

streptavidin-biotin peroxidase method and a Histofine SAB-PO kit (Nichirei, Tokyo, Japan), visualized using diaminobenzidine (DAB) and intensified with osmic acid. Control sections, in which the primary antibody was omitted, were processed in parallel. Sections were counterstained with hematoxylin for 10 sec.

To identify the cytoarchitecture and cortical layers of BA9,³⁴ sections usually adjacent to or within 20 μ m from the immunostained sections were stained with hematoxylin.

Areal density and cell size measurement

PV-, CR-, and CB-IR neurons were analyzed by two investigators (TS and AO). The methods of image and quantitative analysis were identical for each investigation.

Immunoreactive cells were plotted at 4 \times magnification using a Nikon microscope (Eclipse E800) equipped with an Olympus digital camera (DP 50). Using the software Viewfinder Lite ver.1.0 and Studio Lite ver.1.0 (Pixera Japan, Kanagawa, Japan), we obtained a series of contiguous images of the cortex from the pia to the gray/white matter border, from which a single composite image was formed using Adobe Photoshop CS.

Sections stained with hematoxylin for identification were analyzed using Image-J software ver.1.34 to measure cortical and laminar thicknesses and to count the number of cells in each layer. Cortical layers were distinguished on the basis of the differences in the distribution, size and shape of their neurons.³⁴ At each position in which data were acquired, immunoreactive cells were counted for each layer. The density of neuronal profiles was expressed as mean values (\pm SE) per mm² per layer from a total of two 1000- μ m-wide cortical traverses, each from the pial surface to the white matter border. Cortical traverses were located in an area devoid of damage and blood vessels and where the pial surface was parallel to the white matter border.

We used a semiautomated threshold to identify and outline all stained cells within the composite images. The threshold of the light intensity level was selected for each image so that the glia and neurons were well outlined. Neurons were identified by the presence of a stained cytoplasm and by their generally larger shape. Glia were differentiated from neurons by their more rounded and darker appearance, and smaller shape.

For each case and section, the somal size of each cell counted was measured using Image-J software, and each IR-neuron was classified into two classes according to their size. The size range was determined using the mean and SD of the size of the cells of the control subjects as follows: medium (within mean + 1 SD), large (larger than mean + 1 SD).

Statistical analysis

The relative density of labeled neurons from the two cortical traverses was averaged for each cortical layer in each case, and the results were analyzed by two-way ANOVA followed by the Bonferroni or Tamhane test using layers and diagnoses as variables. Following this analysis, the mean densities of PV-, CB-, and CR-IR neurons in each cortical layer for each of the two patient groups were compared with those of the control group by one-way ANOVA, which enabled us to determine disease and laminar specificity.

The demographic and histological variables listed in Table 1, for example age and sex, were considered to be confounders, and were therefore included in the analysis as covariates if they differed between each group at the 10% significance level (ANOVA or χ^2 test) or if they could also be shown empirically to predict densities at the 10% significance level (Spearman's rank correlation). All statistical analyses were carried out using SPSS 12.0 software (SPSS Japan Inc., Tokyo, Japan.).

RESULTS

Identification of adjustment variables

Because no significant group differences were detected for the demographic or clinical variables at the 10% significance level (ANOVA or χ^2 test) (Table 1), these variables were not included in the analysis as covariates.

Neurons and glia

Significant reductions in neuronal density were detected by two-way ANOVA in the BPD subjects ($P = 0.038$) and SCZ ($P = 0.002$) subjects. The neuronal density determined at each layer comparison showed reductions in layer 3 (22%, $P < 0.001$), layer 4 (31%, $P < 0.001$), and layer 5/6 (28%, $P = 0.006$) in the SCZ subjects, and in layer 4 (28%, $P = 0.031$) in the BPD subjects, and even after Abercrombie correction changes in the same direction were estimated. However, no significant differences in the somal size of neurons were observed. There was no change in glial density in any of the layers in the psychiatric disorder groups compared with that in the control group, and no change in glial size was observed.

CBP-IR neurons

CB-IR neurons were present predominantly in layer 2 and the superficial layer 3. The majority of these cells corresponded to non-pyramidal neurons, and showed intense immunoreactivity, and the minority were pyramidal in shape with a low immunoreactivity (Fig. 1). CR-IR neurons also appeared to be non-pyramidal neurons that

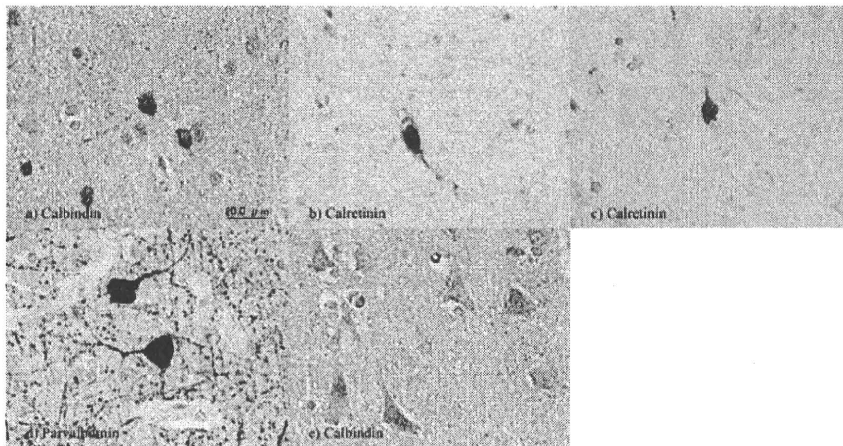


Fig. 1 Cells labeled by immunoreactivity to calbindin in layer 2 (a), calretinin in layer 1 (b) and layer 3 (c), parvalbumin in layer 3 (d), and labeled by low immunoreactivity to calbindin in layer 3 (e) of control subjects (bar = 20 μ m).

