients were medicated with antidepressants, while seven patients were drug naive on admission. We did not control for class of antidepressant medication, and data were obtained in the ordinary clinical setting in Japan. Thus, a variety of antidepressants were medicated; however, switch in medication was avoided for at least 4 days before conducting the DEX/CRH test. ECT was administered six to 10 times (two or three times a week) for each patient with electrical stimuli of 100–110 V for 5–10 s, discharged with bilateral electrodes placed bifrontotemporally. Patients were anesthetized with propofol, and motor convulsion was suppressed with succinylcholine.

DEX/CRH Test

The DEX/CRH test was conducted as described by Zobel *et al* (2001). Subjects were pretreated with an oral dose of

1.5 mg of DEX (Asahikasei Pharmaceutical Corporation, Tokyo, Japan) at 2300 hours. On the next day, a vein was cannulated at 1430 hours to collect blood at 1500, 1530, 1545, 1600, and 1615 hours via an intravenous catheter. Human CRH (100 μg) (hCRH 'Mitsubishi', Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 1500 hours immediately after the first blood collection (protocol 1). In part of patients ($N=8$), a vein was cannulated at 1330 hours to collect blood at 1400, 1415, 1430, 1500, and 1600 hours (protocol 2). hCRH was administered intravenously immediately after the first blood collection at 1400 hours. Subjects rested supine throughout the test in a calm room. Between the collections of blood specimen, the catheter was kept patent by physiological saline infusion at a rate of 50 ml/h. Blood samples were immediately centrifuged and stored at −20°C. Plasma concentrations of ACTH and cortisol were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limits for ACTH and cortisol were 5.0 pg/ml and 1.0 μg/dl, respectively.

Data Presentation for Hormonal Measures

We defined A_0 as the plasma concentration of ACTH in the blood sample obtained at 1500 hours for protocol 1 or 1400 hours for protocol 2, that is, plasma concentrations that were measured at 16 or 15 h after the oral intake of DEX but immediately before the infusion of CRH. A_{30} and A_{60} were defined as the plasma concentrations of ACTH, which were measured at 30 and 60 min after the intravenous infusion of CRH, respectively. A_{dif} denotes the difference between the A_0 and A_{60} values. The C_0, C_{30}, C_{60}, and C_{dif} values were defined similarly for the plasma concentrations of cortisol. These values were available in both protocols 1 and 2, which enabled us to combine data from these protocols and to analyze all the subjects simultaneously. There was no evidence for significant effects on hormonal outcomes due to a 1 h difference between the two protocols (data not shown), combining the data from the two protocols. According to our previous reports (Oshima *et al*, 2000; Kunugi *et al*, 2004), the plasma concentrations of ACTH and cortisol peak at approximately 60 min after infusion of CRH, indicating that the A_{60} and C_{60} values reflect the maximal responses to CRH. The area under the time curve (AUC, arbitrary unit) was calculated according to the trapezoidal rule (A_{auc} for ACTH and C_{auc} for cortisol). A_{auc} was calculated from the A_0, A_{30}, and A_{60} values, and C_{auc} from the C_0, C_{30}, and C_{60} values.

As the plasma cortisol criterion concentration of 5 μg/dl was suggested to be most effective for assessing an abnormal DST result (Carroll, 1982), 'nonsuppressor' was defined to be an individual who showed a C_0 value of $\geqslant 5$ μg/dl irrespective of the C_{60} value. Furthermore, 'intermediate suppressor' was defined *a priori* to be an individual who showed a C_0 value of <5 μg/dl and a C_{60} of $\geqslant 5$ μg/dl. The remaining individuals, who showed both C_0 and C_{60} values of <5 μg/dl, were defined as 'suppressors'. These definitions were almost identical to those described by Kunugi *et al* (2004).

Statistical Analysis

All statistical analyses were made with SPSS for Windows (version 11, SPSS Japan, Tokyo). Intergroup comparisons were made for age and HDRS scores according to *t*-test or one-way ANOVA. The proportion of categorical data, such as gender, was compared according to the χ^2 test for independence. Intergroup comparisons were made for ACTH and cortisol according to the Mann–Whitney *U*-test (comparisons between two groups) or the Kruskal–Wallis *H*-test (comparisons among three groups). Differences between hormonal responses observed on admission and before discharge were tested according to the Wilcoxon paired signed-ranks test. Nonparametric tests were employed because the endocrine values did not always show a normal distribution and part of subjects showed the A_0 and/or C_0 values below detection limits. All *p*-values reported are two-tailed. A value of $p<0.05$ was considered statistically significant.

RESULTS

Hormonal Responses in Patients and Controls

Effects of age and gender. Hormonal responses in patients (on admission) and controls are shown in Table 1. To see the effects of age on hormonal responses, the patient group was dichotomized into the 'young' ($\leqslant 50$ years) and 'aged' (>50 years) patient groups. All controls, whose age ranged between 22 and 48 years, were considered to be 'young' subjects. To see the effects of gender, hormonal values were presented separately by gender. In the patient group, all the mean hormonal values (A_0, A_{60}, A_{dif}, A_{auc}, C_0, C_{60}, C_{dif}, and C_{auc}) were higher in the aged patient group than in the young patient group, although a statistically significant difference was found only for A_0 ($p=0.048$, Mann–Whitney *U*-test) and a nonsignificant trend for C_{auc} ($p=0.055$).

