cultured hippocampal neurons (Fig. 2 and supplemental Fig. 2). In the exocytotic release of neurotransmitter, synaptic vesicle-associated proteins (synapsin I, synaptotagmin, synaptobrevin, synaptophysin, *etc.*) and plasma membrane-associated proteins (syntaxin, SNAP25, *etc.*) are important (38). Indeed, after BDNF application during the *in vitro* maturation period, cultured hippocampal neurons at the mature stage showed more synapsin I-positive presynaptic sites compared with that in the absence of BDNF (Fig. 4A). However, cultured neurons after coapplication with DEX failed to increase synaptic protein (Fig. 2) and synapsin I-positive sites (Fig. 4A). To our knowledge, there is no report that DEX suppresses the expression of presynaptic proteins in an *in vitro* system. Indeed, although sole DEX application at high dose seemed to reduce synaptic proteins compared with control, quantification showed no significance (supplemental Fig. 2). After long exposure, sole DEX treatment decreased the number of synapsin I-positive sites compared with control. Because BDNF expression gradually increases in the development stage (39), the reduction in the number of presynaptic sites by DEX alone might result from inhibition of the endogenous BDNF expression. In our cultures, significant reduction in endogenous BDNF after 72 h exposure of DEX was not observed (supplemental Fig. 2C), suggesting no involvement of endogenous BDNF in synaptic protein expression at DIV4. In the case of a decrease in the number of synapsin I-positive sites after long DEX exposure, down-regulation of endogenous BDNF may be involved in the effect of sole DEX addition. Furthermore, it is possible that the change in the efficiency of secretion of endogenous BDNF after DEX exposure is involved in the effects of sole DEX treatment, although further study is required.

Application of DEX before BDNF addition significantly inhibited the BDNF-up-regulated synaptic proteins, whereas sole DEX treatment had little influence compared with the control, suggesting that glucocorticoid may have a more severe effect on the BDNF-dependent biological effects compared with that in the control condition. Interestingly, DEX affected BDNF-induced neurite outgrowth, especially of glutamatergic neurons (Fig. 1). BDNF is important not only for excitatory but also for inhibitory neurons (40). Indeed, BDNF tended to enhance the neurite outgrowth of both glutamatergic and GABAergic neurons in our cultures. However, inhibition by DEX on the BDNF-enhanced neurite outgrowth of GABAergic neurons was weak, implying that glutamatergic neurons have a higher sensitivity to glucocorticoid.

The inhibitory action of DEX on the BDNF-increased synaptic proteins in the early development stage may result in down-regulation of synaptic function in the mature stage. Exposure of neurons to DEX early in culture reduced the BDNF-enhanced presynaptic activity and glutamate release (Fig. 4C). One possibility is that the activity of individual synapses is down-regulated. However, DEX had no significant influence on exocytotic efficiency in individual synaptic sites (Fig. 4B, FM imaging). As shown in Fig. 4A, immunostaining with anti-synapsin I antibody showed a decrease in the number of synaptic sites. Taking these findings together, it is possible that the number of synaptic connections is decreased.

Generally, if inputs from presynapses are reduced for a long time, the sensitivity of postsynapses will increase plastically to compensate for the reduction of the presynaptic inputs (41). However, DEX decreased both pre- and postsynaptic functions in the present study. BDNF is produced and secreted in a neuronal activity-dependent manner (42–44); thus, continuous weak synaptic activity could result in the reduction of BDNF level as is observed in depressive disorder (19). The down-regulation of BDNF itself or function caused by glucocorticoid exposure in the immature period might make a neuronal system vulnerable to various stresses. Glucocorticoid exposure or stress application during the early postnatal days is suggested to influence later life (15, 30). DEX-injected rats at P1 show a reduction of long-term potentiation in the CA1 region of the hippocampus, and reduction in NR2B level in adulthood occurs (31). Maternal deprivation stress to neonatal (P9) rats suppresses the expression of mRNA of BDNF, NR2A, and NR2B in the hippocampus and prefrontal cortex when they become adults (32). These studies, including our results, support the idea that glucocorticoid exposure during the development of neurons results in inhibition of network establishment (33).

Long-term exposure of glucocorticoid is suggested to reduce GR protein (36). Consistently, DEX-dependent suppression of a developmental increase in the GR expression was confirmed (Fig. 5A). In our system, to reveal the marked inhibitory action of DEX, the high dose of DEX is required, although the dose dependency of DEX in its inhibitory action was observed (Fig. 2). Thus, to clarify the involvement of GR, the siRNA for GR knockdown was applied. We found that DEX failed to inhibit the BDNF-increased synaptic proteins after siRNA application (Fig. 6). Moreover, RU486, a GR antagonist, reversed the inhibitory effect of DEX (Fig. 5B). These results suggest that DEX exerts its inhibitory effect through GR. The high dose required for the present study may be a result of nonspecific binding to extracellular molecules derived from serum in media for plating of cells.

BDNF binds to the TrkB receptor and activates various signaling pathways, including the MAPK and phosphatidylinositol 3-kinase pathways (45). In our study, DEX treatment had no effect on activation of the phosphatidylinositol 3-kinase pathway (Fig. 7B), which is important for neuronal survival (46, 47). Consistently, DEX did not have any effect on neuronal viability (supplemental Fig. 1). Therefore, DEX might inhibit the MAPK pathway, especially. MAPK signaling plays multiple roles in neurons. We recently reported that 17β-estradiol protects cortical neurons against oxidative stress-induced cell death through reduction in the

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activity of MAPK (48). Interestingly, it was reported that an early phase of the MAPK activation contributed to the cellular adaptive response, but the late phase of the signal activation exerted a toxic response to oxidative stress in the HT22 mouse hippocampal cell line (49). With regard to synaptic function, we recently reported that long-lasting (over 9–24 h) activation of the MAPK pathway is important for BDNF-increased presynaptic protein levels and glutamate release (26). In hippocampal neurons, the suppression of BDNF-stimulated MAPK activation was observed in DEX-treated cultures (Fig. 7). Moreover, BDNF-induced increases in synaptic proteins (Fig. 7A), Ca^{2+} influx (Fig. 7C), and glutamate release (Fig. 7D) were inhibited by the MAPK pathway inhibitor U0126. Taking these results together, DEX might influence the action of BDNF via reduction of MAPK activation. In the present study, the influence on the exocytotic efficiency of individual synaptic sites was not significant (Fig. 4). Jovanovic *et al.* (50) reported that acute application of BDNF enhances glutamate release from synaptosomes obtained from cerebral cortex and suggested the involvement of synapsin I phosphorylation through the MAPK pathway. The importance of the MAPK pathway in phosphorylation of synapsin I and exocytosis (triggered by glucose) was also suggested in pancreatic β-calls (51). In their experiment, cell responses through the MAPK pathway after stimulation are acute actions. In our experiment, neuronal function at DIV7 (5 d after BDNF addition) was examined; thus, the difference in experimental conditions including the timescale, maturity, or cell types might contribute to these differences.

The MAPK pathway is also involved in dendritic formation (28). We also reported the importance of the MAPK pathway for dendrite outgrowth of developing cortical neurons (52). Therefore, inhibition of neurite outgrowth with DEX treatment in our study may also be due to reduction in the activity of MAPK signaling. The inhibitory effect of DEX on MAPK activation has recently been reported in the analysis of another growth factor. Platelet-derived neurotrophic factor induced the activation of MAPK/ERK, and pretreatment of DEX inhibited it (53). As expected, DEX inhibited the platelet-derived neurotrophic factor-dependent outgrowth of the neuronal progenitor cell line HIB5. The glucocorticoid-induced leucine zipper protein GILZ is reported to inhibit the phosphorylation of ERK in the T-cell cell line 3DO (54). Furthermore, glucocorticoid induces the expression of MAPK phosphatase-1 (MKP-1), resulting in a decrease in the phosphorylation of MAPK/ERK in the mast cell line RBL-2H3 (55). Therefore, up-regulation of the phosphatase or a similar functional molecule may be involved in our neuronal system. On the other hand, GR function is also suggested to be reduced by MAPK activation (56); thus, it is interesting to study the interaction between the glucocorticoid/GR function and BDNF/MAPK signaling.

In the present study, we focused on the cross-talk between the BDNF function and the effect of glucocorticoid. In particular, the essential role of BDNF involved in synaptic protein expression and function was down-regulated after glucocorticoid exposure. We recently reported that chronic treatment with antidepressant potentiated the BDNF-induced glutamate release via enhancing the activation of BDNF-stimulated intracellular signaling (23). Our current results may provide critical information regarding the interaction of BDNF signaling and stress hormone.

