## Summary

Clinical Effects of High Oral Dose of Donepezil in Patients with Alzheimer's Disease

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A daily dose of 10 mg donepezil for severe Alzheimer's disease (AD) patients was licensed in Japan since the end of August 2007 ; but most Japanese patients with AD are prescribed a daily dose of 5 mg donepezil for more than 4 weeks. In addition, it has been confirmed in foreign countries that a daily dose of 10 mg of donepezil is effective for mild-to-moderate AD patients. We initiated medical treatment in 57 AD patients at a daily dose of 10 mg donepezil and subsequently assessed examined the clinical effects, adverse effects, and the relation with apolipoprotein E4 in these patients. We observed that a daily dose of 10 mg donepezil could stabilize or slow down the progression of cognitive impairment in our patients. The severity of AD, the duration of medication with 5 mg donepezil and the apolipoprotein E genotype did not influence any clinical effects. The incidence of adverse effects in this study was 12.3%, which was considerably lower than the incidence rate of 40% in the previous clinical trial in Japan. These findings suggest that long-term medication with a daily dose of 5 mg donepezil might be associated with fewer adverse effects.

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アルツハイマー病の診断と治療

アルツハイマー病のやさしい理解

# アルツハイマー病の神経伝達系の異常

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Key Words

アルツハイマー病
神経伝達物質
アセチルコリン
セロトニン

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高齢社会のなかでアルツハイマー病（Alzheimer disease；AD）は大きな社会的関心をよんでいるが，現在のところ唯一の治療薬であるドネペジルはその存在価値が大きいといえる．塩酸ドネペジルは，パーキンソン病におけるドパミン系薬剤と同じように，神経伝達物質研究から生まれてきたのはいうまでもない．

本稿では，ADにおける神経伝達物質異常について，歴史的展開から将来にわたる展望までを研究の意義とともに論じてみたい．

## アルツハイマー病研究の歴史

A.Alzheimerにより，50歳代の進行性認知症症例が学会報告されたのが1906年である．その後100年を経て，20世紀はまさにAD研究であったといっても過言ではない．それは科学的研究手法と機器の発展とともに，肉眼的脳研究から始まって顕微鏡レベルでの神経細胞や構成蛋白，そして分子レベル，最後は遺伝子までという神経科学的研究の流れが理解できる．しかし，遺伝子発見まで進んでADの病因・病態解明がすべて解決するというわけではない．そのため，最近ではこれまでに解明された家族性ADの原因遺伝子をもとに，遺伝子発現，エピジェネティクス，プロテオミクス，メタボロミクスなど，遺伝子から蛋白レベルに再び立ち戻って，孤発性ADの解明を目指しているといえる．

## 神経伝達物質研究の意義

神経伝達物質研究は上記のような流れのなかでちょうど中間に位置する．これは1976～1977年に英国の3グループがAD脳におけるアセチリコリン（Ach）作動系障害を報告し，スウェーデンのGottfriesらが生体アミン類障害を報告したことに始まる（詳細は文献[1]を参照されたい）が，わが国AD例死後脳における報告は，1984年になる[2]．

これらの神経伝達物質研究は，それまでの形態学研究をブレークスルーしたという意味をもつ．ADの病態を成因論的にまとめると図1のようになり，形態学的所見とは別な面からADの病態を明らかにした．ここからAD研究は新たな展開をみせ，治療薬や臨床的マーカーの開発につながり，一方では免疫組織化学法の発展とともに神経病理学的にも伝達物質関連研究が行われるようになった．

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## アセチルコリン作動系の障害

1960年代からパーキンソン病でドパミン作動系障害が注目されていた流れのなかで，前述したように1976～1977年にAD患者死後脳において，Ach合成酵素であるcholine acetyltransferase（ChAT）活性が有意に低下していたと報告され，大きな注目を浴びた（表1）[1]．また，Ach分解酵素であるacetylcholinesterase（AchE）の活性も有意に低下している．わが国症例AD脳におけるAch作動系の検討結果でも同様の所見が得られている（図2）[2]．その後，これらの変化はマイネルト基底核から大脳皮質に投射されているAch系障害と関連することも明らかになった．一方，シナプス後である受容体では，ムスカリン受容体については大きな変化はない傾向があり，ニコチン受容体では結合能が低下していると報告されている．