When males and females were compared, hormonal responses to pretreatment with DEX (A_0 and C_0) did not seem to be consistently different in young patients, aged patients, or controls (see Table 1). However, hormonal responses to CRH as manifested by A_{dif} and C_{dif} were consistently higher in females than in males for all the three groups (young patients, aged patients, and controls). Among young patients, C_{dif} was significantly higher in females than in males ($p=0.029$, Mann–Whitney *U*-test), although differences in A_{dif} did not reach statistical significance ($p=0.17$). Similarly, in aged patients, the mean C_{dif} value was significantly higher in females than in males ($p=0.034$). However, differences in mean A_{dif} failed to reach statistical significance ($p=0.14$). Among subjects in the control group, females showed statistically higher A_{dif} values than males, and there was a trend towards an increased C_{dif} value in females than in males.

Patients vs *controls.* Since the above data indicated that age and gender have effects on the DEX/CRH test, young patients (14 men and eight women) only were compared with controls (37 men and 20 women). These two groups were nearly matched for the ratio of males to females ($p=0.91$, χ^2 test), although there was still a significant difference in mean age (41 years SD 7 *vs* 31 years SD 7;

Table 1 Hormonal Responses to the DEX/CRH Test in Patients (on Admission) and Controls (Mean ± SD)

		ACTH (pg/ml)				Cortisol (μg/dl)			
	N	A_0	A_{60}	A_{dif}	A_{auc}	C_0	C_{60}	C_{dif}	C_{auc}
Patients									
Total	61	8.7±8.2	27.4±29.3	18.7±24.8	1227±1230	3.2±3.8	8.4±7.1	5.2±4.9	365±344
Young total	22	6.6±3.5	20.9±19.4	14.3±16.4	958±937	2.1±1.0	6.6±5.8	4.5±5.3	268±243
Young male	14	6.8±4.1	18.3±19.9	11.6±16.3	870±998	1.8±0.8	4.8±4.9	3.0±4.6	190±180
Young female	8	6.4±2.6	25.4±19.1	19.0±16.6	1112±862	2.6±1.1	9.7±6.3	7.1±5.9	404±290
Aged total	39	9.9±9.8	31.1±33.3	21.2±28.4	1379±1356	3.9±4.5	9.4±7.7	5.6±4.7	420±381
Aged male	10	9.9±7.3	21.5±21.0	11.6±14.3	1211±1278	3.8±4.8	6.8±6.6	3.1±3.5	355±385
Aged female	29	9.9±10.6	34.5±36.3	24.5±31.3	1437±1399	3.9±4.5	10.3±7.9	6.4±4.8	442±384
Controls									
Total	57	6.0±1.5	13.8±9.3	7.8±8.6	638±317	1.5±0.8	3.9±3.9	2.4±3.8	155±131
Young male	37	6.1±1.5	12.4±8.9	6.3±8.1	598±321	1.6±0.9	3.2±2.6	1.6±2.4	139±102
Young female	20	5.8±1.6	16.4±9.6	10.6±9.2	713±303	1.3±0.3	5.2±5.4	3.9±5.3	186±170

$p<0.001$ by *t*-test). Although the values for ACTH (A_0, A_{60}, A_{dif}, and A_{auc}) were consistently higher in young patients than in controls (see Table 1), none of the differences reached statistical significance ($p=0.41$ for A_0, $p=0.27$ for A_{60}, $p=0.13$ for A_{dif}, $p=0.74$ for A_{auc}, Mann–Whitney *U*-test). However, the values for cortisol were significantly higher in young patients than in controls ($p=0.004$ for C_0, $p=0.033$ for C_{60}, $p=0.036$ for C_{auc}), except for C_{dif} ($p=0.14$).

One might suspect, however, that the significant differences observed in cortisol measures were attributable simply to the differential age distributions between young patients and controls. To resolve this issue, *post hoc* analyses were performed, selecting 18 patients and 18 controls matched for both age and gender (12 males and six females; mean age 38 years (SD 6) for patients and controls). Results in these subjects were essentially unchanged; cortisol measures were significantly higher in patients than in controls ($p=0.009$ for C_0, $p=0.044$ for C_{60}, $p=0.040$ for C_{auc}), except for C_{dif} ($p=0.21$). This ensures that enhanced cortisol responses to the DEX/CRH test in patients were not attributable simply to differential age distribution between patients and controls.