Table 2 Densities (mean \pm SE cells/mm²) of calcium-binding-protein-immunoreactive neurons in BA9 in schizophrenia (SCZ), bipolar disorder (BPD), and control (CON) groups

Calcium-binding protein	Cortical layer	Diagnosis					
		CON (n = 5)		BPD (n = 5)		SCZ (n = 7)	
		Medium	Large	Medium	Large	Medium	Large
Calbindin	1	3.40 \pm 1.69	0.51 \pm 0.51	0	0	0.84 \pm 0.60	0
	2	64.48 \pm 7.61	11.32 \pm 4.01	46.20 \pm 5.47	2.35 \pm 1.13	32.29 \pm 7.33*	0.30 \pm 0.30
	3	32.14 \pm 13.03	2.21 \pm 0.67	13.27 \pm 5.09	0.54 \pm 0.41	13.80 \pm 3.98	0.71 \pm 0.29
	4	15.54 \pm 9.81	3.27 \pm 2.87	3.53 \pm 2.17	0.36 \pm 0.36	2.91 \pm 1.24	0.28 \pm 0.28
	5/6	7.34 \pm 4.14	1.29 \pm 1.29	2.07 \pm 1.05	0.38 \pm 0.25	2.82 \pm 1.51	0.07 \pm 0.07
Calretinin	1	18.98 \pm 5.09	3.51 \pm 1.37	20.15 \pm 7.02	2.25 \pm 0.95	4.03 \pm 1.92	1.03 \pm 1.03
	2	56.38 \pm 6.90	5.52 \pm 1.63	56.46 \pm 9.07	17.11 \pm 3.76*	29.47 \pm 7.94	2.78 \pm 1.44
	3	22.74 \pm 4.81	4.26 \pm 1.23	21.08 \pm 2.92	7.01 \pm 1.80	10.52 \pm 2.86	1.29 \pm 0.59
	4	7.59 \pm 3.21	1.87 \pm 0.88	6.18 \pm 2.94	0.68 \pm 0.68	4.80 \pm 2.13	1.11 \pm 0.96
	5/6	1.64 \pm 0.74	0.12 \pm 0.12	1.93 \pm 0.72	0.34 \pm 0.14	0.68 \pm 0.19	0.17 \pm 0.17
Parvalbumin	1	1.82 \pm 0.91	0	2.40 \pm 1.61	0	0.65 \pm 0.65	0
	2	36.33 \pm 6.06	1.51 \pm 0.69	28.79 \pm 3.19	3.03 \pm 1.17	19.41 \pm 5.31	0
	3	41.15 \pm 3.92	9.28 \pm 2.39	35.81 \pm 3.89	9.48 \pm 3.64	30.53 \pm 3.47	3.33 \pm 1.12
	4	57.60 \pm 6.84	12.48 \pm 3.79	60.25 \pm 4.10	11.57 \pm 3.33	45.10 \pm 3.59	2.87 \pm 1.37
	5/6	20.82 \pm 2.63	2.00 \pm 0.89	20.43 \pm 2.99	4.36 \pm 1.70	12.92 \pm 1.67	0.62 \pm 0.24

* $P < 0.05$.

were present in all the layers, but were predominantly present in the superficial layers such as layers 2 and 3 (Fig. 1). PV-IR neurons were mainly distributed from the intermediate to inferior layers, and consisted of some morphologically distinctive neurons, including small ovoid-type and large multipolar neurons (Fig. 1). A plexus of PV-IR material was also distributed throughout the neuropil of layers 3, 4, and 5/6 and consisted of stained processes and puncta, which have been identified as terminals principally found on dendritic spines.³⁵ Summaries of the mean densities and sizes for each neuronal subpopulation in each layer are shown in Table 2 and Figure 2.

CBP-IR neurons were classified into medium and large classes according to size at the data acquisition points using mean + 1 SD of the size of the cells of the control subjects as follows: CB-IR neurons, 318 μ m²; CR-IR neurons, 231 μ m²; PV-IR neurons, 533 μ m².

Density

Neuronal density was reduced in the SCZ and BPD subjects, and this variable was included in ANOVA as a covariate for evaluating CBP-IR neuron density; however, no significant correlations were obtained between neuronal density and CBP-IR neuron density, and therefore CBP-IR neuron density was analyzed by ANOVA. Before the classification according to cell size, no significant differences were detected by two-way ANOVA between the control group and the psychiatric disorder groups, but there was a trend toward reductions in CB-IR ($P = 0.061$), CR-IR ($P = 0.061$) and PV-IR ($P = 0.093$) neuron densities in the SCZ group. The total CBP-IR neuron density in each layer was estimated, and the CB-IR neuron density in layer 2 (57%, $P = 0.007$), and PV-IR neuron density in layer 4 (32%, $P = 0.031$) in the SCZ group were reduced compared with

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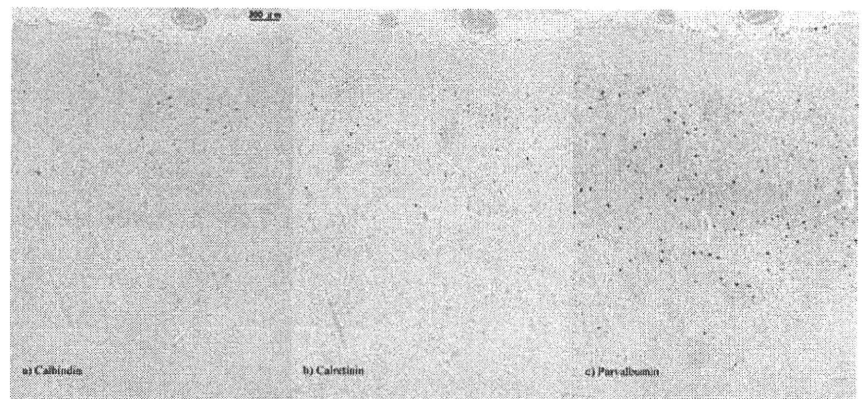


Fig. 2 Composite image showing calcium-binding protein-immunoreactive neurons. The composite images were made up of a series of contiguous images obtained individually at $\times 4$ magnification, that were merged to form a single large image. (bar = 200 μm)

those in the control group. In the BPD group, no significant difference was noted. After classifying the cells by size, medium CB-IR neuron density was found to be reduced in layer 2 in the SCZ subjects (50%, $P = 0.018$), and large-CR-IR neuron density in layer 2 in the BPD subjects (68%, $P = 0.015$) was increased compared with those in the control subjects (Table 2). No differences in the density of any PV-IR neuron types were detected in the BPD or SCZ subjects, but trends toward decreases in large-PV-IR-neuron density in layer 4 (77%, $P = 0.075$) and in medium-PV-IR-neuron density in layer 5/6 (38%, $P = 0.089$) in the SCZ subjects were noted.

Abercrombie correction

After Abercrombie correction, the estimated CBP-IR neuron density ratios indicated the same changes as those described above. These were a reduction in medium-class CB-IR neuron density ratio in layer 2 in the SCZ subjects ($P = 0.024$), a trend toward a reduction in large-class PV-IR neuron density ratio in layer 4 in the SCZ subjects ($P = 0.075$), and an increase in large-class CR-IR neuron density ratio in layer 2 in the BPD subjects ($P = 0.017$). However, there were no significant changes in the ratios of the total counts of PV-IR neurons in layer 4, and of medium-class PV-IR neurons in layer 5/6.