MATERIALS AND METHODS

Chemicals

DEX was dissolved in dimethylsulfoxide (DMSO; Wako Pure Chemical Industries, Ltd.). Thus, the effect of DMSO (vehicle) was checked, and we confirmed that DMSO alone did not have any effects compared with no treatment (data not shown). RU486 was obtained from LKT Laboratories (St. Paul, MN). With regard to intracellular signaling inhibitors, U0126, a MAPK pathway inhibitor, was purchased from Promega (Madison, WI) and used at a final concentration of 10 μM. Other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Regeneron Pharmaceutical Co., Takeda Chemical Industries (Osaka, Japan), and Sumitomo Co. Ltd. (Osaka, Japan) donated the BDNF. BDNF was applied to neurons at a final concentration of 100 ng/ml.

Cell Culture

Dissociated hippocampal neurons prepared from postnatal 2-d-old rats (SLC, Shizuoka, Japan) were maintained as reported previously (27). All animals were treated according to the institutional guideline for care and use of animals. Hippocampal cells were plated on polyethyleneimine-coated culture dishes (Corning, Corning, NY), plates (Corning), or coverglasses (Matsunami, Osaka, Japan) attached to flexiPERM (Vivascience, Gottingen, Germany). The cell density was $5 \times 10^5/cm^2$ for Western blot analysis and glutamate release detection, $2 \times 10^5/cm^2$ for Ca^{2+} imaging, or $5 \times 10^4/cm^2$ for immunocytochemistry and exocytosis imaging. The culture medium consisted of 5% fetal bovine serum, 5% heat-inactivated horse serum, and 90% of a 1:1 mixture of DMEM and Ham's F-12 medium (Invitrogen, Carlsbad, CA). Four hours after cell plating, the medium was exchanged to Neurobasal-A medium (Invitrogen) containing 2% B27 supplement (Invitrogen) and 0.5 mM L-glutamine (Invitrogen).

Immunocytochemistry

Two days after cell plating (DIV2), BDNF was applied with or without pretreatment with 10 μM DEX for 24 h. Forty-eight hours after BDNF application, cultured cells were fixed with 4% paraformaldehyde for 30 min and then rinsed three times with PBS. Cells were then permeabilized with 0.1% Triton X-100 and 5% heat-inactivated horse serum in PBS for 20 min at room temperature. Afterward, the fixed cells were incubated with first antibodies overnight at 4 C. We used anti-MAP2 (1:1000; Sigma), anti-GAD (1:2000; Sigma), anti-glutamate (1/8000; Sigma), and anti-synapsin I (1:2000; Chemicon, Temecula, CA) antibodies. Alexa fluor 488- or Alexa fluor 594-conjugated antimouse IgG (1:1000; Invitrogen Molecular Probes, Tokyo, Japan) or antirabbit IgG (1:1000; Invitrogen Molecular Probes) were used as secondary

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antibodies. Immunoreactivity was observed with a fluorescence microscope (Axiovert 200; Zeiss, Tokyo, Japan), and the obtained images were analyzed with the Slide Book 3.0 software (Intelligent Imaging Innovations Inc., Denver, CO). To estimate the neurite outgrowth, the number of primary dendrites per cell after immunostaining was examined (52). MAP2 and GAD (or glutamate) double-positive dendritic neurites was examined. The number of primary dendrites (from the cell body) that were two times the length of the cell diameter or longer was counted. The number of presynaptic sites was estimated by counting the synapsin I-positive dots on dendrite shafts per 50-μm length.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) Assay

MTT assay (mitochondrial-dependent conversion of tetrazolium salts) was performed as reported previously (57). In brief, after treatment with 10 μM DEX (DIV1) and/or BDNF (DIV2), cultured hippocampal neurons were incubated with MTT solution at DIV4. Two hours later, cultures were lysed, and the metabolic activity of the mitochondria was estimated to determine the cell viability.

Western Blotting

Cells were lysed in SDS lysis buffer containing 1% SDS, 20 mM Tris-HCl (pH 7.4), 5 mM EDTA (pH 8.0), 10 mM NaF, 2 mM Na_3VO_4, 0.5 mM phenylarsine oxide, and 1 mM phenylmethylsulfonyl fluoride. After boiling for 5 min, lysates were centrifuged at 15,000 rpm for 60 min at 4 C, and the supernatants were collected for analysis. The protein concentration of the supernatants was quantified by BCA Protein Assay kit (Pierce, Rockford, IL), and the same amount of total protein was assayed for each Western blot. For primary antibodies, anti-NR2A (1:500; Sigma), anti-NR2B (1:500; Sigma), anti-GluR1 (1:1000; Chemicon), anti-synapsin I (1:2000; Chemicon), anti-synaptotagmin (1:3000; Transduction Laboratories, Lexington, KY), anti-SNAP25 (1:1000; Synaptic Systems, Gottingen, Germany), anti-TUJ1 (1:5000; Berkeley Antibody Co., Berkeley, CA), anti-pERK (1:1000; Cell Signaling Technology, Beverly, MA), anti-ERK (1:1000; Cell Signaling), anti-Akt (1:1000; Cell Signaling), anti-pAkt (1:1000; Cell Signaling), anti-GR (1:500; Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-MR (1:500; Santa Cruz), and anti-BDNF (1:200; Santa Cruz) antibodies were used. The changes in protein expression are indicated as a ratio that was normalized to sole BDNF application in each experiment. The n indicates the number of experiments performed with separate cultures.

Imaging of Intracellular Ca^{2+}

Ca^{2+} imaging analysis was carried out as reported previously (27). Briefly, cultured cells maintained on polyethyleneimine-coated coverglasses attached to flexiPERM were incubated for 1 h at 37 C with 10 μM Fluo-3 AM (Molecular Probes, Eugene, OR) diluted in HEPES-buffered Krebs Ringer assay buffer (KRH; 130 mM NaCl, 5 mM KCl, 1.2 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 10 mM glucose, 1% BSA, and 25 mM HEPES, pH 7.4). The dye intensity was monitored using a fluorescent microscope (Axiovert 200 controlled by Slide Book 3.0). The emitted fluorescence was guided through a ×20 objective. Image data were obtained every 2 sec. High KCl (50 mM, final concentration) solution or glutamate (1 μM, final concentration) was applied by bath application to trigger cell depolarization. Data were stored and analyzed with the Slide Book 3.0. The experiment was performed at least three times with separate cultures, and the reproducibility was confirmed. Representative data from neurons in a sister culture are shown in the figures.

Exocytosis Imaging

The efficiency of exocytosis in synaptic sites was measured with FM-43 fluorescent imaging as reported previously (58). Briefly, after washing three times with KRH buffer, FM-43 dye (2 μM in KRH) was loaded for 30 min at 37 C. After washing cells three times with KRH, the fluorescence was monitored using a fluorescent microscope. The emitted fluorescence was guided through a ×40 objective. Image data were obtained every 2 sec. The exocytosis was evoked by 50 mM (final concentration) KCl. The efficiency of exocytosis was estimated as the quenching ratio of fluorescence before and after the stimulation. The fluorescence elimination was expressed by the value of (F0 − F)/F0, where F0 is basal fluorescence 4 sec before KCl stimulation and F is fluorescence 20 sec after the stimulation.

Detection of Amino Acid Neurotransmitters

The amounts of amino acids released from cultured hippocampal neurons were measured as described previously (59). Briefly, amino acids released into KRH assay buffer were measured by HPLC (Shimadzu Co., Kyoto, Japan). Initially, KRH assay buffer was collected without stimulation (1 min); that is, the amount of glutamate in the sample was considered a basal release. Next, 50 mM (final concentration) KCl was added to cultures for 1 min, and the samples were collected as KCl-stimulated samples. The amount of released glutamate is indicated as a relative release amount *vs.* basal release in control (without DEX and BDNF). Representative data from a sister culture are shown in the figures. The n indicates the number of wells for each experimental condition on a plate. The reproducibility was confirmed.

siRNA

SiRNA transfection was performed as reported (60). We used 21-nucleotide siRNA duplexes with two 3′ overhanging nucleotides of the rat GR mRNA coding region (1801–1819, 5′-TGACCACACTCAACATGTT-3′, NM_012576). Sense (5′-UGACCACACUCAACAUGUUTT-3′) and antisense (5′-AACAUGUUGAGUGUGGUCATT-3′) strands were chemically synthesized by Nippon EGT Co., Ltd. (Toyama, Japan). The siRNA (GCGCGCUUUGUAGGAUUCG) named ScrambleII from Dharmacon Research Inc. (Lafayette, CO) was used as a control. Transfection of both siRNAs (final 100 nM) was performed using Lipofectamine 2000 reagent (Invitrogen, Tokyo, Japan). We carried out the siRNA transfer 24 h before DEX exposure. After the addition of 10 μM DEX (at DIV2) and/or BDNF (at DIV3), the DIV5 hippocampal neurons were lysed for Western blotting.