Clinical variables and DEX/CRH test results. Possible relationships between clinical variables and DEX/CRH test results were examined in the patient group. There was a significant or a nonsignificant trend towards a positive correlation between HDRS scores on admission and some hormone values (A_{60}: Pearson's $r=0.27$, $p=0.035$; A_{dif}: $r=0.27$, $p=0.038$; A_{auc}: $r=0.25$, $p=0.055$; C_{60}: $r=0.23$, $p=0.079$; C_{auc}: $r=0.24$, $p=0.059$), suggesting that pituitary–adrenal responses to the DEX/CRH test tend to become higher as severity of depression increases. When confounding factors of age and gender were examined, however, there was a highly significant positive correlation between age and HDRS scores ($r=0.39$, $p=0.002$), although there was no significant difference in mean HDRS score between males and females ($t=-1.2$, $df=59$, $p=0.23$). Thus, the observed correlation between HDRS scores and the DEX/CRH test might be explained, at least in part, by the correlation between HDRS scores and age in our sample. No significant difference was found in hormonal measures between patients on medication and patients without medication on admission (Table 2). When the diagnosis groups (unipolar depression with single episode, recurrent unipolar depression, and bipolar disorder) were compared, ACTH responses differed consistently with a significant difference (A_{dif}) and a nonsignificant trend (A_0 and A_{60}) (Table 2). Against our expectation, ACTH responses were high in the decreasing order of patients with unipolar depression with single episode, those with bipolar disorder, and those with recurrent unipolar depression. Comparisons of confounding factors of age, gender, and HDRS score revealed no significant difference among these three diagnosis groups (age: $p=0.35$ by one-way ANOVA; gender: $p=0.37$ by χ^2 test; HDRS score: $p=0.52$ by one-way ANOVA).

Repeated Assessments of the DEX/CRH Test

Among patients, 35 were repeatedly assessed with the DEX/CRH test on admission and before discharge. The mean HDRS scores as well as plasma concentrations of ACTH and cortisol before and after treatment are shown in Table 3. HDRS scores lowered substantially due to treatment. All values of ACTH and cortisol decreased significantly after treatment. When suppression status was examined, the distributions of nonsuppressors, intermediate suppressors, and suppressors changed significantly after treatment (14, 51, and 34%, respectively, on admission, in contrast to 11, 14, and 74%, respectively, before discharge; $p=0.004$ by the Wilcoxon paired signed-ranks test). No significant correlation was found between changes in HDRS scores

Table 2 Relationships between Clinical Variables and Hormonal Responses to the DEX/CRH Test in the Patient Group (on Admission) (Mean ± SD)

		ACTH (pg/ml)				Cortisol (μg/dl)			
	N	A_0	A_{60}	A_{dif}	A_{auc}	C_0	C_{60}	C_{dif}	C_{auc}
Medication									
With medication	54	8.7 ± 8.3	27.7 ± 30.4	19.0 ± 25.9	1194 ± 1200	3.1 ± 3.5	8.2 ± 7.1	5.1 ± 4.9	350 ± 326
Without medication	7	8.8 ± 8.0	25.4 ± 21.2	16.7 ± 14.8	1481 ± 1523	4.4 ± 5.6	10.1 ± 7.9	5.6 ± 5.7	483 ± 476
p-value#		$p = 0.73$	$p = 0.96$	$p = 0.85$	$p = 0.75$	$p = 0.27$	$p = 0.51$	$p = 0.82$	$p = 0.63$
Diagnosis									
UP single episode	29	10.3 ± 10.5	33.2 ± 31.0	22.9 ± 23.0	1543 ± 1494	3.3 ± 4.0	9.2 ± 7.1	6.0 ± 4.8	399 ± 363
UP recurrent	25	7.1 ± 5.5	20.5 ± 28.7	13.3 ± 28.0	849 ± 790	3.4 ± 3.9	7.1 ± 7.7	3.7 ± 4.8	321 ± 355
BP	7	7.7 ± 4.3	28.3 ± 21.3	20.7 ± 12.2	1269 ± 1067	2.5 ± 1.3	9.6 ± 5.5	7.1 ± 5.1	380 ± 221
p-value##		$p = 0.086$	$p = 0.071$	$p = 0.036$	$p = 0.10$	$p = 0.89$	$p = 0.29$	$p = 0.095$	$p = 0.39$

UP = unipolar; BP = bipolar.
#Mann–Whitney *U*-test.
##Kruskal–Wallis *H*-test.

Table 3 HDRS Scores and Hormonal Measures in 35 Patients who were Repeatedly Assessed (Before and After Treatment) with the DEX/CRH Test (Mean ± SD)

		ACTH (pg/ml)				Cortisol (μg/dl)			
	HDRS	A_0	A_{60}	A_{dif}	A_{auc}	C_0	C_{60}	C_{dif}	C_{auc}
Total (N = 35)									
Before treatment	28.8 ± 9.1	8.1 ± 5.5	27.5 ± 28.2	19.4 ± 26.5	1176 ± 971	3.3 ± 3.4	8.9 ± 7.1	5.6 ± 5.0	384 ± 325
After treatment	6.5 ± 4.1	5.7 ± 2.3	14.9 ± 12.4	9.2 ± 11.8	682 ± 529	2.1 ± 1.8	5.3 ± 6.5	3.2 ± 5.4	236 ± 273
p-value#	$p < 0.001$	$p = 0.022$	$p = 0.007$	$p = 0.012$	$p = 0.007$	$p = 0.005$	$p = 0.002$	$p = 0.013$	$p = 0.002$
Pharmacotherapy alone (N = 23)									
Before treatment	29.3 ± 9.6	7.6 ± 4.8	28.7 ± 32.0	21.1 ± 31.0	1171 ± 989	3.2 ± 3.7	8.7 ± 7.7	5.6 ± 5.3	374 ± 344
After treatment	6.8 ± 4.7	5.9 ± 2.9	16.4 ± 14.1	10.5 ± 13.3	746 ± 622	2.4 ± 2.2	6.3 ± 7.7	3.9 ± 6.3	277 ± 323
p-value#	$p < 0.001$	$p = 0.14$	$p = 0.078$	$p = 0.083$	$p = 0.048$	$p = 0.19$	$p = 0.048$	$p = 0.13$	$p = 0.042$
ECT and pharmacotherapy (N = 12)									
Before treatment	27.8 ± 8.4	9.0 ± 6.8	25.0 ± 19.8	16.0 ± 15.1	1186 ± 979	3.6 ± 2.7	9.3 ± 6.2	5.7 ± 4.6	403 ± 299
After treatment	6.1 ± 2.9	5.3 ± 0.7	12.2 ± 8.1	6.8 ± 8.2	559 ± 261	1.7 ± 0.5	3.5 ± 2.9	1.8 ± 2.9	158 ± 115
p-value#	$p = 0.002$	$p = 0.11$	$p = 0.041$	$p = 0.050$	$p = 0.041$	$p = 0.006$	$p = 0.021$	$p = 0.038$	$p = 0.012$

