Ⅲ. 研究成果の刊行に関する一覧表

英文業績

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kohda K, Jinde S, Iwamoto K, Bundo M, Kato N, Kato T	Maternal separation stress drastically decreases expression of transthyretin in the brains of adult rat offspring.	International Journal of Neuropsychopharmacology	4	1-8	2005
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佐々木司	大学・大学院および職場における広汎性発達障害.	精神科			印刷中
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ARTICLE

Maternal separation stress drastically decreases expression of transthyretin in the brains of adult rat offspring

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Abstract

Adversity in early life has been recognized as a risk factor for psychiatric disorders. In experimental animals, maternal separation (MS) during the neonatal period has been shown to be critical for susceptibility to stress in adult offspring. In this study, we used DNA microarray analysis of rat hippocampal samples to investigate differential gene expression caused by 8-hour MS (MS-8h) every other day during the neonatal period. We found 15 up-regulated and 9 down-regulated genes. We added samples from a daily 15-minute MS (MS-15m) group and performed quantitative real-time PCR to validate the results. Expression of transthyretin (TTR), which is specifically expressed in the choroid plexus (CP), was drastically reduced in the MS-8h group. Two other CP-enriched genes, angiotensin I converting enzyme I and insulin-like growth factor II (IGF-II), were also significantly down-regulated in the MS-8h rats, while significant reduction of IGF-II expression was also found in the MS-15m group. These MS-induced differential gene expressions could be involved in the molecular mechanisms of stress susceptibility. Our findings indicate that the CP, in addition to the neuronal and glial system, might play an important role in determining stress susceptibility.

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Key words: Choroid plexus, DNA microarray, hippocampus, maternal separation, transthyretin.

Introduction

Adversity in early life has been recognized as a critical factor determining susceptibility to psychiatric disorders as well as physical illnesses in humans. Maltreatment, such as abuse and neglect, increases the risk for depression and anxiety disorders (Bifulco et al., 1991; Brown et al., 1999; Holmes and Robins, 1987, 1988). In experimental animals, consequences of stress exposure in the early postnatal period have been extensively investigated, commonly using methods of maternal separation (MS) or neonatal handling (Meaney, 2001). MS is thought to increase an animal's susceptibility to stress. In previous studies, MS rats have shown enhanced hypothalamo–pituitary–adrenal (HPA) responses to stress in adulthood, probably due to increased expression of corticotropin-releasing factor (CRF) in the hypothalamus and decreased expression of glucocorticoid receptors (GR) in the hippocampus (Francis et al., 2002; Ladd et al., 2004; Liu et al., 2000; Patchev et al., 1997; Plotsky and Meaney, 1993), both of which should result in attenuated feedback in the HPA system. These rats reportedly showed increased fearfulness and cognitive deficits (Caldji et al., 2000; Patchev et al., 1997). On the other hand, postnatal handling, which involves brief mother–pup separation, induced precisely the opposite effects: enhanced inhibition of the HPA response, increased GR in the hippocampus, and reduced CRF in the hypothalamus (Meaney, 2001). These findings suggest that aversive rearing environments in the early neonatal period, especially maternal maltreatment, contribute to stress susceptibility through plastic changes of the pup brain that persists throughout the lifespan (Francis et al., 1999; Liu et al., 1997).

The hippocampus is known to control the feedback loop of the HPA axis and to be vulnerable to stress (McEwen, 1999). Therefore, in this study, our

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aim was to comprehensively search for genes differentially expressed in the hippocampus that might generate stress susceptibility, utilizing DNA microarray in MS rats. Among several differentially expressed genes, we found a drastic decrease in expression of transthyretin (TTR) in the 8-hour MS (MS-8h) rats; this is a gene that is specifically expressed in the choroid plexus (CP) in the brain. Angiotensin I converting enzyme I (ACE) and insulin-like growth factor II (IGF-II), also enriched in the CP, were significantly down-regulated. These findings indicate that the CP, in addition to the neuronal and glial system, might play a role in determining stress susceptibility.

Materials and methods

Animals and maternal separation

Fisher 344 rats were purchased from SLC (Hamamatsu, Japan) and bred in our facility under controlled illumination (12 h/12 h, lights on at 08:00 hours) and ambient temperature (22–23 °C). Each pregnant female rat was housed individually with free access to food and water. New-born litters were culled to eight pups on postnatal day 1 (PD1). Between PD2 and PD10, all the MS-8h pups were separated from their dams for 8 h (11:00 to 19:00 hours) every other day (Patchev et al., 1997). As for the 15-minute MS (MS-15m) group, these pups were removed daily from their mother for 15 min between PD1 and PD14. The pups were placed individually in a plastic container during separation. The control pups remained in their home cage without any manipulation. All rats were weaned in postnatal week (PW) 3 and housed individually in PW7. Body weight (BW) of the pups was measured on PD8, 14, 28, 42, 56 and 70. Although both male and female pups were involved in the MS protocol, only male rats were subjected to the following experimental procedures.

DNA microarray analysis

In PW13, the male rats were sacrificed. Hippocampal slices of 500 μm thickness were made in a choline solution containing (in mM) 124 choline-Cl, 3 KCl, 2 $CaCl_2$, 4 $MgSO_4$, 1.25 NaH_2PO_4, and 10 D-glucose at 4 °C using a rotary slicer (Dosaka, Kyoto, Japan). After overnight incubation in RNAlater (Ambion, Austin, TX, USA) at 4 °C, slices were frozen and stored at −80 °C.

The sample preparation procedures for DNA microarray analysis have been described previously (Iwamoto et al., 2004). Briefly, total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) and purified with a RNeasy column (Qiagen, Valencia, CA, USA). Purity and integrity of total RNA were checked by OD measurement.

DNA microarray assay was performed using RNA derived from individual rats according to the protocols of the manufacturer (Affymetrix, Santa Clara, CA, USA). We used 10 μg of total RNA to synthesize cDNA. Biotinylated cRNA was generated from the cDNA. The cRNA was fragmented and applied to a Test2Chip (Affymetrix) to assess sample quality. For DNA microarray assay, rat U34A (Affymetrix) was used. The hybridization signal was scanned with a HP GeneArray scanner (Hewlett-Packard, Palo Alto, CA, USA) and processed using GeneSpring software (Silicon Genetics, Redwood City, CA, USA).

For normalization, the expression values of the genes were divided by the median value. We defined the differentially expressed genes based on the following criteria: (i) 1.5-fold or greater change in the mean expression level and (ii) $p < 0.05$ in the two-tailed Welch test.

Quantitative real-time PCR (qPCR)

We carried out qPCR to quantify mRNA obtained from individual rats with an ABI PRISM 7900HT (Applied Biosystems, Foster City, CA, USA) following the protocols of the manufacturer. Measurement was done in duplicate. Normalization was performed by calculating the ratio to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or prostaglandin D synthase (PGDS). Primers were obtained from Applied Biosystems ('Assays-on-Demand').

Statistical analysis

The BW and qPCR data were analysed using one-way ANOVA with Tukey post-hoc tests. Differences were considered significant when $p < 0.05$.

Results

Hippocampal samples derived from the MS-8h and control rats were subjected to DNA microarray analysis to study differential gene expression caused by MS in the early neonatal period. Filtering the microarray data based on the criteria described in the Materials and methods section, we obtained 15 up-regulated and 9 down-regulated genes (Tables 1 and 2 respectively). The most notable finding was a 4.85-fold decrease in TTR expression in the MS-8h group, whereas the fold changes of other genes were <2 (Table 2, Figure 1a).