DISCUSSION

IR neurons

In this study, we found significant reductions in the density of CB-IR neurons in layer 2 and PV-IR neurons in layer 4 of the PFC in SCZ subjects (in the between-layer comparison). In addition, when CBP-IR neurons were divided into two classes according to their size, a reduced density of medium-class CB-IR neurons in layer 2 in the SCZ subjects was also observed. We found no significant changes in either of the two types of PV-IR density, but there was a

trend toward a reduction in large-PV-IR neuron density in layer 4 in the SCZ subjects. These results confirm those of previous studies on the PFC, which showed reductions in the density of CB-IR neurons^{28,29} or PV-IR cells²⁸⁻³¹ and suggested no significant changes in CR-IR neuron density in the SCZ subjects.^{31,36} This study supports the evidence that there is a deficit in GABAergic neurotransmission in SCZ.

No significant changes in CBP-IR neuron density between layers in the BPD subjects were found, which is consistent with the results of a report showing no changes in CBP-IR neuron density³³ in the dorsolateral PFC in BPD subjects. However, when IR cells were classified by size, there was an increased density of large CR-IR neurons in layer 2 in the BPD subjects, and also notably a non-significant but 28% reduction in CB-IR neuron density in the BPD subjects compared with the case of the control subjects (Table 2), which confirms the findings of a previous study.²⁸ These results suggest alterations in the cellular organization of CR-IR and CB-IR cells, and it is possible that an increase in CR-IR neuron density may be secondary to a reduction in CB-IR neuron density, or vice versa. Because we found no differences in neuronal density in layer 2 in the SCZ or BPD subjects, our findings on CBP-IR neurons may not depend on a reduction in cell number but rather on a decrease in protein expression.

Because non-suicidal subjects with psychiatric disorders were compared with control subjects, the findings in this study are free from additional effects of suicidal symptoms and actions. Suicide is the most serious outcome of mental disorders, and suicide cases usually present emotional instability and other severe symptoms immediately before death. Most previous studies compared psychiatric sample groups including suicide subjects, who amounted to half the total number of subjects or more, with control groups without any cases of suicide. When suicidal subjects were excluded, influence of mental state before suicide and of suicide actions would be avoided. The deficits in the

subpopulations of GABAergic neurons that were observed in this study may reflect cytoarchitectural abnormalities which constitute vulnerability to SCZ or BPD, but as our data are still preliminary, so a definite conclusion should be left to more rigorous and large-scale studies.

In addition, the distribution of the CBP-IR neurons classified in two types according to their size was examined, and a speculation about differences in IR-neuron activity between groups is possible. Because neuronal somal size is considered to be correlated with the extent of a cell's dendritic arborization,^{37,38} a reduced neuronal somal size suggests diminished neuronal activity, and the changes in cellular organization also indicate alterations in neurotransmission. In this regard, the reductions of medium-class CB-IR neurons and large-class PV-IR neurons suggest deficits in neurotransmission between proximal neurons and between distal neurons, respectively, in SCZ in PFC (BA9), and the increase in large CR-IR neuron density may be related to the potentiation of GABAergic transmission in BPD.

Methodological and confounding factors

We determined two-dimensional, not three-dimensional, cell density. The chief problem with two-dimensional counting is that there is a possibility of overcounting and creating a bias. However, this bias can be corrected using formulae.³⁹ Although unbiased three-dimensional counting is superior when there are group differences in cell size, two-dimensional counting methods, which use large sampling frames, provide more accurate estimates of cell density than their three-dimensional counterparts if the confounding effect of cell size is correctly adjusted for using Abercrombie correction.³⁹ Another potential advantage of two-dimensional counting is that three-dimensional counting, which uses large sampling frames, makes inappropriate assumptions on complete spatial randomness for neurons, which could bias results.⁴⁰ The results of this analysis did not differ from those carried out on unadjusted data.

Because it is difficult to perform immunocytochemistry with three types of antibody at a time in thick sections which are typically used in three-dimensional counting, we used the antibody for each CBP in three separate sections. The thicknesses of these serial sections were all 4 μm , 16 μm in total, such that the size is smaller than the diameter of one neuron. Therefore, it is safely assumed that all three CBP-IR neurons are distributed in the column in which a single neuron exists.

In this study, the influence of major potential confounders on measures of CBP-IR neurons was examined. No significant differences at the 10% significance level (ANOVA or χ^2 test) were detected for the demographic or clinical

variables shown in Table 1. There were also no significant differences and correlation in some other potential confounders such as postmortem interval and time from fixation to specimen making (data not shown). Therefore our data are presumably specific to each diagnostic group and not artifacts of these confounders.

Our present data suggest that there are GABAergic dysfunctions in schizophrenia and bipolar disorder. However, because GABAergic neurons are subdivided by other coexisting proteins such as somatostatin, vasointestinal polypeptide (VIP), and cholecystokinin (CCK), it is necessary to investigate these alternate subtypes of GABAergic neurons to comprehensively determine the nature of GABAergic abnormalities in these disorders.

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Relationship between age at onset and magnetic resonance image-defined hyperintensities in mood disorders

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Abstract

Objectives: To examine in patients with mood disorders the relationship of age at onset with the location and degree of MRI-defined brain hyperintensities.

Method: Fifty-two patients diagnosed as having mood disorders and 14 controls participated in the study. Brain MR images were analyzed according to semiquantitative ratings for the anatomical distribution and severity of T2-weighted hyperintensities. We compared these hyperintensities among the three age- and sex-matched groups of late-onset mood disorder patients (LOM), early-onset mood disorder patients (EOM), and controls. The time since the onset of disorder was significantly longer in the EOM than in the LOM group. We also conducted linear multiple regression analysis using the severity of hyperintensities as dependent variable to determine whether the clinical features correlate with vascular pathology.

Results: As for deep white matter hyperintensity (DWMH), LOM exhibited higher ratings than EOM; as for brain areas, significant between-group differences were detected in the bilateral frontal areas and in the left parieto-occipital area. No significant difference was observed between EOM and controls. As for periventricular hyperintensity, there was no difference among the three groups. We obtained a significant regression model to predict DWMH ratings; age, number of ECTs, and LOM were selected as significant variables.

Conclusion: The present study suggests that the time since the onset of disorder does not affect the development of white matter lesions, but that white matter lesions are associated with late-onset mood disorders. The frontal areas and the left parieto-occipital area would be important for the development of late-onset mood disorders.