#*p*-value was obtained by the Wilcoxon paired signed-ranks test.

and changes in any of hormonal measures in the 35 patients.

Restoration from HPA axis abnormalities was examined separately in patients who underwent ECT and in patients who underwent pharmacotherapy alone. Figure 1 shows time-course changes of the mean plasma concentrations of cortisol in patients who underwent ECT ($N = 12$) and in patients who did not ($N = 23$). As shown in Figure 1, the magnitude of hormonal response reduction appeared to be greater in patients who underwent ECT in addition to pharmacotherapy compared with those who underwent pharmacotherapy alone. As shown in Table 3, indeed, changes in hormone concentrations between pre- and post-treatment were significant only for A_{auc}, C_{60}, and C_{auc} in the pharmacotherapy group, while all values of both ACTH and cortisol, except for A_0, changed significantly in the ECT group.

DISCUSSION

We assessed the DEX/CRH test as a state-dependent marker to monitor HPA axis abnormalities in depression. We found that elderly and female individuals tended to show increased pituitary–adrenal responses to the DEX/CRH test compared to young and male individuals, respectively. We

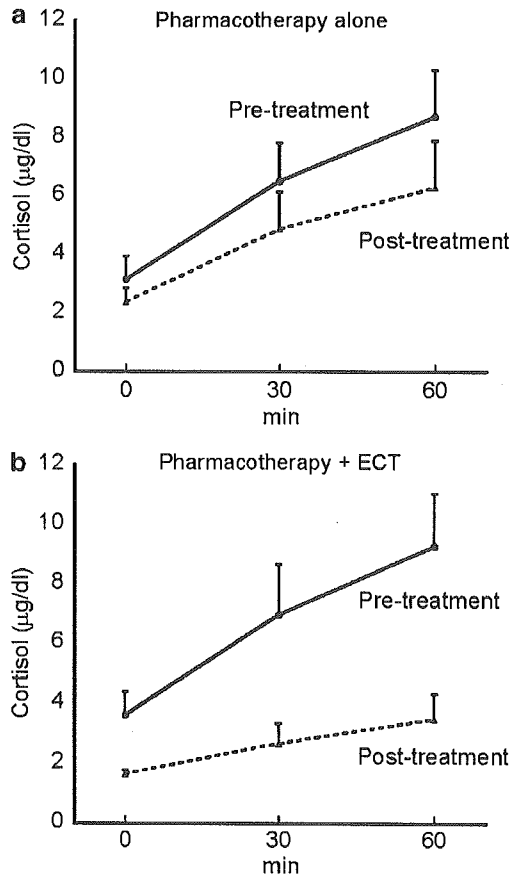

Figure 1 Time-course curves of cortisol responses to the DEX/CRH test before and after treatment in the pharmacotherapy group (a) and the ECT group (b). The *X*-axis represents time after CRH infusion. Error bars represent SEs.

confirmed that pituitary-adrenal responses to the DEX/CRH test are enhanced in depressed patients compared to controls. Severity of depression correlated with the DEX/CRH test results, although this was explained, at least in part, by a positive correlation between age and severity in our patients. Medication *per se* was unrelated to DEX/CRH test results. For patients who were repeatedly assessed with the DEX/CRH test, hormonal responses were reduced after treatment. This reduction was observed predominantly in patients who underwent ECT in addition to pharmacotherapy rather than in those who underwent pharmacotherapy alone.

