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including the small case number and retrospective design. In addition, this study focused on patients who already had persistent MRSA bacteremia, and therefore its findings should not be generalized to all patients with CRBSI. Further studies are needed to determine which patients require immediate catheter removal to improve outcomes.

CONCLUSIONS

In summary, CRBSI and persistent bacteremia caused by MRSA in elderly patients is associated with high rates of morbidity and mortality. This study found no survival advantage associated with early catheter removal.

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ACKNOWLEDGMENTS

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this paper.

Author Contributions: All the authors contributed to the composition of this letter.

Sponsor's Role: None.

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THE TAKEDA THREE COLORS COMBINATION TEST: AN EASY AND QUICK SCREENING FOR ALZHEIMER'S DISEASE

To the Editor: Alzheimer's disease (AD) is the most common type of dementia and is prevalent worldwide.[1] With a projected increase in the AD population,[2] early detection has become increasingly important, promoting demand for screening tests with adequate sensitivity. This study explored whether the Takeda Three Colors Combination Test (TTCC) is a feasible and simplified screening test for early detection of AD and examined its effectiveness.

METHODS

TTCC involves three colored wooden cards (red, blue, and yellow) and a model figure with a diagram of the three colored squares in a certain configuration (Figure 1). The model figure is presented to the examinee for 5 seconds and then hidden. After a simple interference task (backward digit span), the examinee is required to arrange the three cards to match the configuration shown in the model figure. If the examinee makes exactly the same configuration as shown in the model figure, he or she is assessed as being normal, whereas if the examinee failed, AD is suspected.

Subjects consisted of two groups. Those with mild AD (n = 91) met the AD criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*[3] and had a Mini-Mental State Examination (MMSE)[4] score greater than 20 and a Clinical Dementia Rating (CDR)[5] score of 0.5 or 1.0. Exclusion criteria were mixed dementia, active neurological disease, and other acute somatic diseases. The control group (n = 75) comprised people who had no complaint of memory lapse, no history of psychiatric or neurological disease, a MMSE score of 26 or greater and a CDR score of 0. All subjects were aged 60 and older and had normal visual acuity. The object and methods of the research were explained to the subjects (or their families), and their consent was obtained in advance of their participation.

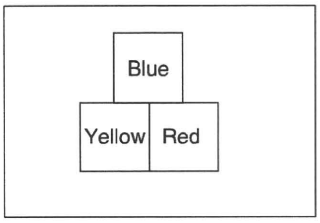

Figure 1. Arrangement of three colored squares that should be remembered and reproduced later after an interference task using three colored wooden cards.

Cognitive functioning was assessed in all subjects in the following order: TTCC, MMSE, CDR, and TTCC again, with the second TTCC administered within 30 minutes of the first. The examiner was blind to which group the examinees belonged to. TTCC sensitivity and specificity were calculated using data from the first trial. The difference between the control group and group with mild AD was examined using logistic regression analysis with likelihood ratio test to adjust for other factor using group as a dependent variable and TTCC results, age, and sex as independent variables.

RESULTS

For the control group, 65 responded correctly, and 10 responded incorrectly. For the group with mild AD, 14 responded correctly and 77 responded incorrectly. The sensitivity of TTCC in detecting mild AD based on this result was 85%, and the specificity was 87%. The results of logistic regression analysis revealed that TTCC results was the significant factor. The odds ratio of incorrect response to TTCC was 32.0 (95% confidence interval (CI) = 13.1–78.1, $P<.001$) for mild AD compared with control.

The phi coefficient was 0.76 ($P<.001$), and the consistency percentage between the first and second trials was 88%. Spearman rank correlation analysis, using MMSE scores as an external standard, revealed a significant correlation between the MMSE and the TTCC ($\rho = 0.58$, $P<.001$). TTCC was implemented within 2 minutes for all subjects. No refusal or resistance to this test was observed for any of the subjects.

DISCUSSION

The results of logistic regression analysis suggest that people who respond incorrectly to the TTCC are 32 times as likely to have cognitive impairment as those who respond correctly. As for the power of the TTCC to detect mild AD, sensitivity was 85% and specificity was 87%. The sensitivity of MMSE is considered to be low for detecting mild forms of dementia,[6] a limitation that can be seen when sensitivity falls to as much as 44%[7] or 54%[8] for dementia groups scoring more than 20. The findings of the current study indicate that the TTCC's sensitivity to mild AD is satisfactory. TTCC's fidelity in detecting impairment in visuospatial memory, which is frequently seen at the early stage of AD, associated with reduction in the blood flow in the parietal lobe and hippocampus,[9,10] may explain the relatively high sensitivity for mild AD shown in the test.

With regard to reliability, the retest showed a high concordance rate and correlation coefficient, which implies good test–retest reliability of the TTCC. Concurrent validity was established using the MMSE as an external criterion, showing high correlation between TTCC and MMSE scores. The administration of the TTCC can be conducted in a short time, with no prior training required, and assessment is easy, making it suitable as a screening test for general practitioners and examinees in various clinical settings.

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ACKNOWLEDGMENTS

We would like to deeply thank the staff of Tottori Seikyo Hospital for help with this study.

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this paper.

Supported by Grants-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan: 21730550.

Author Contributions: All of the authors contributed to the design, data analysis, and preparation and writing of the letter.

Sponsor's Role: None.

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Hippocampal Astrocytes are Necessary for Antidepressant Treatment of Learned Helplessness Rats

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ABSTRACT: The astrocyte is a major component of the neural network and plays a role in brain function. Previous studies demonstrated changes in the number of astrocytes in depression. In this study, we examined alterations in the number of astrocytes in the learned helplessness (LH) rat, an animal model of depression. The numbers of activated and nonactivated astrocytes in the dentate gyrus (molecular layer, subgranular zone, and hilus), and CA1 and CA3 regions of the hippocampus were significantly increased 2 and 8 days after attainment of LH. Subchronic treatment with imipramine showed a tendency (although not statistically significant) to decrease the LH-induced increment of activated astrocytes in the CA3 region and dentate gyrus. Furthermore, subchronic treatment of naïve rats with imipramine did not alter the numbers of activated and nonactivated astrocytes. However, the antidepressant-like effects of imipramine in the LH paradigm were blocked when fluorocitrate (a reversible inhibitor of astrocyte function) was injected into the dentate gyrus or CA3 region. Injection of fluorocitrate into naive rats failed to induce behavioral deficits in the conditioned avoidance test. These results indicate that astrocytes are responsive to the antidepressant-like effect of imipramine in the dentate gyrus and CA3 region of the hippocampus. © 2010 Wiley-Liss, Inc.

KEY WORDS: learned helplessness (LH); astrocyte; depression; hippocampus; behavior

INTRODUCTION

Depression is related to neuroplasticity, including neurotrophins, cell proliferation, dendritic branching, and synaptogenesis. Neuroplasticity involves the interaction between astrocytes and neurons (Haber et al., 2006). Astrocytes provide trophic support for neurons, neuronal migration, and inflammatory processes for maintenance of the neural network. Thus, astrocytes provide neurons with glutamine for the synthesis of glutamate or γ-aminobutyric acid (GABA) and contribute to the removal of glutamate released during neuronal activity (Willoughby et al., 2003). Astrocytes enhance synaptic activity and promote synaptogenesis (Slezak and Pfrieger, 2003). Astrocytes regulate potassium and calcium during and after stress (Lian and Stringer, 2004). To maintain homeostasis, astrocytes respond to neuroactive compounds including neurotransmitters, neuropeptides, growth factors, cytokines, small molecules, and toxins (Barres et al., 1990; Hosli and Hosli, 1993). Therefore, it is likely that astrocytes play a role in the mechanism of depression.

Postmortem brains of depressed patients demonstrated neuropathological changes in the prefrontal cortex and hippocampus (reviewed by Harrison, 2002). Reductions in the number of astrocytes in the prefrontal cortex (Ongur et al., 1998, Rajkowska et al., 1999, Miguel-Hidalgo et al., 2000, Cotter et al., 2002), amygdala (Bowley et al., 2002), and hippocampus (Müller et al., 2001) in depression were reported. These changes may contribute to the reduction in volume of the hippocampus and dysfunction of neuronal circuits in major depression (reviewed by Rajkowska et al., 1999).

The hippocampus is a candidate site for the impaired functions associated with depression (Duman et al., 1997). It is well documented that patients with depression show a reduction in hippocampal volume (Sheline et al., 1996; Bremner et al., 2000). Among the causes, reduction in hippocampal volume could be due to decreases in neurotrophic factors or neurogenesis (Pezawas et al., 2004; David et al., 2009).

The learned helplessness (LH) paradigm is an animal model of depression (Seligman and Beagley, 1975). In this paradigm, an animal is initially exposed to uncontrollable stress. When the animal is later placed in a situation in which shock is controllable (escapable), the animal has a difficulty in acquiring the escape responses. Thus, LH animals showed increased numbers of escape failures in a two-way conditioned avoidance test. This escape deficit is reversed by chronic antidepressant treatment (Shirayama et al., 2002; Iwata et al., 2006).

In this study, we investigated the role of astrocytes in the hippocampus of LH rats using immunohistochemical methods and behavioral studies. We examined the effects of LH training on the number of activated and nonactivated astrocytes as reflected by the number of glial fibrillary acidic protein (GFAP: a marker of astrocytes) positive cells in the hippocampus. Activated astrocytes are characterized by cellular hypertrophy. Next, we examined the effects of subchronic treatment with imipramine on the number of GFAP-positive cells in the hippocampus of LH rats. Finally, we examined the effects of infusion of fluoro-

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Accepted for publication 4 March 2010
DOI 10.1002/hipo.20803
Published online in Wiley InterScience (www.interscience.wiley.com).

citrate (a reversible inhibitor of astrocyte function) into the hippocampus of LH rats on the antidepressant-like effects of imipramine. Fluorocitrate is uploaded into astrocytes and impairs TCA cycle. We chose the dose and time course of fluorocitrate injection on a basis of a previous study demonstrating that astrocyte almost recovered at 24 h after injection of fluorocitrate and high concentration of fluorocitrate has a possibility to cause irreversible damage to astrocyte (Paulsen et al., 1987).