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Keywords: Cerebrovascular lesion; Bipolar disorder; Major depressive disorder; Illness vulnerability; Risk factors; Hypertension

1. Introduction

Regarding major depression, there are differences in various clinical indices depending on the age at onset, such as clinical symptoms (Baldwin and Tomenson, 1995; Salloway et al., 1996; Krishnan et al., 1995), degree of cognitive impairment (Salloway et al., 1996; Alexopoulos et al.,

1993), clinical course (Hickie et al., 1997; Alexopoulos et al., 1996), and suicide risk (Lyness et al., 1992). Hopkinson (1964) reported that the risk of depression in the first-degree relatives of depressed patients was 20% in the early-onset depression group compared with 8.3% in the late-onset depression group over 50 years of age. Because similar conclusions have been made by Schultz (1951) and Post (1975), it may be assumed that genetic factors exert greater effects in early-onset depression than in late-onset depression.

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Bipolar affective disorders (BPADs) have also been studied in terms of subgroups divided according to the age at onset (Leboyer et al., 2005). Strober (1992) reviewed eight studies and found that relatives of early-onset BPAD patients are 1.1–3.9 times more likely to develop affective disorder than are the relatives of late-onset BPAD probands, indicating that the onset of early-onset BPADs is strongly associated with genetic factors.

Physical factors, particularly organic brain factors, are highly involved in mood disorders occurring at older ages (Rodin and Voshart, 1986). In the late 1980s, it was reported that patients with late-onset depression had significantly more complications of cerebrovascular disorders including asymptomatic cerebral infarction than those without any depression (Krishnan et al., 1988; Baldwin, 1993; Soares and Mann, 1997). It was previously reported that there were many patients who developed depression after having a stroke (poststroke depression) (Folstein et al., 1977). These observations led to the view that the pathophysiology of late-onset depression may be closely associated with cerebrovascular conditions; hence, the concept of vascular depression was proposed by Alexopoulos et al. (1997) and Krishnan et al. (1997). In large-scale epidemiologic studies that followed, de Groot et al. (2000) found in their Rotterdam Scan Study that subjects with severe white matter lesions showed a 3–5-fold risk of depression. There has been reported of an increase in the severity of deep white matter lesions in BPADs as well. They are more frequently observed in late-onset than in early-onset BPADs (Altshuler et al., 1995).

Factors such as age (de Leeuw et al., 2001; Manolio et al., 1994), elevated blood pressure (de Leeuw et al., 2002), diabetes (Longstreth et al., 2001), and cardiac arrhythmia (Ylikowski et al., 1995) have been reported as risk factors of white matter lesions. In the general population, however, there is less evidence that vascular risk factors are a major cause of depression (Thomas et al., 2004). There have been reports that there is no link between depression symptoms and elevated blood pressure (Jones-Webb et al., 1996; Friedman and Bennet, 1977) nor between a history of hypertension or of coronary heart disease and a history of depression (Steffens et al., 2002). In the Rotterdam Study, however, Tiemeier et al., who examined vasomotor reactivity in the middle cerebral artery using CO₂ inhalation, reported that the depression group exhibited a lower vasomotor reactivity than healthy controls (Tiemeier et al., 2002) and that arterial stiffness significantly correlated with the severity of depression (Tiemeier et al., 2003). Thomas et al. (2001) found in their postmortem study that atheromas in major vessels (coronary arteries, carotid arteries, and aortas) were significantly more severe in the depression group than in the normal group.

In summary, differences in pathogenesis and pathophysiology presumably exist between the early-onset and the late-onset mood disorder groups. Also, depression itself can be a risk factor for vascular lesions (Baldwin, 2005), which led to the hypothesis that depression exacerbates

cerebrovascular lesions with aging (Lenze et al., 1999). However, there have been few studies in which subjects within age-matched groups were compared, that is, between patients with late-onset mood disorders, those with early-onset ones, and normal elderly subjects with regard to brain areas.

In this study, we hypothesized that patients with late-onset mood disorders would show more severe cerebrovascular lesions, especially in the frontal area, than those with early-onset mood disorders in the same present age range and with longer illness period than those with late-onset mood disorders, because there have been a number of studies indicating frontal lobe dysfunction in depressed patients. Therefore, we compared MRI-defined subcortical high intensities with regard to brain areas by dividing the mood disorders patients of the same age range into the late-onset and early-onset mood disorder groups and by including normal elderly subjects as the control. We also conducted linear multiple regression analysis using the severity of subcortical lesions as dependent variable to determine whether the clinical features of mood disorders and medical comorbidities correlate with vascular pathology.

2. Subjects and method

2.1. Subjects

This is a retrospective study conducted in the Department of Neuropsychiatry, Gunma University Hospital in Japan. One hundred and three outpatients or inpatients with mood disorders underwent MRI scan from 2003 to 2006. Patients who met the DSM-IV criteria for any types of dementia ($n = 15$) or showed symptoms of cerebral infarction ($n = 8$) or were aged fewer than 50 ($n = 28$) were excluded. After this exclusion, subjects consisted of 52 patients who met the DSM-IV criteria for major depressive disorder (24 females, 18 males; 9 mild, 18 moderate, 12 severe with psychotic feature, 3 severe without psychotic feature) or bipolar I disorder (1 females, 5 males) or bipolar II disorder (1 females, 3 males). In addition, we added to the study group 14 psychiatrically normal elderly controls older than 50 years old (EC; 8 females, 6 males) according to a comprehensive history and psychiatric interview. By setting the age at onset of 50 years as the cut-off for late onset vs. early onset, we classified 29 patients into the late-onset mood disorder (LOM) group and 23 patients into the early-onset mood disorder (EOM) group.

The characteristics of the subjects are shown in Table 1. There were no significant differences in age (one-way ANOVA: $F = 1.81$, $df = 2$, $P = 0.172$) or gender (two-tailed chi-square test: $\chi^2 = 2.18$, $df = 2$, $P = 0.337$) among the LOM, EOM and EC groups. Ages at onset were significantly lower in the EOM group than those in the LOM group (two-tailed nonpaired t -test: $t = 9.39$, $df = 36.26$, $P < 0.001$). There was also no significant difference in the percentage of patients with BPADs between the LOM

Table 1
Characteristics of subjects

	Patient			Controls
	Combined	Late onset	Early onset	
<i>N</i>	52	29	23	14
Female, <i>n</i> (%)	26 (50.0)	17 (58.6)	9 (39.1)	8 (57.1)
Age, mean year (SD)	60.8 (7.0)	62.2 (5.3)	58.9 (8.5)	63.1 (9.4)
Age at onset, mean year (SD)	49.5 (13.2)	59.0 (6.2) ^a	37.6 (9.4)	–
Bipolar, <i>n</i> (%)	10 (19.2)	5 (17.2)	5 (21.7)	–

^a Late onset vs. early onset: $T = 9.39$, $df = 36.26$, $P < 0.001$.

and EOM groups (two-tailed Fisher's exact test: $P = 0.734$).