Effects of Age and Gender

In our patients, all the mean plasma concentrations of hormones were higher in the aged patient group (>50 years) than in the young patient group ($\leqslant 50$ years), although a statistically significant difference was found only for A_0 and a nonsignificant trend for C_{auc}. Previous studies have provided conflicting results as to the possible effects of age on hormonal responses to the DEX/CRH test. von Bardeleben and Holsboer (1991) reported that the cortisol response to the DEX/CRH test increased with age in patients with depression but was absent in controls. Heuser *et al* (1994a) examined healthy subjects and found that hormonal responses to the DEX/CRH test were enhanced in older subjects (aged 60–84 years) compared to younger subjects (22–48 years). In line with this, Kudielka *et al* (1999) found that healthy elderly women (60–75 years) exhibited a markedly enhanced cortisol response to the DEX/CRH test compared to young controls (20–31 years). In contrast, a more recent study found no significant effect of age on hormonal responses to the DEX/CRH test in acutely depressed inpatients (Kunzel *et al*, 2003). Studies in rats have provided evidence that aged animals tend to show enhanced hormonal responses to the DEX/CRH test compared to young animals, and that vasopressin is involved in these age-associated changes (Hatzinger *et al*, 1996, 2000; Revskoy and Redei, 2000). Our results support the possibility that older age is associated with enhanced pituitary–adrenal responses to the DEX/CRH test, at least in depressed patients.

With respect to gender, female depressed and normal subjects were reported to have increased hormonal responses to the DEX/CRH test in comparison with male counterparts (Heuser *et al*, 1994a; Kunzel *et al*, 2003). Our results give further support for this gender difference. Based on our data, responses to pretreatment with DEX (A_0 and C_0) did not seem to be consistently different between males and females among young patients, aged patients, and controls (Table 1). However, hormonal responses to the subsequent injection of CRH as manifested by A_{dif} and C_{dif} were consistently higher in females than in males for all the three groups. These results suggest that gender difference in hormonal responses to the DEX/CRH test might be attributable predominantly to that in hormonal responses to CRH.

Patients *vs* Controls

When young patients were compared with controls, all hormonal responses of both ACTH and cortisol were consistently higher in patients on admission than in controls (Table 1). The differences in C_0, C_{60}, and C_{auc} were statistically significant. The differences in A_0, A_{60}, A_{dif}, A_{auc}, and C_{dif} failed to reach statistical significance, which was probably attributable to the lack of statistical power due to the small number of young patients ($N=22$). Even when *post hoc* analyses were performed in 18 patients and the same number of controls, matched for both age and gender, results were similar. Overall, our data provide further evidence for enhanced HPA activity detected with the DEX/CRH test in depressed patients.

Clinical Variables and DEX/CRH Test

We found a positive correlation between HDRS scores on admission and hormonal values, suggesting that pituitary-adrenal responses to the DEX/CRH test tend to become higher as severity of depression increases. This observation is in accordance with previous studies (von Bardeleben and Holsboer, 1991; Rybakowski and Twardowska, 1999; Kunzel *et al*, 2003). In our sample, however, there was a highly significant correlation between HDRS scores and age ($p=0.002$), which was stronger than the correlation between HDRS scores and hormonal measures, indicating

that the observed correlation between HDRS scores and the DEX/CRH test might be explained, at least in part, by the correlation between HDRS scores and age. Zobel *et al* (2004) recently reported that a decrease in cortisol response to the DEX/CRH test was more closely related to an improvement in specific brain functions (especially working memory) than to the global severity of depression.

Hormonal measures were not significantly different between patients on medication and patients without medication on admission, indicating that the fact of being on medication *per se* was unrelated to DEX/CRH test results. This observation is in line with the finding of Kunzel *et al* (2003), that is, the presence or absence of antidepressant treatment, the type of antidepressant treatment, or the number of ineffective antidepressant treatment trials during the index episode had no effect on hormonal responses to the DEX/CRH test. One exceptional drug is mirtazapine, an antidepressant known to acutely inhibit cortisol secretion in healthy subjects; the drug was shown to attenuate rapidly HPA axis hyperactivity in depressed patients via direct pharmacoendocrinological effects, which was not necessarily related to clinical improvement (Schule *et al*, 2003).

With respect to possible differences in DEX/CRH test results among the diagnosis groups (unipolar depression with single episode, recurrent unipolar depression, and bipolar disorder), ACTH responses were high in the decreasing order of patients with unipolar depression with single episode, those with bipolar disorder, and those with recurrent unipolar depression. This finding was unexpected because at least three research groups had obtained evidence that HPA abnormalities as detected with the DEX/CRH test became more pronounced as the number of previous episodes increased (Hatzinger *et al*, 2002; Kunzel *et al*, 2003; Gervasoni *et al*, 2004). Inconsistency between previous studies and ours might be attributable to the effects of confounding factors of age, gender, and HDRS score. However, we found no significant difference in any of these variables among the three diagnosis groups. Alternatively, differences in ACTH responses may have occurred by chance, considering that there was no significant difference in cortisol responses among the diagnosis groups. Further investigations are required to draw any conclusion.

Repeated Assessments of the DEX/CRH Test

When depressed patients were repeatedly assessed with the DEX/CRH test on admission and before discharge, all the measures of ACTH and cortisol were significantly reduced after treatment. Our results are consistent with previous studies reporting a reduction in hormonal responses to the DEX/CRH test after successful pharmacotherapy with antidepressants (Holsboer-Trachsler *et al*, 1991; Heuser *et al*, 1996; Deuschle *et al*, 1997; Baghai *et al*, 2002; Frieboes *et al*, 2003), suggesting that alterations in the HPA axis as detected with the DEX/CRH test are, at least in part, state dependent.

