symptoms correlate with psychosocial factors, and (6) to clarify the factors related to PTSD evaluations in female juvenile offenders who have never been under psychiatric medication in Japan.

Methods

Subjects

The subjects were 64 female juvenile offenders consecutively recruited from a female juvenile detention center in Japan as follows: from October 2004 to June 2006, 181 delinquent adolescents were incarcerated in a detention centre. Among these offenders, we excluded those who had already received neuroleptics (i.e., major tranquilisers, antidepressants, lithium, methylphenidate, and anticonvulsants) or those who were in a severe physical or psychiatric condition. That design was intended to avoid bias caused by medications which induce reduction of symptoms, when a structured interview was conducted for determining natural prevalence, to obtain reliable informed consent, and to consider the physical situations under a burden of this investigation. Seventeen cases (9%) were excluded on the basis of medication, and the total number of final candidates who received randomisation was 164. No subjects were excluded because of severe physical or psychiatric illness, and only subjects with a psychiatric history were included in the study. Finally, 64 subjects completed the initial screening interview and reporting questionnaires; however, two subjects refused to participate in the succeeding comprehensive interview. The subjects' ages ranged from 16 to 19 years (mean = 17.2, S.D. = 1.0) and the ethnicity of all the subjects was Japanese. Before incarceration, approximately half (55%) of the offenders were not living with their immediate family. Sixty-one percent of the offenders had dropped out of school before grade 10 (16 years old), and 33% had not been admitted to high school (15–18 years old). The other offenders are currently enrolled in high school.

Regarding their offence profile, 41% of the offenders were detained for drug-related crimes, 30% for violent crimes (e.g., assault, robbery), and 22% for pre-delinquent behaviour (e.g., prostitution or 'sugar daddy business'). Approximately 10% of the female delinquents were multiple offenders, and 60% had been arrested at least twice.

Procedures

This investigation was conducted as part of the regular medical service for maintaining the mental health of offenders in reformatory schools. Written informed consent was obtained from all the subjects, and the institutional head and chief director of the correction centre (Haruna Joshi Gakuen, covered by the Tokyo Regional Office of Correction Bureau, Ministry of Justice, Japan) approved the study. The subjects were individually approached by the first author (M.A.), who explained the nature of the study and provided an information sheet and a consent form. The interviewers (M.A., T.U. and Y.I.) emphasised that the procedure was voluntary and that the subjects could withdraw at any time. All the subjects were interviewed within approximately one month of their detention. During assessment, each interviewer was unaware of the subjects' offence and socio-demographic information. Within one week of their interview, the participants were asked to complete five self-rating questionnaires.

Measures

General. The interviewers assessed the background characteristics corresponding to the subjects' demographics, history of use of any illegal drugs, and trauma exposure of the subjects. Information on age, criminal history, recidivism history, family composition, living conditions, history of psychiatric visits and admission to a psychiatric hospital, family alcohol or drug problems, educational attainment and intelligence quotient (IQ; already measured in a juvenile classification home) was recorded.

As regards their history of illegal drug use, the subjects were asked whether they had used any of the following illegal drugs: stimulants, cocaine, anaesthetics, hallucinogens, inhalants, marijuana or psychotropic drugs. Information on the start and frequency of drug use, and the dose of the drug use was also obtained.

The traumatic event checklist of the Clinician-Administered PTSD Scale for DSM-IV (CAPS; Blake et al., 1995) was used to obtain the subjects' trauma history. The subjects were asked whether they had experienced any of the 12 possible traumatic events on the list and whether they had experienced any trauma in addition to those on the list. Information on the onset, frequency and duration of each trauma was also obtained.

Structured interviews. Consequently, psychiatric diagnosis was determined using the Japanese version of the Mini-International Neuropsychiatric Interview for Children and Adolescents (MINI-kid). We measured CAPS score only for the subjects who fulfilled the criteria of PTSD, as determined using the MINI-kid.

The Mini-International Neuropsychiatric Interview (MINI) was developed by Sheehan et al. (1998); it is organised into diagnostic modules. On the other hand, the MINI-kid was developed for children and adolescents; it is used in screening 23 axis-I DSM-IV disorders. For most modules of MINI, two to four screening questions are used to rule out the diagnosis when answered negatively. Positive responses to screening questions are examined by further investigation of other diagnostic criteria. We obtained permission to use the official Japanese version from Dr Otsubo (Showa University, Japan), the original translator of the MINI-kid.

CAPS is a structured clinical interview designed for assessing adults for the 17 symptoms of PTSD outlined in DSM-IV along with five associated features (i.e., guilt, dissociation, derealisation, depersonalisation, and reduction in awareness of surroundings). CAPS provides a means of evaluating self-reports of exposure to potential criterion-A events, current and/or lifetime DSM-IV diagnosis of PTSD, the frequency and intensity of each symptom, the impacts of the 17 PTSD symptoms on social and occupational functions, and the

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

overall severity of PTSD. CAPS consists of standardised prompt questions, supplementary follow-up (probe) questions, and behaviourally anchored five-point rating scales corresponding to the frequency and intensity of each symptom assessed. The Japanese version is currently used widely, and we administered it with permission from Dr Asukai.

Before the investigation, raters were trained using the standard manual of the MINI-kid (Otsubo et al., 2005). The CAPS interview took about 2 hours, and the raters were also trained using a videotape of the Japanese version of CAPS (Asukai, Hirohata, Kato, & Konishi, 2003).

Self-rating questionnaires. Five questionnaires were used in the study, which included the Japanese version of the DSM Scale for Depression (DSD), the Japanese version of the Barratt Impulsiveness Scale 11th version (BIS-11), Eating Attitudes Test-26 (EAT-26), the Parental Bonding Instrument (PBI) and the Impact of Event Scale-Revised (IES-R).

DSD (Roberts, Roberts, & Chen, 1995) is used in dimensionally evaluating depressive symptoms and diagnose major depressive episode according to the DSM criteria. The questionnaire for this scale is based on the *Diagnostic Statistical Manual for Mental Disorders*, 4th edition, with 27 items for identifying depression symptoms such as 'feel very sad'. EAT-26 (Garner, Olmsted, Bohr, & Garfinkel, 1982) is used in assessing a broad range of symptoms and provides a total score for disturbed eating attitudes and behaviours. It contains three factors as follows: dieting, bulimia and food preoccupation, and oral control. BIS-11 (Patton, Stanford, & Barratt, 1995) is a short questionnaire designed for measuring impulsiveness and has three factors, namely, motor impulsivity, no planning, and inappropriate attention. It has 30 items and impulsiveness level is calculated by summing the scores for each item. PBI (Parker, Tupling, & Brown, 1979) has been widely used in evaluating the parental situations of subjects all over the world. It was developed to assess paternal and maternal parenting attitudes recognised by offenders. It provides two dimensional scores, namely, care and overprotection. IES-R (Weiss & Marmar, 1997) was used to assess only the participants who had experienced traumatic events, and these offenders were asked about their most stressful event. The IES-R has 22 items, seven of which have been added to the original 15 items of IES. These assess hyperarousal symptoms such as anger and irritability, heightened startle response, difficulty in concentrating and hypervigilance, and the intrusion scale assesses a dissociative-like reexperience and true flashbacks. Eight items are used in assessing avoidance according to DSM-IV. Respondents are asked to rate each item according to the past seven days. The reliability and validity of each Japanese version has already been confirmed (Doi, Roberts, Takeuchi, & Suzuki, 2001; Ujiie & Kono, 1994; Someya et al., 2001; Kitamura & Suzuki, 1993; Asukai et al., 2002).

Statistical analysis

We used descriptive statistics, that is, the χ^2 test and analysis of variance (ANOVA), to investigate the associations of the respective evaluable factors with PTSD diagnosis or exposures only to a traumatic event; logistic regression analysis to estimate associations and risks for the prediction of a PTSD diagnosis (PTSD score, 1 point) among the subjects who had trauma exposure using all factors as independent variables, and multiple linear regression analysis to determine correlated factors with the IES-R scores using dimensional scores as independent variables; non-paired *t*-test (two-tailed) to characterise CAPS ratings in detail in female offenders with PTSD; and Bonferroni's correction to avoid α error with multiple comparisons. A probability level of .05 or less was considered statistically significant. We used the Japanese version of SPSS for statistical analysis (SPSS Japan, Inc.).

Results

Trauma exposure and PTSD prevalence

Figure 1 shows the statistics of trauma exposures in the juvenile female offenders; 76.5% of the participants experienced a traumatic event. Most of the participants were exposed to multiple types of traumas, with sexual abuse being the most frequently reported trauma (54.7%). Being a victim of violence (45.3%), being confronted with traumatic news and childhood maltreatment, excluding neglect, (32.8%) were also frequently reported.