MATERIALS AND METHODS

Animal and Treatments

Animals-use procedures were in accordance with the Tottori University Guide for the Care and Use of Laboratory Animals and were approved by the Tottori University Animal Care and Use Committee. Male Sprague Dawley rats (250–300 g) were used. The animals were housed under a 12 h light/dark cycle with free access to food and water.

Fluorocitrate was dissolved in 0.1 M HCl, precipitated by addition of a few drops of 0.1 M Na_2SO_4, then buffered with 0.1 M Na_2HPO_4 and centrifuged at 1,000*g* for 5 min, and the supernatant was diluted with 0.9% saline.

Learned Helplessness Paradigm

LH behavioral tests were performed using the Gemini Avoidance System (San Diego, CA). This apparatus has two compartments divided by a retractable door. On days 1 and 2, rats were subjected to 60 inescapable electric foot shocks (0.65 mA, 30 s duration, averaging 20–40 s). On day 3, a two-way conditioned avoidance test was performed as a post-shock test to determine if the rats showed the predicted escape deficits. This screening session consisted of 30 trials in which electric foot shocks (0.65 mA, 6 s duration, at random intervals [mean of 30 s]) was preceded by a 3 s conditioned stimulus tone that remained on until the shock was terminated. Rats with more than 20 escape failures in the 30 trials were regarded as having reached criterion. Approximately 65% of the rats reached this criterion. For antidepressant treatment, LH rats or naïve rats were treated with imipramine (20 mg/kg, i.p., once daily) or saline for 7 days (from day 4 to day 10).

Rats were anesthetized with pentobarbital sodium solution (50 mg/kg, intraperitoneal injection, Abbott Laboratories) and surgery was performed using a stereotaxic apparatus (Narishige, Tokyo). Rats received bilateral microinjection of fluorocitrate (0.1 or 0.5 nmol/side) or 0.9% saline on day 4 and day 7 (two times, first and forth days during antidepressant treatment for 7 days) because disruption of astroglial metabolism by fluorocitrate lasts for more than 24 h (Paulsen et al., 1987). A total volume of 1.0 µl was infused into each side of hippocampal regions over 15 min and the injection syringe was left in place for an additional 5 min to allow for diffusion. The coordinates for the dentate gyrus (DG) and CA3 relative to the bregma according to the atlas of Paxinos and Watson (1997) were as follows: −3.8 anteroposterior (AP), ±2.0 lateral, −3.2 dorsoventral (DV) from dura (DG); and −3.6 AP, ±3.8 lateral, −3.0 DV from dura (CA3). The placements of injection cannula in the hippocampus are shown in Figure 1. On day 11, a two-way conditioned avoidance test was performed.

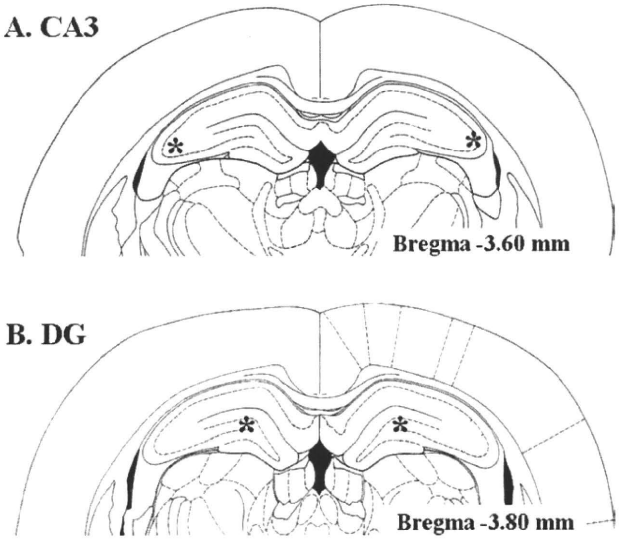

FIGURE 1. A schematic representation of microinjection sites within the CA3 region of hippocampus (A) and the dentate gyrus of hippocampus (B).

Immunohistochemistry

We performed three experimental procedures. In one, rats were killed two days after the acquisition of LH (Experiment 1). Next, LH rats were killed 24 h after subchronic treatment with imipramine for 7 days (Experiment 2). In addition, naïve rats were killed 24 h after subchronic treatment with imipramine for 7 days (Experiment 3).

All rats were placed under deep pentobarbital anesthesia (50 mg/kg, i.p.) and killed via intracardial perfusion with 4% paraformaldehyde in 0.1 M PBS, pH 7.4. Brains were removed, postfixed overnight in the same fixative at 4°C, and stored at 4°C in 30% sucrose. Serial coronal sections of the brains were cut (35 µm sections) on a Microslicer® (DTK-1000, Dosaka EM, Kyoto, Japan), and sections were stored at 4°C in 0.1 M PBS containing 0.1% sodium azide.

GFAP immunohistochemistry was investigated as described below. Free-floating sections were washed three times for 5 min in 0.1 M PBS and then incubated for 10 min in 0.1 M PBS containing 0.6% hydrogen peroxide to eliminate endogenous peroxidases. After washing three times for 5 min in 0.1 M PBS, sections were then incubated for 1 h in 0.1 M PBS containing 2% bovine serum albumin (BSA), 5% normal goat serum, and 0.2% Triton X-100 for blocking. For GFAP immunostaining, a primary GFAP mouse monoclonal antibody (1:1,000; Chemicon, Temecula, CA) was used. The secondary antibody was biotinylated horse antimouse (Vector Laboratories, Burlingame, CA). Amplification was done with an avidin-

biotin complex (Vectastain Elite ABC kit; Vector Laboratories) and was visualized with DAB (Vector Laboratories).

Three slices of the same region of the hippocampus were selected, and the number of GFAP-positive astrocytes per square on both sides (6 sites) was counted. Star-shaped astrocytes were visualized by GFAP immunostaining in the dentate gyrus (molecular layer, subgranular zone, and hilus), and CA3 and CA1 regions of the hippocampus (Fig. 2). Astrocytes were classified as activated or nonactivated astrocytes by measuring the size of the cell body, and the length and thickness of the dendrites was calculated (Fig. 3). Reactive astrocytes are recognized by GFAP labeling and extended lengths of processes (Viola et al., 2009). We differentiated activated astrocytes from nonactivated astrocytes mainly by dimension rather than by length of longest process. The numbers of activated astrocytes and the combined activated and nonactivated astrocytes were counted.

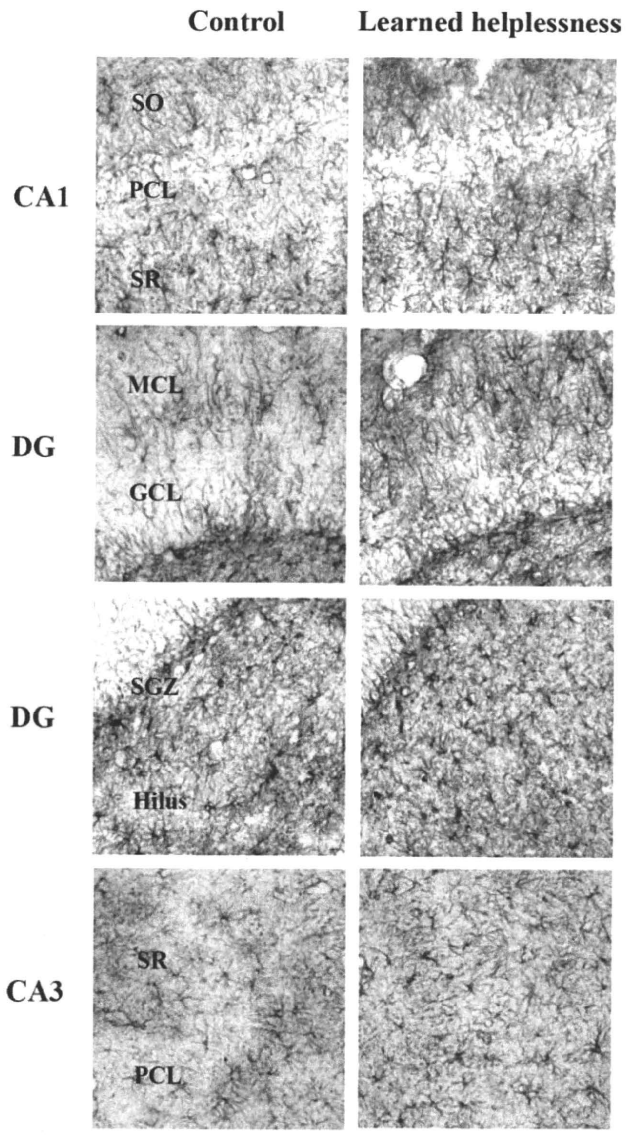

FIGURE 2. GFAP immunostaining in the hippocampus. GCL, granule cell layer; ML, molecular layer; PCL, pyramidal cell layer; SGZ, subgranular zone; SR, stratum radiatum; SO, stratum oriens.

Date Analysis

Statistical differences among more than three groups were estimated by a one-way ANOVA, followed by Scheffe's test. For comparison of the mean values between the two groups, statistical evaluation was done using the two-tailed Student's *t*-test. The criterion of significance was $P < 0.05$.

RESULTS

Increased Number of Activated Astrocytes after Attainment of LH and Attenuating Effects of Imipramine

The numbers of activated astrocytes were significantly increased in the CA1, molecular layer, subgranular zone, hilus, and CA3 regions 2 and 8 days after the attainment of LH (Fig. 4). The magnitude of the change in the number of activated astrocytes was bigger 8 days after the attainment of LH than 2 days after (Fig. 4). However, subchronic treatment with imipramine showed a tendency (although not statistically significant) to decrease the LH-induced increment rate of activated astrocytes at the subgranular zone ($P = 0.054$), hilus ($P = 0.085$), and CA3 region ($P = 0.074$). Furthermore, subchronic treatment of naïve rats with imipramine did not alter the numbers of activated astrocytes in the regions examined (Fig. 4).