When suppression status was examined, the distributions of nonsuppressors, intermediate suppressors, and suppressors significantly changed after treatment (14, 51, and 34%, respectively, on admission; 11, 14, and 74%, respectively, before discharge) ($p = 0.004$). However, the difference in the rate of nonsuppressors (defined as C_0 of $\geqslant 5\,\mu g/dl$) alone (14 *vs* 11%) was not statistically significant in our patients. This finding indicates that pretreatment with DEX alone (ie conventional DST) lacks sensitivity to monitor changes in HPA abnormalities during the treatment course of depressed patients and that the combination of CRH with DEX (ie DEX/CRH test) is much more sensitive. The definition of 'intermediate suppressors' (defined as C_0 of $<5\,\mu g/dl$ and C_{60} of $\geqslant 5\,\mu g/dl$) might be useful in future studies and in the clinical setting.

When patients who underwent pharmacotherapy alone and patients who underwent ECT in addition to pharmacotherapy were examined separately, the magnitude of hormonal response reduction appeared to be greater in the latter than in the former, suggesting that ECT has an additional effect on restoration from HPA axis abnormalities besides the effect of pharmacotherapy. This finding is consistent with the results from several conventional DST studies that reported a positive correlation between hormonal responses to ECT and the normalization of the HPA system (Albala *et al*, 1981; Papakostas *et al*, 1981; Grunhaus *et al*, 1987; Devanand *et al*, 1991). However, controversial results have also been reported (Coryell, 1986; Fink *et al*, 1987). This inconsistency in the literature may be due in part to the immediate effects of ECT, which activates HPA axis (Aperia *et al*, 1984; Swartz and Chen, 1985). Devanand *et al* (1991) measured plasma cortisol concentrations in depressed patients who had undergone ECT three times, that is, pre-ECT, immediately post-ECT, and 1 week after ECT. They found that plasma cortisol concentrations decreased significantly from pretreatment to immediately post-treatment and then further decreased during the first week after the ECT course. Based on this observation, Devanand *et al* (1991) pointed out that it may be necessary to wait 1 or more weeks in repeated DST studies in patients receiving ECT. We conducted the post-treatment DEX/CRH test before discharge, but not immediately after ECT, which might have reduced the possible effects of postictal increases in plasma ACTH and cortisol concentrations.

Proposal for a Simpler Test Protocol

In our comparisons between patients and controls, cortisol responses were more clearly different from ACTH responses between the two groups. Furthermore, ACTH and cortisol responses generally tend to parallel as pointed out by Heuser *et al* (1994b). Therefore, the measurement of ACTH might add little information. In our study, core results were derivable from the C_0, C_{60}, and C_{dif} values. A simpler test protocol might hence be available which measures plasma cortisol concentrations only twice at 1500 hours (immediately before CRH injection) and 1600 hours (1 h later). Such a simpler protocol might save time and cost without losing essential information to be obtained from the DEX/CRH test and might be practical in the clinical setting. However, the current results were based on observations on in-patients with major depressive episode who were acutely ill, and data on chronic depression or minor depression are unavailable in the current study. Further studies are warranted to draw conclusions as to the appropriateness of such a simple test protocol.

Limitations

The present study involves several limitations. First, the sample size was not very large, which might have given rise to type II errors. Second, all patients were in-patients and acutely ill; hence, severe forms of depression might be over-represented in our sample. It is possible that the sensitivity of the DEX/CRH test might be lower in outpatients and patients with chronic depression as, respectively, reported by Gervasoni *et al* (2004) and Watson *et al* (2002). Third, the majority of patients were medicated with antidepressants, which might have influenced the DEX/CRH results. Although antidepressants *per se* have been shown not to influence test results (Heuser *et al*, 1996; Kunzel *et al*, 2003), it is possible that the effects of drug treatment already emerged in part of patients. Furthermore, drugs may have exerted some effects on the degradation rate of DEX and in turn influenced DEX/CRH test results. However, a recent study of Watson *et al* (2004) compared DEX levels between patients and controls and found that there was no difference in DEX levels between the two groups, suggesting that an effect of psychotropic medication on cortisol output via DEX metabolism appears unlikely. Finally, the detection limits for the hormone assay may have reduced the sensitivity, particularly ACTH responses, of the DEX/CRH test.

Conclusions

In conclusion, we obtained additional evidence for enhanced HPA axis activity, which was detected with the DEX/CRH test when Japanese patients with major depressive episode were compared with controls. Age, gender, but not the fact of being on medication *per se*, are shown to be associated with DEX/CRH test results. Repeated DEX/CRH tests on admission and before discharge provided clear evidence that the DEX/CRH test is a sensitive test as a state-dependent marker to monitor HPA axis abnormalities in Japanese patients with depression. Furthermore, our results suggest that normalization of HPA axis occurs with clinical responses to ECT as well as pharmacotherapy in depressed patients.

ACKNOWLEDGEMENTS

We thank Ms Kazuko Sakai for her substantial assistance. This study was supported in part by a grant from the Mitsubishi Pharma Corporation and by a research grant from the Ministry of Health, Labor, and Welfare of Japan.