As evaluated using the MINI-kid, 21 (32.8%) of the juvenile female delinquents were diagnosed as currently having PTSD, whereas 43.7% were diagnosed as currently not having PTSD despite having experienced traumatic events. Afterwards, CAPS was used to assess the 21 subjects; 19 completed the interview but two were unable to complete the interview owing to mental instability. Fifteen (29.7%) of these 19 subjects were diagnosed as having full PTSD, two as suffering from partial PTSD, and the other two as currently not having PTSD.

PTSD and comorbidity

Table 1 shows a comparison of comorbid psychiatric diagnosis among the female offenders with PTSD, without PTSD and without trauma exposure. Those with PTSD have significantly higher comorbidities with depression ($\chi^2 = 12.1$, $p = .002$), panic disorder ($\chi^2 = 14.8$, $p = .001$), agoraphobia ($\chi^2 = 8.3$, $p = .016$), separation anxiety disorder ($\chi^2 = 13.0$, $p = .002$), social phobia ($\chi^2 = 17.7$, $p = .000$), obsessive-compulsive disorder ($\chi^2 = 9.0$, $p = .011$), conduct disorder ($\chi^2 = 6.2$, $p = .045$) and psychotic disorder (current episode) ($\chi^2 = 7.3$, $p = .027$) than those not exposed to trauma. Those with PTSD were more likely to report comorbidities of panic disorder, social anxiety disorder, social phobia and psychotic disorder including a lifetime episode ($\chi^2 = 8.0$, $p = .018$) than those without PTSD. In addition, those with PTSD indicated a significantly higher risk of suicidal

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

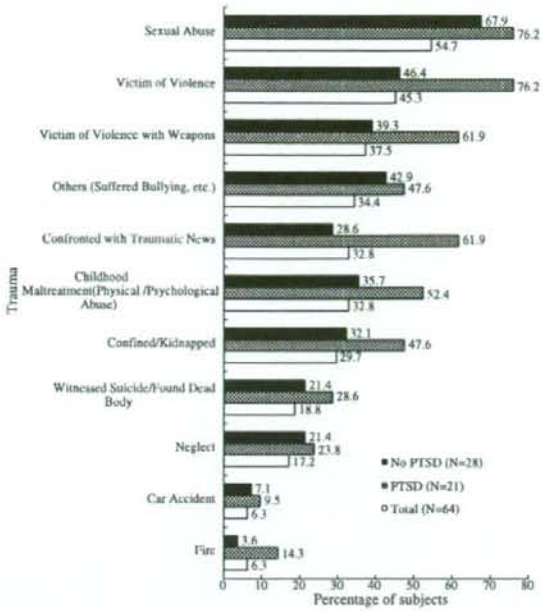

Figure 1 Trauma exposure of female offenders with and without PTSD for each trauma type. Overall, 76.5% of the subjects experienced a traumatic event. Most of the participants were exposed to multiple types of trauma, with sexual abuse being the most frequently reported type of trauma (54.7%)

tendency than those without trauma experience ($\chi^2 = 9.3$, $p = .009$).

Comparisons of self-questionnaires

Table 2 shows a comparison of the mean scores in the self-rating questionnaires (DSD, EAT-26, BIS-11 and PBI) between the female offenders with PTSD, without PTSD, and without trauma experience by one-way ANOVA and post-hoc comparison. The female offenders with PTSD showed significantly higher scores in DSD ($F[2,60] = 8.4$, $p < .01$), total EAT-26 ($F[2,61] = 6.8$, $p < .01$), and two subscales of EAT-26 (diet factor ($F[2,61] = 4.6$, $p < .05$) and bulimia/food preoccupation factor ($F[2,61] = 6.2$, $p < .01$)) than those without PTSD or trauma experience. The oral control subscale scores in EAT-26 of the female offenders with PTSD were significantly higher than those of the female offenders without trauma experience ($F[2,61] = 3.4$, $p < .05$). There were no statistically significant differences in impulsiveness and parental attitude among the groups.

Prediction of PTSD diagnosis and symptomatology

Logistic regression analysis of all the factors including categorical and dimensional items such as independent variables enabled us to identify three significant predictors and respective risks for the development of PTSD among 47 subjects who had trauma exposure (Table 3). The scores in DSD significantly predicted the development of PTSD ($p < .01$), and its odds ratio was 1.1. Additionally, lower maternal protection and maternal care scores assessed using the PBI were selected as risk factors for PTSD diagnosis. This logistic model was statistically highly significant ($\chi^2 = 15.8$, $p = .001$).

To determine the predictive factors of the severity of PTSD-related symptoms, stepwise multiple regression analysis was conducted with IES-R total score as a dependent variable. A statistically significant model was obtained with two correlation factors ($R^2 = .66$, $F[2,45] = 43.3$, $p < .001$), and the DSD ($\beta = .73$, $p < .001$) and EAT-26 oral control scores ($\beta = .17$, $p < .08$) were entered as significant related factors.

Features of PTSD in female offenders determined using CAPS

To classify the characteristics of PTSD symptom profiles according to the type of traumatic event or comorbidity, we compared CAPS score including the frequency and intensity scores of three criteria between the subjects with and without comorbidity, and those with and without respective trauma experiences. Table 4 shows detailed comparisons only of the variables that were statistically significant as determined by Bonferroni's correction ($p < .0062$; .05/repeated numbers).

The comorbidity of panic disorder significantly increased the intensity scores of criteria B and

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

Table 1 Comparison of comorbid psychiatric diagnoses of female offenders with PTSD, without PTSD and without trauma exposure

Diagnosis (determined using MINI-kid)	I PTSD ($N = 21$) N (%)	II No PTSD ($N = 28$) N (%)	III No Tex ($N = 15$) N (%)	$X^2(df = 2)$
Depression	11 (52.4)	7 (25.0)	0	12.1**(I > III)a
Dysthymia	7 (33.3)	6 (21.4)	1 (6.7)	3.6
(Hypo)manic episode				
Current	3 (14.3)	1 (3.6)	0	3.7
Past	12 (57.1)	14 (50.0)	4 (26.7)	3.5
Panic disorder	9 (42.9)	2 (7.1)	0	14.8***(I > II, I > III)a
Agoraphobia	7 (33.3)	3 (10.7)	0	8.3* (I > III)a
Separation anxiety disorder	11 (52.4)	6 (21.4)	0	13.0**(I > II, I > III)a
Social phobia	11 (52.4)	3 (10.7)	0	17.7***(I > II, I > III)a
Specific phobia	5 (23.8)	4 (14.3)	2 (13.3)	1.0
OCD	8 (38.1)	4 (14.3)	0	9.0*(I > III)a
Alcohol				
Abuse	14 (66.7)	18 (64.3)	7 (46.7)	1.7
Dependence	16 (76.2)	16 (57.1)	7 (46.7)	3.5
Substance				
Abuse	13 (61.9)	14 (50.0)	11 (73.3)	2.3
Dependence	10 (47.6)	12 (42.9)	9 (60.0)	1.2
Tic disorders				
Tourette	1 (4.8)	0	0	2.1
Motor	0	1 (3.6)	0	1.3
Vocal	0	0	0	–
Transient	1 (4.8)	0	0	2.1
ADHD				
Combined	8 (38.1)	5 (17.9)	1 (6.7)	5.5
Inattentive	0	4 (14.3)	2 (13.3)	3.2
Hyperactive/Impulsive	1 (4.8)	1 (3.6)	0	.7
Conduct disorders	17 (81.0)	22 (78.6)	7 (46.7)	6.2*(I > III, II > III)a
Oppositional defiant disorder	1 (4.8)	2 (7.1)	0	1.1
Psychotic disorder				
Current	9 (42.9)	5 (17.9)	1 (6.7)	7.3*(I > III)a
Lifetime	11 (52.4)	6 (21.4)	2 (13.3)	8.0*(I > II)a
Mood disorders with psychotic features	2 (9.5)	2 (7.1)	0	1.4
Anorexia nervosa	3 (14.3)	2 (7.1)	3 (20.0)	1.6
Bulimia nervosa	5 (23.8)	1 (3.6)	2 (13.3)	4.5
Generalised anxiety disorder	1 (4.8)	1 (3.6)	0	.7
Adjustment disorders	0	1 (3.6)	0	1.3
Pervasive developmental disorder	0	2 (7.1)	0	2.7
Suicidal tendency	15 (71.4)	13 (46.4)	3 (20.0)	9.3**(I > III)a

Note. PTSD = posttraumatic stress disorder; Tex = trauma exposure; OCD = obsessive-compulsive disorder; ADHD = attention deficit/hyperactivity disorder. a: Significant difference between groups by Fisher's exact probability test (two-sided); *$p < .05$; **$p < .01$; ***$p < .001$, two-tailed.