Statistical Analysis

Data shown in the present study are expressed as mean ± SD. For statistical analysis comparing two groups, Student's *t* test was used. For multiple groups, analysis using nonparametric Kruskal-Wallis test and Mann-Whitney *U* test were carried out. To analyze the large sample number ($n > 30$), ANOVA was also performed. All analyses were conducted using the Statistical Package for Social Science (SPSS) version 11.0 (SPSS Japan, Tokyo, Japan). *P* values $< 5\%$ were considered significant.

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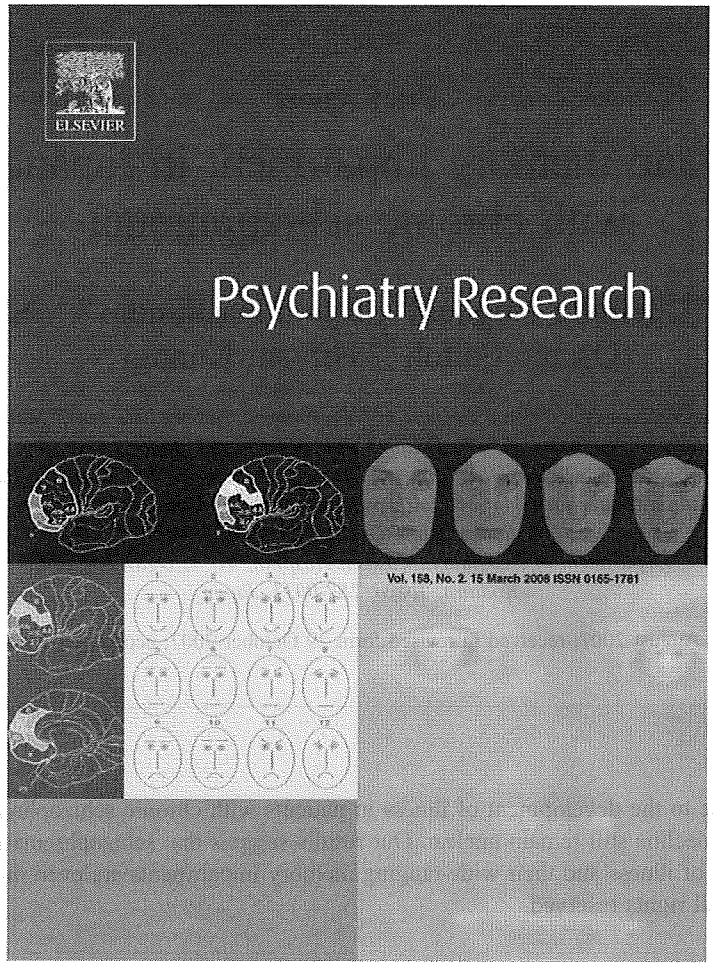

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Brief report

IQ decline and memory impairment in Japanese patients with chronic schizophrenia

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Abstract

The extent of IQ decline due to the development of illness in patients with chronic schizophrenia and the degree of memory impairment relative to such IQ decline still remain unclear. Our results suggest that schizophrenia patients experience marked IQ decline due to the development of illness and their wide-ranging memory impairments are even more severe than the IQ decline.
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Keywords: Schizophrenia; IQ; Memory

1. Introduction

Cognitive impairment is a core feature of schizophrenia, with a great impact on patients' daily lives. Those therapies that have the potential to improve cognitive deficits of patients with schizophrenia, including cognitive remediation therapy (Medalia et al., 1998; Wykes et al., 2003), as well as the favorable effects of atypical antipsychotic drugs on cognition (Bilder et al., 2002; Harvey et al., 2006; Keefe et al., 2006), have been attracting increasing attention from researchers and clinicians. From this viewpoint, the precise delineation of cognitive impairments in schizophrenia patients is essential.

Intellectual deficits in patients with chronic schizophrenia have been reliably identified (Heinrichs and Zakzanis, 1998; Dickinson et al., 2004) with some ongoing debate as to "whether it is possible to be schizophrenic yet neuropsychologically normal" (Palmer et al., 1997; Kremen et al., 2000; Wilk et al., 2005); however, the extent of IQ decline caused by the development of schizophrenia remains unclear because the premorbid IQ scores of persons who later develop schizophrenia are lower than

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those of their peers (Fuller et al., 2002; Reichenberg et al., 2005). Impairments in memory, working memory, and attention in patients with schizophrenia are well documented (Aleman et al., 1999; Silver et al., 2003; Hori et al., 2006), but the relationship of these cognitive deficits to the possible decline in IQ has not been established. Here we assessed cognitive functions including intellectual and wide-ranging memory functioning in patients with chronic schizophrenia in relation to age- and premorbid IQ-matched healthy controls.

2. Materials and methods

Eighty-two patients who met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia participated in this study. All patients were receiving antipsychotic drugs at the National Center of Neurology and Psychiatry (NCNP), Musashi Hospital and were clinically stable at the time of the neuropsychological tests. Eighty-two age- and premorbid IQ-matched healthy volunteers were recruited from hospital staff and their associates and also from the community. Healthy participants were interviewed by a research psychiatrist using the Japanese version of the Mini-International Neuropsychiatric Interview (MINI, Sheehan et al., 1998) to confirm the absence of any psychiatric illnesses. A portion of the subjects were from our previous sample (Hori et al., 2006). Written informed consent was obtained from all subjects prior to their inclusion in the study. The study was approved by the ethics committee of the NCNP.

Premorbid IQ was estimated with the Japanese Adult Reading Test (JART, Matsuoka et al., 2002; 2006), a Japanese version of the National Adult Reading Test (NART, Nelson and Wilson, 1991). This test is considered to provide an estimate of premorbid IQ in schizophrenia patients (Uetsuki et al., 2006), which is consistent with the original NART (Crawford et al., 1992; O'Carroll et al., 1992). In this test, subjects were required to read out 100 idioms of Han-Chinese characters (Japanese kanji characters). JART-estimated premorbid IQ was calculated for each subject according to previous reports (Matsuoka et al., 2002, 2006). The full version of the Wechsler Memory Scale-Revised (WMS-R, Wechsler, 1987; Sugishita, 2001) was administered to all participants. Outcome measures of the WMS-R were verbal memory, visual memory, delayed recall, auditory attention, visual attention, verbal working memory, and visual working memory. To precisely assess subjects' current intellectual function, a full version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Wechsler, 1981; Shinagawa et al., 1990) was administered, yielding age-corrected indices of verbal, performance, and full-scale IQs.

Schizophrenic symptoms were assessed by an experienced research psychiatrist in 46 of the 82 patients using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Daily doses of antipsychotics and anticholinergic antiparkinsonian drugs were converted to chlorpromazine equivalents (CPZeq) and biperiden equivalents (BPDeq), respectively, using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999; Minzenberg et al., 2004).

Results are reported as mean ± standard deviation (S.D.). Demographic characteristics and cognitive test results were compared between groups. We used *t*-test or analysis of variance (ANOVA) to compare mean scores and the χ^2 tests to compare categorical variables. Analysis of covariance (ANCOVA) was used to compare means between groups, controlling for confounding variables. Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

Male/female ratios of patients and controls were 48/34 and 25/57, respectively, indicating that the patient group had a greater representation of males ($\chi^2(1)=13.06$, $P<0.001$). The mean ages of the patients and controls were 44.3±13.8 and 44.2±14.9, respectively ($t=0.05$, $df=162$, $P=0.96$). The mean years of education of the patients and controls were 13.4±2.5 and 14.1±2.2, respectively ($t=1.88$, $df=162$, $P=0.06$). The JART-predicted premorbid IQ scores of patients and controls were 102.2±11.6 and 102.3±7.4, respectively ($t=0.46$, $df=137.8$, $P=0.96$). Of the 82 patients, 56 were outpatients and 26 were inpatients. The mean age of illness onset was 24.7±8.8. Illness duration was 19.6±13.7 years, demonstrating that our patients were in the chronic phase of schizophrenia. CPZeq and BPDeq were 781.7±710.1 and 2.2±2.0, respectively. PANSS positive, negative, and total scores were 13.9±6.7, 19.1±7.1, and 62.1±17.9, respectively.

Verbal, performance, and full-scale IQs of patients with schizophrenia and healthy controls are presented in Supplementary Table 1. ANOVA showed that these three IQ indices in patients were significantly lower than those in controls (all $P<0.001$). The VIQ/PIQ ratios of patients and controls were 1.08±0.18 and 0.95±0.11, respectively ($F=22.5$, $df=1,160$, $P<0.001$, by ANCOVA with gender as a covariate). Scores of 13 subscales of the WMS-R in patients and controls are also shown in Supplementary Table 1. Patients performed significantly

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more poorly than controls on all these cognitive domains (all $P<0.001$), except for auditory attention ($P=0.15$). Fig. 1(a) shows mean scores of the patients and controls on JART-estimated IQ, WAIS-R full-scale IQ, and the main three memory indices of the WMS-R. Dips of current IQ and all memory domains in patients are apparent, although the two groups are matched for the JART-estimated premorbid IQ.