Combined Numbers of Activated and Nonactivated Astrocytes After Attainment of LH and Effects of Imipramine

Significant increases in the total numbers of both activated and nonactivated astrocytes were found in the CA1 and CA3 regions, but not in the molecular layer, subgranular zone, and hilus two days after the attainment of LH (Fig. 5). The total numbers of both activated and nonactivated astrocytes were significantly increased in all regions examined 8 days after the attainment of LH (Fig. 5). Furthermore, subchronic treatment with imipramine did not alter the combined numbers of either activated or nonactivated astrocytes in LH rats (Fig. 5). Additionally, subchronic treatment of naïve rats with imipramine did not alter the total numbers of activated or nonactivated astrocytes in the regions examined (Fig. 5).

Effects of Inhibition of Hippocampal Astrocyte Function of LH Rats on Conditioned Avoidance Test

The antidepressant-like effects of imipramine were significantly blocked in the LH paradigm when fluorocitrate was injected into the dentate gyrus (Fig. 6). Similarly, the antide-

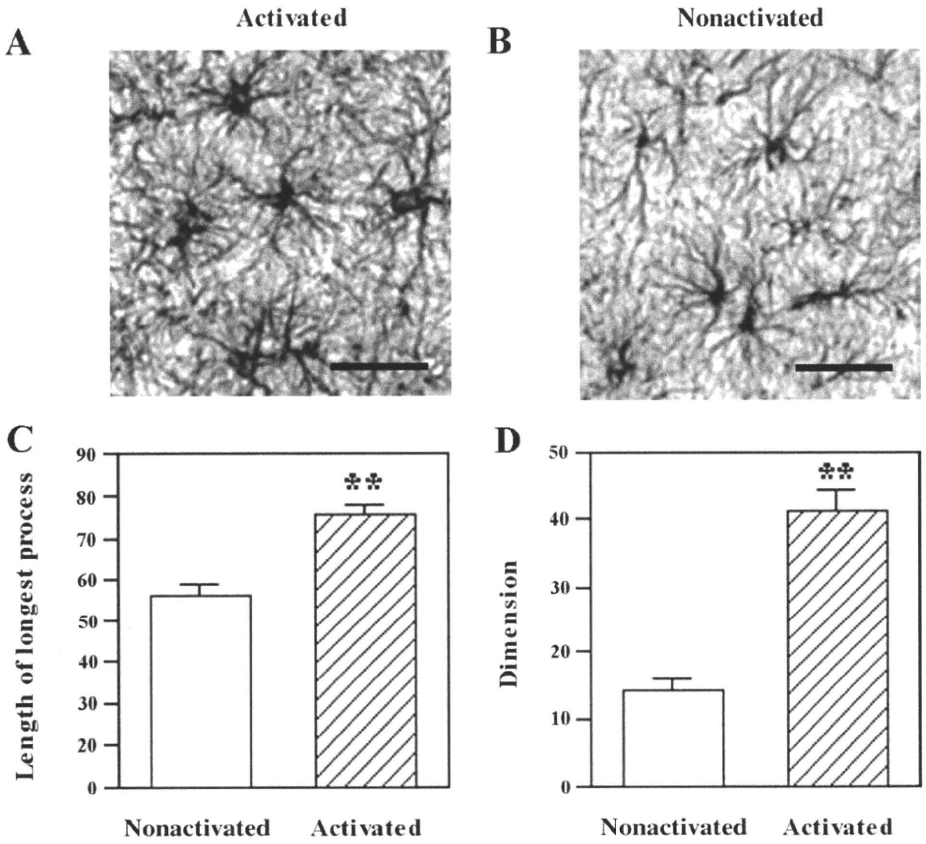

FIGURE 3. Representative images of GFAP immunoreactive activated (A) and nonactivated (B) astrocytes. Scale bar: 25 μm. The lengths of the longest processes (C) and dimensions (D) of activated and nonactivated astrocytes are indicated. The results are expressed as mean ± SEM. Sample number = 10. $^{**}P < 0.01$ when compared with controls (Student's *t*-test).

pressant-like effects of imipramine were significantly blocked when fluorocitrate was injected into the CA3 region of the hippocampus (Fig. 7). Meanwhile, injection of fluorocitrate into the dentate gurus or CA3 region of naïve rats failed to induce behavioral deficits in the conditioned avoidance test (Figs. 6 and 7).

DISCUSSION

The main finding of this study was that the numbers of activated astrocytes and the combined numbers of activated and nonactivated astrocytes were increased in the regions examined 2 and 8 days after the attainment of LH. Thus, LH continued to activate and induce astrocytes. This result was in a good agreement with previous studies. For example, repeated immobilization stress increased αB-crystallin, which is localized in astrocytes and increases in reactive astrocytes, in the hippocampus through activation of astroglia (Yun et al., 2002). Chronic restraint stress increased glia-specific excitatory amino acid transporter (GLT-1) in the dentate gyrus and CA3 region of the hippocampus (Reagan et al., 2004). Subchronic treatment of amphetamine, which induces not only pleasure but also stress, including supersensitivity, increased GFAP levels in the rat hippocampus (Frey et al., 2006). Chronic unpredictable stress increased levels of immunoreactivity of GFAP in the ventral tegmental area (Ortiz et al., 1996). Therefore, the LH condition and repeated stress produce similar glial changes in the brain.

The prevailing view about the response of astrocytes to injury is that the appearance of reactive astrocytes impedes the regenerative process of scar tissue formation. Therefore, the increases in the numbers of activated and nonactivated astrocytes 2 and 8 days after the attainment of LH are associated with impairments in neuronal function, which may be the cause of LH. However, it is likely that astrocytes play a role in neuroprotection and the regenerative process after neuronal impairment (Eddleston and Mucke, 1993). Cytokine-stimulated astrocytes promoted the recovery of CNS function (Liberto et al., 2004). Thus, the LH paradigm induced increases in the number of activated and nonactivated astrocytes, which could be a compensatory response to stress. In support of this, environmental enrichment, which increases

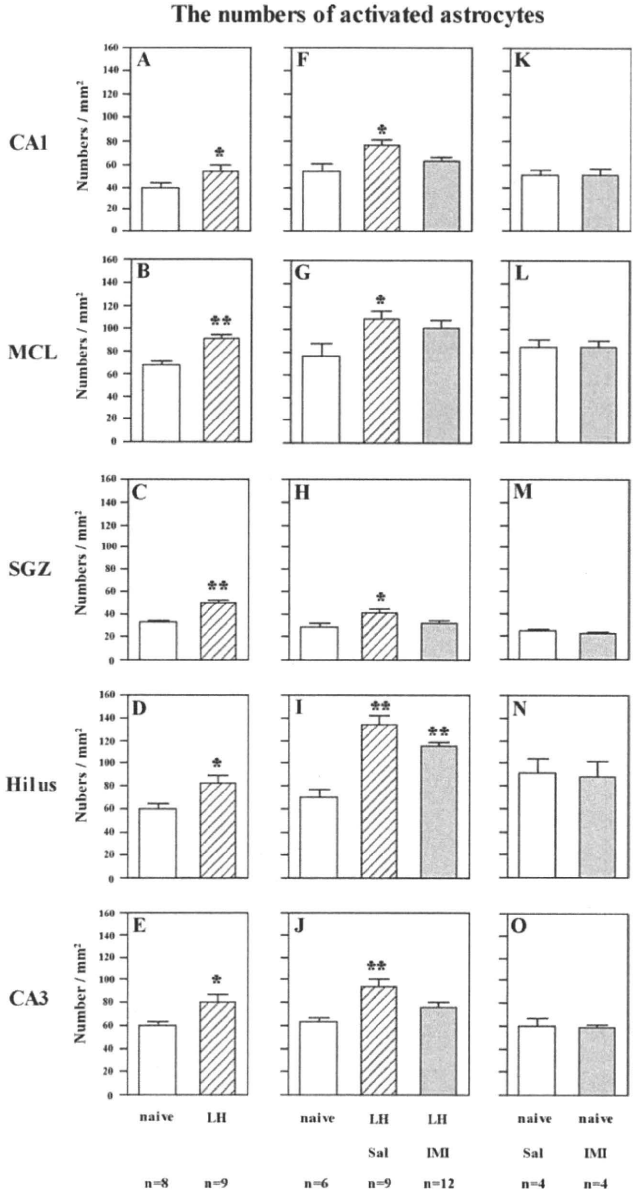

FIGURE 4. The number of activated astrocytes in the CA1, granule cell layer, hilus and CA3 regions of hippocampus of LH rats and effect of antidepressant drug. (A) $t = 2.145$, $P = 0.0487$; (B) $t = 4.618$, $P = 0.0003$; (C) $t = 5.360$, $P = 0.0001$; (D) $t = 2.661$, $P = 0.0178$; (E) $t = 2.55$, $P = 0.0222$; (F) $F(2,24) = 4.821$, $P = 0.0174$; (G) $F(2,24) = 3.732$, $P = 0.0388$; (H) $F(2,24) = 5.292$, $P = 0.0125$; (I) $F(2,24) = 23.26$, $P < 0.0001$; (J) $F(2,24) = 6.190$, $P = 0.0068$. (K) $t < 0.001$, $P > 0.9999$; (L) $t = 0.29$, $P = 0.9775$; (M) $t = 1.243$, $P = 0.2603$; (N) $t = 0.171$, $P = 0.8696$; (O) $t = 0.234$, $P = 0.8225$. *$P < 0.05$; **$P < 0.01$ when compared with controls (Student's t-test or ANOVA followed by Scheffe's test).

neurogenesis in the hippocampus, produced an increase in the ramification of astrocytic processes and the number and length of primary processes extending in the hippocampus (Viola et al., 2009). Furthermore, an increase in the number of GFAP-labeled astrocytes was seen in the cingulate of postpartum rats (Salmaso et al., 2009). Therefore, an alternative explanation may be that neuroplasticity including nerve growth, neuroprotection, and regeneration requires astrocytes.