The Institutional Review Board of Gunma University Hospital approved this study, and written informed consent was obtained from all the subjects and/or their families.

2.2. MRI procedure and image analysis

We obtained the following images of the subjects using a 1.5-T MAGNETOM Symphony Maestro class, (Siemens Medical Solutions, Erlangen, Germany): axial T_2 -weighted images (TR 3800ms; TE 90ms) and axial T_1 -weighted images (TR 500ms; TE 14ms). The slices of images were 5-mm thick, with a 2.0-mm interslice gap, and the images of the entire brain parenchyma were obtained. An experienced psychiatrist (K.T. or A.O.) independently evaluated the MR images without knowledge of subjects' status. Each T_2 -weighted MR image was evaluated in terms of periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH) on the basis of Fazekas criteria (Fazekas et al., 1987), obtaining semiquantitative ratings for brain hyperintensities. Typical images of Faze-

kas criteria are shown in Fig. 1. PVH was rated as follows: 0 = absent, 1 = "caps" or pencil-thin lining, 2 = smooth "halo", and 3 = irregular PVH extending into the deep white matter. DWMH was rated as follows: 0 = absent, 1 = punctate foci, 2 = beginning confluence of foci, and 3 = large confluent area. To understand the functional anatomical role of the lesions, the regions of interest were classified into the following four areas in each hemisphere. Eight areas were thus evaluated similarly to the four-stage Fazekas DWMH criteria. The areas of interest were the following: (1) the frontal area (right and left, FR and FL, respectively), the frontal lobe anterior to the central sulcus; (2) the parieto-occipital area (right and left, POR and POL, respectively), consisted of the parietal and occipital lobe together; (3) the temporal area (right and left, TR and TL, respectively), the temporal lobe (the border between the parieto-occipital and temporal lobes was approximated as the line drawn from the posterior part of the Sylvian fissure to the trigone areas of the lateral ventricles); and (4) the basal ganglia (right and left, BGR and BGL, respectively), including the striatum, globus pallidus, thalamus, internal and external capsules, and insula. Periventricular lesions were not added to the respective regional scores in order to focus on short association fibers within hemispheres and to exclude commissural fibers and projection fibers that are more densely found in the periventricular area. The interrater reliability exceeded 0.95. There was no discrepancy of more than 1 point between the two evaluators with the given criteria.

2.3. Statistical analysis

All analyses were performed using SPSS (SPSS Inc., Chicago, Illinois; version 12.0J). We compared between the LOM and EOM groups using Fisher's exact test in terms of the presence or absence of psychotic features, history of suicide attempt, history of delirium, a family history

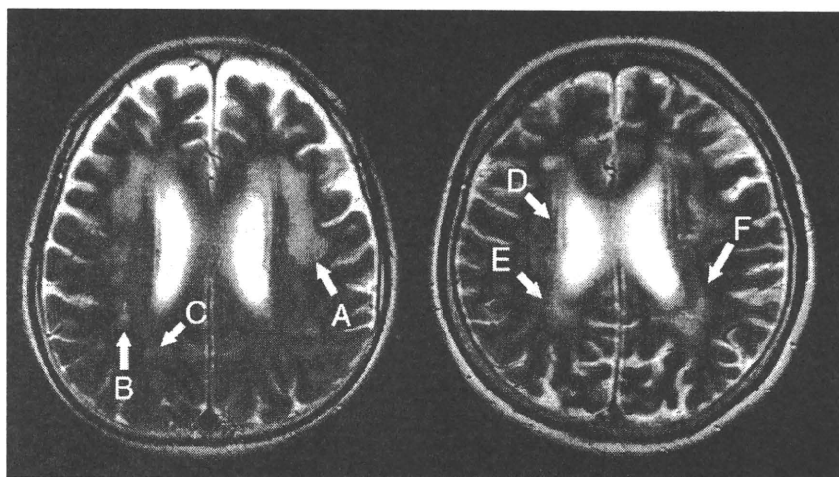


Fig. 1. Typical images of Fazekas ratings. Note. DWMH grade: A = grade 3, B = grade 2, C = grade 1; PVH grade: D = grade 1, E = grade 2, F = grade 3.

of psychiatric disorders within the second-degree, history of electroconvulsive therapy (ECT), habitual smoking, habitual alcohol drinking, obesity (Body Mass Index: BMI > 25), the percentage of patients who have had a history of complications of diabetes mellitus (DM), hypertension (HT), hyperlipemia (HL), ischemic heart disease (IHD) and cardiac arrhythmia (CA). We also compared time since the onset of a disorder, the number of episodes (depressive and manic episodes), the total number of episodes, the number of suicide attempts, the number of ECTs and doses of antidepressants (Imipramine equivalent dose) between the groups using the nonpaired *t*-test. *P*-values were truncated at 0.05 using the two-tailed test in Fisher's exact test and the nonpaired *t*-test. As for the Fazekas ratings of PVH and DWMH in FR, FL, POR, POL, TR, TL, BGR, and BGL in the LOM, EOM, and EC groups, we compared them by one-way ANOVA followed by Tuckey's test.

For these 52 patients, we also conducted linear multiple regression analysis using the DWMH scores as dependent variable. Using stepwise method, independent variables were selected from dimensional scores; age, age at onset, time since the onset of a disorder, the number of episodes (depressive, manic and total episodes), the number of suicide attempts, the number of ECTs and doses of antidepressants, and from categorical scores; LOM, sex, bipolar, psychotic features, delirium, family history of psychiatric disorders, habitual smoking, habitual alcohol drinking, obesity, DM, HT, HL, IHD, CA. Significant

variables were entered if the *P*-value of each variable was under 0.05, and deleted for *P* > 0.10.

3. Results

3.1. Early-onset mood disorders vs. late-onset mood disorders vs. elderly controls

3.1.1. Clinical background and medical comorbidities

The results of comparison of clinical features between the LOM and EOM groups are shown in Table 2. The time since the onset of disorder was significantly longer in the EOM group ($T = -7.36$, $df = 26.62$, $P < 0.001$). The percentage of patients with obesity was higher in the EOM group ($P = 0.015$). There was not any significant difference in the other items.

3.1.2. Fazekas semiquantitative ratings

As for the scores of DWMH and PVH (Fig. 2, Table 3), there was a significant difference in DWMH ($F = 4.67$, $df = 2$, $P = 0.013$) between the groups, but not in PVH ($F = 2.43$, $df = 2$, $P = 0.096$). Pathological changes in LOM were significantly more severe than those in EOM ($P = 0.016$). Compared with EC, LOM showed more severe pathological changes, but not significantly ($P = 0.096$). There was no difference between EOM and EC ($P = 0.946$).