Table 1. Fifteen up-regulated genes due to the 8-hr MS stress

Affymetrix ID	Public ID	Symbol	Gene title	Category/function	Fold change
rc_AA894210_at	AA894210	–	EST198013	Unknown	1.90
X04070_at	X04070	Gjb1	Gap junction membrane channel protein beta 1	Cell contact	1.78
rc_AA875577_at	AA875577	–	Similar to dapper2 (LOC308212), mRNA	Unknown	1.72
rc_AA800551_at	AA800551	Hsj2	DnaJ-like protein	Stress response	1.71
rc_AA866369_at	AA866369	–	Transcribed sequences	Unknown	1.68
rc_AI228407_s_at	AI228407	Adcyap1	Adenylate cyclase activating polypeptide 1	Signal transduction	1.63
rc_AA892637_at	AA892637	Grp58	Glucose regulated protein, 58 kDa	Stress response	1.61
rc_H33461_at	H33461	Oxr1	Oxidation resistance 1	Stress response	1.61
rc_AI639001_at	AI639001	Ptprm	Protein tyrosine phosphatase, receptor-type, M	Signal transduction	1.60
rc_AA894168_at	AA894168	–	Similar to PHD finger protein 3 (LOC363210), mRNA	Unknown	1.60
AA850219_at	AA850219	Anx3	Annexin III (Lipocortin III)	Phospholipid binding protein	1.59
U35371_at	U35371	Cntn4	Contactin 4	Cell contact	1.53
rc_AA875023_at	AA875023	–	Similar to RIKEN cDNA 2410005K17 (LOC362578), mRNA	Unknown	1.53
rc_AA866299_g_at	AA866299	–	Transcribed sequences	Unknown	1.52
AF003835_at	AF003835	Idi1	Isopentenyl-diphosphate delta isomerase	Lipid metabolism	1.50

Differential expression was defined as follows: (i) 1.5-fold or greater change in the mean expression level; (ii) $p<0.05$ by two-tailed Welch test.

Table 2. Nine down-regulated genes due to the 8-hr MS stress

Affymetrix ID	Public ID	Symbol	Gene title	Category/function	Fold change
rc_AA945169_at	AA945169	Ttr	Transthyretin	Carrier protein	4.85
D28560_at	D28560	Enpp2	Ectonucleotide pyrophosphatase/ phosphodiesterase 2	Myelin formation	1.97
U03734_at	U03734	Ace	Angiotensin 1 converting enzyme 1	Renin-angiotensin system	1.92
rc_AA684641_at	AA684641	–	Transcribed sequences	Unknown	1.84
X17012mRNA_s_at	X17012	Igf2	Insulin-like growth factor 2	Signal transduction	1.71
D49847_at	D49847	Grb2	Growth factor receptor bound protein 2	Signal transduction	1.60
U04835_at	U04835	Crem	cAMP responsive element modulator	Transcription	1.58
D85035_g_at	D85035	Dpyd	Dihydropyrimidine dehydrogenase	Pyrimidine metabolism	1.58
X56596_at	X56596	RT1-Bb	RT1 class II, locus Bb	Rat MHC class II	1.51

Differential expression was defined as follows: (i) 1.5-fold or greater change in the mean expression level; (ii) $p<0.05$ by two-tailed Welch test.

Brief daily neonatal handling of rats has the opposite effect of hours-long separation (for review, see Meaney, 2001). We therefore generated the MS-15m group for comparison with the MS-8h rats. We used qPCR to validate the microarray analysis data. Normalized by GAPDH expression level, one-way ANOVA indicated significant difference in TTR expression ($F_{2,12}=8.08$, $p<0.01$). TTR expression in

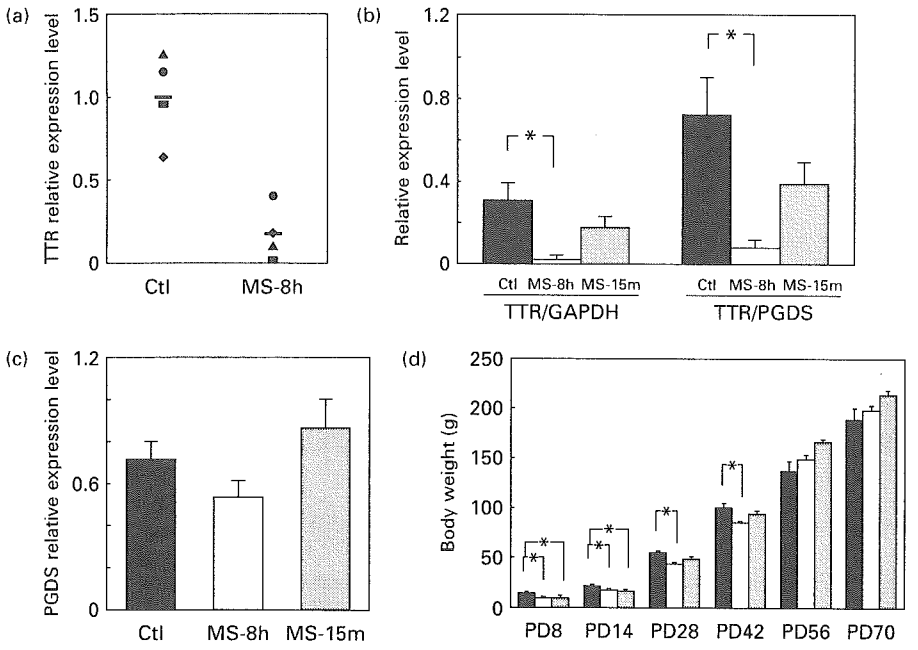

Figure 1. Drastic reduction of TTR expression in MS rats. (a) Distribution of relative TTR expression level of control and MS-8h rats in DNA microarray analysis. In the diagram, the mean value of the control group TTR expression was set as 1. The bars indicate the mean value ($n=4$ for both groups). (b) The difference in TTR expression was validated by qPCR. TTR expression was normalized by GAPDH or PGDS. Drastic decrease of TTR in the MS-8h group was validated in either case ($n=4$–5 per group). (c) Comparison of PGDS expression level in the control, MS-8h, and MS-15m groups ($n=4$–5 per group) by qPCR. There was no statistical difference between the three groups. (d) The effects of MS on body weight (BW) of the pups. On PD8 and PD14, the MS-8h and MS-15m pups weighed significantly less than the control rats. Although the BW of the MS-15 group caught up with the control group by PD28, a significant difference between the MS-8h and control groups remained until PD42. The mean and S.E.M. are shown in (b), (c), and (d). Ctl, control (■); MS-8h, maternal separation for 8 h (□); MS-15m, maternal separation for 15 min (▣). * $p<0.05$.

the MS-8h rats was, indeed, significantly decreased in comparison with controls (post-hoc test, $p=0.008$; Figure 1b). The MS-15m group indicated reduced expression of TTR, although there was not significant difference compared with either controls or MS-8h rats (vs. controls, $p=0.22$; vs. MS-8h, $p=0.12$; Figure 1b) It was previously reported that TTR is expressed almost exclusively in the CP in the brain, but not in neurons (Herbert et al., 1986; Schreiber et al., 1993). Since we did not control the amount of CP included during hippocampal slice preparation, it was possible that this result might have simply reflected variance in the amount of CP contained in the samples. Thus, we normalized the qPCR results by measuring PGDS mRNA, which is also expressed predominantly in the CP (Hayaishi, 1999). To our knowledge, PGDS has never been suggested to correlate with stress reaction or depression. Again, the TTR expression level was significantly lower in the MS-8h group compared with controls ($F_{2,12}=9.44$, $p<0.01$; post-hoc test, $p=0.005$; Figure 1b). In addition, one-way ANOVA revealed that PGDS expression levels were not different among the three groups ($F_{2,12}=0.50$, $p=0.62$; Figure 1c). These data indicate that differential TTR expression resulted from MS, rather than from accidental differences in the amount of CP included in the samples.

The plasma TTR level is known as a sensitive indicator of nutritional state, although the concentration of TTR in the cerebrospinal fluid (CSF) is reportedly less sensitive to dietary changes than is plasma TTR (Dickson et al., 1986; Wade et al., 1988). BW changes were measured to determine whether maternal separation affected gross development of the pups. One-way ANOVA revealed significant difference in BW on PD8 ($F_{2,21}=28.92$, $p<0.001$), PD14 ($F_{2,21}=6.94$, $p<0.05$), PD28 ($F_{2,21}=8.77$, $p<0.005$), and PD42 ($F_{2,21}=4.00$, $p<0.05$) among the control, MS-8h, and MS-15m groups (Figure 1d). Post-hoc tests indicated that rats in the MS-8h and MS-15m groups weighed

significantly less, on average, than those in the control group on PD8 ($p<0.001$ for both groups) and PD14 ($p<0.01$ for MS-8h and $p<0.05$ for MS-15m). By PD28, the control and MS-15m rats did not differ from one another in BW, whereas the MS-8h rats still remained smaller in BW than the controls ($p<0.01$). The BW of the MS-8h group caught up with the controls by PD56 (PD56: $F_{2,21}=3.04$, $p=0.07$; PD70: $F_{2,21}=1.60$, $p=0.22$). These findings suggested that there was no significant difference present in gross development at the time of DNA microarray or qPCR analysis (PW13) among the three groups, although BW gain was delayed in the MS-8h pups for a longer period than in the MS-15m pups. Therefore, the results of TTR expression level should not be affected by the nutritional state of the rats.