However, other animal studies showed contrasting results. For example, a significant deficit in GFAP-immunoreactive cells was found in the prefrontal cortex, amygdala, and hippocampus in the Wistar-Kyoto rat strain (a model of depression)

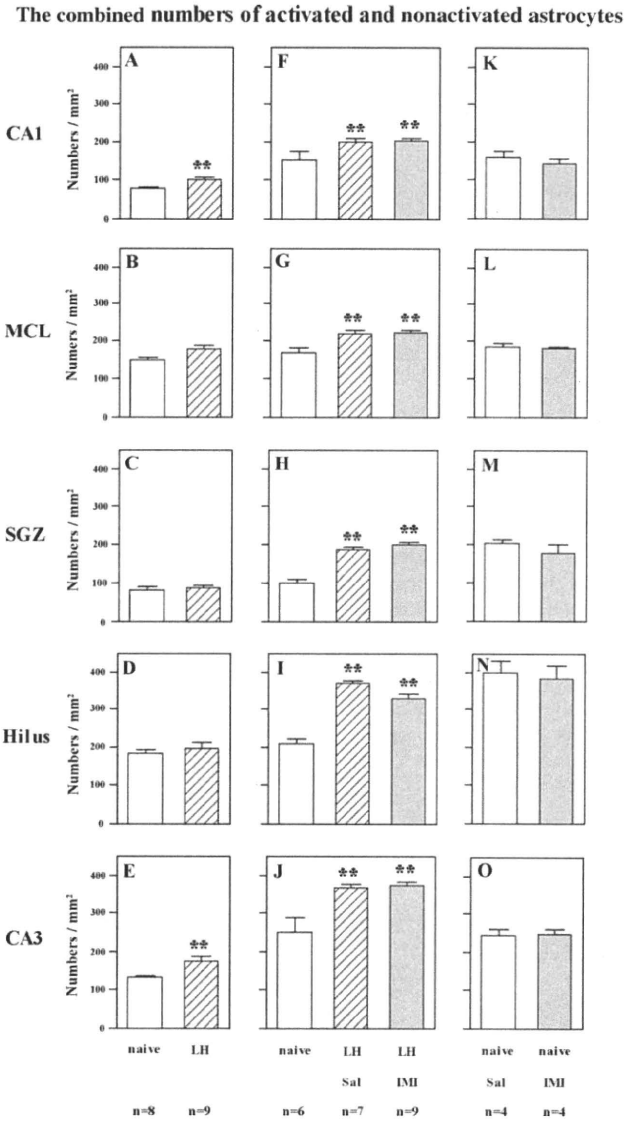

FIGURE 5. The combined number of both activated and non-activated astrocytes in the CA1, granular cell layer, hilus and CA3 regions of the hippocampus of LH rats and effects of antidepressant drug. (A) $t = 3.769$, $P = 0.0019$; (B) $t = 2.090$, $P = 0.0541$; (C) $t = 0.823$, $P = 0.4236$; (D) $t = 0.610$, $P = 0.5508$; (E) $t = 3.447$, $P = 0.0036$; (F) $F(2,24) = 5.541$, $P = 0.0105$; (G) $F(2,24) = 4.042$, $P = 0.0307$; (H) $F(2,24) = 42.545$, $P < 0.0001$; (I) $F(2,24) = 45.749$, $P < 0.0001$; (J) $F(2,24) = 13.822$, $P = 0.0001$. (K) $t = 0.646$, $P = 0.5422$; (L) $t = 0.410$, $P = 0.6940$; (M) $t = 1.014$, $P = 0.3498$; (N) $t = 0.314$, $P = 0.7643$; (O): $t = 0.149$, $P = 0.8864$. *$P < 0.05$; **$P < 0.01$ when compared with controls (Student's t-test or ANOVA followed by Scheffe's test).

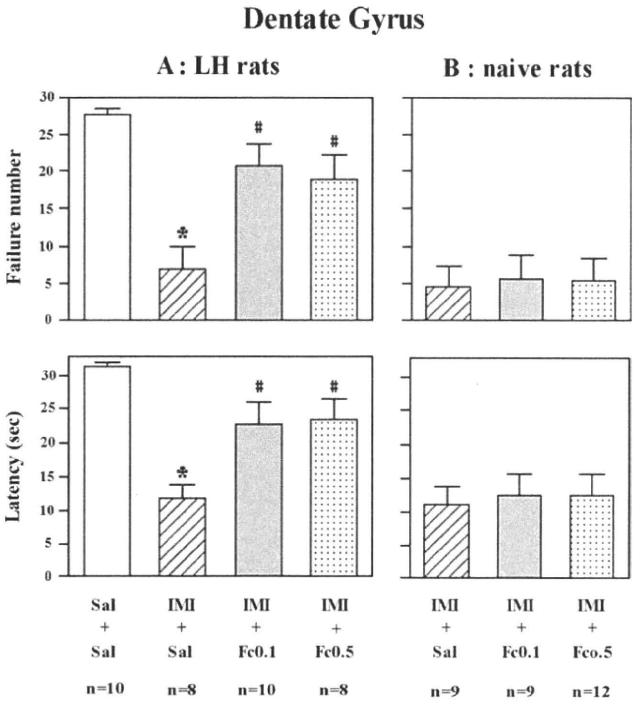

FIGURE 6. Effects of injection of fluorocitrate into the DG of the hippocampus of LH rats receiving imipramine in the conditioned avoidance test. Escape failure number and latency to escape were determined. The results are expressed as mean ± SEM. Shown on the right are the results of fluorocitrate-injection into imipramine-treated normal rats for comparison. Left top, $F(3,32) = 10.554$, $P < 0.0001$; left bottom, $F(3,32) = 10.140$, $P < 0.0001$; right top, $F(2,27) = 0.212$, $P = 0.8104$; right bottom, $F(2,27) = 0.060$, $P = 0.9415$. $^{*}P < 0.05$ when compared with controls (saline+saline-treated LH rats) (ANOVA followed by Scheffe's test). $^{\#}P < 0.05$ when compared with imipramine-treated LH rats (ANOVA followed by Scheffe's test). Fc, fluorocitrate; IMI, imipramine; Sal, saline

(Gosselin et al., 2009). Chronic psychosocial stress decreased both the number and somal volume of astroglia in the hippocampus (Czéh et al., 2006). Furthermore, glial loss in the prefrontal cortex induced depressive-like behaviors (Banasr and Duman, 2008). The precise function of astrocytes needs to be established.

The above discrepancy could be explained by Selye's definition of stress as a response to any demand that produces three stages (alarm, resistance and exhaustion). Our working hypothesis is that astrocytes first respond to alarm, and then become activated, or new astrocytes are induced to increase the numbers at the stage of resistance, and finally astrocytes tire and the numbers of astrocytes decrease at the stage of exhaustion. The increase in the numbers of activated and nonactivated astrocytes in the present study could be considered as a resistant and compensatory mechanism, which is distinct from the final reduction in the number of astrocytes, although this is speculation. Further studies are needed to elucidate this hypothesis.

The next finding is that the reversible impairment of astrocyte function by infusion of fluorocitrate into the dentate gyrus or CA3 region of LH rats blocked the antidepressant-like effects of subchronic treatment with imipramine on the conditioned active avoidance test. Fluorocitrate specifically and reversibly disrupts astroglial metabolism by blocking aconitase, an enzyme integral to the TCA cycle (Paulsen et al., 1987; Hassel et al., 1994; Willoughby et al., 2003). We can assume various mechanisms. First, there is a possibility that the blockade of glutamate uptake and glutamine synthesis lead increased extracellular glutamate levels and decreased extracellular glutamine levels, interrupting the antidepressant effect of imipramine. In support, treatment with antidepressants reduced the serum levels of glutamate, and increased the serum levels of glutamine in major depression patients (Maes et al., 1998). Future study will be needed to elucidate the mechanism of glutamine in the effects of antidepressants. Second, the impairment of astrocytes may increase extracellular serotonin levels like antidepressant drugs because antidepressant drugs inhibit a glial serotonin transporter in the rat brain, increasing extracellular serotonin levels (Bel et al., 1997). It is likely that increases in extracellular serotonin levels remind us of selective serotonin reuptake inhibitor (SSRI) type of antidepressants. However, the present study demonstrated that inhibi-

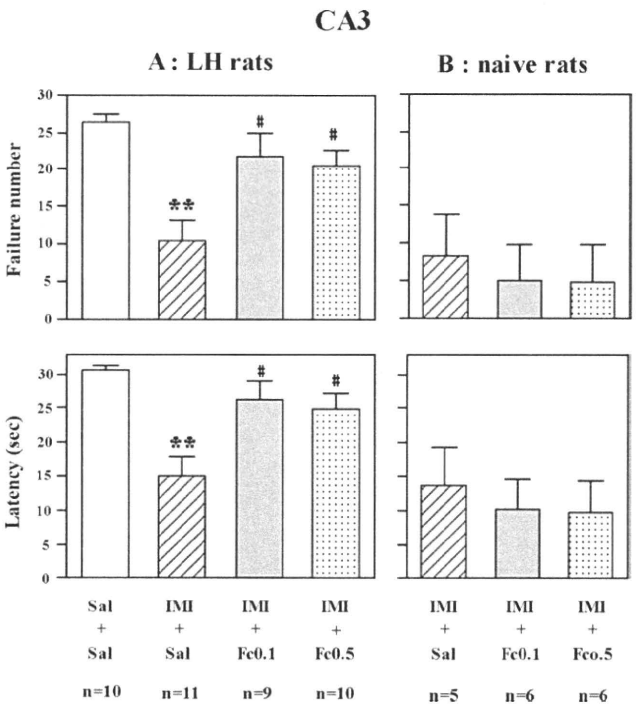

FIGURE 7. Effects of injection of fluorocitrate into the CA3 region of the hippocampus of LH rats receiving imipramine on conditioned avoidance test. Escape failure number and latency to escape were determined. The results are expressed as mean ± SEM. Shown on the right are the results of fluorocitrate-injection into imipramine-treated normal rats for comparison. Left top, $F(3,32) = 10.140$, $P < 0.0001$; left bottom, $F(3,32)$, $P < 0.0001$; right top, $F(2,14) = 0.132$, $P = 0.8778$; right bottom $F(2,14) = 0.199$, $P = 0.8222$. $^{**}P < 0.01$ when compared with controls (saline+saline-treated LH rats) (ANOVA followed by Scheffe's test). $^{\#}P < 0.05$ when compared with imipramine-treated controls (ANOVA followed by Scheffe's test). Fc, fluorocitrate; IMI, imipramine; Sal, saline.