B + C + D of the PTSD subjects. Concerning the differences in the type of traumatic event, the experience of being a victim of violence significantly influenced the intensity scores of criteria D and B + C + D. The experience of witnessing suicide or finding a dead body significantly affected the increases in the frequency scores of criteria B.

Discussion

In this study, we found that experiencing traumatic events is very serious and common in female juvenile delinquents, and that the prevalence of PTSD is remarkably high in juvenile female Japanese offenders. These findings are consistent with those on young females under detention in Western countries (Dixon et al., 2005; Abram et al., 2004; Cauffman et al., 1998).

Previous research studies have shown that female offenders are usually exposed to multiple traumatic events; in particular, sexual abuse is one of the most serious forms of victimisation among female children and adolescents. Many researchers have identified PTSD as a core manifestation of sexual abuse because of the high frequency with which this disorder and related symptoms appear in sexually assaulted children (Kendall-Tackett, Williams, & Finkelhor, 1993). The results of this study also supported the notion that trauma from sexual abuse trauma is obviously high in young female offenders in Japan.

Our findings that female offenders with PTSD show higher psychiatric comorbidity including depression and anxiety disorders are similar to a previous finding in male delinquents (Ruchkin et al., 2002). The other study showed that incarcerated male individuals with PTSD show more pronounced

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

Table 2 Comparison of self-rating questionnaire scores of female offenders with and without PTSD, and without trauma exposure

	I PTSD (n = 21)		II No PTSD (n = 28)		III No Tex (n = 15)			
Variable	Mean	s.d.	Mean	s.d.	Mean	s.d.	F	p value
DSD	64.4	18.9	48.9	15.1	44.5	12.8	8.4 (I > II, I > III)*	.001
EAT-26								
Diet	13.3	9.2	7.3	6.2	6.5	8.5	4.6 (I > II, I > III)*	.014
Bulimia/food preoccupation	4.6	4.8	1.5	2.2	1.3	2.4	6.2 (I > II, I > III)*	.003
Oral control	3.5	2.4	2	2.4	1.5	2.8	3.4 (I > III)*	.040
Total	21.4	13.5	10.8	8.8	9.3	12.6	6.8 (I > II, I > III)*	.002
BIS-11								
Iat	20.7	5.7	19.9	4.6	20.6	3.8	.2	.801
Im	28.5	6.8	27.7	5.3	28.5	5.9	.1	.865
Inp	29.2	4.3	29.9	5.9	32.1	4.9	1.4	.244
Total	78.4	13.1	77.5	13.3	81.3	11.1	.4	.647
PBI								
p-care	20.5	9.2	15.6	9.7	14.1	7.9	2.6	.082
p-op	22.8	7.0	24.3	7.3	25.4	8.1	.6	.545
m-care	13.1	9.9	10.7	9.3	13.5	8.7	.6	.545
m-op	23.3	7.7	26.1	7.0	27.5	8.8	1.4	.245

Note. PTSD = posttraumatic stress disorder; Tex = trauma exposure; DSD = DSM Scale for Depression; EAT-26 = Eating Attitudes Test-26; BIS-11 = Barratt Impulsiveness Scale-11; Iat = attentional impulsiveness; Im = motor impulsiveness; Inp = non-planning impulsiveness; PBI = Parental Bonding Instrument; p-care = paternal care factor; p-op = paternal overprotection factor; m-care = maternal care factor; m-op = maternal overprotection factor.
*Bonferroni's post-hoc multiple comparison.

Table 3 Logistic regression analysis of PTSD diagnosis of female offenders with trauma exposure

Variable	B	Std. error	Odds Ratio	p value	95% CI
DSD Score	.08	.03	1.09	.003	1.03–1.15
PBI Maternal care	−.11	.06	.90	.081	.79–1.01
Maternal op	−.16	.09	.85	.070	.72–1.01

Note. N = 47; Model Fit: χ^2 = 15.8, df = 3, p = .001.
DSD = DSM Scale for Depression; PBI = Parental Bonding Instrument.

distress, anxiety and depression (Steiner, Garcia, & Matthews, 1997). Dixon et al. (2005) reported that female offenders with PTSD more frequently show comorbid depression, anxiety disorders, psychoses and eating disorders than those without PTSD. In particular, depression and mostly panic disorder or social phobia are associated with trauma-related symptoms. Depression is prevalent among female juvenile offenders similarly to the depression observed among the general adolescent population. In adolescence, this depression is often characterised by irritability, aggression or suicidal ideation. Confinement may trigger depressive symptoms; however, these mood swings frequently predate arrest. In consideration of unusual situations in detention centres, incarceration might precipitate major depression among vulnerable individuals. The experience of traumatic events, such as sexual abuse and violence, could enhance vulnerability to psychosocial stressors. Thus, it may be speculated that many female offenders with PTSD easily create a vicious cycle of trauma and depression. In addition, the risk of suicide was obviously high in female delinquents in our study, which is in agreement with the findings of Dixon et al. (2005). Sanislow, Grilo, Fehon, Axelrod, and McGlashan (2003) suggested that it is helpful to examine impulsivity and the history of drug abuse when assessing suicidal risk in detained adolescents. Although in this study we did not present distinct links between suicidal risk and impulsivity or substance use, further analysis of these issues is necessary. Moreover, a study of the prevalence of dissociative disorders in young offenders is also important (Carrion & Steiner, 2000). As a trauma spectrum, dissociation has a special relationship to sexual assault, which is common in female delinquents (Plattner et al., 2003). Dissociation is another important issue to be solved in this series of investigation in Japan.

Dimensional analysis revealed close associations of PTSD with depressive symptoms and eating problems. We emphasise that abnormal eating behaviours including binge eating and purging are relevant symptoms in female delinquents with PTSD. From the significant differences in ANOVA, eating abnormalities as assessed using EAT-26 seem to have a strong relationship with PTSD or trauma-related problems. In addition, only a trauma experience does not reflect the comorbidity of depression and abnormal eating behaviours. Thus, it should be noted that the comorbidities of depression and eating problems are defined by PTSD development.

The results of the logistic and linear regression analyses indicate that depression is the most important symptom that correlates with PTSD development and related symptoms assessed using the IES-R. The correlation between PTSD and depression was previously suggested in several

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

Table 4 Comparison of CAPS score between PTSD offenders with and without comorbid diagnosis, and those with and without respective trauma experiences

	Frequency score				Intensity score			
Comorbidity and trauma	B	C	D	B + C + D	B	C	D	B + C + D
CAPS: Mean (s.d.) ($N = 19$)	10.1 (5.4)	15.5 (6.6)	13.0 (5.2)	38.6 (16.0)	11.2 (5.3)	14.3 (6.0)	10.8 (4.3)	36.4 (14.2)
Panic disorder								
+($N = 8$)	13.6 (5.1)	19.9 (4.3)	15.3 (3.3)	48.8 (11.6)	14.9 (4.8)	18.3 (3.4)	13.4 (2.8)	46.5 (9.6)
−($N = 11$)	7.5 (4.3)	12.4 (6.2)	11.4 (5.9)	31.3 (15.0)	8.5 (4.1)	11.5 (6.0)	9.0 (4.3)	29.0 (12.5)
t (df = 17)	2.8	2.9	1.7	2.7	3.1*	2.9	2.5	3.3*
Victim of violence								
+($N = 15$)	11.3 (5.6)	17.4 (5.7)	14.3 (4.8)	42.9 (14.7)	12.7 (5.0)	16.0 (5.1)	12.2 (3.1)	40.9 (11.4)
−($N = 4$)	5.8 (1.0)	8.5 (4.8)	8.3 (4.3)	22.5 (9.6)	5.5 (1.0)	8.0 (5.4)	5.8 (4.4)	19.3 (10.3)
t (df = 17)	1.9	2.8	2.3	2.6	2.8	2.8	3.4*	3.4*
Witnessing Suicide/Dead Body								
+($N = 5$)	15.6 (4.0)	20.2 (3.9)	16.8 (2.6)	52.6 (9.3)	16.0 (3.1)	19.2 (3.3)	14.8 (1.1)	50.0 (5.3)
−($N = 14$)	8.1 (4.8)	13.9 (6.6)	11.6 (5.3)	33.6 (15.1)	9.5 (5.0)	12.6 (5.9)	9.4 (4.0)	31.5 (13.1)
t (df = 17)	3.2*	2.0	2.1	2.6	2.7	2.4	2.9	3.0