It should be noted that the MS-15m rats showed significantly smaller BW on PD8 and PD14. This suggested that 15-min separation, a standard manipulation for postnatal handling, worked as weak MS stress in this study (see Discussion). Therefore, the results obtained from the MS-15m rats should be consistent with those from the MS-8h.

Among the genes whose expression was decreased significantly in the MS-8h group in DNA microarray analysis, we found ACE and IGF-II. Interestingly, it was previously reported that their expression is enriched in the CP, although not limited to this region (Chai et al., 1987; Newton et al., 2003). Normalized by either GAPDH or PGDS, qPCR revealed the significantly different expression level of ACE ($F_{2,12}=9.15$, $p<0.01$ for ACE/GAPDH; $F_{2,12}=5.08$, $p<0.05$ for ACE/PGDS) and IGF-II ($F_{2,12}=11.45$, $p<0.005$ for IGF-II/GAPDH; $F_{2,12}=10.24$, $p<0.005$ for IGF-II/PGDS; Figure 2a, b). Post-hoc tests indicated that ACE and IGF-II were expressed less in the MS-8h group than in the control group (ACE/GAPDH, $p=0.004$; ACE/PGDS, $p=0.03$; IGF-II/GAPDH, $p=0.002$; IGF-II/PGDS, $p=0.004$), whereas the MS-15m data indicated significant reduction in expression of IGF-II (IGF-II/GAPDH, $p=0.04$; IGF-II/PGDS, $p=0.02$; Figure 2b), but not in ACE (ACE/GAPDH, $p=0.10$; ACE/PGDS, $p=0.12$).

In the MS model, in-situ hybridization studies have indicated that GR expression was down-regulated in the subregions of hippocampus (Francis et al., 2002; Ladd et al., 2000, 2004). We found no significant difference in GR expression between the MS-8h and control groups in DNA microarray analysis (data not shown). In addition, qPCR revealed no significant difference in the MS-8h, MS-15m and control groups ($F_{2,12}=0.27$, $p=0.76$ for GR/GAPDH; data not shown). This apparent discrepancy between the previous reports and the present study is probably due to the fact that we used the whole hippocampi for microarray analysis.

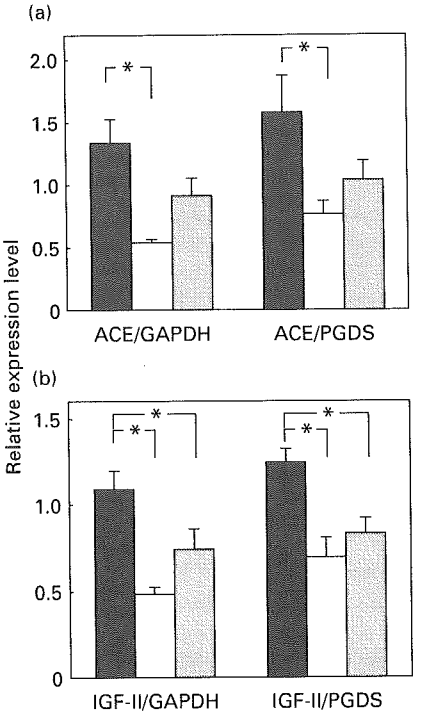

Figure 2. Reduced expression of ACE and IGF-II in the MS rats. The results of qPCR normalized by GAPDH or PGDS are shown. (a) ACE reduction was significant in the MS-8h group, normalized by either GAPDH or PGDS. (b) Expression of IGF-II was significantly decreased in both MS-8h and MS-15m rats. The mean and S.E.M. are shown in the diagrams. Ctl, control (■). MS-8h, maternal separation for 8 h (□). MS-15m, maternal separation for 15 min (▨). * $p<0.05$.

Discussion

Since MS rats show increased fearfulness and attenuated feedback of the HPA axis in adulthood, it is assumed that MS stress produces plastic changes in the brains of pups, leading to stress susceptibility that lasts throughout life. In this study, using hippocampal samples and DNA microarray analysis, we intended to comprehensively search the genes that might be involved in the molecular mechanisms of MS-induced stress susceptibility. We found several differentially expressed genes between the MS-8h and control groups.

Quantitative real-time PCR, indeed, revealed significant decrease of TTR expression in the MS-8h group

in comparison with the control group. Nutritional state should not affect the result, since BW of the MS-8h pups caught up with the other two groups by PD56. In addition, the MS-15m rats also showed reduced, but not significant, TTR expression compared with the control group. This result was rather unexpected, since they reportedly showed the opposite characteristics in behavioural and neuroendocrinological responses to the rats of hours-long MS (Meaney, 2001). In the previous reports, the delay of BW gain was not observed in 15-min separation, whereas hours-long MS resulted in significantly less weight gain (Barna et al., 2003; Huot et al., 2004; Ploj et al., 2003). The significantly smaller BW in the MS-15m rats suggested that 15-min separation did not work as the 'neonatal handling' manipulation, but rather that it worked as MS stress in our study. This might be due to differences of the strains used for the experiments. The parallel changes in expression of TTR, ACE and IGF-II found in the MS-8h and MS-15m groups should be consistent in the view of MS stress in the early postnatal period.

TTR, a carrier protein of thyroxine and retinol, is found in the plasma and CSF (Davis et al., 1970; Hagen and Elliott, 1973; Schreiber, 2002) and its expression is almost specifically restricted in the CP in the brain (Herbert et al., 1986; Schreiber et al., 1993). Since the biologically active compounds triiodothyronine (T3) and retinoic acid are indispensable for brain function and development (Anderson, 2001; Bernal, 2002; Morriss-Kay and Ward, 1999), and TTR is the only thyroid hormone-binding protein found at a substantial level in the CSF (Herbert et al., 1986), it is possible that TTR reduction in MS-8h rats could affect supply of T3 and retinoic acid, which might cause developmental abnormalities and/or dysfunction of the brain.

Using PCR-based subtractive hybridization, increased TTR expression by stress during fear-conditioning training was shown in samples from mouse basolateral amygdala (Stork et al., 2001). Microarray analysis of rat hippocampal samples indicated that single-prolonged stress, a model of post-traumatic stress disorder, also up-regulated TTR expression (Harada et al., unpublished observations). These findings suggest that stress should up-regulate TTR expression. Considering that the MS rats showed greater susceptibility to stress as well as a drastic decrease in TTR, we suggest that TTR might have a protective role against stress in the brain.

One clinical study reported that TTR expression in CSF was significantly reduced in depressed patients (Sullivan et al., 1999), suggesting that TTR reduction might be involved in the pathophysiology of depression. Since attenuated HPA feedback is also thought to be a biological marker of depression (Carroll, 1982), the increased risk of depression by maltreatment during early life might be partly explained by TTR. It was recently reported that TTR-deficient mice showed behavioural alterations, such as decreased immobility in the forced swimming test and increased exploratory activity in the open-field test (Sousa et al., 2004). Although these alterations apparently indicate a resistance to depression and anxiety, TTR seems to be involved in their molecular mechanisms. Notably, norepinephrine (NE) levels were increased in the limbic forebrain in TTR-deficient mice. Since TTR can also bind the oxidation product of NE (Boomsma et al., 1991) and MS rats indicated increased levels of NE in the frontal cortex, hippocampus and hypothalamus after restraint stress (Daniels et al., 2004), it is possible that reduction of TTR affected the function of the NE system in the brain.

ACE and IGF-II expression, which is normally enriched in the CP (Chai et al., 1987; Newton et al., 2003), was significantly decreased in the MS-8h group. Significant reduction of IGF-II was also found in MS-15m rats. Again, these results should reflect MS stress in both groups.

Regarding ACE, its expression is found in various regions in the brain. It is expressed rather strongly in the dentate gyrus in the hippocampus, in addition to the CP (Chai et al., 1987). Other than for blood pressure control, the renin–angiotensin system seems to be involved in depression and/or anxiety. Although ACE inhibitors have been demonstrated to have antidepressant effects in humans and rats (Martin et al., 1990; Vuckovic et al., 1991; Zubenko and Nixon, 1984) and angiotensinogen-deficient mice showed reduced depression-like behaviour (Okuyama et al., 1999b), enhanced anxiety has been observed in mice lacking angiotensin II type-2 receptor (Okuyama et al., 1999a). Reduced expression of ACE in the hippocampus and/or the CP might be involved in enhanced anxiety of the MS rats.