In terms of regional DWMH (Table 3), there was a significant ANOVA results in FR ($F = 4.52$, $df = 2$,

Table 2
Clinical background, medical comorbidities and *P*-values for comparison between late-onset mood disorder ($n = 29$) and early-onset mood disorder ($n = 23$) groups

	LOM	EOM	<i>P</i> -value
<i>N</i>	29	23	
Time since onset of disorder, mean year (SD)	3.3 (4.1)	21.3 (11.2)	<0.001 ^a
Depressive episodes, mean number (SD)	1.6 (0.8)	6.0 (12.4)	0.096 ^a
Manic episodes, mean number (SD)	0.3 (0.9)	1.0 (2.2)	0.195 ^a
Total episodes, mean number (SD)	1.9 (1.4)	7.1 (12.8)	0.063 ^a
With psychotic features, <i>n</i> (%)	12 (41.4)	4 (17.4)	0.077 ^b
Suicide attempts, mean number (SD)	0.2 (0.7)	0.22 (0.4)	0.948 ^a
With suicide attempt history, <i>n</i> (%)	3 (10.3)	5 (21.7)	0.441 ^b
With delirium history, <i>n</i> (%)	5 (17.2)	8 (34.8)	0.201 ^b
Family history of psychiatric disorders, <i>n</i> (%)	9 (31.0)	13 (56.5)	0.092 ^b
ECTs, mean number (SD)	12.8 (48.8)	7.9 (21.0)	0.656 ^a
With ECT history, <i>n</i> (%)	8 (27.6)	7 (30.4)	1.000 ^b
Antidepressant agent, Imipramine equivalent dose mg/day (SD)	116.0 (86.5)	107.2 (91.7)	0.724 ^a
Habitual smoking, <i>n</i> (%)	8 (27.6)	6 (26.1)	1.000 ^b
Habitual alcohol drinking, <i>n</i> (%)	6 (20.7)	5 (21.7)	1.000 ^b
Obesity, <i>n</i> (%) ^c	1 (4.2)	7 (35.0)	0.015 ^b
Medical comorbidities			
DM, <i>n</i> (%)	5 (17.2)	2 (8.7)	0.444 ^b
HT, <i>n</i> (%)	10 (34.5)	5 (21.7)	0.369 ^b
HL, <i>n</i> (%)	1 (3.4)	2 (8.7)	0.577 ^b
IHD, <i>n</i> (%)	1 (3.4)	0 (0.0)	1.000 ^b
CA, <i>n</i> (%)	3 (10.3)	1 (4.3)	0.621 ^b

Note. LOM, late-onset mood disorder group; EOM, early-onset mood disorder group; ECT, electroconvulsive therapy; DM, diabetes mellitus; HT, hypertension; HL, hyperlipemia; IHD, ischemic heart disease; CA, cardiac arrhythmia.

^a *P*-values for two-tailed nonpaired *t*-test.

^b *P*-values for two-tailed Fisher's exact test.

^c Comparison between 24 patients of LOM and 20 patients of EOM.

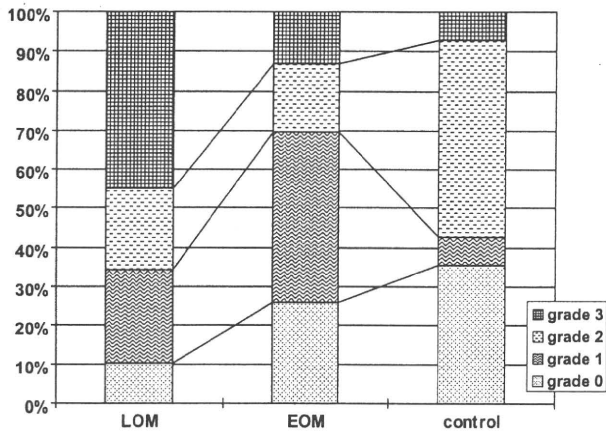


Fig. 2. The distribution of Fazekas DWMH ratings in LOM, EOM, and control groups. Note. LOM, late-onset mood disorder group; EOM, early-onset mood disorder group.

$P = 0.015$), FL ($F = 3.29$, $df = 2$, $P = 0.044$), and POL ($F = 6.35$, $df = 2$, $P = 0.003$). In the post hoc tests between LOM and EOM, significant differences were detected in FR ($P = 0.033$), FL ($P = 0.043$), and POL ($P = 0.003$). Between LOM and EC, a significant difference was detected in FR ($P = 0.048$).

3.2. Linear multiple regression analysis using the DWMH ratings as dependent variable

Results of linear multiple regression analysis are shown in Table 4. We obtained a significant regression model to predict the DWMH ratings ($F = 16.5$, $P < 0.001$); age, number of ECTs, and LOM were selected as significant variables ($t = 5.5$, -3.7 , and 2.5 ; $P < 0.001$, 0.01 , and 0.05 , respectively).

4. Discussion

This study is unique in that we compared white matter lesions with regard to brain areas among the age- and gender-matched LOM, EOM and EC groups that include major depressive disorder and bipolar disorder. As for DWMH, LOM exhibited a higher rating than EOM; as for brain areas, significant between-group differences were detected in bilateral frontal areas and in the left parieto-occipital area. LOM, but not EOM, tended to show more severe pathological changes than the EC group, and there was a significant difference in the severity of changes in the right frontal areas. As for PVH, there was no difference among the three groups. In the linear multiple regression

Table 3

Fazekas ratings and P -values for comparison among late-onset mood disorder ($n = 29$), early-onset mood disorder ($n = 23$), and elderly control ($n = 14$) groups

	LOM	EOM	Controls	One-way ANOVA		Post hoc test P -value (Tukey HSD)		
				F -value	P -value	LOM vs. EOM	LOM vs. controls	EOM vs. controls
DWMH, mean (SD)	2.00 (1.07)	1.17 (0.98)	1.29 (1.07)	4.67	0.013	0.016*	0.096	0.946
PVH, mean (SD)	1.28 (1.22)	0.61 (1.08)	0.71 (1.14)	2.43	0.096	0.105	0.301	0.961
FR, mean (SD)	1.76 (1.18)	1.00 (1.00)	0.93 (0.83)	4.52	0.015	0.033*	0.048*	0.978
FL, mean (SD)	1.72 (1.19)	0.96 (1.07)	1.14 (1.03)	3.29	0.044	0.043*	0.253	0.875
POR, mean (SD)	1.14 (1.06)	0.48 (0.85)	0.86 (0.86)	3.09	0.052	0.041	0.637	0.472
POL, mean (SD)	1.31 (1.07)	0.43 (0.79)	0.64 (0.74)	6.35	0.003	0.003*	0.073	0.782
TR, mean (SD)	0.83 (1.00)	0.48 (0.90)	0.50 (0.52)	1.21	0.305	0.340	0.495	0.997
TL, mean (SD)	0.86 (1.03)	0.57 (0.95)	0.64 (0.63)	0.75	0.498	0.490	0.749	0.967
BGR, mean (SD)	0.62 (0.86)	0.43 (0.79)	0.29 (0.47)	0.97	0.385	0.664	0.380	0.836
BGL, mean (SD)	0.66 (0.97)	0.43 (0.84)	0.21 (0.43)	1.36	0.264	0.618	0.249	0.721