Note. CAPS = Clinician-Administered PTSD Scale for DSM-IV; B = re-experience symptom; C = avoidance/numbing symptom; D = hyperarousal symptom.
Non-paired t-test, two-tailed, *$p < .0062$ (Bonferroni's correction).

studies (e.g., Oquendo et al., 2005), and the risk of developing PTSD diagnosis is relatively high in accordance with an increase in DSD score. In addition, parental attitude assessed using PBI is selected as a relative risk factor. Affectionless-control (low degrees of care and high degrees of protection) has been popular as a candidate risk factor related to the development of several psychological disturbances or psychiatric illness (Parker et al., 1979), and little maternal care could be supported by our findings. Although this study shows that weak involvement with maternal protection may be related to PTSD development, the PBI provides responders' recognition regarding their parents' behaviours retrospectively. Actual parental attitude possibly differs from perceived assessments, and we should mention that the associations found in this study are correlations. Although depression and parenting attitude are important in discriminating a PTSD diagnosis among traumatised adolescents, we have to analyse parental influence by considering the actual familial situations of offenders and implying causations using a prospective procedure. Linear regression analysis also indicates that oral control correlates with trauma symptoms. There are only a few studies of the association of PTSD symptoms with eating problems. Conclusively, eating behaviour should be paid particular attention as one of the factors related to PTSD development. In contrast, impulsivity was not significantly related to IES-R score. This result may be caused by a ceiling effect, that is, the mean scores in the BIS-11 of the PTSD group were originally as high as those of the other groups; therefore, it might have been difficult to determine the statistical significance of differences among the groups even if there was a difference between the offenders and the control subjects. Moreover, some items of BIS are not applicable to adolescents; therefore, other instruments for assessing impulsivity may be more useful to test our hypothesis.

The results of this study indicate that the comorbidity of panic disorder enhances each intensity of PTSD symptoms, particularly the intensity of the re-experience symptom, and there is evidence that panic attacks are closely related to PTSD development (Favarelli, Webb, Ambonetti, Fonnesu, & Sessarego, 1985; Bandelow et al., 2002). Panic attacks are similar to somatic symptoms such as headache, chest pain, dizziness, or gastrointestinal complaints including criterion B (re-experience symptom) in people with PTSD. It is reasonable that the intensity criteria of PTSD had a significantly higher score as a result of the exposure to physical or psychological violence. It is also clear that witnessing suicide or finding a dead body increased the frequency scores of criterion B. We can therefore assume that trauma exposure accompanied by indirect fear of mortality enhances flashbacks or intrusion rather than arousal and avoidance.

The limitations of this study should be mentioned. The sample size of our study is small, and no comparisons with control females were conducted. Further investigation using a larger sample and a control group is required for the generalisation of the findings. We did not assess Axis-I disorders or personality traits because it is difficult to determine personality disorders in juveniles and adolescents. Some young offenders usually have personality deviations; thus, it is important to include personality assessments. Dissociation should also be evaluated as mentioned above. Although the validity of the answers to the questionnaires in this study is supported by the investigators' instruction and confirmation, methodological limitations are common in studies using self-reports. In this study, we used MINI as a screening tool. Although this tool is

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

convenient and comprehensive, it also has some limitations in confirming accurate diagnosis. Other reliable interview methods such as the Schedule for Affective Disorders and Schizophrenia for School-Age Children or the Diagnostic Interview Schedule for Children could also be used if respective Japanese versions become available. We were not able to establish a causal relationship between parental style, depression and PTSD only on the basis of retrospective procedures. The relationships between developmental disorders and offence patterns should be investigated in the future. Finally, we emphasise the need for prospective follow-up studies according to therapeutic approaches.

Conclusion

Incarcerated young female offenders in Japan have very serious psychiatric problems related to trauma exposure. We recommend that female delinquents be provided with not only correctional education but also mental support to prevent PTSD development among offenders in detention centres. For female offenders with psychiatric problems, treatment interventions are essential, and PTSD and comorbidity including depression or eating abnormality must be considered in the overall therapeutic strategy.

Correspondence to

Toru Uehara, Department of Psychiatry and Human Behaviour, Gunma University, Graduate School of Medicine, 3-39-22 Showa, Maebashi, Gunma 371-8511, Japan; Tel/fax: +81 27 220 8186; Email: toruaki@med.gunma-u.ac.jp

References

Abram, K.M., Teplin, L.A., Charles, D.R., Longworth, S.L., McClelland, G.M., & Dulcan, M.K. (2004). Posttraumatic stress disorder and trauma in youth in juvenile detention. *Archives of General Psychiatry, 61*, 403–410.

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* 4th edn (DSM-IV). Washington, DC: APA.

Asukai, N., Hirohata, S., Kato, H., & Konishi, T (2003). Psychometric properties of the Japanese-language version of the Clinician-Administered PTSD Scale for DSM-IV. *Japanese Journal of Traumatic Stress, 1*, 47–53 (in Japanese).

Asukai, N., Kato, H., Kawamura, N., Kim, Y., Yamamoto, K., Kishimoto, J., Miyake, Y., & Nishizono-Maher, A. (2002). Reliability and validity of the Japanese-language version of the Impact of Event Scale-Revised (IES-R.-J): Four studies of different traumatic events. *Journal of Nervous and Mental Disease, 190*, 175–182.

Bandelow, B., Spath, C., Tichauer, G.A., Broocks, A., Hajak, G., & Ruther, E. (2002). Early traumatic life events, parental attitudes, family history, and birth risk factors in patients with panic disorder. *Comprehensive Psychiatry, 43*, 269–278.

Blake, D.D., Weathers, F.W., Nagy, L.M., Kaloupek, D.G., Gusman, F.D., Charney, D.S., & Keane, T.M. (1995). The development of a Clinician-Administered PTSD Scale. *Journal of Traumatic Stress, 8*, 75–90.

Carrion, V.G., & Steiner, H. (2000). Trauma and dissociation in delinquent adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry, 39*, 353–359.

Cauffman, E., Feldman, S.S., Waterman, J., & Steiner, H. (1998). Posttraumatic stress disorder among female juvenile offenders. *Journal of the American Academy of Child and Adolescent Psychiatry, 37*, 1209–1216.

Crimmins, S.M., Cleary, S.D., Brownstein, H.H., et al. (2000). Trauma, drugs and violence among juvenile offenders. *Journal of Psychoactive Drugs, 32*, 43–54.

Dixon, A., Howie, P., & Starling, J. (2005). Trauma exposure, posttraumatic stress, and psychiatric comorbidity in female juvenile offenders. *Journal of the American Academy of Child and Adolescent Psychiatry, 44*, 798–806.

Dixon, A., Howie, P., & Starling, J. (2004). Psychopathology in female juvenile offenders. *Journal of Child Psychology and Psychiatry, 45*, 1150–1158.

Doi, Y., Roberts, R.E., Takeuchi, K., & Suzuki, S. (2001). Multiethnic comparison of adolescent major depression based on the DSM-IV criteria in a U.S.-Japan study. *Journal of the American Academy of Child and Adolescent Psychiatry, 40*, 1308–1315.

Favarelli, C., Webb, T., Ambonetti, A., Fonnesu, F., & Sessarego, A. (1985). Prevalence of traumatic early life events in 31 patients with panic attacks. *American Journal of Psychiatry, 142*, 1493–1495.

Garner, D.M., Olmsted, M.P., Bohr, Y., & Garfinkel, P.E. (1982). The Eating Attitudes Test: Psychometric features and clinical correlates. *Psychological Medicine, 12*, 871–878.

Giaconia, R.M., Reinherz, H.Z., Silverman, A. B., et al. (1995). Traumas and posttraumatic stress disorder in a community population of older adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry, 34*, 1369–1380.

Kendall-Tackett, K.A., Williams, L.M., & Finkelhor, D. (1993). Impact of sexual abuse on children: A review and synthesis of recent empirical studies. *Psychological Bulletin, 113*, 164–180.

Kitamura, T., & Suzuki, T. (1993). A validation study of the Parental Bonding Instrument in a Japanese population. *Japanese Journal of Psychiatry and Neurology, 47*, 29–36.

Oquendo, M., Brent, D.A., Birmaher, B., Greenhill, L., Kolko, D., Stanley, B., Zelazny, J., Burke, A.K., Firinciogullari, S., Ellis, S.P., & Mann, J.J. (2005). Posttraumatic stress disorder comorbid with major depression: Factors mediating the association with suicidal behavior. *American Journal of Psychiatry, 162*, 560–566.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., & Kamijima, K. (2005). Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. *Psychiatry and Clinical Neurosciences, 59*, 517–526.