It has been reported that IGF-II is synthesized predominantly in the leptomeninge, the CP, and microvasculatures, while its immunoreactivity has been detected in various areas of the brain (Logan et al., 1994). This finding indicates that IGF-II produced locally in the non-neuronal system probably spreads to and affects the whole brain. Although the physiological roles of IGF-II in the brain are not well understood, it should be noted that electroconvulsive seizure, an established treatment for depression,

induces IGF-II up-regulation in the CP (Newton et al., 2003), suggesting antidepressant-like roles of IGF-II. Persistently lowered levels of IGF-II in MS-8h and MS-15m rats might have a significant role in their stress susceptibility.

In the hippocampal samples used in this study, we found differential expression of several genes induced by MS stress. Surprisingly, TTR, a gene expressed almost specifically in the CP, indicated the most conspicuous decrease. We also found ACE and IGF-II, which are also enriched in the CP, to be significantly down-regulated. Since the CP is thought to support the brain by releasing several trophic polypeptides into the CSF (Chodobski and Szmydynger-Chodobska, 2001), dysfunction of the CP could conceivably play a role in the pathophysiology of MS rats.

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Statement of Interest

None.

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Neuroanatomy in monozygotic twins with Asperger disorder discordant for comorbid depression

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Significant genetic contributions to autism spectrum disorders (ASDs) have been reported.[1] However, specific genetic variants that contribute to ASD have not been conclusively identified. Recently, interest has centered on an approach aimed at identifying potential intermediate phenotypes such as neuroanatomic abnormalities, as such findings may facilitate the endeavor to localize specific genetic variants.[2] Here we report common and distinct neuroanatomic abnormalities of a pair of monozygotic twins concordant for Asperger disorder (ASP) but discordant for psychiatric comorbidity. The current study applied individual whole-brain voxel-based morphometry (VBM) to quantitatively identify neuroanatomic abnormalities.

Participants. A pair of 22-year-old male twins with ASP was recruited from the Department of Neuropsychiatry, Hospital of Tokyo University, Japan (see table E-1 on the *Neurology* Web site at www.neurology.org). Diagnosis of ASP was determined for each patient according to the Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV) (reference E-1) and further confirmed according to the International Classification of Diseases-10 (reference E-2) criteria through a consensus of two trained child psychiatrists. DNA fingerprint probes were used to establish zygosity, using an eight-probe single-locus DNA profile. DNA testing was performed to rule out fragile X syndrome. Although both the twins showed normal intelligence, one of them had current major depression as a psychiatric comorbidity (for detailed clinical characteristics of each twin, see table E-1). Eighty-two Japanese men without neuropsychiatric disorder served as a comparison sample (mean [SD] age = 28.9 [4.0] years, range 22 to 39 years). The participants were interviewed by trained psychiatrists and screened for the presence or absence of DSM-IV axis I disorder (reference E-3). All subjects were right-handed based on the Edinburgh Inventory (reference E-4). The Ethical Committee of the Faculty of Medicine, University of Tokyo, approved of this study. After a complete explanation, written informed consent was obtained from all participants.

MRI acquisition and analysis. The methods of MRI acquisition and image processing have been described in detail elsewhere.[3] In brief, the MRI data with $0.9375 \times 0.9375 \times 1.5$–mm voxels were obtained from all subjects using a 1.5 T scanner. Processing of the acquired images was similar to that described in our previous study[3] except that, rather than SPM99, the current study employed SPM2, which includes spatial normalization using study-specific customized template, tissue segmentation with extracting nonbrain voxels and smoothing with 12-mm full width at half-maximum. Furthermore, global gray matter, white matter, and CSF volumes were calculated from the optimized VBM procedure.[4] Statistical comparisons of the processed images between the twin pair (n = 2) and controls (n = 82) and between each twin (n = 1) and controls (n = 82) were performed using an analysis-of-covariance model with age and intracranial volumes as confounding covariates. For individual VBM, a statistical analysis method similar to that of a previous study[5] was employed. Significance levels were set at corrected $p < 0.05$.

Results. Significantly reduced gray matter voxel densities were found in the left superior temporal gyrus including superior temporal sulcus (STS), left fusiform gyrus, right amygdala, and right prefrontal cortex (PFC) in twins with ASP as compared with control subjects. Individual VBM revealed reduced gray matter densities in the left STS, fusiform, and right PFC commonly in both twins. In contrast, the reduced gray matter densities in the right amygdala were evident in the twin with comorbid depression but not in the co-twin without mood disorder. No significant group difference in voxel density was detected for other gray matter regions or any of the white matter regions (figure, page 492).

Discussion. Both of the monozygotic twins concordant for ASP had significantly smaller than normal left STS, left fusiform gyrus, and right PFC, regions important for social cognition and behavior (reference E-5). The current findings are generally consistent with previous structural MRI studies in persons with ASD, although some inconsistencies exist in the literature.[6] These findings further suggest a contribution of shared genetic factors to underlying the structural abnormalities in ASD. Of particular interest, however, reduction of the amygdala was evident only in the twin with comorbid depression. Here the difference in age distribution between the twins and the control group and the medication effect on the depressed twin should be considered. Taking into account the age-associated decrease in brain volume[4] and neurotrophic effects of lithium and antidepressants, (reference E-6) however, the elimination of these effects would likely only strengthen the statistical difference. Taken together with the amygdala volume reduction reported in some forms of depression and anxiety (reference E-7), our results have an important implication for the interpretation of structural abnormality of amygdala, which has been extensively demonstrated in adults with ASD (reference E-8). Our results are also in accordance with recent animal studies[7] suggesting a role of the amygdala in abnormal fear and anxiety rather than abnormal social behavior in ASD.

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the August 9 issue to find the title link for this article.

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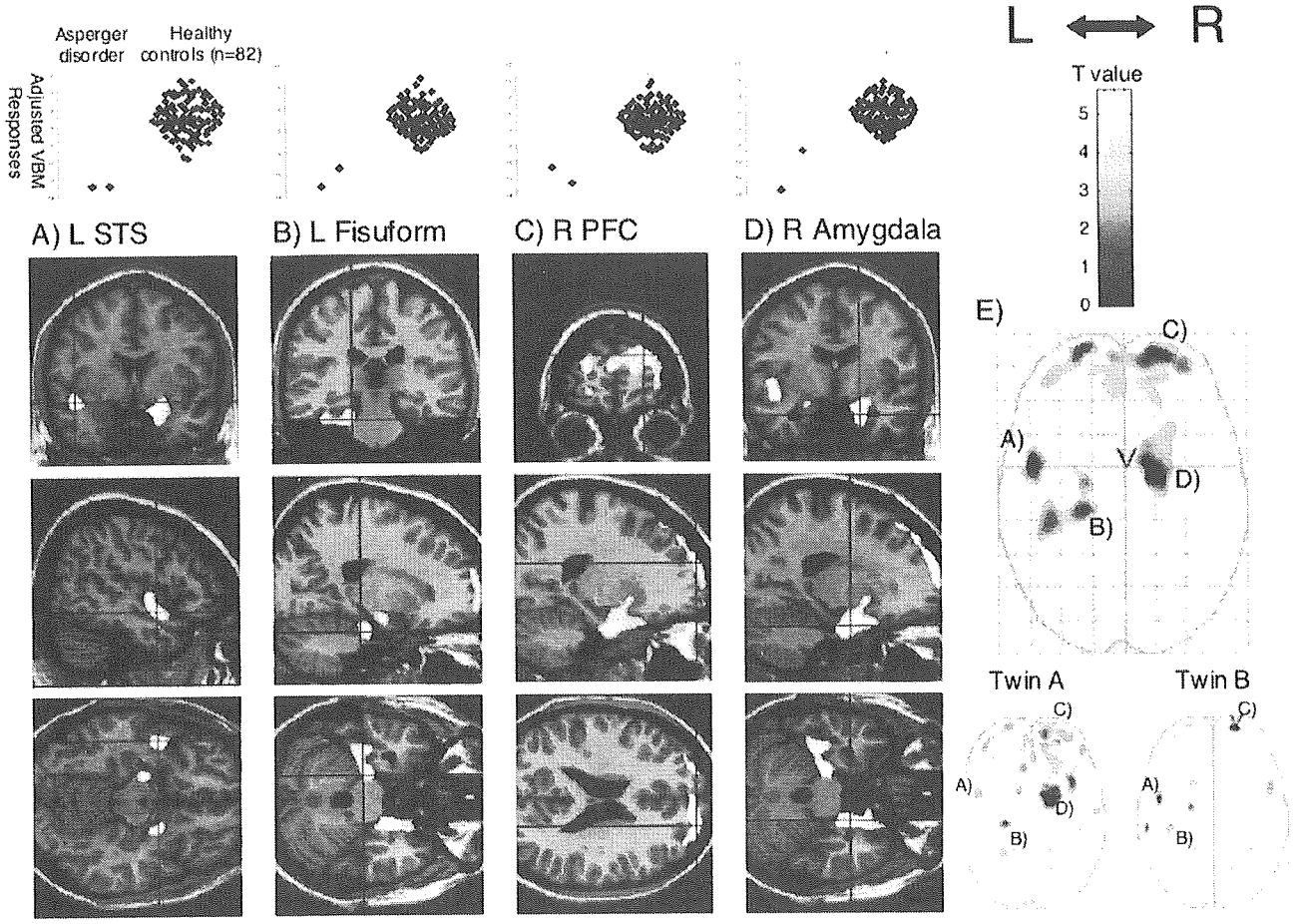