Note. LOM, late-onset mood disorder group; EOM, early-onset mood disorder group; DWMH, deep white matter hyperintensity; PVH, periventricular hyperintensity; FR, right frontal area; FL, left frontal area; POR, right parieto-occipital area; POL, left parieto-occipital area; TR, right temporal area; TL, left temporal area; BGR, right basal ganglia; BGL, left basal ganglia.

* One-way ANOVA $P < 0.05$ and post hoc test $P < 0.05$.

Table 4

Prediction of the DWMH ratings

	Dependent variable	Variables entered	Standardized coefficients	t	R^2	F
$n = 52$	DWMH ratings				0.52	16.5***
$df (1,44)$		Age	0.61	5.5***		
		number of ECTs	-0.40	-3.7**		
		LOM group	0.27	2.5*		

Note. Multiple linear regression, stepwise method, DWMH: deep white matter hyperintensities, ECT: electro convulsive therapy, LOM: late-onset mood disorder.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

analysis using the DWMH ratings as dependent variable, age, number of ECTs and LOM were observed as significant independent variables. The observation that about 80% of patients examined in the present study suffered from major depressive disorder suggests that the findings in this study largely reflect the factors associated with major depressive disorder.

There are many reports that DWMHs are more severe in late-onset depression than in normal controls (Greenwald et al., 1996; Kumar et al., 1997; Tupler et al., 2002), which agrees with our results. As for PVH, we found no significant difference between the mood disorders and the control subjects, which also agrees with many previous studies (Greenwald et al., 1996; Lenze et al., 1999; Tupler et al., 2002). However, some previous studies showed that there is a difference (Coffey et al., 1993; Kumar et al., 1997; Iidaka et al., 1996). Discrepancies between studies may be due to differences in sample size, chronological age of subjects, or other sampling characteristics.

Few studies have made a comparison between late-onset and early-onset depression patients of the same generation. Fujikawa et al. (1993) compared between the late and early-onset depression groups, reporting that the late-onset depression group has a higher rate of white matter lesions than the early-onset depression group. Salloway et al. (1996) reported that DWMH as well as PVH were significantly more severe in the late-onset depression group than in the early-onset depression group. Greenwald et al. (1996) reported that elderly depressed patients manifested significantly more severe hyperintensity ratings in the subcortical gray matter than age-matched controls, but between the early and late-onset groups whose age and cerebrovascular disease risk were identical, both DWMH and PVH showed no differences. However, Tupler et al. (2002) reported that although there was some difference in age between the groups, DWMHs were more severe with the late-onset than in the early-onset patients and controls, whereas no difference was noted for PVH. Among these reports, our results agree most closely with those of Tupler et al. (2002).

There were even fewer studies that compared between age-matched early- and late-onset groups with regard to brain areas. In our present study, LOM showed significantly more severe white matter lesions in the bilateral frontal areas and the left parieto-occipital area than did EOM. Tupler et al. (2002) reported that the lesions in the left middle and posterior areas correlated with their late-onset group, which agrees with our results. However, Tupler et al. (2002) also reported that there was no difference in the severity of white matter lesions in either frontal lobe areas between the late- and early-onset groups. Although they did not classify their patients according to age at onset, Taylor et al. (2003) reported that in the depression group, the severity of white matter lesions in the bilateral frontal areas and the left middle area correlated with age and that, in normal controls, the severity of white matter lesions in bilateral middle areas, but not

in any frontal areas, showed the same correlation, as shown by statistical parametric mapping. The topographical pattern shown in their depression group is close to that in our LOD group, who showed more severe pathological changes than EOD and EC in similar brain regions. We consider it as a merit that Fazekas scale is easily applicable to daily clinical settings. However, Fazekas scale is more coarse tools to quantify the high intensity lesions than modern neuroimaging programs that generate volumetric data. This point is the limitation of the present study.

As for the frontal lobe in depressed patients, there have been several studies indicating differences from controls, such as volume decrease (Coffey et al., 1993; Kumar et al., 1998), glucose metabolism deterioration determined by positron emission tomography (PET) (Videbech, 2000; Drevets, 2001), aberration in phospholipid or energy metabolism in magnetic resonance spectroscopy (MRS) (Kumar et al., 2002), and cognitive dysfunction determined by neuropsychological studies (Fossati et al., 2002). The frontal lobes have mutual fiber communications with the subcortical nuclei, such as the thalamus, basal ganglia, and the amygdaloid body, with five independent parallel circuits proven, which Tekin and Cummings (2002) organized and classified into two groups: (1) the dorsolateral prefrontal and lateral orbitofrontal subcortical circuits and (2) the medial orbitofrontal and anterior cingulate circuits. Projections from the mesencephalon or the subcortical gray matter to the frontal lobes are related to the emotional processing of information and the maintenance of emotional state, and some investigators postulate that these neural networks may be disturbed in depression (Brody et al., 2001). Therefore, it is possible that functional deficits in the frontal lobes are caused by subcortical lesions within these circuits, resulting in secondary functional deficits in the frontal areas that trigger mood disorders.

As for the percentage of patients with medical comorbidities, such as diabetes, hypertension, hyperlipemia, ischemic heart disease, cardiac arrhythmia, habitual alcohol drinking and habitual smoking, that are risk factors for cerebrovascular lesions, there was no difference between LOM and EOM. In addition, such factors were not selected as significant variables in the linear multiple regression analysis using the DWMH ratings as dependent variable. These results might be due to small sample size of this study. Also, our data lack the exact history and duration of these comorbidities. Otherwise, these reports, together with those by Tiemeier et al. (2002, 2003) and Thomas et al. (2001), indicate the importance of investigating not only the presence/absence of hypertension but also other indices such as arterial stiffness and atheroma when considering risk factors for late-onset mood disorders. Therefore more detailed investigation of risk factors for cerebrovascular lesions may be needed in further studies.