tion of astrocytes function blocks the beneficial effects of antidepressant drugs on the LH paradigm. Thus, serotonin would be irrelevant for the effects of fluorocitrate. Finally, astrocytes secrete physiologically active agents including brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) (Chen et al., 2006). It is well documented that infusion of BDNF into the hippocampus produced antidepressant-like effects (Shirayama et al., 2002) and that antidepressants treatment increased GDNF mRNA and GDNF release, promoting neuronal survival and protection from the damaging effects of stress (Hisaoka et al., 2001). Meanwhile, L-deprenyl, an inhibitor of monoamine oxidase B, which is predominantly localized in astrocytes, potentiates the reaction of astrocytes to mechanical lesions, and increases basic fibroblast growth factor (bFGF) production (Biagini et al., 1994). Furthermore, intracerebroventricular administration of FGF2 exerted antidepressant-like effects (Turner et al., 2008). Taken together, BDNF, GDNF, and FGF could play an important role in antidepressant effects of imipramine. Future study needs to elucidate the relationships of antidepressant drugs with glutamine, BDNF, GDNF, and FGF in astrocytes.

In conclusion, the numbers of activated and nonactivated astrocytes were significantly increased in the hippocampus after the attainment of LH. Subchronic treatment with imipramine showed a tendency (although not statistically significant) to decrease the numbers of activated astrocytes at the subgranular zone, hilus, and CA3 region. However, subchronic treatment of naïve rats with imipramine failed to induce changes in the numbers of astrocytes. Finally, the antidepressant-like effects of imipramine were significantly blocked in the LH paradigm when fluorocitrate was injected into the dentate gurus or CA3 region, whereas injection of fluorocitrate into naive rats failed to induce behavioral deficits in the conditioned avoidance test. These results suggest that hippocampal astrocytes contribute to the pathophysiology of depression.

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Neuroscience Research xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Research

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A multi-channel near-infrared spectroscopy study of prefrontal cortex activation during working memory task in major depressive disorder

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ARTICLE INFO

Article history:
Received 10 September 2010
Received in revised form 7 December 2010
Accepted 5 January 2011
Available online xxx

Keywords:
Near-infrared spectroscopy
Working memory
Major depressive disorder
Prefrontal cortex
2-Back task
Cerebral blood volume

ABSTRACT

Many neuropsychological studies demonstrate impairment of working memory in patients with major depressive disorder (MDD). However, there are not enough functional neuroimaging studies of MDD patients seeking for the underlying brain activity relevant to working memory function. The objective of this study is to evaluate prefrontal hemodynamic response related to working memory function in patients with MDD. Twenty-four subjects with MDD and 26 age- and gender-matched healthy subjects were recruited for the present study. We measured hemoglobin concentration changes in the prefrontal and superior temporal cortical surface areas during the execution of working memory task (WM; 2-back, letter version) using 52-channel near-infrared spectroscopy (NIRS), which enables real-time monitoring of task-related changes in cerebral blood volumes in the cortical surface areas. MDD patients showed a smaller increase in lateral prefrontal and superior temporal cortex activation during the 2-back task and associated poorer task performance than healthy controls. The results coincided with previous findings in terms of working memory deficits and prefrontal cortex dysfunction in MDD patients, but contradicted with some previous fMRI studies that suggested increased cortical activity during the working memory task in patients with depression. The contradiction may, in part, be explained by a relatively low level of cognitive demand imposed on the subjects in the present study.

1. Introduction

Working memory is an extensively researched psychological concept related to the temporary storage and processing of information (Baddeley, 1992, 2003). Intact working memory is essential for everyday functioning. Working memory tasks require several cognitive processes, such as online monitoring, continuous updating, manipulating stored information, and decision making, which might all be affected by major depressive disorder (MDD). Many neuropsychological studies demonstrate impairment of working memory in patients with MDD (Rose and Ebmeier, 2006; Harvey et al., 2004; Porter et al., 2003; Landro et al., 2001; Nebes et al., 2000; Elliott et al., 1996; Beats et al., 1996; Channon et al., 1993). However, some other studies failed to find significant differences between patients with MDD and normal controls (Elderkin-Thompson et al., 2003; Sweeney et al., 2000; Zakzanis et al., 1998; Purcell et al., 1997). The inconsistency presumably owes much to the difference in the patients' clinical characteristics, the cognitive demand of the various neuropsychological tests applied in the studies.

The hemodynamic responses related to the neural activity underlying working memory processes have been widely investigated using neuroimaging (fMRI and PET) techniques (Owen et al., 2005; Wager and Smith, 2003). In healthy subjects, the *n*-back task activated a bilateral network consisting of ventrolateral prefrontal cortex (VLPFC) and dorsolateral prefrontal cortex (DLPFC), frontal poles, lateral premotor cortex, dorsal cingulate and medial premotor cortices, and medial and lateral posterior parietal cortices (Owen et al., 2005). Recently, a number of studies have used fMRI and other imaging techniques to study brain activation associated with working memory function in patients with MDD. However, the findings have been inconsistent. There is one study that demonstrated significantly greater activation in the dorsolateral cortex in MDD patients than in healthy controls (Matsuo et al., 2007), whereas another study showed no difference in the prefrontal activation between MDD patients and healthy controls (Barch et al., 2003). Interestingly, in both studies, there was no significant between-group difference in the performance level. Moreover, a number of neuroimaging studies targeting working memory have demonstrated *load-related* hyperfrontality in MDD patients com-

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doi:10.1016/j.neures.2011.01.001

Please cite this article in press as: Pu, S., et al., A multi-channel near-infrared spectroscopy study of prefrontal cortex activation during working memory task in major depressive disorder. Neurosci. Res. (2011), doi:10.1016/j.neures.2011.01.001

pared with that in healthy controls (Harvey et al., 2005; Walsh et al., 2007; Fitzgerald et al., 2008), namely, MDD patients showed more increased cerebral activation in association with a higher level of complexity than healthy controls. The authors suggest that patients with MDD require greater resources to maintain task performance with increasing cognitive demand. Walter et al. (2007) assessed patients' neural response to correct trials only in a working memory task and found increased DLPFC activation. In their study, however, patients with MDD showed less accurate performance than healthy controls in a task with higher complexity and increased activation was not seen when incorrect trials were included in the analysis. This result suggests that increased cortical response is associated with matched performance level and may enable identification of where patients' performance is impaired.

In this regard, it is noteworthy that better working memory performance is associated with increased prefrontal activation in healthy subjects (Courtney et al., 1998; Ungerleider et al., 1998; Courtney et al., 1996; Leung et al., 2002; Sakai et al., 2002). Although the relationship between working memory performance and prefrontal activation in patients with MDD is not so clear, cortical response may be attenuated compared with that in healthy controls as a function of the extent of impaired performance of the patients.

In this study, we examined hemodynamic response in the fronto-temporal regions during engagement in working memory task in patients with MDD using multi-channel near-infrared spectroscopy (NIRS). Multi-channel NIRS (ETG-4000, Hitachi Medical Co.), a recently developed functional neuroimaging technology, enables the non-invasive detection of spatiotemporal characteristics of brain function near the brain surface using near-infrared light (Strangman et al., 2002a; Boas et al., 2004). NIRS has enabled bedside measurement of the concentrations of oxygenated ([oxy-Hb]) and deoxygenated hemoglobin ([deoxy-Hb]) in micro-blood vessels. Assuming that hematocrit is constant, the changes in [oxy-Hb], [deoxy-Hb] and also [total Hb] (summation of [oxy-Hb] and [deoxy-Hb]) are correlated with the changes in the regional cerebral blood volume (rCBV) as shown by simultaneous NIRS and positron emission tomography (PET) measurements (Hock et al., 1997; Villringer et al., 1997; Ohmae et al., 2006). In contrast to other neuroimaging methodologies such as fMRI, PET, electroencephalography (EEG) and magnetoencephalography (MEG), NIRS can be measured under a more restraint-free environment that is especially suitable for psychiatric patients. Indeed, NIRS has been used to assess brain functions in many psychiatric disorders (Matsuo et al., 2003; Suto et al., 2004; Kameyama et al., 2006). Moreover, unlike fMRI, which mainly represents the blood oxygenation level-dependent (BOLD) effect in the draining vein, NIRS is more likely to measure the changes in rCBV in distensible capillary vessels. Although the two methods target distinct aspects of hemodynamic response, the findings in terms of cortical activation obtained by simultaneous recordings of fMRI and NIRS are generally in agreement (Lee et al., 2008; Strangman et al., 2002b).

The goal of this study was to compare brain activation, measured by NIRS, as well as behavioral performance in patients with MDD and age- and gender-matched healthy controls during engagement in working memory task. From the hypothesis that patients with MDD require more resources to maintain the task performance, we predicted that patients with MDD would show either (1) increased prefrontal activation associated with comparable task performance or (2) decreased or equivalent activation associated with impaired task performance compared with the healthy controls.