Figure. Morphological abnormalities in twins with Asperger disorder. (Left bottom, A through D) Gray matter voxels with reduced density in the twins with Asperger disorder as compared with normal control subjects (n = 2 vs n = 82) were rendered onto orthogonal slices of the normal template MR images. Voxel threshold: uncorrected p< 0.001; significantly abnormal regions: left superior temporal gyrus including superior temporal sulcus (A): peak coordinate at (x, y, z): (−49, 2, -13), spatial extent k = 1,934, Z(2,81) = 4.94, corrected p = 0.015; left fusiform gyrus (B): (−23, −23, −26), Z = 4.97, k = 3,590, corrected p = 0.013; right prefrontal cortex (C): (20, 61, 23), Z = 5.01, k = 9,617, corrected p = 0.011; right amygdala (D): (18, -3, -26), Z = 5.14, k = 6,514, corrected p = 0.006. (Left top, A through D) Plots of adjusted voxel-based morphometry responses at each brain region. (E) Statistical parametric maps in the axial projection showing gray matter voxels with reduced density in the twins (n = 2; upper map) and each twin (lower maps) as compared with normal controls (n = 82). A significant reduction in the right amygdala (D) found in Twin A was absent in Twin B. Voxel threshold: uncorrected p< 0.001.

A patient with left ventricular thrombus and recurrent stereotypic TIAs

Christine M. Bower, MD; Lola Morgan, MD; and Bruce Ovbiagele, MD

Stereotyped TIAs are presumed to occur secondary to a fixed flow-limiting stenosis of medium/large vessels in the cervicocephalic arterial tree[1] or in situ disease of small deep penetrating arteries in the brain.[2] We report the unusual case of a patient with recurrent stereotypic TIAs associated with the presence of a left ventricular thrombus and with delayed focal ischemia on the T1-weighted MRI sequence.

Case report. A 60-year-old nonsmoking Filipino man, with an unremarkable medical history, reported three distinct episodes of sudden-onset right-sided weakness and numbness and difficulty with expression. These episodes occurred every 2 hours over a 6-hour period. The first two spells lasted 10 minutes, and the third spell lasted 20 minutes.

On admission, his blood pressure was 155/90 mm Hg and pulse was 61 beats/min. Otherwise, general and neurologic exams were normal. Brain CT showed no evidence of infarct. Because of the temporal and stereotypic nature of his spells, we felt that the patient had a fixed flow-limiting stenosis in his left internal carotid or middle cerebral arteries causing hemodynamic compromise. The patient was admitted to the intensive care unit and placed on a heparin drip. MRI of the brain showed a mild hyperintensity on diffusion-weighted imaging (DWI) with corresponding apparent diffusion coefficient hypointensity in the left head and body of the caudate and a portion of the anterior internal capsule (figure, A and B). There was no corresponding signal change on T1-weighted, T2-weighted, or fluid-attenuated inversion recovery images. MR angiograms of the neck and circle of Willis were within normal limits (see the figure, C and D). Despite his stereo-

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Delayed automatic detection of change in speech sounds in adults with autism: A magnetoencephalographic study

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Abstract

Objective: Autism is a form of pervasive developmental disorder in which dysfunction in interpersonal relationships and communication is fundamental. This study evaluated neurophysiological abnormalities at the basic level of language processing, i.e. automatic change detection of speech and non-speech sounds, using magnetoencephalographic recording of mismatch response elicited by change in vowels and tones.

Methods: The auditory magnetic mismatch field (MMF) was evaluated in 9 adults with autism and 19 control subjects using whole-head magnetoencephalography. The MMF in response to the duration change of a pure tone or vowel /a/ and that in response to across-phoneme change between vowels /a/ and /o/, were recorded.

Results: The groups were not significantly different in MMF power under any conditions. However, the autism group showed a left-biased latency prolongation of the MMF particularly under the across-phoneme change condition, and this latency delay was significantly associated with greater symptom severity.

Conclusions: These results suggest that adults with autism are associated with delayed processing for automatic change detection of speech sounds. These electrophysiological abnormalities at the earliest level of information processing may contribute to the basis for language deficits observed in autism.

Significance: These results provide the first evidence for delayed latency of phonetic MMF in adults with autism.

Keywords: Autism; Magnetoencephalography (MEG); Mismatch negativity (MMN); Phoneme; Speech sound; Tone; Vowel

1. Introduction

Autism is a pervasive developmental disorder associated with aberrant social skills, deficient language, abnormal attention, and stereotyped repetitive behaviors (American Psychiatric Association, 1994). Fundamental cognitive deficits in autism are characterized by a lack of normal attentional preference to socially relevant stimuli (Rapin, 1997). For example, individuals with autism spent more time looking at objects and less time looking at people (Swettenham et al., 1998). Moreover, children with autism

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oriented more poorly to social (both speech and non-speech) than to non-social stimuli (Dawson et al., 1998). However, brain functional basis for deficits in socially relevant auditory stimuli such as speech sounds in autism has been poorly understood. At the earliest stage, i.e. the level of auditory sensory processing, speech sound processing requires the discrimination of phonemes; a process that requires the categorization of the simplest unit of speech sounds according to their acoustic features. Such a process can be indexed by the auditory mismatch negativity (MMN) elicited by speech sounds (Näätänen et al., 2001).

The MMN or its magnetic counterpart (magnetic mismatch field; MMF) is an event-related potential (ERP) or magnetic field peaked at approximately 100–200 ms after the onset of a physically deviant auditory stimulus in identical and repeated sequence (Hari et al., 1984; Näätänen et al., 1978). Näätänen (1992) noted that MMN (MMF) reflects the detection of mismatches between the deviant stimuli and the neural trace encoding the physical features of the standard stimuli and that MMN (MMF) can be elicited even under passive conditions when subjects ignore the stimuli entirely. Thus, MMN (MMF) can be considered an index of the process of automatic detection of acoustic change in humans. A number of researchers have recently extended their investigations into MMN (MMF) in response to speech sound discrimination (reviewed in Näätänen, 2001). Magnetoencephalography (MEG) (Alho et al., 1998a; Koyama et al., 2000; Näätänen et al., 1997; Rinne et al., 1999) and positron emission tomography (PET) (Tervaniemi et al., 2000) studies have demonstrated that the left auditory cortex is predominantly activated during the automatic processing of speech sounds (vowel or consonant–vowel syllables) in normal subjects. Moreover, Kraus (1998) suggested that phonetic MMN showed an increase as a result of cognitive discrimination training; thus it may be an index of language-related plasticity in the central nervous system.

A review of the previous literature on MMN or MMF in individuals with autism, identified 5 studies that employed ERPs (MMN) (Čeponienė et al., 2003; Ferri et al., 2003; Gomot et al., 2002; Kemner et al., 1995; Seri et al., 1999) and one that employed MEG (MMF) (Tecchio et al., 2003); the results of these studies are mixed (reviewed and discussed in detail in the Discussion section). The subjects in all 5 ERP studies were children with autism, and the MEG study by Tecchio et al. employed autism individuals with a broader range of ages (8–32 years). Moreover, only two ERP studies (Čeponienė et al., 2003; Kemner et al., 1995) used speech sounds to elicit MMN: Kemner et al. (1995) reported preserved MMN amplitude in response to change between /ay/ and /oy/ sounds in children with autism; however, no analysis of latency data was reported; Čeponienė et al. (2003) reported intact MMN amplitude and latency elicited by change in vowels as well as simple and complex tones in children with autism. Thus, to date, no studies have evaluated MMN/MMF specifically in adults with autism; no studies have used MEG to record mismatch response to speech sounds in autism. Importantly, no studies have explored the relationship between MMN/MMF indices and clinical symptoms in autism. Additionally, all 5 studies that evaluated tonal MMN/MMF in autism (Čeponienė et al., 2003; Ferri et al., 2003; Gomot et al., 2002; Seri et al., 1999; Tecchio et al., 2003) measured mismatch response to frequency change (frequency MMN/MMF), with none of them assessing MMN/MMF in response to duration change of tones (duration MMN/MMF).