As for the percentage of patients with obesity, there was significant difference between LOM and EOM. Depression and obesity frequently co-exist, and obesity can follow depression that occurred earlier in life (Bornstein et al.,

2006). Medications, particularly those commonly used in psychiatry and neurology, are a significant iatrogenic source of overweight and obesity (Aronne and Segal, 2003). In these regards, the higher percentage of patients with obesity in the EOM group might be caused by psychiatric medication during a longer period since the onset of a mood disorder.

There was negative correlation between the number of ECTs and the DWMH ratings. This result might be explained by the possibility that, because patients with severe DWMH rating have a poor response to ECT (Simpson et al., 1998; Steffens et al., 2001) and are predisposed to delirium (Figiel et al., 1990), frequent ECTs were avoided in the treatment for these patients, and the number of ECTs turned out to be lower in this population.

In summary, these findings in this study suggest that, in mood disorder patients, the time since the onset of disorder does not affect the development of white matter lesions, but that white matter lesions are associated with late-onset mood disorders. As for brain regions, it was also speculated that the frontal areas and the left parieto-occipital areas are important for the development of the late-onset type.

Our present findings indicate that various pre-existing risk factors including aging and (here undetected) medical comorbidities may have caused cerebrovascular lesions in these regions, which then resulted in depressive phenotype of LOM, and that early-onset type may be more closely associated with non-vascular factors like genetic elements. Prospective studies are needed to investigate whether cerebrovascular lesions surely induce depressive phenotype in later life. Furthermore, as the symptom spectrum of late onset mood disorders may be accompanied by cognitive impairment not closely analyzed in this study, the effect of these lesions on cognitive function should be examined.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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

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Abstract

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

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

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

Information processing deficits underlying these cognitive dysfunctions in major depressive disorder patients can be examined electrophysiologically using event-related potentials (ERPs). For example, among late ERPs, the P300 component has often been reported to be reduced in amplitude and delayed in peak latency in major depressive disorder patients (Karaaslan, Gonul, Oguz, Erdinc, & Esel, 2003; Kawasaki, Tanaka, Wang, Hokama, &

Hiramatsu, 2004; Papageorgiou et al., 2004; Roschke & Wagner, 2003; Urretavizcaya et al., 2003), and the results are interpreted as reflecting abnormalities in the capacity of attentional operation resource (Kok, 2001). However, because late ERPs such as P300 are often vulnerable to motivational factors and task involvement of the participants, the interpretation of these findings in major depressive disorder patients is difficult.

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

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

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

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

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

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

between major depressive disorder patients and healthy volunteers because most voxel-based morphometry studies showed no anatomical differences in temporal structures between major depressive disorder patients and healthy volunteers (Beyer & Krishnan, 2002).

Methods

Participants

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

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

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

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

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

Task Procedures

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

Table 1. Characteristics of Participants

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

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

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

F3 = 2750, and F4 = 3600 Hz;/o/: F0 = 140, F1 = 480, F2 = 770, F3 = 2820, and F4 = 3600 Hz. The frequency of the pure-tone stimuli was 1000 Hz, nearly equal to the central frequency of the formants of the vowel stimuli. The order of the two conditions was counterbalanced across the subjects.

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

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

Data Acquisition

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

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

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

Data Analysis

Magnetic counterpart of global field powers (mGFPs) of P1m and MMNm. The mismatch reaction is defined as the difference between the magnetic field of the standard tone and the evoked field of the deviant tone. The magnetic fields of standard tones were subtracted from those of deviant tones for each channel, and the root mean squares of the differences over the 54 channels positioned over the temporal region (Figure 1) were calculated for each subject as the magnetic counterpart of the global field power (mGFP) of the mismatch reaction, separately for each condition and hemisphere, using the formula shown in Figure 2 (Kreitschmann-Andermahr et al., 1999; Lehmann & Skrandies, 1980). The peak latency of P1m was determined as the maximum amplitude of the individual mGFP curve of standard stimuli for

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

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

each condition between 40 and 100 ms (Tervaniemi et al., 1999). The individual curves of MMNm were calculated by subtracting the wave at each channel elicited in response to the standard tone from that elicited in response to deviant tones under the same experimental condition (Alho et al., 1998). The peak latency of MMNm was determined as the maximum amplitude of the individual mGFP curves between 100 and 250 ms (Kasai et al., 2003; Tervaniemi et al., 1999). The individual curve whose peak amplitude could not be found in the designated periods of those of P1m and MMNm was excluded from analysis.

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

Statistical Analyses

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

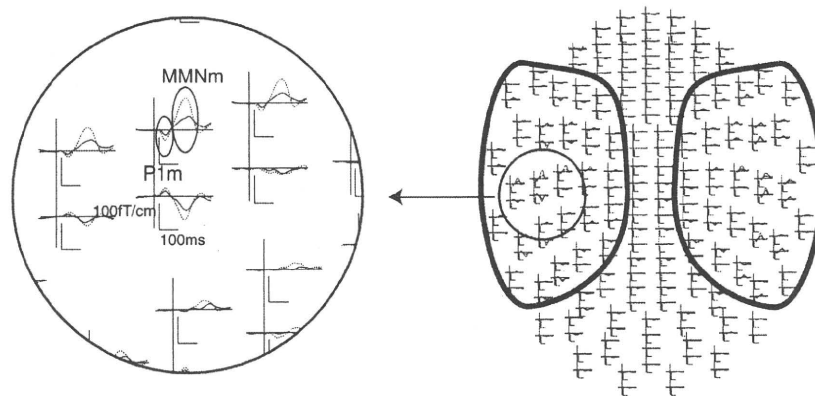


Figure 2. Formula for calculating mGFP. In this formula, n is the number of channels and u is the amplitude.

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

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

Results

Behavioral Data

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

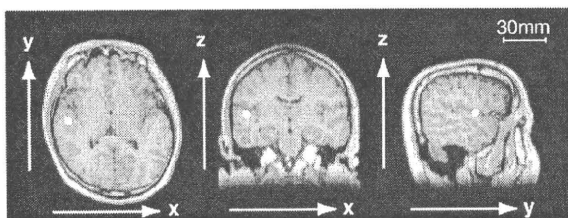


Figure 3. ECD location of MMNm of healthy volunteers for the pure-tone duration change condition in the left hemisphere. The arrows in this figure indicate each axis. The x -axis defines the right and left directions, the y -axis defines the anterior and posterior directions, and the z -axis defines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

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

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

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

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

Dipole Analysis

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

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

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

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

** $p < .01$.

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

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

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

Correlational Analysis

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

Discussion

Summary of Results

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

Comparison with Previous Studies

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