To control for the current IQ and gender effects on these test results, ANCOVA was used with full-scale IQ and gender as covariates. It revealed that patients performed significantly more poorly than controls on verbal memory, visual memory, delayed recall, visual attention, and verbal working memory, even after controlling for full-scale IQ and gender (Supplementary Table 1). To confirm these results, additional comparisons were made between patients whose current IQ scores were within normal limit (IQ-WNL patients, defined as WAIS-R full-scale IQ ≥ equal to or greater than 85; $n=46$) and total controls ($n=82$). Fig. 1(b) summarizes the results, showing that there was no difference in current IQ between IQ-WNL patients (mean IQ: 98.85±8.55) and controls (mean IQ: 101.95±11.30), while these patients still showed significantly lower scores on all three memory indices compared with controls. On the other hand, the JART-estimated premorbid IQ of IQ-WNL patients was significantly higher than that of controls.

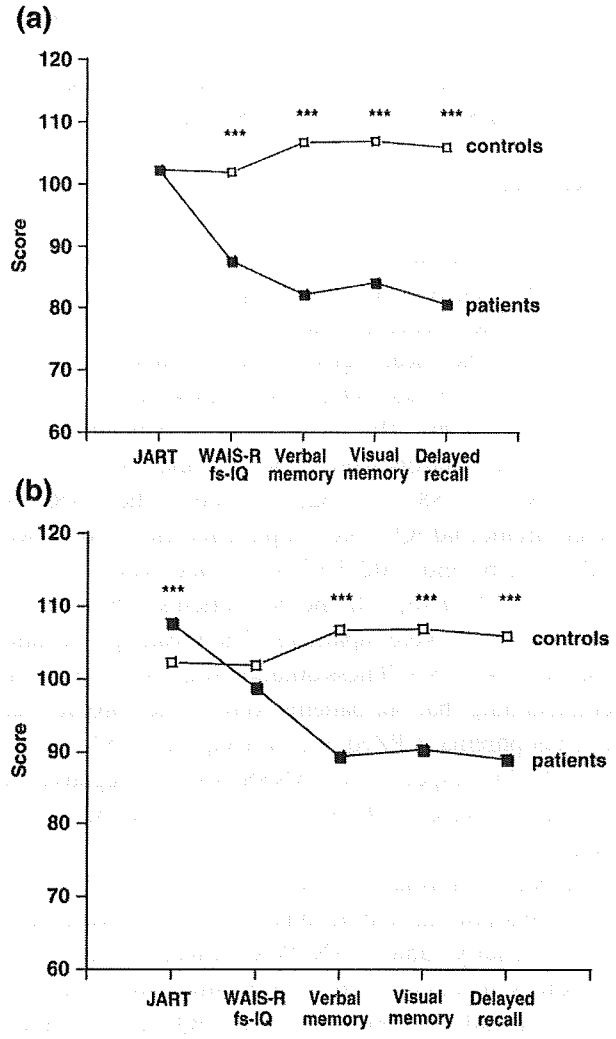

Fig. 1. Mean scores of patients and controls on JART IQ, WAIS-R full-scale IQ, Verbal memory, Visual memory, and Delayed recall indices (WMS-R). (a) total patients ($n=82$) vs. total controls ($n=82$) and (b) IQ-WNL patients (defined as WAIS-R full-scale IQ ≥ 85, $n=46$) vs. total controls ($n=82$).***$P<0.001$.

4. Discussion

In the present study we examined intellectual and memory functions in patients with chronic schizophrenia relative to age- and premorbid IQ-matched healthy controls. Our results confirmed that patients with chronic schizophrenia have wide-ranging cognitive impairments, consistent with the literature on schizophrenia.

The relationship of the development of schizophrenia to declining IQ scores has been confounded by findings that premorbid intelligence itself is likely to be lower in persons who later develop schizophrenia than in their peers (Fuller et al., 2002; Reichenberg et al., 2005). To address this issue, we employed a premorbid IQ-matched case-control sample. Although the cross-sectional nature of the present study does not allow any definite conclusions to be drawn concerning the time when the IQ decline actually occurred (i.e., during the prodromal stage, immediately after illness onset, or during the chronic course of illness), the observed differences in current IQs between patients and controls provide evidence for marked IQ decline due to the development of schizophrenia. Means of estimated premorbid IQ and current full-scale IQ in patients were 102.20 and 87.68, respectively, suggesting an approximate 1 S.D. decline in IQ score related to the development of illness. On the other hand, the subgroup of patients whose current IQ was within normal limits (and thus similar to that of controls) showed significantly higher premorbid IQ as estimated by the JART than controls (Fig. 1(b)), which favors the view that even neuropsychologically normal patients with chronic schizophrenia have compromised cognitive functioning relative to their presumed premorbid level of intellectual function (Kremen et al., 2000). Furthermore, in the present study performance IQ of the patients was more severely impaired than verbal IQ, congruent with prior reports (Heinrichs and Zakzanis, 1998).

Pervasive memory impairment in patients with schizophrenia relative to premorbid IQ-matched controls was

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found, and most deficits remained significant even after current IQ was controlled for, supporting that memory impairment is a core feature of schizophrenia (Saykin et al., 1991; Heinrichs and Zakzanis, 1998; Aleman et al., 1999). The marked impairment in verbal memory is consistent with numerous studies (e.g., Saykin et al., 1991; Heinrichs and Zakzanis, 1998). Although visual memory deficits in schizophrenia have attracted less attention from researchers than verbal memory, several studies have reported substantial impairment of visual memory (Saykin et al., 1991; Aleman et al., 1999), consistent with the present study. The pronounced impairment in delayed recall observed here is also in line with prior reports (Aleman et al., 1999; Dickinson et al., 2004). Deficits of verbal and spatial working memory in schizophrenia tapped by the Wechsler digit span backward and spatial span backward subtests, respectively, are fairly consistent findings (Conklin et al., 2000; Silver et al., 2003; Dickinson et al., 2004), which were replicated in the current study. Previous studies have reported that the performance on the forward digit span task of schizophrenia patients is significantly poorer than that of healthy people, indicating impaired attentional function in schizophrenia (Conklin et al., 2000; Silver et al., 2003). The findings of the present study, by contrast, suggest that auditory attention as measured by the forward digit span subtest is preserved in schizophrenia. The discrepant findings regarding auditory attention in the present study relative to previous ones might be due in part to the distinct matching status between patients and controls regarding education and premorbid IQ.

In conclusion, our results suggest that patients with chronic schizophrenia have substantially lower intellectual function relative to their presumed premorbid level and that their memory impairment is even more severe than the IQ decline. To definitively delineate the lifetime course of cognitive decline in schizophrenia, longitudinal studies that range from childhood to the chronic phase are needed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2007.11.002.

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Progressive changes of white matter integrity in schizophrenia revealed by diffusion tensor imaging

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Abstract

Recent magnetic resonance imaging (MRI) studies using diffusion tensor imaging (DTI) have suggested reduced fractional anisotropy (FA) in the white matter (WM) of the brain in patients with schizophrenia. We tried to examine whether such reduction in FA exists and whether such changes in FA progress in an age-dependent manner in a Japanese sample of chronic schizophrenia. FA values were compared between 42 patients with chronic schizophrenia and 42 controls matched for age and gender, by using DTI with voxel-by-voxel and region-of-interest analyses. Correlations of FA values with age and duration of illness were examined. Patients with schizophrenia showed lower FA values, compared to controls, in the widespread WM areas including the uncinate fasciculi and cingulum bundles. A significant group-by-age interaction was found for FA in the WM, i.e., age-related reduction of FA was more pronounced in schizophrenics than in controls. A significant negative correlation between FA and duration of illness was also found in the WM. Our data confirmed decreased FA in schizophrenics, compared to controls in the widespread WM areas. Such decreased FA values in schizophrenia might be attributable, at least in part, to progressive changes after the onset of the illness.
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Keywords: Magnetic resonance imaging (MRI); DTI; Fractional anisotropy (FA); Aging

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1. Introduction

Diffusion tensor imaging (DTI) (Basser et al., 1994), a newly developed method to estimate the white matter (WM) integrity, provides information about the diffusion of water in biological tissues. In the WM, water diffusion is highly anisotropic, with greater diffusion in the direction parallel to axonal tracts. Thus, diminished anisotropy of water diffusion has been proposed to reflect compromised WM integrity (Lim et al., 1999). Fractional anisotropy (FA) (Basser, 1995) is a quantitative measure of diffusion anisotropy acquired from DTI.