Accordingly, the goal of this study was to investigate, using a whole-head MEG, whether or not a reduction and/or latency prolongation in magnetic mismatch field elicited by across-category change of speech sounds is present in adults with autism. Additionally, we also measured duration MMF using tonal and vowel stimuli. The use of a whole-head MEG instead of a scalp EEG has two advantages. First, the use of a whole-head MEG enables independent investigation of left and right hemispheric functions, because, in contrast to electrical fields, magnetic fields are not influenced by intervening tissues of different conductivities. Second, MEG selectively detects electrical currents tangential to the scalp, whereas EEG is more sensitive to radially oriented currents. Thus, MMF generated in the superior temporal plane constituting the auditory cortex could be selectively detected by MEG recording, while MMF from other generators such as the frontal component (Alain et al., 1998; Alho et al., 1994; Giard et al., 1990; Kasai et al., 1999; Liasis et al., 2001; Umbricht et al., 2000), having preferentially radially oriented currents (Giard et al., 1990; Kasai et al., 1999), is largely filtered out.

2. Methods

2.1. Subjects

Nine right-handed (Edinburgh Inventory [Oldfield, 1971] with laterality index ≥ 0.8 as the cut-off for right-handedness) adults with autism were recruited from the Outpatient Clinic, Department of Neuropsychiatry, University Hospital of Tokyo, Japan. Six were male and 3 were female, and the mean age was 27.2 (SD 7.7). Nineteen age-, gender-, and handedness-matched healthy subjects (mean age 27.3; SD 7.0; 13 males and 6 females) participated in the study. Diagnosis of autism was made according to the DSM-IV (American Psychiatric Association, 1994) criteria for autistic disorder and confirmed using the Childhood Autistic Rating Scale—Tokyo Version (CARS-TV) (Kurita et al., 1989) administered by an experienced child psychiatrist (O.H.). Scores for all subjects in the patient group (mean 33.7 [SD 1.2]) were above the cut-off point of >27 for adult criteria of autism (Mesibov et al., 1989). All subjects with autism were able to construct 3 word sentences. Patient's IQs (mean 57.2 [SD 15.0]) were evaluated using the Wechsler Adult Intelligence Scale-Revised (WAIS-R)

Table 1
Subject information (autism group)

Subject no.	Gender	Age	CARS	IQ			Medication (mg/day)			EEG abnormality
				Verbal	Performance	Total	Neuroleptics	Antiepileptic drugs	Anticholinergic drugs	
1	M	33	33.5	NA[a]	NA[a]	38	Haloperidol 0.75	None	Biperiden 1	No
2	F	31	36.5	NA[a]	NA[a]	48	Haloperidol 2.25	Valproate 600	Biperiden 2	No
3	F	27	34.5	62	52	54	Haloperidol 0.75	None	Biperiden 1	No
4	M	20	33.0	73	73	68	Haloperidol 0.75	Valproate 600	Trihexyphenidyl 2	No[b]
5	F	20	34.0	NA[a]	NA[a]	37	Haloperidol 1.5	None	Biperiden 2	No
6	M	15	33.0	52	99	71	None	None	None	No
7	M	38	33.0	70	91	77	None	None	None	No
8	M	26	32.5	56	52	51	None	None	None	No
9	M	35	33.5	80	64	71	Bromperidol 3	None	Trihexyphenidyl 2	No

CARS, Childhood Autism Rating Scale; EEG, electroencephalogram; M, male; F, female.
[a] Verbal and non-verbal IQs were not available because the data were based on the Tanaka–Binet Test. Otherwise, the IQs were evaluated using the Wechsler Adult Intelligence Scale-Revised (WAIS-R).
[b] This subject had a history of generalized tonic–clonic seizures in his childhood, although no epileptiform EEG activities had been previously detected.

(Wechsler, 1981; Japanese standardized version, Shinagawa et al., 1990) or the Tanaka–Binet Intelligence Scale (Japanese standardized version of the Stanford–Binet test, Tanaka Institute for Education, 1987) (Table 1). No individuals with autism showed current electroencephalogram (EEG) abnormalities. One subject had a history of generalized tonic–clonic seizures in childhood, although no epileptiform EEG activities had been previously detected. Six of the individuals were treated with neuroleptics to reduce the occurrence of disabling self-injurious behavior. Mean haloperidol equivalent dose (Inagaki et al., 1999a) was 1.0 mg/day (SD 1.1). These 6 individuals also received anticholinergic drugs; mean biperiden equivalent dose (Inagaki et al., 1999b) of 0.89 mg/day (SD 0.78), to prevent the occurrence of Parkinsonism secondary to the neuroleptics. No symptoms of Parkinsonism were clinically observed. No individuals with autism received anxiolitics or hypnotics, but two were treated with sodium valoproate (600 mg/day) as a mood stabilizer.

The first language of all participants in both groups was Japanese. The exclusion criteria for both groups were a history of electroconvulsive therapy, neurological illness, traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min, substance use or addiction, and presence of hearing or vision impairment. No individuals with autism showed evidence of tuberous sclerosis. An additional exclusion criterion for the control group was a history of psychiatric disease in themselves, or a family history of axis I disorder in their first-degree relatives.

This study was approved by the Ethical Committee of the Faculty of Medicine, University of Tokyo. After a complete explanation of the study, written informed consent was obtained from all the control subjects. Written informed consent was also obtained from all the autism participants as well as from their parents.

2.2. Task procedures

The subjects were presented with sequences of auditory stimuli consisting of standard (probability 90%) and deviant ($P=10\%$) stimuli delivered randomly, except that each deviant stimulus was preceded by at least one standard stimulus. The interstimulus interval (ISI) was 510 ± 20 ms. The stimuli were delivered binaurally through plastic tubes. The subjects were instructed to watch a silent film to help distract them from the stimuli.

The experiment consisted of 3 conditions. The first condition was to elicit MMF in response to a duration decrement of pure-tone stimuli (tone-duration condition; standard, 100 ms duration; deviant, 50 ms duration). The second condition was to elicit MMF in response to a duration decrement of vowel stimuli (phoneme-duration condition; standard, Japanese vowel /a/ with a 150 ms duration; deviant, /a/ with a 100 ms duration). The last condition was to elicit MMF in response to a vowel across-category change (across-phoneme condition; standard, Japanese vowel /a/ with a 150 ms duration; deviant, /o/ with a 150 ms duration). These vowel stimuli were spoken by a native-Japanese-speaking actor, digitized using the NeuroStim system (NeuroScan Inc., USA), and edited to have a duration of 100 or 150 ms, loudness of 70 dBSPL and rise/fall time of 10 ms. The frequency spectra for the vowels were as follows: /a/, formant (F) $0=140$ Hz, $F1=760$, $F2=1250$, $F3=2750$, and $F4=3600$; /o/, $F0=140$ Hz, $F1=480$, $F2=770$, $F3=2820$, and $F4=3600$. The order of the 3 conditions was counterbalanced across the subjects.

2.3. Data collection and processing

The recording and analysis procedures were the same as those described elsewhere (Kasai et al., 2001, 2002, 2003). Magnetic fields were recorded in a magnetically shielded room (NKK Plant Engineering Co., Japan) with a 122 channel magnetometer (Neuromag Ltd., Finland; Knuutila et al., 1993). This whole-head magnetometer consists of 61 dual-sensor units, each with two orthogonal planar gradiometers for recording maximal signals directly above the source (Hämäläinen et al., 1993). The subjects sat on a chair with their 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 by recording the magnetic fields produced by currents fed into 3 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. One electrode was placed at the outer canthus and another one below the left eye to monitor eye movements.