2. Subjects and methods

2.1. Subjects

Twenty-four patients with MDD and 26 healthy controls participated in the study (Table 1). The patients were recruited from the outpatients at Tottori University Hospital, and were diagnosed using the criteria of Diagnostic and Statistical Manual of Mental Disorders, the fourth edition, text revision (DSM-IV-TR, American Psychiatric Association 2000).

To obtain detailed information on psychiatric symptoms, the participants were questioned using a structured interview, the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). None of the subjects had clinical evidence of other central nervous system disorders based on history and medical examination. Patients with previous head trauma, stroke, electroconvulsive therapy, and current or previous history of substance abuse were excluded from the study. Twenty-four individuals (12 male and 12 female) meeting these criteria participated in the investigation. All the patients with MDD were in a depressed mood state. Within the MDD sample, 13 patients were taking selective serotonin reuptake inhibitors (SSRIs), 8 were taking serotonin noradrenaline reuptake inhibitors (SNRIs) and 3 were taking tricyclic antidepressants.

Individuals who were appropriate age and gender matches for the MDD patients participated as controls in the present study. Inclusion criteria for controls were similar to those for the patient sample, although controls were additionally required to have no previous or current psychiatric illnesses. Twenty-six individuals

Table 1
Demographic characteristics of the subjects and scores of BDI, HAMD and task performance (given values are means with standard deviations in parentheses).

	Major depression disorder ($N=24$)	Normal controls ($N=26$)	Group difference P-value
Gender (f/m)	12 f/12 m	18 f/8 m	0.16
Age (years)	47.9 (13.9)	42.4 (9.3)	0.10
Duration of illness (years)	4.0 (4.9)	N/A	
Age of onset (years)	43.0 (14.3)	N/A	
Number of depressive episodes	1.8 (1.4)	N/A	
Beck Depression Inventory (BDI)	22.1 (12.7)	8.0 (8.0)	<0.001
Hamilton Depression Rating Scale (HAMD)	20.3 (9.2)	N/A	
Task performance			
Reaction time (RT; ms)	739.4 (220.4)	678.1 (179.4)	0.32
Accuracy (%)	77.4 (0.30)	96.5 (0.08)	<0.01
Sensitivity A′	0.87 (0.28)	0.99 (0.02)	<0.05
Antidepressants (imipramine equivalents) (mg/day)	101.0 (57.1)	N/A	
Other drugs			
Anxiolytics	5	N/A	
Hypnotics	12		
Anxiolytics and hypnotics	3		

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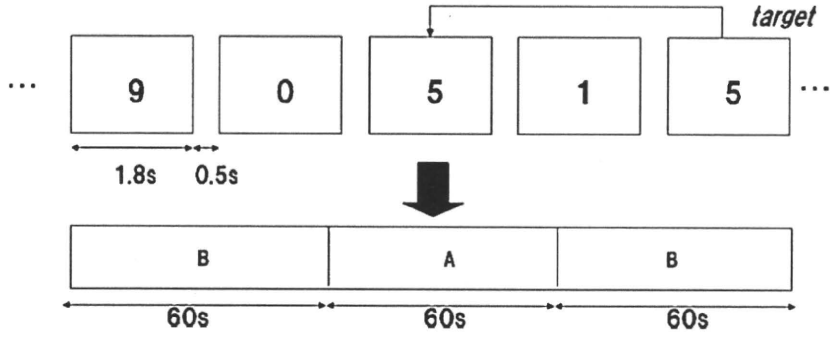

Fig. 1. The task design of 2-back task. A: Activation condition: 2-back. B: Baseline condition: 0-back, "9" as target.

(8 male and 18 female) meeting these criteria were selected to participate in the study.

All participants were right-handed with criteria of more than 80% by the Edinburgh Inventory Index (Oldfield, 1971). All subjects gave their consent in a written form after receiving comprehensive information on the study protocol. The study was approved by the ethics committee of Tottori University Faculty of Medicine.

2.2. Assessment of clinical evaluation

Prior to scanning, all subjects undertook a self-assessment of depression severity using the Beck Depression Inventory (BDI, Beck et al., 1961). In addition, only the patients were assessed for depression severity using the Hamilton Rating Scale for Depression (HAMD, Hamilton, 1960) by two trained psychiatrists.

2.3. NIRS measurements

2.3.1. Activation task

We used a 2-back task with a blocked periodic BA design (Fig. 1) to activate brain regions specialized for maintenance components of verbal working memory, as originally described by Cohen et al. (1994). Two contrasting conditions were visually presented in 60-s periods to subjects on a computer screen placed approximately one meter away from the subjects' eyes. During the period of the baseline (B) condition, subjects viewed a series of figures (0–9), which appeared one at a time, and were required to press a button with their right index finger whenever the figure "9" appeared. During the period of the activation (A) condition (2-back), subjects again viewed a series of figures (0–9) and were required to press a button with their right index finger if the currently presented figure was the same as that presented two trials previously (e.g., 5–1–5, but not 2–6–3–2 or 7–7). The working memory task consisted of a 60-s pre-task period (baseline (B) condition), a 60-s 2-back task period (activation (A) condition), and a 60-s post-task period (baseline (B) condition). Each period comprised 25 stimuli (5 targets, stimulus duration 1.8 s, stimulus onset asynchrony (SOA) = 2.3 s). Behavioral performance on 2-back task during measurement was monitored in terms of reaction time (RT) to target figures, accuracy (number of target figures correctly identified) and sensitivity A′ (Grier, 1971). All subjects received identical training prior to measurement.

2.3.2. NIRS machine

The 52-channel NIRS machine (ETG-4000) measures relative changes of [oxy-Hb] and [deoxy-Hb] using two wavelengths (695 and 830 nm) of infrared light on the basis of the modified Beer–Lambert law (Yamashita et al., 1996). In this system, these [Hb] values include differential pathlength factor (DPF). The distance between pairs of source-detector probes was set at 3.0 cm and each measuring area between pairs of source-detector probes was defined as 'channel'. It is considered that the machine measures points at 2–3 cm depth from the scalp, that is, the surface of the cerebral cortex (Okada and Delpy, 2003; Toronov et al., 2001). The probes of the NIRS machine were fixed with thermoplastic 3×11 shells, with the lowest probes positioned along the Fp1–Fp2 line according to the international 10–20 system used in electroencephalography. The arrangement of the probes enabled the measurement of [Hb] values from bilateral prefrontal and superior temporal cortical surface regions. The correspondence of the probe positions and the measuring area on the cerebral cortex has been reported elsewhere (Okamoto et al., 2004). It was approximated by superimposing the measuring positions on MRI of a three-dimensionally reconstructed cerebral cortex made by averaging 17 healthy volunteers' brain images normalized to the MNI152 standard template (Fig. 2).

The rate of data sampling was 0.1 s. The obtained data were analyzed using the "Integral mode"; the pre-task baseline was determined as the mean over a 10-s period just prior to the task period, and the post-task baseline was determined as the mean over the last 5 s of the post-task period; linear fitting was applied to the data between these two baselines. A moving average method using a window width of 5 s was applied to remove any short-term motion artifacts. However, a moving average method alone could not remove all the artifacts and, thus, we applied a semi-automatic method for removing those data with significant artifacts. First, we applied the algorithm developed by Takizawa et al. (2008) that enables a fully automatic rejection of data with artifacts separately for each channel using quantitative evaluation, although the algorithm appeared to even reject data without artifacts. Therefore, in the next step, two researchers, who were both blind to the clinical background of the data, judged whether or not to save those data rejected by the algorithm through consultation. Consequently, the number of averaged data for each channel did not vary widely within and between the two diagnostic groups (MDD: $N = 20$–24 [mean = 22.7, SD = 1.28]; control: $N = 23$–26 [mean = 24.9, SD = 1.00]).

2.4. Data analysis

First, the performance level was compared between the two groups using the Wilcoxon rank sum test. Next, for the analysis of the hemodynamic response data, [Hb] variables, which are specifically [oxy-Hb], [deoxy-Hb] and [total Hb] concentrations, of each channel were averaged for the two time segments (pre- and post-task baseline and task period). We focused on [oxy-Hb] concentrations, since [oxy-Hb] change (task period – pre- and post-task baseline period) is assumed to more directly reflect cognitive activation than [deoxy-Hb] change as shown by a stronger correlation with blood-oxygenation level-dependent signal measured by fMRI (Strangman et al., 2002b). The mean [oxy-Hb] changes were compared between the two groups (MDD and control) for each channel using Student's t-test. Since we performed 52 t-tests,