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

Parker, G., Tupling, H., & Brown, L. B. (1979). A parental bonding instrument. *British Journal of Medical Psychology, 52*, 1–10.

Patton, J.H., Stanford, M.S., & Barratt, E.S. (1995). Factor structure of the Barratt Impulsiveness Scale. *Journal of Clinical Psychology, 51*, 768–774.

Plattner, B., Silvermann, M.A., Redlich, A.D., Carrion, V.G., Feucht, M., Friedrich, M.H., & Steiner, H. (2003). Pathways to dissociation: Intrafamilial versus extrafamilial trauma in juvenile delinquents. *Journal of Nervous and Mental Disease, 191*, 781–788.

Roberts, R.E., Roberts, C.R., & Chen, Y.W. (1995). *Ethnocultural differences in prevalence of adolescent depression.* 123rd Annual Meeting of American Public Health Association, San Diego.

Ruchkin, V.V., Schwab-Stone, M., Koposov, R., Vermeiren, R., & Steiner, H. (2002). Violence exposure, posttraumatic stress, and personality in juvenile delinquents. *Journal of the American Academy of Child and Adolescent Psychiatry, 41*, 322–329.

Sanislow, C.A., Grilo, C.M., Fehon, D.C., Axelrod, S.R., & McGlashan, T.H. (2003). Correlates of suicide risk in juvenile detainees and adolescent inpatients. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*, 234–240.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G.C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry, 59*(Suppl. 20), 22–33; Quiz 34–57.

Someya, T., Sakado, K., Seki, T., Kojima, M., Reist, C., Tang, S., & Takahashi, S. (2001). The Japanese version of the Barratt Impulsiveness Scale, 11th version (BIS-11): Its reliability and validity. *Psychiatry and Clinical Neurosciences, 55*, 111–114.

Steiner, H., Garcia, I., & Matthews, Z. (1997). Posttraumatic stress disorder in incarcerated juvenile delinquents. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*, 357–365.

Ujiie, T., & Kono, M. (1994). Eating Attitudes Test in Japan. *Japanese Journal of Psychiatry and Neurology, 48*, 557–565.

Weiss, D.S., & Marmar, C.R. (1997). The Impact of Event Scale – Revised. In J. Wilson & T. M. Keane (Eds.), *Assessing psychological trauma and PTSD* (pp. 399–411). New York: The Guilford Press.

Yoshinaga, C., Kadomoto, I., Otani, T., Sasaki, T., & Kato, N. (2004). Prevalence of post-traumatic stress disorder in incarcerated juvenile delinquents in Japan. *Psychiatry and Clinical Neurosciences, 58*, 383–388.

Manuscript accepted 05 July 2007

© 2007 The Authors
Journal compilation © 2007 Association for Child and Adolescent Mental Health.

Neuropathology 2008; **28**, 143–150 doi:10.1111/j.1440-1789.2007.00867.x

Original Article

Changes in density of calcium-binding-protein-immunoreactive GABAergic neurons in prefrontal cortex in schizophrenia and bipolar disorder

Tsutomu Sakai,[1] Akihiko Oshima,[1] Yusuke Nozaki,[1] Itsuro Ida,[2] Chie Haga,[3] Haruhiko Akiyama,[3] Yoichi Nakazato[4] and Masahiko Mikuni[1]

Departments of [1]Psychiatry and Human Behavior and [4]Human Pathology, Gunma University Graduate School of Medicine, [2]Takasaki National Hospital, Gunma, and [3]Tokyo Institute of Psychiatry, Tokyo, Japan

There is evidence that GABAergic neurotransmission is altered in mental disorders such as schizophrenia (SCZ) and bipolar disorder (BPD). The calcium-binding proteins (CBPs) calbindin (CB), calretinin (CR), and parvalbumin (PV) are used as markers of specific subpopulations of cortical GABAergic interneurons. We examined the post-mortem prefrontal cortical region (Brodmann's area 9) of patients with SCZ and BPD, and of age-matched control subjects, excluding suicide cases. The laminar density of neurons immunoreactive (IR) for three CBPs, namely CB, CR, and PV, was quantified. The densities of CB-IR neurons in layer 2 and PV-IR neurons in layer 4 in the SCZ subjects decreased compared with those in the control subjects. When CBP-IR neurons were classified according to their size, a reduction in the density of medium CB-IR neurons in layer 2 in SCZ subjects and an increase in the density of large CR-IR neurons in layer 2 in BPD subjects were observed. These results suggest that alterations in specific GABAergic neurons are present in mental disorders, and that such alterations may reflect the vulnerability toward the disorders.

Key words: bipolar disorder, calbindin, calretinin, parvalbumin, schizophrenia.

Correspondence: Tsutomu Sakai, MD, Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan. Email: sakaitsu@med.gunma-u.ac.jp

Received 13 August 2007; revised 19 September 2007; accepted 1 October 2007.

© 2007 Japanese Society of Neuropathology

INTRODUCTION

GABAergic neurons provide both inhibitory and disinhibitory modulation of cortical and hippocampal circuits and contribute to the generation of oscillatory rhythms, discriminative information processing and the gating of sensory information within the corticolimbic system. In previous studies, it was suggested that these functions are altered in schizophrenic (SCZ) subjects.[1–3] GABAergic function also contributes to the control of impulsive and aggressive behaviors, and drugs such as carbamazepine, valproate, and lithium carbonate, which have been reported to change the levels of GABA and glutamic acid decarboxylase (GAD) activity,[4–7] have been used as mood stabilizers in the treatment of bipolar disorder (BPD) to reduce impulsive and aggressive behaviors. These drugs have also been used as adjunct therapy to antipsychotics in the treatment of SCZ.[8–10] In these reports, it was suggested that GABAergic neurotransmission is altered in mental disorders such as SCZ and BPD.

In the prefrontal cortex (PFC) of SCZ subjects, a reduced number of neurons expressing the mRNA for the 67-kDa isoform of GAD,[11,12] and a high density of $GABA_A$ receptor subunits[13,14] have been reported, whereas in the anterior cingulate cortex (ACC) of SCZ subjects, an increased number of $GABA_A$ receptors,[15] and increases in the size of GAD_{65}-immunoreactive (IR) terminals[16] are indicated. The high-intensity immunoreactivity of $GABA_A$ receptor subunits[13,14] in PFC and a reduction in the density of GAD_{65}-IR terminals in PFC and ACC[16] have been also described in BPD subjects. These findings suggest that there is a specific deficit in GABAergic inhibitory neurons in these disorders.

Cortical GABAergic cells can be categorized by the colocalization of neuropeptides, including somatostatin, cholecystokinin, neuropeptide Y, and vasoactive intestinal polypeptide.[17–19] Somatostatin, neuropeptide Y, vasoactive intestinal polypeptide and cholecystokinin concentrations are reduced in SCZ subjects,[20] and neuropeptide Y mRNA expression is reduced in BPD subjects.[21,22]

GABAergic neurons can also be classified by the presence of the calcium-binding proteins (CBPs) parvalbumin (PV), calbindin (CB), and calretinin (CR).[23,24] CB, PV and CR are present in non-pyramidal GABAergic neurons, which participate in various primate cortical circuits that may differ depending on the species, cortical area and layer in which they are located. CR is found in double-bouquet neurons, bipolar cells and Cajal-Retzius cells, CB is found in neurogliaform neurons and double-bouquet cells, and PV is found in chandelier and wide arbor (basket) neurons, and each calcium-binding protein is expressed in separate populations of prefrontal cortical neurons.[25,26]

Previous studies in which these CBPs were used as markers of GABAergic neurons in the prefrontal cortex did not show consistent results: a trend towards increases in the densities of CR-IR and PV-IR neurons;[27] reductions in the densities of CB-IR neurons[28,29] and PV-IR neurons in the PFC of SCZ subjects;[28–31] and no changes in the density of CR-IR[29,31] or PV-IR[32] neurons. Similarly, a decrease in the density of CB-IR neurons[28] and no change in CBP-IR neuron density[33] were also found in the PFC of BPD subjects. However, in most of these studies, SCZ and BPD subjects including suicide subjects were examined; control subjects were not suicidal. Moreover, the number of reports on CBP-IR neurons in the postmortem brain of BPD subjects is still small.

Therefore, the following questions arise. (i) If these studies excluded suicide subjects, would there be any alterations in density and distribution of CBP-IR neurons in subjects with these disorders? (ii) Are there consistent changes in the postmortem tissue of subjects with BPD? (iii) If the CBP-IR neurons are classified according to size, are there alterations in the cellular distribution in the PFC in subjects with these disorders?

To address these points, we quantified the densities of interneurons immunoreactive for PV, CB, and CR in the prefrontal cortical region of subjects with SCZ and BPD, and of age-matched control subjects, excluding suicide subjects.