MEG epochs were averaged separately for standard and deviant stimuli online. The duration of the averaging period was 512 ms, including a 64 ms prestimulus baseline. The recording bandpass was 0.03–100 Hz, with a sampling rate of 500 Hz. The first 10 stimuli were automatically excluded from averaging. Epochs coinciding with electrooculogram movement or MEG exceeding 150 μV or 3000 fT/cm were also excluded from averaging. Each condition lasted until 100 deviant stimuli without contamination of artifacts were acquired. Averaged responses were digitally filtered with a bandpass of 1–30 Hz.

2.4. MMF measurement

For each subject in each condition, equivalent current dipoles (ECDs) for MMF were calculated primarily according to the method used by Alho et al. (1998b). Briefly, the MMF was determined from the difference curves obtained by subtracting the response to standard stimuli from that to deviant stimuli. ECDs were then determined using a least-squares fit at 2 ms intervals from 100 to 250 ms. The calculation was performed separately for each hemisphere (a subset of 44 channels over the temporal brain areas), utilizing a spherical head model in which the center of the model sphere was placed 45 mm above the origin of the coordinate system (Alho et al., 1998b). ECDs with a maximal goodness of fit (GOF) $\geq 60\%$ were included in the analysis. In this procedure, we reduced the number of channels to 28–43 when the dipole was not calculated or a certain channel had a considerable number of artifacts. The mean GOFs under the 3 conditions and in the two hemispheres ranged from 78.3 to 85.7% for the autism group and 72.5 to 85.7% for the control group, and did not differ between groups for any condition or hemisphere (Mann–Whitney's U test, $Ps > 0.23$).

The subjects for whom ECDs were not reliably calculated for at least one hemisphere were 6/19 for the control group and 9/9 for the autism group. In the control group, visual inspection of the signals for these cases indicated that MMF was strongly lateralized to one hemisphere, possibly resulting in the failure to calculate ECDs in the other hemisphere. In the autism individuals, there were no gross artifacts or noises superimposed on averaged response curves that would account for the failure to calculate ECDs. In theory, the ECD is stronger when neuronal activities are synchronized and regionalized (Kasai et al., 2002, 2003). Thus, an alternative explanation for the failure to calculate ECDs may be that some individuals with autism had deficits in the synchronization and regionalization of the neuronal population involved in MMF generation.

To utilize the data of all the subjects in the statistical analyses, the magnitude of MMF responses was reassessed by applying global field power (GFP; Lehmann and Skrandies, 1980) to the analysis of MEG data (magnetic counterpart of GFP [mGFP]; Kasai et al., 2001, 2002, 2003; Kreitschmann-Andermahr et al., 1999; Rosberg et al., 2000). First, for each subject, the mGFP was calculated separately for each condition and hemisphere using the same 44 channels as those used in the dipole analysis. In this procedure, the number of channels was reduced to 28–43 when a certain channel had a considerable number of artifacts. The peak latency of MMF for each subject was determined based on the individual mGFP curve as a function of time. Second, the grand mean mGFP curves were plotted. The MMF power for each subject was then determined as the mean mGFP within a 100 ms window around the peak latency of the grand mean mGFP. This 100 ms window was chosen because it was within the length of clear evocation of MMF (Fig. 1). Our previous studies have shown the mGFP power/latency to be a good substitute for ECD strength/latency (Kasai et al., 2001, 2002, 2003).

2.5. Statistical analysis

Group differences in dipole strength and location were tested using Mann–Whitney U test. The t tests were performed for the group comparison of MMF power or latency for each condition and hemisphere. Since there were 12 multiple comparisons, the level for significance was set at $P = 0.0042$ (Bonferroni correction). Moreover, since there was unequal sample size and, in two conditions unequal variance (Levene's test for equality of variance; tone-duration, left hemisphere: $P = 0.02$; phoneme-duration, left hemisphere: $P = 0.008$; other conditions: $P > 0.28$) between the groups, we also performed non-parametric Mann–Whitney U test for confirmation purpose. Spearman's rho was calculated for correlations between MMF indices and total CARS scores in the autism group. Additionally,

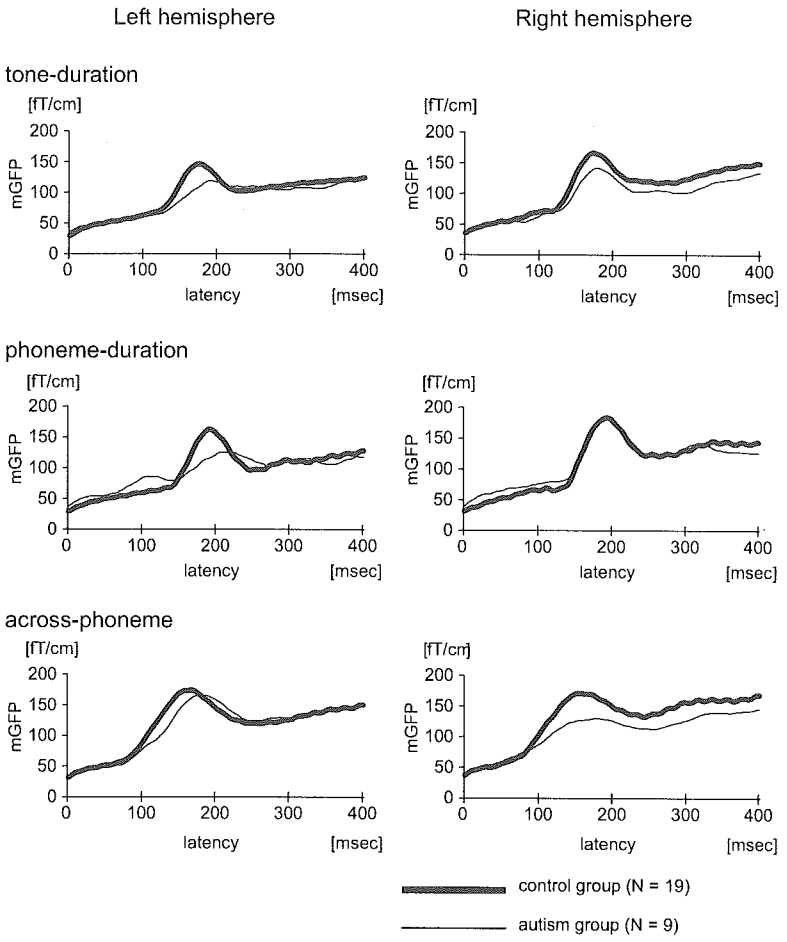

Fig. 1. Grand mean magnetic counterpart of global field power (mGFP) waveforms under the tone-duration (top), the phoneme-duration (middle), and the across-phoneme (bottom) change condition for each hemisphere. Thick lines are for the control group and thin lines for the autism group.

Spearman's correlations between MMF indices and age (for each of both groups), IQ scores (autism group only) or neuroleptics and anticholinergic dose (autism group only) were calculated to test for potential confounding status of these indices.

3. Results

3.1. Dipole analysis

An example of dipole locations superimposed on a subject's magnetic resonance imaging is shown in Fig. 2. For this subject, MMF for each condition was located in the vicinity of the posterior superior temporal gyrus in each hemisphere, coinciding with previous reports on source localization of MMF in response to pure tones (Alho et al., 1998b) or speech sounds (Alho et al., 1998a).

Group differences in dipole strengths or locations were not statistically significant for any condition or hemisphere after Bonferroni correction (the level of significance was $P=0.002$; 24 comparisons) (Table 2).

3.2. MMF power and latency

The t tests showed that the autism group was associated with significantly delayed latency of MMF under the across-phoneme condition in the left hemisphere ($t[26]=3.11$, $P=0.004$) (Table 3). Additionally, these results were confirmed by Mann–Whitney U test which showed a significance in the left hemisphere of across-phoneme condition ($Z=-2.46$, $P=0.014$), while other conditions/hemisphere did not reach significance ($P>0.07$).