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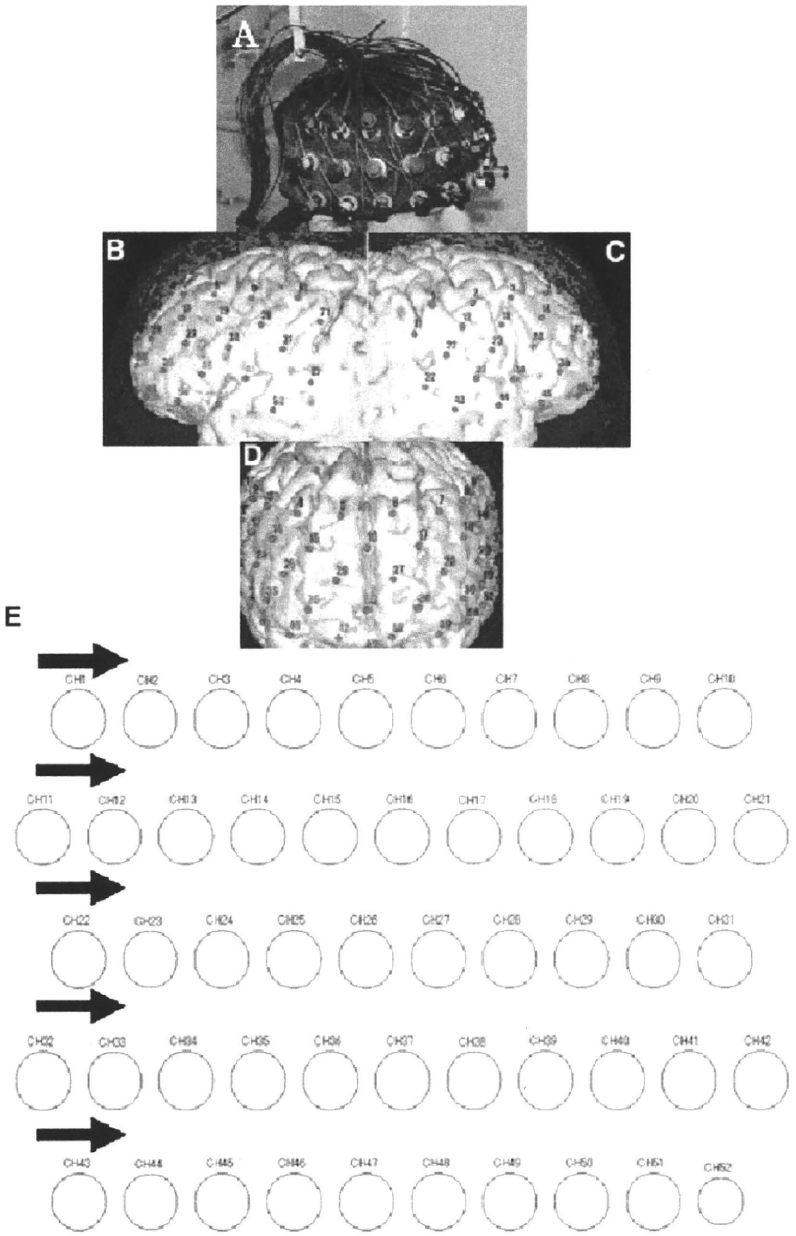

Fig. 2. Probe setting and measurement points for 52-channel near-infrared spectroscopy (NIRS). (A) The probes with 3 × 11 thermoplastic shells were placed over a subject's bilateral frontal regions. (B–D) The 52 measuring positions of the NIRS machine are superimposed on 3D-reconstructed cerebral cortical surface from magnetic resonance imaging (MRI) made by averaging 17 healthy volunteers' brain images normalized to the MNI152 standard template. The channel numbers are indicated above the measuring points. (E) The 52 measuring areas are labeled ch1–52 from the right posterior to the left anterior.

the correction for multiple comparisons was made using false discovery rate (FDR). We set the value of q specifying the maximum FDR to 0.05, so that there were no more than 5% false-positives on average (Singh and Dan, 2006). In case there was a significant between-group difference in the performance level (sensitivity A′), we performed additional analyses of co-variance (ANCOVA) using the performance level (sensitivity A′) as a covariate to the [oxy-Hb] changes, also applying FDR correction.

For MDD patients, Spearman's rhos were calculated for each channel to assess the relationship between the mean [oxy-Hb] changes and the clinical characteristics such as duration of illness, age of onset, number of depressive episodes, HAMD and BDI scores. We again adopted an FDR-based procedure for the multiple testing correction in correlational analyses for 52 channels and identified those channels for which r values reached a significance level of $P<0.05$ (FDR-corrected). Additionally, we investigated the relationship between [oxy-Hb] changes and performance level (reaction time, accuracy, sensitivity A′) and age in total samples using Spearman's rhos. Finally, we examined the relationship between [oxy-Hb] changes and the daily dose levels of antidepressants (imipramine equivalents) and also compared [oxy-Hb] changes between patients taking SSRIs and those taking SNRIs. Statistical analyses were performed using SPSS 13.0 software.

3. Results

3.1. Task performance

The response sensitivity A′ ($P<0.05$) and accuracy ($P<0.01$) on the 2-back task during NIRS measurement were significantly worse

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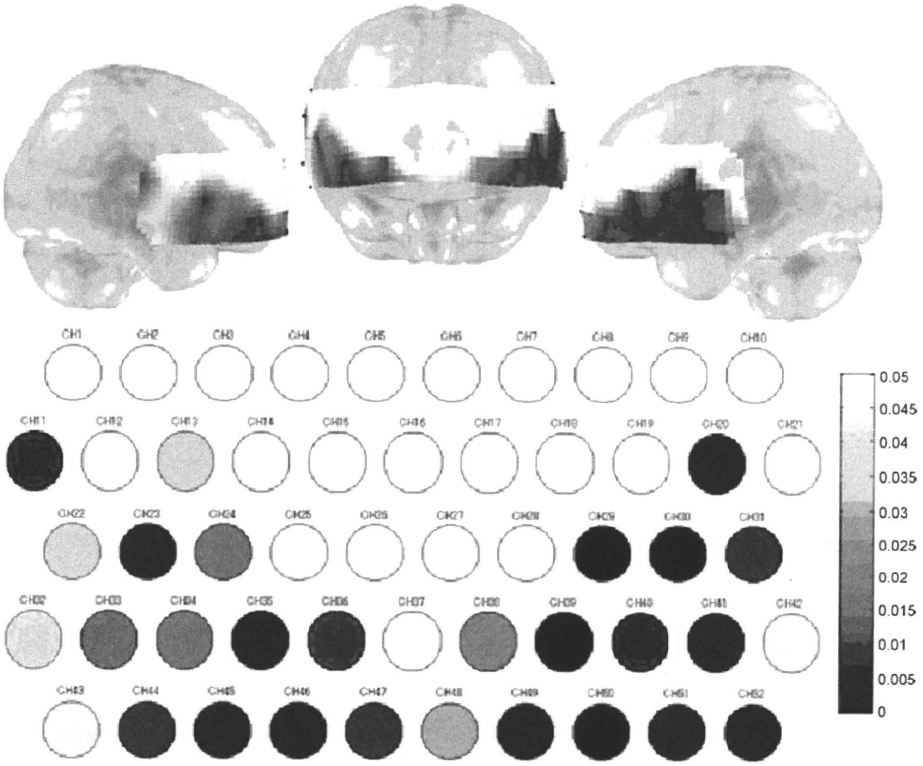

Fig. 3. *P*-value significance map of *t*-tests for [oxy-Hb] activation in patients with MDD compared with healthy controls using FDR correction. The numbered dots on the lower panel indicate 52 channels, which are projected to the 3D brain model in the upper panel.

in the MDD group than in the healthy controls. There was no significant between-group difference in reaction time (Table 1).

3.2. *[Hb] changes during task (Fig. 3)*

MDD patients were associated with a significantly smaller increase in [oxy-Hb] than controls at 29 channels (ch11, ch13, ch20, ch22–24, ch29–36, ch38–52; FDR-corrected *P*: 0.001–0.030). Fig. 3 is a *P* significance map of the *t*-tests, that is, MDD versus control, which shows significant group differences in a broad area.

The between-group differences in the [oxy-Hb] changes remained significant after correcting for performance levels in 22 channels (ch11, ch20, ch23–24, ch29–31, ch33–36, ch39–41, ch44–47, ch49–52; FDR-corrected *P*: 0.001–0.018) with ANCOVA using sensitivity A′ as a covariate to the [oxy-Hb] changes.

3.3. *Correlations between [oxy-Hb] change and clinical variables, performance level, age and drugs*

The mean [oxy-Hb] change in neither channel during the task period was significantly correlated with sensitivity A′ (rho = −0.16–0.28, n.s.), accuracy (rho = −0.14–0.30, n.s.), reaction time (rho = −0.33–0.39, n.s.), age (rho = −0.21–0.39, n.s.) for the total sample and also with age of onset (rho = −0.02–0.55, n.s.), duration of illness (rho = −0.61–0.14, n.s.), number of depressive episodes (rho = −0.42–0.19, n.s.), BDI (rho = −0.45–0.47, n.s.), HAMD (rho = −0.53–0.05, n.s.) for patients with MDD.

The mean [oxy-Hb] change in neither channel during the task period was significantly correlated with imipramine equivalents (mg/day). The mean [oxy-Hb] change in either channel did not significantly differ between the patients taking SSRIs ($n = 13$) and those taking SNRIs ($n = 8$) (Student's *t* test, $P = 0.06–0.99$, n.s.).

4. Discussion

The primary objective of this study was to examine whether the hemodynamic response in the fronto-temporal region during working memory processing differs between MDD patients and healthy subjects. Partially consistent with our prediction, we found that patients with MDD showed smaller hemodynamic response and worse task performance during engagement in working memory task than healthy controls. However, the finding contradicts several previous studies.

A number of previous studies demonstrated increased cortical activity in a depressed group using an *n*-back working memory task (Harvey et al., 2005; Walsh et al., 2007; Fitzgerald et al., 2008; Matsuo et al., 2007) and some demonstrated a higher linear load-response in patients with MDD than the normal controls, indicating that hyperfrontality in MDD was more evident in higher cognitive demanding condition. In addition, Barch et al. (2003) reported no significant difference between patients with MDD and normal controls in the neural response in the prefrontal cortex (PFC) elicited by the 2-back task using words, which was considered to be much easier than those adopted in the studies showing hyperfrontality presumed by the high performance level in both controls and patients with MDD. On the other hand, using a different cognitive task with high complexity ("Tower of London" task), Elliott et al. (1997) showed reduced neural response in cortical regions, particularly in VLPFC and DLPFC for patients with MDD compared with healthy controls, where patients' performance was impaired. It therefore seems to be an inverted-U relationship between neural response and cognitive demand in patients with MDD.