The normally aging brain exhibits an assortment of micro- and macroscopic changes in the WM as well as the cerebral cortex. Histological studies demonstrate a decrease in myelin density and in the number of myelinated fibers (Meier-Ruge et al., 1992). Postmortem brain (Meier-Ruge et al., 1992) and volumetric neuroimaging studies (Christiansen et al., 1994; Salat et al., 1999) have suggested that WM changes are more prominent than cortical changes with aging, at least during certain segments of the age span and in certain regions of the brain. For example, volume loss in prefrontal WM is disproportionately greater than that in prefrontal cortex with late aging {comparison of elderly adults aged 60–75 with those aged >85 years (Salat et al., 1999)}. Several DTI studies have demonstrated age-related reductions of WM anisotropy in the genu of the corpus callosum (Pfefferbaum et al., 2000b), anterior WM (Pfefferbaum et al., 2000a; O'Sullivan et al., 2001), periventricular WM (Nusbaum et al., 2001), and the prefrontal WM (Nusbaum et al., 2001; Pfefferbaum et al., 2005; Salat et al., 2005).

Regarding schizophrenia, impairments of the neural connectivity between certain cortical areas, such as frontal and temporal areas, have been implicated in the pathophysiology of the disease (Frith and Dolan, 1996; Andreasen et al., 1997; Bullmore et al., 1997). Indeed, volumetric magnetic resonance (MR) studies and pathological studies demonstrated abnormalities of the WM in schizophrenia (Miyakawa et al., 1972; Cannon et al., 1998; Davis et al., 2003; Ho et al., 2003; Uranova et al., 2004). Changes in WM integrity in schizophrenia has relevance to the neural disconnection model of the disorder and may provide a basis for focal abnormalities as well. Several previous DTI studies in chronic schizophrenia showed decrease of FA in schizophrenics mainly in the front-temporal white matter and corpus callosum (Buchsbaum et al., 1998; Lim et al., 1999; Agartz et al., 2001; Burns et al., 2003). Furthermore, FA decrease in patients with first episode schizophrenia might be less pronounced compared to chronic patients (Price et al., 2005; Szeszko et al., 2005), suggesting that the decreased FA in schizophrenics might be attributed, at least in part, to progressive and exaggerated age-dependent changes in schizophrenics rather than neurodevelopmental abnormalities in the WM. To date, there is only one cross-sectional study with a small sample size investigating age-related FA changes in schizophrenia that demonstrated an age-related FA increase in schizophrenics (Jones et al., 2006).

The present study was aimed to examine whether patients with chronic schizophrenia do have reduced FA values compared to controls and whether such changes in FA progress in an age-dependent manner.

2. Methods

2.1. Subjects

Table 1 shows the characteristics of participants of this study. Forty-two patients with chronic schizophrenia were recruited at the National Center of Neurology and Psychiatry, Tokyo, Japan. Consensus diagnosis was made for each patient by at least two trained psychiatrists according to the DSM-IV criteria (American Psychiatric Association, 1994), based on all available information, including clinical interviews, medical records and other research assessments. All patients were stable and/or partially remitted and had been taking antipsychotic medication at the time of MR measurement and neuropsychological tests. Forty-two healthy volunteers who had no current or past contact to any psychiatric services served as controls. All the subjects were biologically unrelated Japanese. After description of the study, written informed consent was obtained from every subject. The study protocol was approved by the ethics committee of the National Center of Neurology and Psychiatry, Tokyo, Japan. Exclusion criteria for all the participants included asymptomatic or symptomatic cerebral infarctions detected by T2 weighted MRI, serious neurological or endocrine disorder, any medical condition that could potentially affect the central nervous system, or mental retardation according to DSM-IV criteria.

2.2. Image acquisition

MR studies were performed on a 1.5 tesla Magnetom Vision Plus system (Siemens, Erlangen, Germany). Axial DTI scans aligned to the plane containing anterior and posterior commissures were acquired with a pulsed-

Table 1
Characteristics of participants

	Controls	Schizophrenics	Two sample *t*-test	(Two-tailed; df=82)
			t	*P*
Number of subjects	42	42		
Gender (male/female)	26/16	26/16		
Handedness (right/left)	41/1	41/1		
Age (years)	39.2 (9.0)	40.0 (9.3)	−0.42	0.68
Range of age (years)	22–59	22–59		
Education (years)	17.1 (3.5)	13.0 (2.9)	8.1	<0.001
Full-scale IQ (WAIS-R)	114.3 (11.6)	86.0 (21.3)	6.0	<0.001
Age of onset		23.3 (7.0)		
Duration of illness (years)		16.8 (9.0)		
Duration of hospitalization (months)		31.2 (61.3)		
Dose of total antipsychotic drugs (mg/day, chlorpromazine equivalent)		1005.1 (735.3)		
Dose of typical antipsychotic drugs (mg/day, chlorpromazine equivalent)		694.8 (748.3)		
Dose of atypical antipsychotic drugs (mg/day, chlorpromazine equivalent)		310.3 (464.2)		

Mean (S.D.).
WAIS-R: Wechsler Adult Intelligence Scale-Revised.

gradient, spin-echo, single-shot echo planar imaging (EPI) sequence (TR/TE=4000/100 ms; acquisition matrix, 256×256; NEX=4, FOV 240 mm; b=1000 s/mm^2; 20 slices, slice thickness 5 mm, gap 1.5 mm). Diffusion was measured along six non-collinear directions. For each of six gradient directions, four acquisitions were averaged. Four acquisitions without diffusion weighting (b=0) were also averaged. Additionally, a three dimensional volumetric acquisition of a T1-weighted gradient echo sequence with a gapless series of thin sagittal sections using an MPRage sequence (TR/TE=11.4/4.4 ms; flip angle, 15 degree; acquisition matrix, 256×256; NEX=1, FOV 315 mm; slice thickness 1.23 mm) was acquired for evaluating the volume of grey matter (GM), WM and cerebrospinal fluid (CSF) space.

2.3. Image processing

FA images for each subject were computed from seven diffusion images acquired as above by an in-house script on Matlab 6.5 software (Mathworks, Inc., MA, USA). Then, the FA images were spatially-normalized using high-dimensional-warping algorithm (Ashburner et al., 1999) and were matched to the FA template image. To make the FA template image, we warped FA images of 4 normal subjects (other than 42 control subjects) to the single-subject T1 template (skull stripped image) using spatial normalization function of SPM2 and averaged the 4 warped FA images. The transformed FA images were smoothed with a Gaussian kernel. The filter size (full-width at half-maximum: FWHM) was varied from zero to 16 mm in steps of 2 mm to validate the consistency of results of SPM analyses, because a previous study (Jones et al., 2005) reported that the statistical results of SPM analyses were differed depending on filter size. For measuring the volume of GM, WM and CSF space, an additional function of an optimized VBM script (http://dbm.neuro.uni-jena.de/vbm) was used (Good et al., 2001).

2.4. Statistical analysis

2.4.1. Voxel-by-voxel analysis

The resultant FA images were analyzed using statistical parametric mapping with the framework of the General Linear Model in SPM2 (Wellcome Department of Cognitive Neurology, London, UK) (Friston et al., 1995). We constituted following three

Table 2
The relationship between smoothing kernel sizes (FWHM) and number of resels in our sample

FWHM (mm)	Number of resels
None	12460.4
2×2×2	5131.1
4×4×4	1720.2
6×6×6	706.0
8×8×8	289.4
10×10×10	119.7
12×12×12	52.1
14×14×14	24.4
16×16×16	12.4

statistical analyses: 1) a two-sample *t*-test for estimating group differences (controls versus schizophrenics), 2) a correlational analysis between age and FA values in both controls and the schizophrenics and 3) a correlational analysis of FA values with duration of illness, age of onset, duration of hospitalization, and daily dose of