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Acta Psychiatr Scand 2005: 112: 257–265
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DOI: 10.1111/j.1600-0447.2005.00625.x

ACTA PSYCHIATRICA SCANDINAVICA

HPA axis normalization, estimated by DEX/CRH test, but less alteration on cerebral glucose metabolism in depressed patients receiving ECT after medication treatment failures

Yuuki N, Ida I, Oshima A, Kumano H, Takahashi K, Fukuda M, Oriuchi N, Endo K, Matsuda H, Mikuni M. HPA axis normalization, estimated by DEX/CRH test, but less alteration on cerebral glucose metabolism in depressed patients receiving ECT after medication treatment failures.
Acta Psychiatr Scand 2005: 112: 257–265. © 2005 Blackwell Munksgaard.

Objective: To examine the clinical effects of electroconvulsive therapy (ECT) on depressed patients with medication treatment failures, we investigated the alterations in hypothalamic-pituitary-adrenocortical (HPA) function and regional cerebral metabolism rate of glucose (rCMRGlu) after ECT in these patients.
Method: Before and after ECT, the combined dexamethasone/corticotrophin-releasing hormone (DEX/CRH) test was administered to seven patients who were referred for ECT. In the same patients, ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) was also assessed.
Results: Cortisol response in the DEX/CRH test significantly decreased after a successful ECT. A significant hypometabolism in various frontal regions and hypermetabolism in the parietal regions of these patients when compared with controls remained after ECT.
Conclusion: Depressed patients who failed trials of antidepressant medication showed a remission with ECT that was accompanied by resolution of HPA dysregulation. However, measures of cerebral brain metabolism did not resolve.

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Key words: electroconvulsive therapy; medication treatment failures; hypothalamic-pituitary-adrenocortical function; dexamethasone/corticotrophin-releasing hormone test; ^{18}F-fluorodeoxyglucose positron emission tomography

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Accepted for publication August 3, 2005

Significant outcomes

- ECT treatments were effective to depressed patients who failed trials of antidepressant medication and showed high cortisol response in the DEX/CRH test.
- Cortisol response in the DEX/CRH test was normalized after a successful course of ECT.
- Depressed patients with medication treatment failures presented abnormalities of rCMRGlu in the examination by ^{18}F-FDG PET. These abnormalities did not resolve about 1 month after ECT treatments despite clinical remission.

Limitations

- This study was small sample size and open design.
- Depressed subjects were maintained on medication, and not drug free.
- Because we failed to perform the DEX/CRH test and ^{18}F-FDG PET simultaneously, we could not assess the relationship between neuroendocrinological alterations and regional brain activities.

Introduction

A poor response to antidepressant drug medication is often a critical issue in the treatment of a major depressive episode (MDE). Despite the various antidepressant agents including selective serotonin reuptake inhibitors and serotonin and norepinephrine reuptake inhibitors available today, the response rate to these drugs is as low as approximately 50% in some recent meta-analyses of placebo-controlled trials (1, 2). Clinical surveys also indicate that about 30–50% of the patients with MDE fail to respond adequately to antidepressant agents (3, 4). Medication treatment failures pose a clinical challenge not only because of its relatively high prevalence but also because of its deleterious effects on the patient's psychosocial functioning (5).

Electroconvulsive therapy (ECT) has so far been the most firmly established treatment for medication treatment failures. The efficacy and safety of ECT in depressed patients with medication treatment failures has been documented in randomized (paroxetine–) controlled studies (6). A meta-analytic review of randomized controlled trials that compared ECT with simulated ECT or placebo or antidepressant drugs has revealed a significant superiority of ECT, and it was concluded that it is a valid therapeutic tool for the treatment of depression including severe and refractory forms (7).

The pathophysiology of depression with medication treatment failures remains largely unclear and the mechanisms of action of ECT to these patients have not been established. As for the neuroendocrine function of patients with MDE, the hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis has been frequently observed. For example, plasma cortisol levels elevated and non-suppressors in the dexamethasone suppression test (DST) was seen in depressed patients. As one of the mechanisms of action of ECT, Fink and Ottosson (8) and Fink (9) hypothesized that ECT stimulated the hypothalamus to normalize HPA axis function. The DST has become one of the most frequently used neuroendocrine tests to assess the HPA axis function in depression, although one major drawback of the DST for clinical purposes was its low sensitivity (10). In previous reports of the effects of ECT on DST results, some reports have suggested that the normalization of a previously non-suppresive DST parallels a successful course of ECT (11, 12), while others failed to demonstrate an association between DST results and the outcome of ECT (13, 14). Recently, the combined dexamethasone/corticotrophin-releasing hormone (DEX/CRH) test has been reported to be more sensitive to MDE than DST (15). Typically, there is an overshoot of cortisol after CRH administration despite DEX pretreatment in depressed patients, which is not observed in healthy controls (16). Previous studies have shown that successful treatment with antidepressant medications leads to a gradual normalization of cortisol secretion during the DEX/CRH test (17). To our knowledge, there has been no report examining the effect of ECT on DEX/CRH test results.