METHODS AND MATERIALS

Participants

Human brain specimens from Brodmann's area 9 (BA9) were obtained from the Tokyo Institute of Psychiatry and Matsuzawa Hospital (Tokyo, Japan). The samples were obtained from 19 subjects (5 control, 7 SCZ, and 5 BPD subjects). Diagnoses were made according to DSM-IV criteria. Detailed case summaries were provided with demographic and clinical information (see Table 1 for group summary details). Subjects with a past history of neurological diseases and those whose death was caused by suicide were excluded. This study was approved by the Ethical Committee of Tokyo Metropolitan Matsuzawa Hospital, and the specimens were provided with the consent of the patients before death or of the family.

Immunocytochemistry

Hemispheres were fixed in 10% formalin and cut in frontal sections of roughly 1 cm thickness. The slices were embedded in paraffin. From these embedded blocks, serial sections of 4 μm thickness were prepared.

Three tissue sections per subject were used in each of the three investigations. Deparaffinized sections were incubated with either polyclonal anti-CB (1 : 100 Sigma, St Louis, MO, US), polyclonal anti-CR (1 : 1000 Sigma), or polyclonal anti-PV (1 : 800 Abcam, Cambridge, UK) antibodies overnight at 4°C. Sections were processed using the

Table 1 Group summaries of demographic and clinical information on the brains donated by the Tokyo Institute of Psychiatry and Matsuzawa Hospital

Variable	Group		
	Control	BPD	SCZ
Demographics			
Age at death in years (mean ± SD)	56.8 ± 5.81	54.6 ± 9.86	47.4 ± 7.63
Gender (male : female)	2 : 3	1 : 4	3 : 4
Clinical factors			
Cause of death	3a, 2b	4a, 1d	2a, 1c, 3d, 1e
Duration of disorder in years (mean ± SD)	—	21.0 ± 18.3	29.0 ± 7.02
Past alcohol/drug abuse or dependence (no : yes)	4 : 1	5 : 0	6 : 1

The cause of death is categorized under the following headings: a, heart and respiratory failure; b, liver failure; c, renal failure; d, cancer; and e, thyroid crisis. BPD, bipolar disorder, SCZ, schizophrenia.

© 2007 Japanese Society of Neuropathology

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

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

Areal density and cell size measurement

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

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

Sections stained with hematoxylin for identification were analyzed using Image-J software ver.1.34 to measure cortical and laminar thicknesses and to count the number of cells in each layer. Cortical layers were distinguished on the basis of the differences in the distribution, size and shape of their neurons.[34] At each position in which data were acquired, immunoreactive cells were counted for each layer. The density of neuronal profiles was expressed as mean values (± SE) per mm^2 per layer from a total of two 1000-μm-wide cortical traverses, each from the pial surface to the white matter border. Cortical traverses were located in an area devoid of damage and blood vessels and where the pial surface was parallel to the white matter border.

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

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

Statistical analysis

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

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

RESULTS

Identification of adjustment variables

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

Neurons and glia

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

CBP-IR neurons

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

© 2007 Japanese Society of Neuropathology

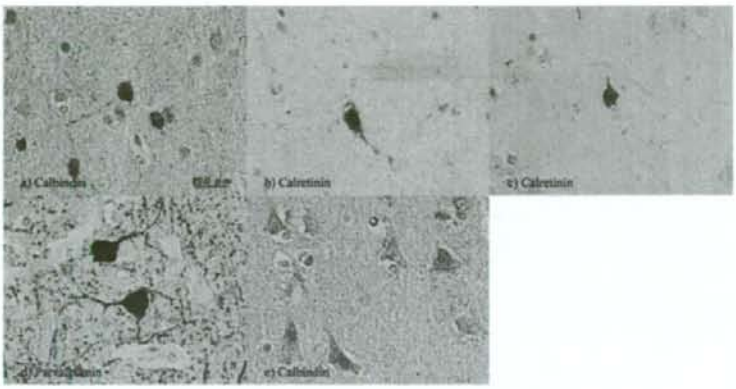

Fig. 1 Cells labeled by immunoreactivity to calbindin in layer 2 (a), calretinin in layer 1 (b) and layer 3 (c), parvalbumin in layer 3 (d), and labeled by low immunoreactivity to calbindin in layer 3 (e) of control subjects (bar = 20 μm).

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

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

*$P < 0.05$.

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

CBP-IR neurons were classified into medium and large classes according to size at the data acquisition points using mean + 1 SD of the size of the cells of the control subjects as follows: CB-IR neurons, 318 μm²; CR-IR neurons, 231 μm²; PV-IR neurons, 533 μm².

Density

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

© 2007 Japanese Society of Neuropathology

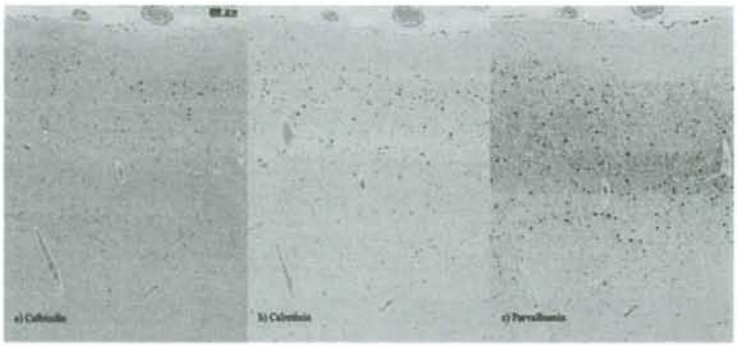

Fig. 2 Composite image showing calcium-binding protein-immunoreactive neurons. The composite images were made up of a series of contiguous images obtained individually at ×4 magnification, that were merged to form a single large image. (bar = 200 μm)

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

Abercrombie correction

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

DISCUSSION

IR neurons

In this study, we found significant reductions in the density of CB-IR neurons in layer 2 and PV-IR neurons in layer 4 of the PFC in SCZ subjects (in the between-layer comparison). In addition, when CBP-IR neurons were divided into two classes according to their size, a reduced density of medium-class CB-IR neurons in layer 2 in the SCZ subjects was also observed. We found no significant changes in either of the two types of PV-IR density, but there was a trend toward a reduction in large-PV-IR neuron density in layer 4 in the SCZ subjects. These results confirm those of previous studies on the PFC, which showed reductions in the density of CB-IR neurons[28,29] or PV-IR cells[28–31] and suggested no significant changes in CR-IR neuron density in the SCZ subjects.[31,36] This study supports the evidence that there is a deficit in GABAergic neurotransmission in SCZ.

No significant changes in CBP-IR neuron density between layers in the BPD subjects were found, which is consistent with the results of a report showing no changes in CBP-IR neuron density[33] in the dorsolateral PFC in BPD subjects. However, when IR cells were classified by size, there was an increased density of large CR-IR neurons in layer 2 in the BPD subjects, and also notably a non-significant but 28% reduction in CB-IR neuron density in the BPD subjects compared with the case of the control subjects (Table 2), which confirms the findings of a previous study.[28] These results suggest alterations in the cellular organization of CR-IR and CB-IR cells, and it is possible that an increase in CR-IR neuron density may be secondary to a reduction in CB-IR neuron density, or vice versa. Because we found no differences in neuronal density in layer 2 in the SCZ or BPD subjects, our findings on CBP-IR neurons may not depend on a reduction in cell number but rather on a decrease in protein expression.

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

© 2007 Japanese Society of Neuropathology

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

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

Methodological and confounding factors

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

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

In this study, the influence of major potential confounders on measures of CBP-IR neurons was examined. No significant differences at the 10% significance level (ANOVA or χ^2 test) were detected for the demographic or clinical variables shown in Table 1. There were also no significant differences and correlation in some other potential confounders such as postmortem interval and time from fixation to specimen making (data not shown). Therefore our data are presumably specific to each diagnostic group and not artifacts of these confounders.

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

ACKNOWLEDGMENTS

We thank Dr Tsuchiya K of the Department of Laboratory Medicine and Pathology, Tokyo Metropolitan Hospital, Tokyo, Japan for the analysis of the brain tissues, and also Yokota M, Yamazaki H, and Isoda K of the Department of Pathology, Gunma University Graduate School of Medicine, Gunma, Japan for technical assistance. This study was partly supported by Research Grants from the Japanese Ministry of Health, Labor and Welfare.