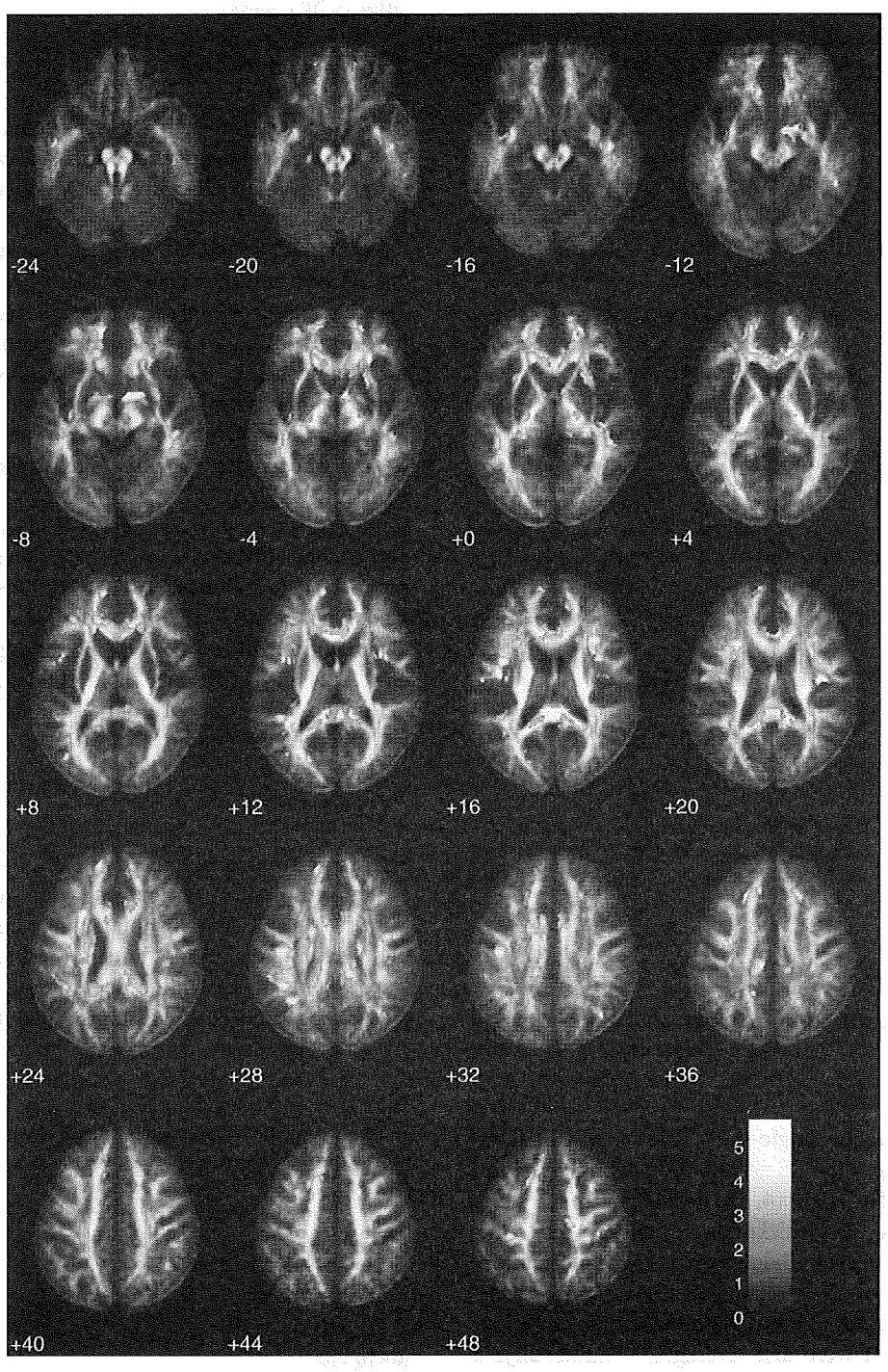

Fig. 1. Comparison in FA values between patients with schizophrenia and controls. The SPM {t} is displayed onto axial FA template images. The WM areas in which significantly lower FA values in patients compared with controls were observed, including the bilateral frontal and temporal WM, uncinate fasciculi, cingulum bundles, and genu and splenium of the corpus callosum ($P<0.001$, uncorrected).

antipsychotic drugs in the schizophrenics. In all the three analyses, relative WM volume (WM volume divided by the summation of GM, WM and CSF volumes) and WAIS-R (Wechsler Adult Intelligence Scale-Revised) full-scale IQ score were treated as nuisance variables. The former was included for eliminating the possible effect of WM volume change associated with aging on the FA values through partial voluming from non-WM voxels. The latter was included to allow for the effects of IQ, because there was some evidence which suggested DTI measures were correlated with cognitive decline in elderly (O'Sullivan et al., 2004). We additionally conducted the analyses without these two nuisance variables to check whether there were any differences in the results with or without nuisance variables in the statistical models. To estimate population effects (diagnostic effects), we used a single-subject, condition (controls or schizophrenics) and covariate (no covariate of interest) model for the SPM analysis. In the second analysis, we applied the single subject condition (controls or schizophrenics) and covariate (interaction with condition, covariate of interest; age) model. A single-subject, covariate only model was applied in the third analysis. For these three analyses, we set masking threshold for FA values of 0.2 for excluding voxels containing partial volume of WM and other tissues. Since the previous study demonstrating a positive correlation between FA values and age in schizophrenics reported mean FA values of around 0.4 (Jones et al., 2006), we additionally set masking threshold for FA values of 0.35 for examining correlation between age and FA values of more anisotropic WM structure in the second analysis. For the evaluation of the statistical models, we used Wake Forest University Pickatlas (Maldjian et al., 2003) to pick up cerebral WM in the Montreal Neurological Institute (MNI) space. We used uncorrected $P<0.001$ as a statistical threshold to search significant differences. As demonstrated in Table 2, the number of resels differed profoundly depending on smoothing kernel sizes (FWHM) and the statistical results with correction for multiple comparisons could change dramatically relying on number of resels. On the other hand, SPM results without correction for multiple comparisons were essentially unchanged regardless of smoothing kernel size (data not shown). Therefore, we did not perform correction for multiple comparisons. The resultant set of t values constituted statistical parametric map (SPM {t}). We employed the filter size of 6 mm for presentation of the results considering for the original voxel dimensions of acquired data {0.94 mm × 0.94 mm × (5.00+1.50) mm}.

2.4.2. ROI analysis

To ensure the robustness of the results of the voxel-by-voxel analyses, we additionally performed ROI analyses. We used MarsBar (http://marsbar.sourceforge.net/) for extracting region of interest (ROI) containing all the voxels classified as WM with Wake Forest University Pickatlas from spatially normalized and smoothed FA images and calculated mean FA values of the ROI. Then, we performed correlational analyses of mean FA values with the same variables in voxel-by-voxel analysis using Statistical Package for Social Science (SPSS), 1) in both controls and schizophrenics, 2) in controls and 3) in schizophrenics. We constituted a General Linear Model for the first analysis and entered diagnosis-by-age interaction effects into the statistical model to examine if there were any diagnosis-by-age interaction effects. For the second and third analyses, Pearson's correlation coefficients between mean WM FA values and covariates were calculated.

3. Results

3.1. Voxel-by-voxel analyses

3.1.1. Comparison between schizophrenics and controls

Schizophrenics demonstrated significantly lower FA values in widespread WM areas, compared with controls. These WM areas included bilateral frontal and temporal lobes, uncinate fasciculi (external capsules), cingulum bundles, and genu and splenium of corpus

Table 3
The summary of the WM areas in which significantly lower FA values in patients compared with controls were observed

Anatomical regions	t-value	MNI coordinates		
	(Voxel level)	x	y	z
Rt frontal lobe white matter	4.34	22.5	52.5	−4.5
Lt frontal lobe white matter	5.43	−13.5	49.5	−6
Rt temporal lobe white matter	4.25	48	−33	−7.5
Lt temporal lobe white matter	4.19	−45	−31.5	−10.5
Rt uncinate fasciculus (external capsule)	4.00	33	12	−1.5
Lt uncinate fasciculus (external capsule)	3.84	−33	12	−1.5
Rt cingulate bundle	4.23	6	6	33
Lt cingulate bundle	4.32	−7.5	6	30
genu of corpus callosum	3.79	6	24	10.5
splenium of corpus callosum	4.18	−3	−33	19.5

callosum (Fig. 1, Table 3). There would be increased possibility of alpha errors because we did not perform correction for multiple comparisons. However, our results were in well concordance with the results of the previous studies (Buchsbaum et al., 1998; Lim et al., 1999; Agartz et al., 2001; Burns et al., 2003; Kubicki