Regional cerebral blood flow (rCBF) and cerebral metabolic rate of glucose (rCMRGlu) in MDE has also been extensively analyzed using neuroimaging techniques, such as single-photon emission computerized tomography (SPECT) and positron emission tomography (PET), to investigate the pathophysiologic mechanisms underlying the disorder and the physiologic basis of clinical responses to antidepressant treatment (18). Definitive evidence has not been determined, however, on the precise regions and functional changes that are responsible for inducing and regulating normal and pathologic mood (19). Nevertheless, several important findings suggesting the possibility that different topographies might be related to treatment response have been reported. Although neuroimaging studies have focused primarily on major depression in general rather than on depression with medication treatment failures specifically, the findings may be usefully applied in depression with medication treatment failures. Several abnormalities have been found in depressed patients, including a decreased rCBF and rCMRGlu in the dorsolateral prefrontal cortex and the caudate nucleus, an increased rCBF and rCMRGlu in the amygdala, orbital and ventrolateral prefrontal cortex (18, 20, 21). Supporting evidence of critical target sites in the brain mediating antidepressant effects is further provided by functional imaging studies of depressed patients studied before and after various forms of antidepressant treatment. However, there is considerable variability in both site and direction of changes across studies (22). SPECT and PET studies on the effect of ECT on brain activities in depressed patients have led to conflicting results, ranging from overall decrease (23–25) to increase (26).

Aims of the study

To address the above issues, the authors conducted the present study to examine, in a naturalistic and prospective manner, i) the clinical effects of ECT on depressed patients with medication treatment fail-

ures and ii) the changes in HPA function and regional brain activities after ECT in these patients.

Material and methods

Subjects

The subjects were seven depressed patients (three females; mean age: 58.0 years; standard deviation, SD: 9.4) and 10 healthy control subjects (five females; mean age, 60.8 years; SD, 7.9). All the subjects were right handed. Table 1 summarizes the demographic and clinical characteristics of the subjects.

All the patients were in-patients of the Department of Neuropsychiatry, Gunma University Hospital in Japan. They were diagnosed according to the DSM-IV criteria and had a current DSM-IV diagnosis of an MDE. Among them, four patients met the criteria for major depressive disorder, two patients met the criteria for bipolar I disorder, and one patient met the criteria for bipolar II disorder. Patients were excluded from the study if they had a significant medical illness, current active alcohol or substance abuse, a history of seizure disorder, cerebrovascular disease or traumatic brain injury with loss of consciousness.

All the patients had not been responded adequately to two or more different antidepressant treatments for more than 4 weeks, and were referred for ECT. The dose of antidepressants used for the patients was 162.5–342.5 mg/day of the imipramine equivalent. The equivalent dose of antidepressants was estimated by modifying the Japanese conversion table of antidepressants (27). Cases of bipolar disorder (cases 5, 6, and 7) had received lithium and/or valproate medication in addition to antidepressants medication. Except for cases of bipolar I disorder (cases 6 and 7), patients had received antidepressants at a minimum dose of 150 mg/day of the imipramine equivalent for more than 4 weeks. Case 6 had had mood shifts three times (depressive phase, manic phase and depressive phase) within this 1 year and he had received antidepressants at a minimum dose of 150 mg/day of imipramine equivalent for more than 4 weeks in a previous depressive episode. Case 7 had not at all responded to previous successful antidepressant treatments in the current episode.

Ten healthy subjects participated in this study as controls for PET study. The healthy subjects were required to have no current or past psychiatric history and to meet the exclusion criteria for the patient group.

Table 1. Demographic and clinical characteristics of subjects and clinical outcome

Case no.	Age (year)	Sex	Diagnosis (DSM-IV)	Antidepressants used before ECT (IMP equivalent of maximum dose) (mg/day)	Duration of index episode (month)	No. of ECT	HDRS score			
							Pre-ECT	First PET	Post-ECT	Second PET
Patients										
1	53	M	Major depressive disorder, single episode, with psychotic features	IMP, CMP, AMT, MPT, SPD, MNP (300)	12	12	37	37	3	2
2	73	F	Major depressive disorder, single episode	CMP, MAS, SPD, MNP (342.5)	9	12	34	32	4	8
3	69	F	Major depressive disorder, single episode, with psychotic features	AMT, MAS, TZD, SPD, FVM, PXT, MNP (200)	14	14	24	24	3	3
4	55	M	Major depressive disorder, recurrent	CMP, MPT, TZD, PXT (237.5)	7	20	36	27	3	3
5	53	F	Bipolar II disorder, most recent episode depressed	CMP, AMT, AXP, MPT, TZD, SPD, PXT, MNP (225)	19	6	18	18	6	2
6	47	M	Bipolar I disorder, most recent episode depressed	MAS, TZD (200)	1	8	21	14	3	3
7	56	M	Bipolar I disorder, most recent episode depressed	TZD, MNP (162.5)	2	18	35	25	8	4
Mean	58.0	M 4/F 3		238.2	9.1	12.9	29.3	25.3	4.3	3.6
SD	9.4			62.6	6.5	5.0	8.0	7.8	2.0	2.1
Control										
Mean	60.8	M 5/F 5								
SD	7.9									

ECT, electroconvulsive therapy; HDRS, Hamilton Depression Rating Scale; IMP, imipramine; CMP, clomipramine; AMT, amitriptyline; AXP, amoxapine; MPT, maprotiline; MAS, mianserin; TZD, trazodone; SPD, sulpiride, FVM, fluvoxamine; PXT, paroxetine; MNP, milnacipran.