REFERENCES

1. Saccuzzo DP, Braff DL. Information-processing abnormalities: trait- and state-dependent components. *Schizophr Bull* 1986; **12**: 447–459.
2. Braff DL, Heaton R, Kuck J *et al.* The generalized pattern of neuropsychological deficits in outpatients with chronic schizophrenia with heterogeneous Wisconsin Card Sorting Test results. *Arch Gen Psychiatry* 1991; **48**: 891–898.
3. Blackwood DH, St Clair DM, Muir WJ, Duffy JC. Auditory P300 and eye tracking dysfunction in schizophrenic pedigrees. *Arch Gene Psychiatry* 1991; **48**: 899–909.
4. Otero Losada ME, Rubio MC. Acute and chronic effects of lithium chloride on GABA-ergic function in the rat corpus striatum and frontal cerebral cortex. *Naunyn Schmiedebergs Arch Pharmacol* 1986; **332**: 169–172.
5. Ahluwalia P, Grewaal DS, Singhal RL. Brain gabaergic and dopaminergic systems following lithium treatment and withdrawal. *Prog Neuropsychopharmacol* 1981; **5**: 527–530.
6. Gottesfeld Z. Effect of lithium and other alkali metals on brain chemistry and behavior. I. Glutamic acid and GABA in brain regions. *Psychopharmacologia* 1976; **45**: 239–242.

© 2007 Japanese Society of Neuropathology

7. Weiss S, Kemp DE, Bauce L, Tse FW. Kainate receptors coupled to the evoked release of 3H-gamma-aminobutyric acid from striatal neurons in primary culture: potentiation by lithium ions. *Mol Pharmacol* 1990; **38**: 229–236.
8. Citrome L, Levine J, Allingham B. Changes in use of valproate and other mood stabilizers for patients with schizophrenia from 1994 to 1998. *Psychiatr Serv* 2000; **51**: 634–638.
9. Citrome L, Jaffe A, Levine J, Allingham B. Use of mood stabilizers among patients with schizophrenia, 1994–2001. *Psychiatr Serv* 2002; **53**: 1212.
10. Berle JO, Spigset O. Are mood stabilizers beneficial in the treatment of schizophrenia? *Tidsskr Nor Laegeforen* 2005; **125**: 1809–1812.
11. Akbarian S, Kim JJ, Potkin SG *et al.* Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch Gen Psychiatry* 1995; **52**: 258–266.
12. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Arch Gen Psychiatry* 2000; **57**: 237–245.
13. Ishikawa M, Mizukami K, Iwakiri M, Hidaka S, Asada T. Immunohistochemical and immunoblot study of GABA (A) alpha1 and beta2/3 subunits in the prefrontal cortex of subjects with schizophrenia and bipolar disorder. *Neurosci Res* 2004; **50**: 77–84.
14. Impagnatiello F, Guidotti AR, Pesold C *et al.* A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proc Natl Acad Sci USA* 1998; **95**: 15718–15723.
15. Benes FM, Vincent SL, Alsterberg G, Bird ED, SanGiovanni JP. Increased GABAA receptor binding in superficial layers of cingulate cortex in schizophrenics. *J Neurosci* 1992; **12**: 924–929.
16. Benes FM, Todtenkopf MS, Logiotatos P, Williams M. Glutamate decarboxylase (65) -immunoreactive terminals in cingulate and prefrontal cortices of schizophrenic and bipolar brain. *J Chem Neuroanat* 2000; **20**: 259–269.
17. Somogyi P, Hodgson AJ, Smith AD, Nunzi MG, Gorio A, Wu JY. Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. *J Neurosci* 1984; **4**: 2590–2603.
18. Demeulemeester H, Vandesande F, Orban GA. Immunocytochemical localization of somatostatin and cholecystokinin in the cat visual cortex. *Brain Res* 1985; **332**: 361–364.
19. Demeulemeester H, Vandesande F, Orban GA, Brandon C, Vanderhaeghen JJ. Heterogeneity of GABAergic cells in cat visual cortex. *J Neurosci* 1988; **8**: 988–1000.
20. Gabriel SM, Davidson M, Haroutunian V *et al.* Neuropeptide deficits in schizophrenia vs. Alzheimer's disease cerebral cortex. *Biol Psychiatry* 1996; **39**: 82–91.
21. Caberlotto L, Hurd YL. Reduced neuropeptide Y mRNA expression in the prefrontal cortex of subjects with bipolar disorder. *Neuroreport* 1999; **10**: 1747–1750.
22. Kuromitsu J, Yokoi A, Kawai T *et al.* Reduced neuropeptide Y mRNA levels in the frontal cortex of people with schizophrenia and bipolar disorder. *Brain Res Gene Expr Patterns* 2001; **1**: 17–21.
23. Celio MR. Calbindin D-28k and parvalbumin in the rat nervous system. *Neuroscience* 1990; **35**: 375–475.
24. Demeulemeester H, Arckens L, Vandesande F, Orban GA, Heizmann CW, Pochet R. Calcium binding proteins and neuropeptides as molecular markers of GABAergic interneurons in the cat visual cortex. *Exp Brain Res* 1991; **84**: 538–544.
25. DeFelipe J. Types of neurons, synaptic connections and chemical characteristics of cells immunoreactive for calbindin-D28K, parvalbumin and calretinin in the neocortex. *J Chem Neuroanat* 1997; **14**: 1–19.
26. Conde F, Lund JS, Jacobowitz DM, Baimbridge KG, Lewis DA. Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefrontal cortex: distribution and morphology. *J Comp Neurol* 1994; **341**: 95–116.
27. Tooney PA, Chahl LA. Neurons expressing calcium-binding proteins in the prefrontal cortex in schizophrenia. Prog Neuropsychopharmacol. *Biol Psychiatry* 2004; **28**: 273–278.
28. Beasley CL, Zhang ZJ, Patten I, Reynolds GP. Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biol Psychiatry* 2002; **52**: 708–715.
29. Reynolds GP, Zhang ZJ, Beasley CL. Neurochemical correlates of cortical GABAergic deficits in schizophrenia: selective losses of calcium binding protein immunoreactivity. *Brain Res Bull* 2001; **55**: 579–584.
30. Beasley CL, Reynolds GP. Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophr Res* 1997; **24**: 349–355.
31. Reynolds GP, Beasley CL. GABAergic neuronal subtypes in the human frontal cortex – development and deficits in schizophrenia. *J Chem Neuroanat* 2001; **22**: 95–100.
32. Woo TU, Miller JL, Lewis DA. Schizophrenia and the parvalbumin-containing class of cortical local circuit neurons. *Am J Psychiatry* 1997; **154**: 1013–1015.

© 2007 Japanese Society of Neuropathology

33. Reynolds GP, Beasley CL, Zhang ZJ. Understanding the neurotransmitter pathology of schizophrenia: selective deficits of subtypes of cortical GABAergic neurons. *J Neural Transm* 2002; **109**: 881–889.
34. Rajkowska G, Goldman-Rakic PS. Cytoarchitectonic definition of prefrontal areas in the normal human cortex. I. Remapping of areas 9 and 46 using quantitative criteria. *Cereb Cortex* 1995; **5**: 307–322.
35. DeFelipe J, Jones EG. Parvalbumin immunoreactivity reveals layer IV of monkey cerebral cortex as a mosaic of microzones of thalamic afferent terminations. *Brain Res* 1991; **562**: 39–47.
36. Daviss SR, Lewis DA. Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. *Psychiatry Res* 1995; **59**: 81–96.
37. Hayes TL. Lewis DA. Hemispheric differences in layer III pyramidal neurons of the anterior language area. *Arch Neurol* 1993; **50**: 501–505.
38. Lund JS, Lund RD, Hendrickson AE, Bunt AH, Fuchs AF. The origin of efferent pathways from the primary visual cortex, area 17, of the macaque monkey as shown by retrograde transport of horseradish peroxidase. *J Comp Neurol* 1975; **164**: 287–303.
39. Abercrombie M, Johnson ML. Quantitative histology of Wallerian degeneration. I. Nuclear population in rabbit sciatic nerve. *J Anat* 1946; **80**: 37–50.
40. Benes FM, Lange N. Two-dimensional versus three-dimensional cell counting: a practical perspective. *Trends Neurosci* 2001; **24**: 11–17.