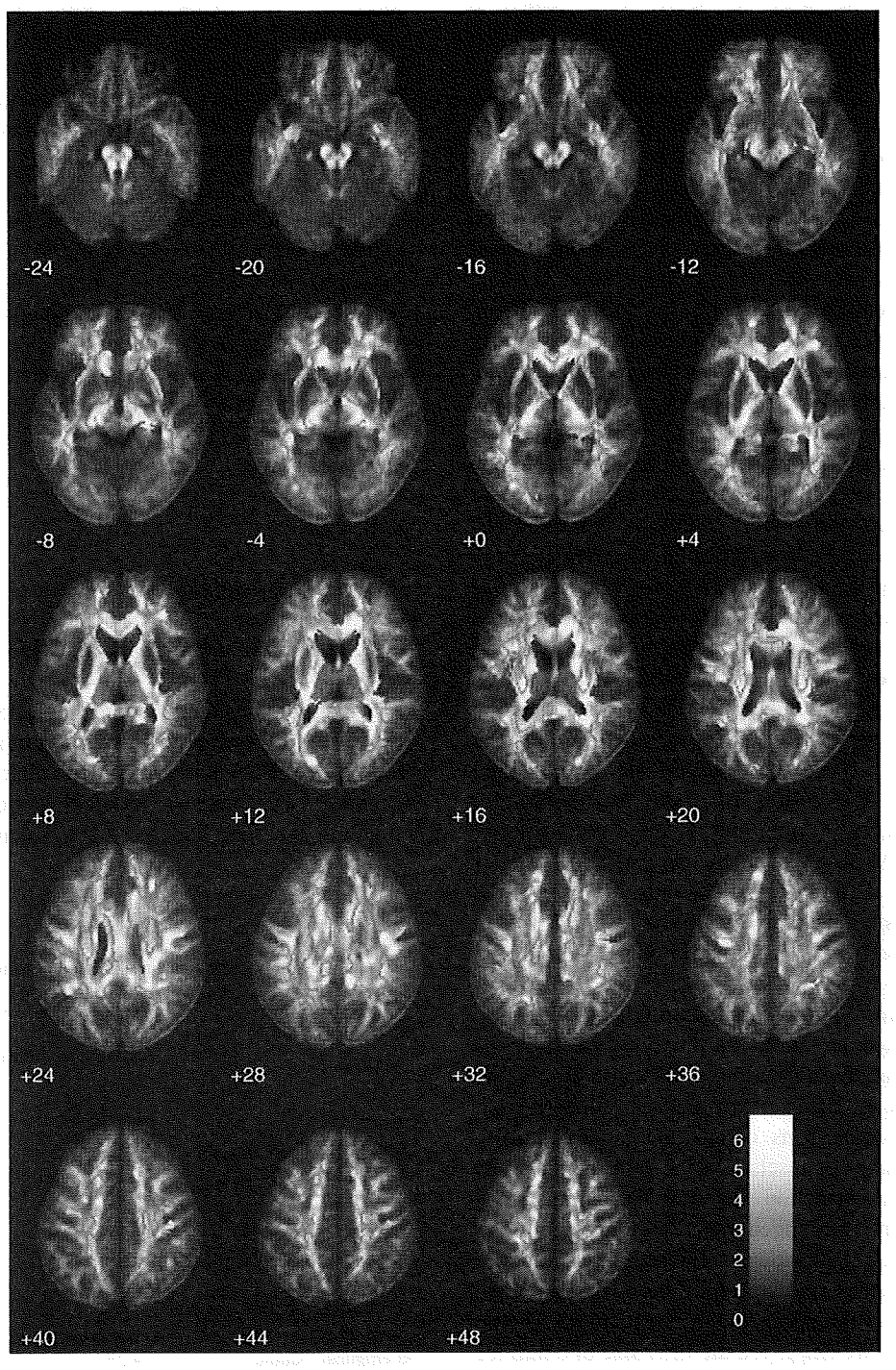

Fig. 2. Correlational analysis between FA values and age with 0.2 as a masking threshold in schizophrenics. The SPM {t} is displayed onto axial FA template images. The widespread WM areas showed a significant negative correlation between FA values and age in schizophrenics ($P<0.001$, uncorrected).

et al., 2003). Therefore, we might be able to regard the results of these previous studies as a priori hypotheses. There were no areas of significantly higher FA values in patients compared with controls even at a lenient threshold ($P<0.05$, uncorrected). In these results of the analysis without nuisance variables in the statistical

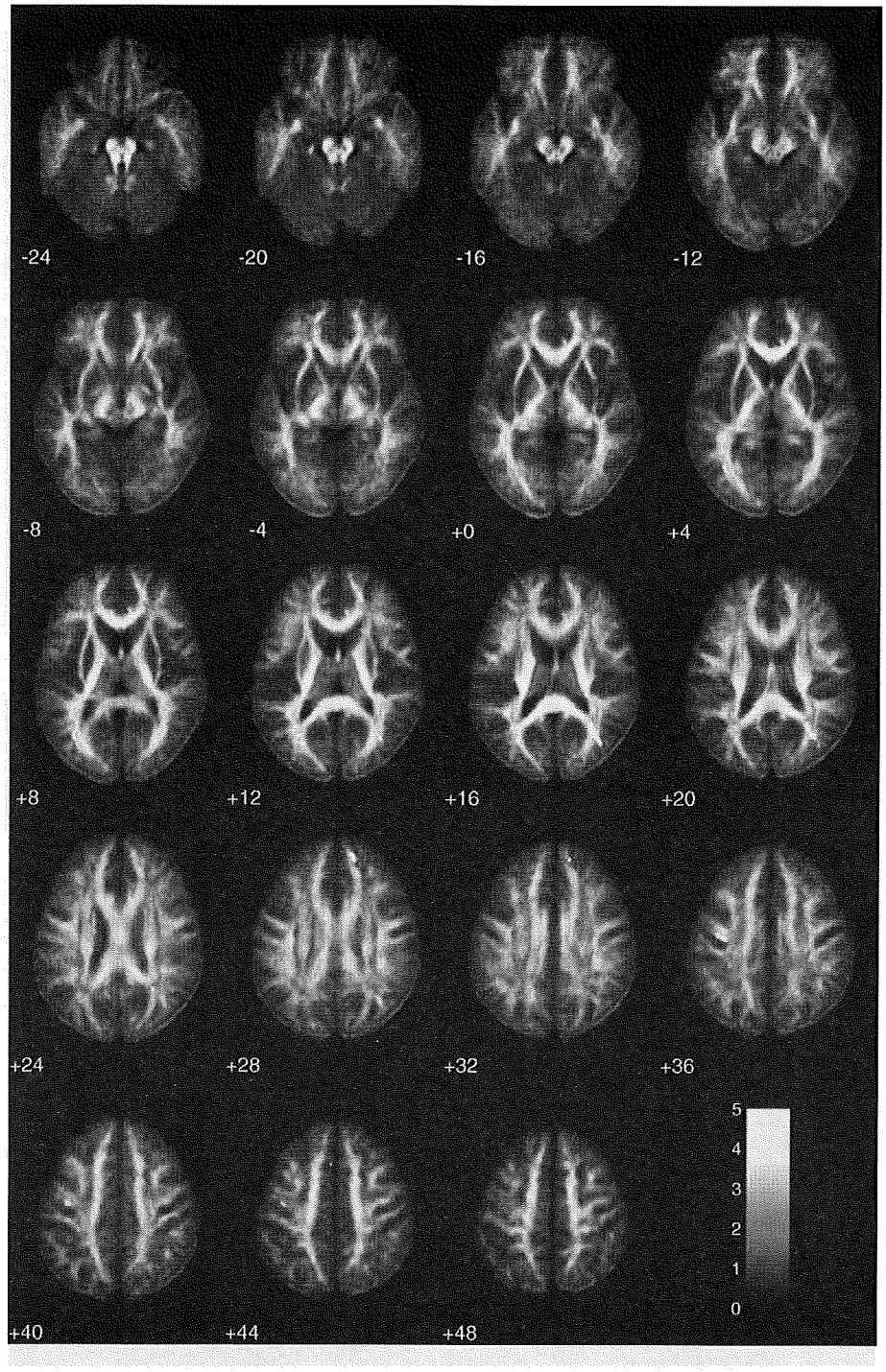

Fig. 3. Correlational analysis between FA values and age with 0.2 as a masking threshold in controls. The SPM {t} is displayed onto axial FA template images. The WM areas showed a significant negative correlation between FA values and age in controls ($P<0.001$, uncorrected), including right prefrontal {(15.0, 49.5, 30.0) in MNI coordinates, $t=5.03$}, left frontal {(−37.5, −15.0, 34.5), $t=4.51$} and bilateral temporo-occipital WM {(31.5, −60.0, 16.5), $t=4.75$; (−30.0, −60.0, 15.0), $t=4.47$}.

models, the distributions of the statistically significant areas were essentially unchanged compared to the results with nuisance variables although the spatial extents of the statistically significant areas were slightly larger (data not shown), which was the case with the results of other two analyses.