3.3. Correlational analyses

Autism individuals' MMF latency in the left hemisphere under the across-phoneme condition showed a significant positive correlation with scores for CARS (rho$=0.672$, $N=9$, $P=0.047$). Importantly, MMF latency in the left hemisphere under the across-phoneme condition in

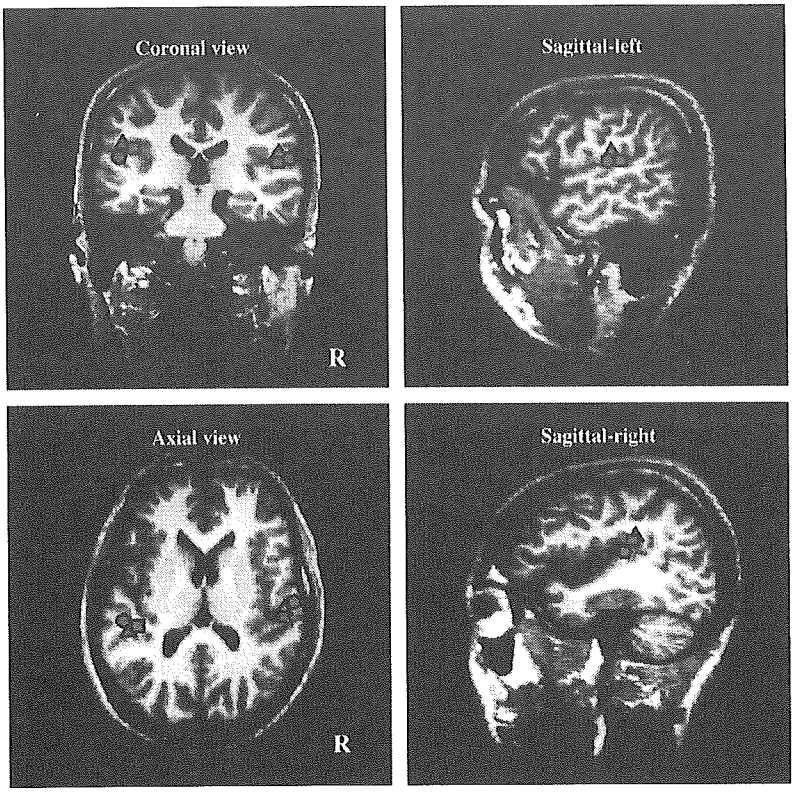

Fig. 2. Locations of equivalent current dipoles (ECDs) under each condition in each hemisphere of a control subject superimposed on a magnetic resonance imaging slice of this subject. Circle: ECD location of MMF under tone-duration condition; triangle: that under phoneme-duration condition; and square: that under across-phoneme condition.

the autism group was not significantly correlated with age (rho = −0.126, $N=9$, $P=0.75$), IQ (rho = −0.285, $N=9$, $P=0.46$), or dose of medication (neuroleptics: rho = 0.173, $N=9$, $P=0.66$; anticholinergic drugs: rho = 0.419, $N=9$, $P=0.26$).

Additional significant results were as follows: control subjects' MMF power under the across-phoneme condition showed a significant negative correlation with age (left hemisphere: rho = −0.552, $N=19$, $P=0.014$; right hemisphere: rho = −0.693, $N=19$, $P=0.001$); autism individuals' MMF latency in the right hemisphere under the phoneme-duration condition showed a significant negative correlation with IQ (rho = −0.689, $N=9$, $P=0.04$); patient's MMF latency in the left hemisphere under the phoneme-duration condition showed a significant negative correlation with neuroleptic dose (rho = −0.676, $P=0.046$) and anticholinergic drugs (rho = −0.698, $P=0.037$).

4. Discussion

The present findings represent the first physiological evidence, derived from whole-head MEG, of delayed automatic processing of change in speech sounds predominantly in the left temporal area in adults with autism. Moreover, to our knowledge, this is the first study that linked MMN/MMF abnormalities to clinical severity in autism. This study suggests that language-related dysfunction in autism may be present at the early stage of auditory processing of relatively simple stimuli such as phonemes, and not just at stages involving higher-order semantic processes. In this study, the autism group showed neither abnormal power nor lateralization for any type of MMF, while MMF latency was prolonged in the left hemisphere during across-category change detection of vowels. Although conclusions must be speculative, it may be that adults with autism have difficulties in rapid evaluation of change in speech sounds, particularly that mediated in the left auditory cortex. These results are also consonant with PET (Boddaert et al., 2003; Zilbovicius et al., 2000) and single photon emission computed tomography (Hashimoto et al., 2000; Ohnishi et al., 2000) studies that report temporal lobe hypoperfusion in autism.

Additionally, the results of the present study indicate that adults with autism did not show marked deficits in duration MMF in response to tones and vowels, although this discussion should be regarded as tentative since there appeared to be a statistically non-significant difference in the left hemisphere. Since no previous study has measured duration MMN/MMF in autism, future studies should assess

Table 2
Equivalent current dipole strength and location in control subjects and autism patients

	Control group			Autism group			Group comparison[a]	
	N	Mean	SD	*N*	Mean	SD	*Z*	*P*
ECD strength (nAm)								
Pure (L)	17	22.2	11.3	5	20.5	9.1	−0.39	0.70
Pure (R)	16	27.3	11.9	8	30.9	6.9	−0.92	0.36
a/a (L)	17	28.3	16.4	3	27.7	26.5	−0.58	0.56
a/a (R)	19	32.8	19.4	7	34.4	9.7	−0.84	0.40
a/o (L)	19	29.0	9.9	7	28.4	15.9	−0.35	0.73
a/o (R)	18	27.9	11.8	5	33.2	16.2	−0.82	0.41
ECD location[b] (mm)								
x-axis								
Pure (L)	17	−53.5	7.7	5	−49.1	6.8	−1.06	0.29
Pure (R)	16	52.7	10.8	8	49.7	8.5	−0.73	0.46
a/a (L)	17	−55.0	8.5	3	−54.5	11.2	−0.21	0.83
a/a (R)	19	51.3	10.6	7	53.4	9.2	−0.26	0.79
a/o (L)	19	−54.0	8.8	7	−49.4	10.9	−0.90	0.37
a/o (R)	18	53.5	6.4	5	56.3	5.7	−0.89	0.37
y-axis								
Pure (L)	17	6.2	8.5	5	6.6	6.5	−0.04	0.97
Pure (R)	16	11.7	8.1	8	4.4	7.5	−2.08	0.04
a/a (L)	17	8.7	8.0	3	−0.9	6.8	−1.85	0.06
a/a (R)	19	13.1	11.6	7	11.3	8.2	−0.20	0.84
a/o (L)	19	7.7	7.6	7	4.1	8.5	−0.84	0.40
a/o (R)	18	13.5	12.2	5	6.4	5.8	−1.53	0.13
z-axis								
Pure (L)	17	65.5	10.5	5	66.7	11.2	−0.04	0.97
Pure (R)	16	66.3	9.4	8	61.8	11.4	−1.13	0.26
a/a (L)	17	60.5	9.8	3	61.4	8.0	−0.16	0.87
a/a (R)	19	65.4	8.7	7	69.3	7.2	−1.19	0.24
a/o (L)	19	64.1	10.9	7	66.1	10.9	−0.14	0.89
a/o (R)	18	64.7	11.7	5	57.8	16.8	−1.04	0.30

ECD, equivalent current dipole; pure, tone-duration condition; a/a, phoneme-duration condition; a/o, across-phoneme condition; L, left hemisphere; R, right hemisphere.

[a] Mann–Whitney *U* test. The level of significance was $P=0.002$ (Bonferroni correction; 24 comparisons).

[b] The coordinate system was defined so that the *x*-axis passes through the preauricular points, with the positive *x*-axis pointing to the right. The *y*-axis passes the nasion, pointing anteriorly, and the *z*-axis points upwards.

Table 3
Global field power and latency of the magnetic mismatch field

	Autism group ($N=9$)		Control group ($N=19$)		Effect size	*T* test (df = 26)	
	Mean	SD	Mean	SD		*T* value	*P* value
Global field power (fT/cm)							
Tone-duration (left)	106	26	119	33	0.39	−0.98	0.34
Tone-duration (right)	117	34	134	40	0.43	−1.11	0.28
Phoneme-duration (left)	115	36	126	40	0.28	−0.67	0.51
Phoneme-duration (right)	147	32	148	34	0.03	−0.06	0.95
Across-phoneme (left)	149	65	153	47	0.09	−0.2	0.84
Across-phoneme (right)	124	47	154	45	0.67	−1.63	0.11
Peak latency (ms)							
Tone-duration (left)	194	30	177	16	1.06	2.02	0.054
Tone-duration (right)	181	11	175	12	0.50	1.24	0.23
Phoneme-duration (left)	194	36	189	9	0.56	0.59	0.56
Phoneme-duration (right)	193	12	193	14	0.00	0.11	0.91
Across-phoneme (left)	186	23	161	19	1.32	3.11	0.004[a]
Across-phoneme (right)	172	35	162	29	0.34	0.74	0.46

df, degree of freedom.

[a] Significantly delayed in the autism group. Statistically significance level was $P=0.0042$ (Bonferroni correction for 12 comparisons).