In the present study, there was no significant correlation between hemodynamic response and performance level, suggesting that the patients failed to recruit additional neural resources to attain higher performance level. As the patients with poor performance level showed similar degree of activation as those with

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high performance level, it can also be assumed that the attenuated [oxy-Hb] change was not due to the patients' disengagement in the task per se. We speculate that the 2-back task adopted in the present study posed relatively small amount of cognitive load on the subjects, which can be presumed by the high performance level of the normal controls, and also the 2-back task using numerical figures (0–9) may well be considered simple, compared to the task using letters adopted in most studies showing hyperfrontality in patients with MDD. Moreover, the period of 2-back task to be engaged in was as short as 60 s, which also implies relatively small load on memory and attentional system. According to the inverted-U relationship between neural response and cognitive demand in patients with MDD, it was suggested that the attenutated [oxy-Hb] change in patients with MDD reflected the failure of recruiting neural resources to attain comparable performance as normal controls even in low cognitive demanding condition.

It is possible that the different results between the present study and previous studies arise from patient characteristics, age or severity of illness. In fact, the mean age of the subjects in the present study (47.9 ± 13.9) was older than those in the previous studies, which were mostly within the range of 30–40 years. Although this is highly speculative, poor vasomotor function associated with presumably higher incidence of microvascular dysregulation in older patients with MDD may have made it difficult to recruit additional neural resources. It may be assumed that NIRS, which mainly measures the rCBV in the distensible capillary vessels, is more sensitive to vasomotor function than fMRI (Matsuo et al., 2005). These issues should be addressed systematically in future research.

One of the shortcomings of NIRS is that it cannot measure the rCBV change in deeper region of the brain such as limbic regions. In depression, DLPFC is thought to inhibit emotional responses through its efferent connections to limbic regions. Previous fMRI studies using a cognitive challenge showed decreased activity in limbic regions including medial prefrontal cortex (MPFC) while DLPFC and other cognitive regions were activated (Pochon et al., 2002). Harvey et al. (2005) demonstrated a trend for a greater decrease of activity in MPFC in control subjects compared to patients with MDD. In the study, the activity gap between cortical and limbic regions increased as the task increased in complexity and the authors suggested that the activity gap may affect the processing efficiency. The hypofrontality as well as poorer performance level in patients with MDD in the present study may have been due to smaller activity gap between cortical and limbic regions, although we should await future studies using other neuroimaging methods to measure the neural activity in limbic regions.

Previous fMRI studies using 2-back task cited in our manuscript did not find significant relationship between neural response and clinical symptoms. We also failed to find any relationship between [oxy-Hb] change and BDI or HAMD scores, but not surprisingly because we speculate that [oxy-Hb] change should be related to cognitive function rather than mood state. A range of neurocognitive functions including verbal memory (Sternberg and Jarvik, 1976), attention (Porter et al., 2003), working memory (Barch et al., 2003; Elliott et al., 1996) and executive function (Elliott et al., 1997) have been shown to be affected in patients with MDD. Moreover, these deficits are now being recognized to be independent of the disturbance of mood, although not entirely. Because cognitive deficits exist even when patients are euthymic (Kennedy et al., 2007) and are closely linked to social function (Jaeger et al., 2006), interventions targeting these deficits seem to be urgently required. Further studies are warranted to elucidate the relationship between [oxy-Hb] change elicited by the 2-back task and cognitive function as well as social function using neuropsychological test batteries and social function measurements.

Contributors

S. Pu designed the study, wrote the protocol, collected the data, statistically analyzed the data, and wrote the first draft of the manuscript. T. Yamada was involved in patient recruitment and data collection; he also contributed to writing the final version of the manuscript. K. Yokoyama, H. Matsumura, H. Kobayashi and N. Sasaki were involved in working out the study design and data collection. H. Mitani and A. Adachi were involved in data collection. K. Kaneko contributed to writing the final version of the manuscript. K. Nakagome was involved in working out the study design, writing the protocol and contributed to writing the final version of the manuscript.

Conflict of interest

All the authors declare that they have no conflicts of interest with respect to this study or its publication.

Role of funding source

Tottori University (Dr. Nakagome) and Hitachi Group (Advanced Research Laboratory, Hitachi Medical Corporation) have had an official contract for a collaborative study on the clinical application of near-infrared spectroscopy in psychiatric disorders since 2006. Hitachi Group has provided a material support. In addition, the present study has been funded by the Research Grant (20B-1) for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare.

Acknowledgements

The authors thank all the participants in this study.

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Please cite this article in press as: Pu, S., et al., A multi-channel near-infrared spectroscopy study of prefrontal cortex activation during working memory task in major depressive disorder. Neurosci. Res. (2011), doi:10.1016/j.neures.2011.01.001

研究報告

うつ病女性患者への集中内観療法による介入研究*

ストレス対処行動の変化

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抄録 精神医学 52:393-400 2010

精神疾患の発症や持続にストレス対処行動の関与が指摘されている。うつ病女性患者のストレス対処行動について，対処行動調査票(CISS)日本語版を用いて健常女性対照群と比較検討し，さらに集中内観療法によりその対処行動に変化が生じるか検討した。うつ病群では，対処行動として情緒優先対処をとりやすく，抑うつ尺度であるSDSと情緒優先対処の得点との間に正の相関が認められた。集中内観療法直後は課題優先対処が有意に上昇し，情緒優先対処は有意に低下した。SDSは，集中内観療法直後には有意に低下していた。以上より，集中内観療法により抑うつ症状および対処行動に変化が認められ，集中内観療法は対人関係療法，認知療法，行動療法などと同様に，女性に関してはうつ病の治療・再発予防効果が期待できる精神療法である可能性が示唆された。

Key words

Depression, Intensive Naikan therapy, Stress coping, Coping inventory for stressful situations (CISS), Female patients

はじめに

ストレス対処行動とは外的・内的なストレスによる精神的・身体的健康への影響を調節しようとする認知的・行動的な対処様式であり，日常我々が経験するストレスの重要な調節因子である。そのため不適切なストレス対処行動は気分障害や不安障害などの精神疾患の病態形成に深くかかわると指摘されている[14]。それゆえ，今まで精神疾患におけるストレス対処行動の役割に関するさまざまな研究がなされており，心的外傷後ストレス障害[5,17]，摂食障害[18,28]，パニック障害[2]などの発症

2007年6月4日受稿，2009年9月18日受理

* An Intervention Study of Intensive Naikan Therapy to Female Patients with Depression : Change in stress-coping behavior

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0488-1281/10/¥500/論文/JCOPY

や維持にストレス対処行動が関与していることが指摘されている。大うつ病性障害[3,20]においても同様の検討がなされており，うつ病女性患者群では情緒優先対処行動が多く，精神的に安定している女性群では課題優先対処行動が多いことが報告されている。また，中島[19]は高校生を対象に行った研究で，大うつ病エピソードの既往がある群は情緒優先対処得点が有意に高いことから，情緒優先対処行動が大うつ病エピソード発症のリスクとなる可能性を示唆した。ストレス対処行動が不適切であれば，心身の不安定さが増幅され，問題となる心身の症状や行動が持続・増悪することとなるので，ストレス対処行動を適切に行うことにより心身を健康に保つことができると考えられる。

日本で生まれた精神療法である内観療法には，内観を指導する施設に寝泊まりして集中して行う「集中内観療法(以下，集中内観)」と在宅で毎日行う「日常内観療法(以下，日常内観)」がある。通常，集中内観を経験した後に引き続き日常内観を継続することとなっている。集中内観の効果の研究として，性格特性や感情の評価などについての報告は多いが[9,24]，ストレス対処行動について評価尺度を用いた研究はほとんど存在しない。そこで今回我々は，男性より環境からのストレスを受けやすいと言われている女性において，うつ病に罹患している患者を対象に集中内観を行うことによりストレス対処行動に変化が生じるかを検討するため，集中内観前後に coping inventory for stressful situations (CISS)[6]（「ストレス状況に対する対処行動調査票」[10]）を用いてストレス対処行動の変化を比較検討した。

対象と方法

1. 対象

本研究の対象は，2005年10～12月にICD-10の診断基準の気分障害(F3)のカテゴリーで，うつ病エピソード(F32)か反復性うつ病性障害(F33)と診断され，国見統合医療研究所附属病院緑が丘心療内科(以下，緑が丘心療内科)に入院して集中内観を行った女性患者10名(すべて moderate depressive episode：SDS得点で評価)である。全症例の平均年齢50.8±17.0(29～80)歳，平均発病年齢47.6±14.9歳，平均罹病期間(今回のエピソードの期間)14.6±9.58か月，平均病相回数1.7±1.63であった。各症例に集中内観開始前に内観療法の概略について説明し，治療に対する動機づけを行ったうえで，研究参加への同意が得られた患者を対象とした。対照群はチラシで募集したボランティアの健常女性10名であり，平均年齢は49.3±13.7(29～74)歳であった(内観療法は高齢者ほど効果が乏しくなるので，年齢を80歳までと制限した)。

2. 集中内観の技法

緑が丘心療内科における集中内観の流れを概説する。まず集中内観を主治医に勧められたか，自ら集中内観を受けようと希望した患者は，医師より集中内観が適応であると判断された場合にのみ入院して集中内観を行う。

集中内観を受ける患者は，まず集中内観の進め方や，それによって期待できる効果についての説明を受け，動機づけが行われたうえで集中内観に導入される。治療薬については，集中内観開始の2週間前から終了時までは内容を変更しない。

緑が丘心療内科で行っている集中内観は鳥取大学医学部精神科で施行されている吉本原法を踏襲している。集中内観開始直前の初日，面接者と患者で集まりミーティングを行い，各自の集中内観に対する動機を言語化させ，他の患者の治療動機や目標などを聴くことで治療意欲を高める。また，面接者は集中内観の心構えを伝え，各患者の治療意欲が高まるよう激励する。

集中内観は，6：00～21：00，病院施設内和室の屏風の中で患者が座位を保ち，以下で説明する内観3項目について内観する。9：00～17：00は1～2時間に1回の間隔で1日6回，内観内容についての面接を面接者が患者に対して行う。また6：00～9：00と17：00～就寝まで(21：00)に，病棟内自室(大部屋のベッドをカーテンで間仕切りした中，または和室の屏風の中)で行われた内観に対しては，面接は行われず，内観内容の記録