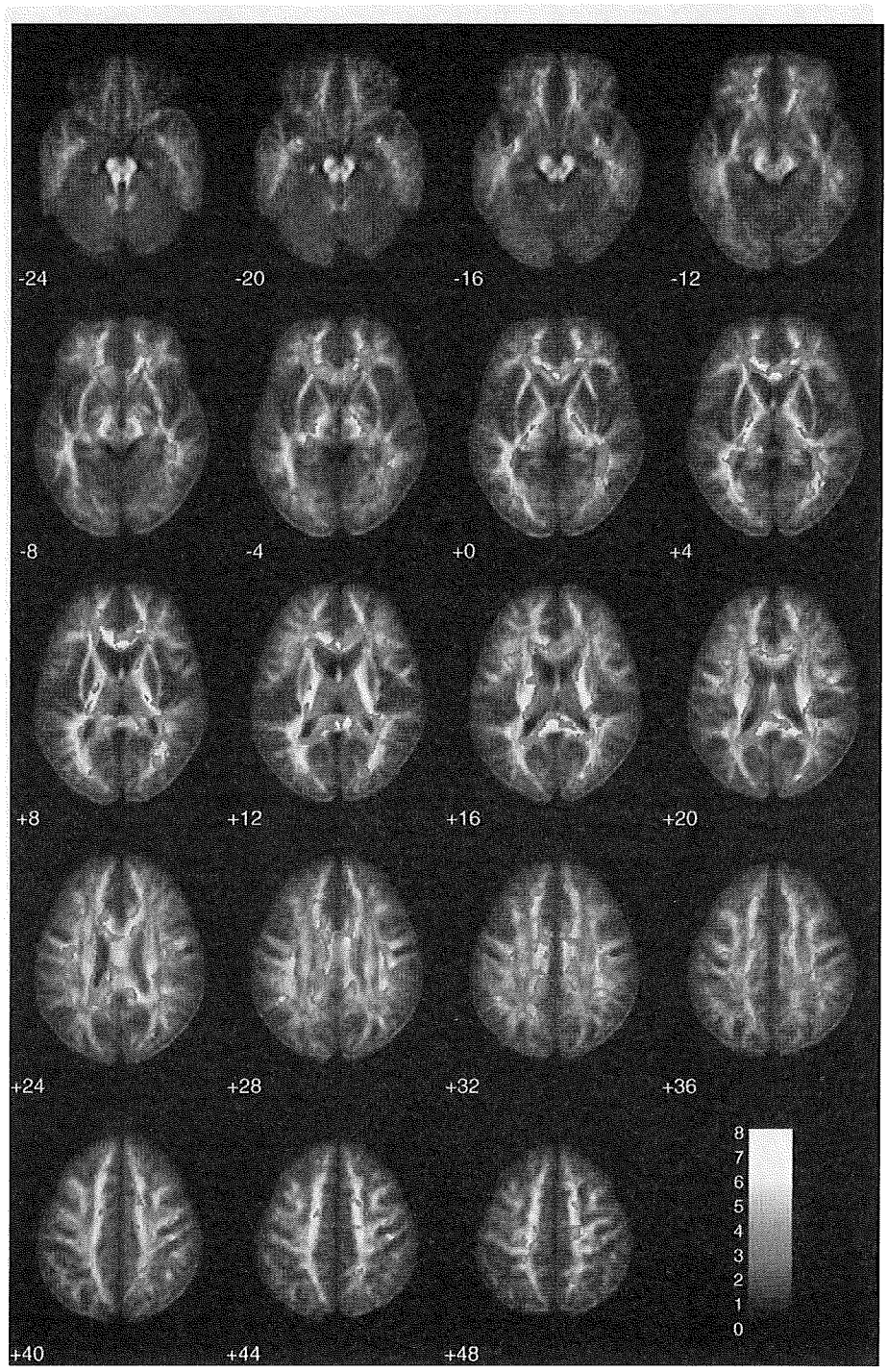

Fig. 4. Correlational analysis between FA values and duration of illness with 0.2 as a masking threshold in schizophrenics. The SPM {t} is displayed onto axial FA template images. The widespread WM areas showed a significant negative correlation between FA values and duration of illness in schizophrenics ($P<0.001$, uncorrected).

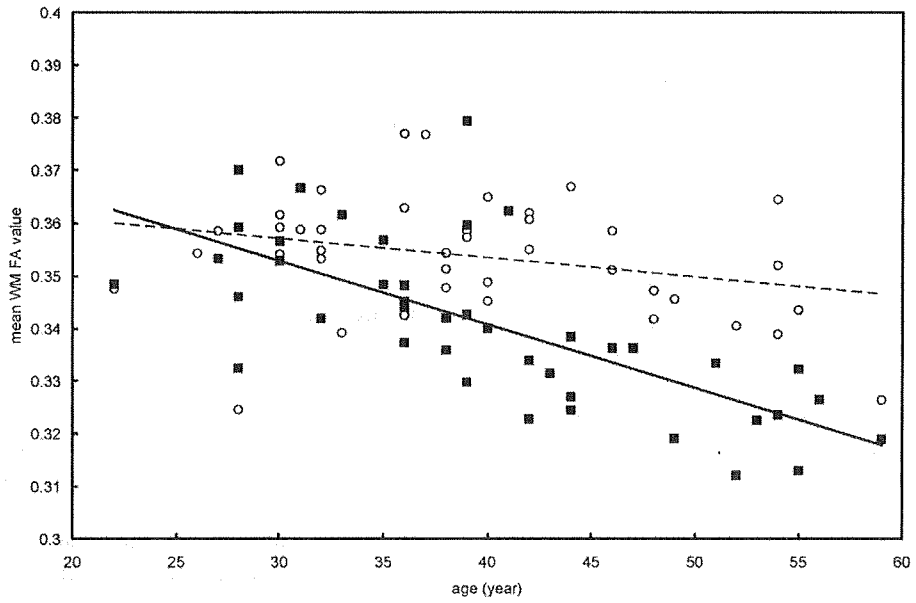

Fig. 5. A scatter plot between age and mean WM FA value when masking threshold for FA values was set to 0.2. Filled squares represent schizophrenics and open circles represent controls. The solid line indicates a regression line for schizophrenics ($y=-0.0012\times+0.3888$, $R^2=0.49$, test for regression slope: df=40; $t=-6.24$; $P<0.0001$). The dashed line indicates a regression line for controls ($y=-0.0004\times+0.3679$, $R^2=0.083$, test f or regression slope: df=40; $t=-1.90$; $P=0.065$). A significant diagnosis-by-age interaction effect (general linear model: $P=0.009$) was noted.

3.1.2. *Correlational analysis in schizophrenic and control groups*

As the results of the second analysis considering aging effects, a significant negative correlation with age was observed in the FA values of widespread, almost diffuse WM areas in the schizophrenic group (Fig. 2), while in the control group, only FA values in right prefrontal {(15.0, 49.5, 30.0) in MNI coordinates, $t=5.03$}, left frontal {(−37.5, −15.0, 34.5), $t=4.51$} and bilateral temporo-occipital WM {(31.5, −60.0, 16.5), $t=4.75$;

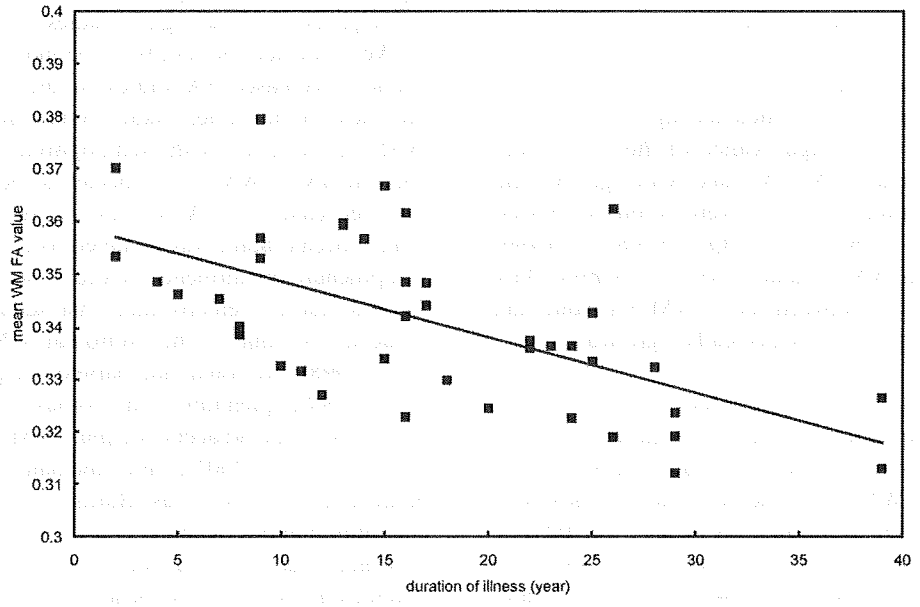

Fig. 6. A scatter plot between duration of illness and mean WM FA value when masking threshold for FA values was set to 0.2. Filled squares represent schizophrenics. The solid line indicates a regression line for schizophrenics ($y=-0.0011\times+0.3590$, $R^2=0.36$, test for regression slope: df=40; $t=-4.78$; $P<0.0001$).