© 2007 Japanese Society of Neuropathology

Available online at www.sciencedirect.com
ScienceDirect
Journal of Psychiatric Research 42 (2008) 443–450
JOURNAL OF PSYCHIATRIC RESEARCH
www.elsevier.com/locate/jpsychires

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

K. Takahashi [a], A. Oshima [a,*], I. Ida [b], H. Kumano [a], N. Yuuki [c], M. Fukuda [a], M. Amanuma [d], K. Endo [d], M. Mikuni [a]

[a] *Department of Psychiatry and Human Behavior, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan*
[b] *Takasaki National Hospital, Gunma, Japan*
[c] *Gunma Prefectural Psychiatric Medical Center, Gunma, Japan*
[d] *Department of Diagnostic Radiology and Nuclear Medicine, Gunma University Graduate School of Medicine, Gunma, Japan*

Received 22 December 2006; received in revised form 3 May 2007; accepted 3 May 2007

Abstract

Objectives: To examine in patients with mood disorders the relationship of age at onset with the location and degree of MRI-defined brain hyperintensities.
Method: Fifty-two patients diagnosed as having mood disorders and 14 controls participated in the study. Brain MR images were analyzed according to semiquantitative ratings for the anatomical distribution and severity of T2-weighted hyperintensities. We compared these hyperintensities among the three age- and sex-matched groups of late-onset mood disorder patients (LOM), early-onset mood disorder patients (EOM), and controls. The time since the onset of disorder was significantly longer in the EOM than in the LOM group. We also conducted linear multiple regression analysis using the severity of hyperintensities as dependent variable to determine whether the clinical features correlate with vascular pathology.
Results: As for deep white matter hyperintensity (DWMH), LOM exhibited higher ratings than EOM; as for brain areas, significant between-group differences were detected in the bilateral frontal areas and in the left parieto-occipital area. No significant difference was observed between EOM and controls. As for periventricular hyperintensity, there was no difference among the three groups. We obtained a significant regression model to predict DWMH ratings; age, number of ECTs, and LOM were selected as significant variables.
Conclusion: The present study suggests that the time since the onset of disorder does not affect the development of white matter lesions, but that white matter lesions are associated with late-onset mood disorders. The frontal areas and the left parieto-occipital area would be important for the development of late-onset mood disorders.
© 2007 Elsevier Ltd. All rights reserved.

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

1. Introduction

Regarding major depression, there are differences in various clinical indices depending on the age at onset, such as clinical symptoms (Baldwin and Tomenson, 1995; Salloway et al., 1996; Krishnan et al., 1995), degree of cognitive impairment (Salloway et al., 1996; Alexopoulos et al., 1993), clinical course (Hickie et al., 1997; Alexopoulos et al., 1996), and suicide risk (Lyness et al., 1992). Hopkinson (1964) reported that the risk of depression in the first-degree relatives of depressed patients was 20% in the early-onset depression group compared with 8.3% in the late-onset depression group over 50 years of age. Because similar conclusions have been made by Schultz (1951) and Post (1975), it may be assumed that genetic factors exert greater effects in early-onset depression than in late-onset depression.

* Corresponding author. Tel.: +81 27 220 8190; fax: +81 27 220 8187.
E-mail address: aoshima@med.gunma-u.ac.jp (A. Oshima).

0022-3956/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2007.05.003

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

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

Factors such as age (de Leeuw et al., 2001; Manolio et al., 1994), elevated blood pressure (de Leeuw et al., 2002), diabetes (Longstreth et al., 2001), and cardiac arrhythmia (Ylikowski et al., 1995) have been reported as risk factors of white matter lesions. In the general population, however, there is less evidence that vascular risk factors are a major cause of depression (Thomas et al., 2004). There have been reports that there is no link between depression symptoms and elevated blood pressure (Jones-Webb et al., 1996; Friedman and Bennet, 1977) nor between a history of hypertension or of coronary heart disease and a history of depression (Steffens et al., 2002). In the Rotterdam Study, however, Tiemeier et al., who examined vasomotor reactivity in the middle cerebral artery using CO_2 inhalation, reported that the depression group exhibited a lower vasomotor reactivity than healthy controls (Tiemeier et al., 2002) and that arterial stiffness significantly correlated with the severity of depression (Tiemeier et al., 2003). Thomas et al. (2001) found in their postmortem study that atheromas in major vessels (coronary arteries, carotid arteries, and aortas) were significantly more severe in the depression group than in the normal group.

In summary, differences in pathogenesis and pathophysiology presumably exist between the early-onset and the late-onset mood disorder groups. Also, depression itself can be a risk factor for vascular lesions (Baldwin, 2005), which led to the hypothesis that depression exacerbates cerebrovascular lesions with aging (Lenze et al., 1999). However, there have been few studies in which subjects within age-matched groups were compared, that is, between patients with late-onset mood disorders, those with early-onset ones, and normal elderly subjects with regard to brain areas.

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

2. Subjects and method

2.1. Subjects

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

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

Table 1
Characteristics of subjects

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

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

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

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

2.2. MRI procedure and image analysis

We obtained the following images of the subjects using a 1.5-T MAGNETOM Symphony Maestro class, (Siemens Medical Solutions, Erlangen, Germany): axial T_2-weighted images (TR 3800ms; TE 90ms) and axial T_1-weighted images (TR 500ms; TE 14ms). The slices of images were 5-mm thick, with a 2.0-mm interslice gap, and the images of the entire brain parenchyma were obtained. An experienced psychiatrist (K.T. or A.O.) independently evaluated the MR images without knowledge of subjects' status. Each T2-weighted MR image was evaluated in terms of periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH) on the basis of Fazekas criteria (Fazekas et al., 1987), obtaining semiquantitative ratings for brain hyperintensities. Typical images of Fazekas criteria are shown in Fig. 1. PVH was rated as follows: 0 = absent, 1 = "caps" or pencil-thin lining, 2 = smooth "halo", and 3 = irregular PVH extending into the deep white matter. DWMH was rated as follows: 0 = absent, 1 = punctate foci, 2 = beginning confluence of foci, and 3 = large confluent area. To understand the functional anatomical role of the lesions, the regions of interest were classified into the following four areas in each hemisphere. Eight areas were thus evaluated similarly to the four-stage Fazekas DWMH criteria. The areas of interest were the following: (1) the frontal area (right and left, FR and FL, respectively), the frontal lobe anterior to the central sulcus; (2) the parieto-occipital area (right and left, POR and POL, respectively), consisted of the parietal and occipital lobe together; (3) the temporal area (right and left, TR and TL, respectively), the temporal lobe (the border between the parieto-occipital and temporal lobes was approximated as the line drawn from the posterior part of the Sylvian fissure to the trigone areas of the lateral ventricles); and (4) the basal ganglia (right and left, BGR and BGL, respectively), including the striatum, globus pallidus, thalamus, internal and external capsules, and insula. Periventricular lesions were not added to the respective regional scores in order to focus on short association fibers within hemispheres and to exclude commissural fibers and projection fibers that are more densly found in the periventricular area. The interrater reliability exceeded 0.95. There was no discrepancy of more than 1 point between the two evaluators with the given criteria.

2.3. Statistical analysis

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

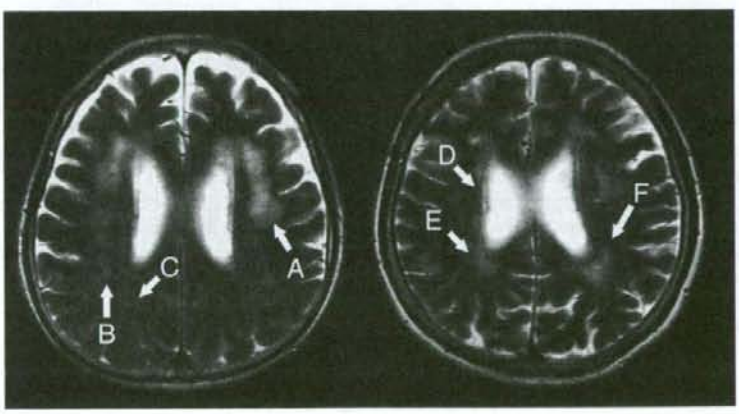

Fig. 1. Typical images of Fazekas ratings. *Note.* DWMH grade: A = grade 3, B = grade 2, C = grade 1; PVH grade: D = grade 1, E = grade 2, F = grade 3.

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

For these 52 patients, we also conducted linear multiple regression analysis using the DWMH scores as dependent variable. Using stepwise method, independent valuables were selected from dimensional scores; age, age at onset, time since the onset of a disorder, the number of episodes (depressive, manic and total episodes), the number of suicide attempts, the number of ECTs and doses of antidepressants, and from categorical scores; LOM, sex, bipolar, psychotic features, delirium, family history of psychiatric disorders, habitual smoking, habitual alcohol drinking, obesity, DM, HT, HL, IHD, CA. Significant variables were entered if the *P*-value of each variable was under 0.05, and deleted for $P > 0.10$.

3. Results

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

3.1.1. Clinical background and medical comorbidities

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

3.1.2. Fazekas semiquantitative ratings

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

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

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

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

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

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

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

[c] Comparison between 24 patients of LOM and 20 patients of EOM.