これらの成果をもとに，AchE阻害によるAD治療薬が開発された一方で，脳脊髄液や血清でのAch関連物質が診断マーカーとなりうるかなどが検討されたが，後者のほうは臨床で応用できるほどの成果は得られていな

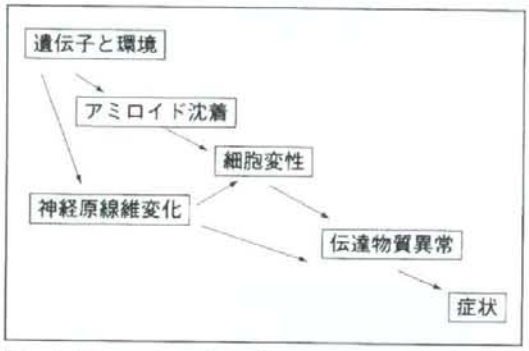


**図1 アルツハイマー病の成り立ち**

**表1 アルツハイマー病脳における神経伝達物質関連マーカーの変化[1]**

| | | | |
|---|---|---|---|
| **1. コリン作動系4** | | **4. ドパミン作動系** | |
| コリンアセチルトランスフェラーゼ活性 | ↓ | ドパミン濃度 | ↓ or→ |
| アセチルコリンエステラーゼ活性 | ↓ or→ | ホモバニリン酸（HVA）濃度 | ↓ |
| アセチルコリン合成率 | ↓ | チロシンヒドロキシラーゼ活性 | ↓ or→ |
| コリンの再取り込み | ↓ | 黒質における神経細胞数 | → |
| マイネルト基底核における神経細胞数 | ↓ | $D_1$受容体結合能 | → |
| ニコチン性受容体結合能 | ↓ | $D_2$受容体結合能 | ↓ |
| ムスカリン性受容体結合能 | →or↓ | **5. 遊離アミノ酸類** | |
| **2. セロトニン作動系** | | グルタミン酸濃度 | ↓ |
| セロトニン濃度 | ↓ | アスパラギン酸濃度 | → |
| 5-ヒドロキシインドール酢酸（5-HIAA） | ↓ | GABA濃度 | →or↓ |
| トリプトファンヒドロキシラーゼ活性 | ↓ | タウリン濃度 | →or↓ |
| セロトニンの取り組み | ↓ | その他アミノ酸濃度 | → |
| 縫線核における神経細胞数 | ↓ | **6. 神経ペプチド類** | |
| セロトニン（5-HT1）受容体結合能 | ↓ | ソマトスタチン | ↓ |
| セロトニン（5-HT2）受容体結合能 | ↓ | コルチコトロピン放出因子（CRF） | ↓ |
| **3. ノルアドレナリン作動系** | | サブスタンスP | ↓ or→ |
| ノルアドレナリン濃度 | ↓ | ニューロペプチドY | →or↓ |
| 3-メトキシ-ヒドロキシフェニルエチレングリコール4-(MHPG）濃度 | ↓ or→or↑ | バソアクティブインテスティナルポリペプチド（VIP） | →or↓ |
| ドパミン$\beta$ハイドロキシラーゼ活性 | ↓ | バソプレッシン | →or↓ |
| ノルアドレナリン再取り込み | ↓ | コレシストキニン | →or↓ |
| 青斑核における神経細胞数 | ↓ | ニューロテンシン | ↓ |
| 受容体結合能（$\alpha_1$，$\alpha_2$，$\beta$） | → | サイロトロピン放出ホルモン（TRH） | ↓ |

患者剖検脳で得られたおもな神経伝達物質関連マーカーの異常を正常対照群と比べて有意に低下（↓），有意に上昇（↑），有意差なし（→）で表している．

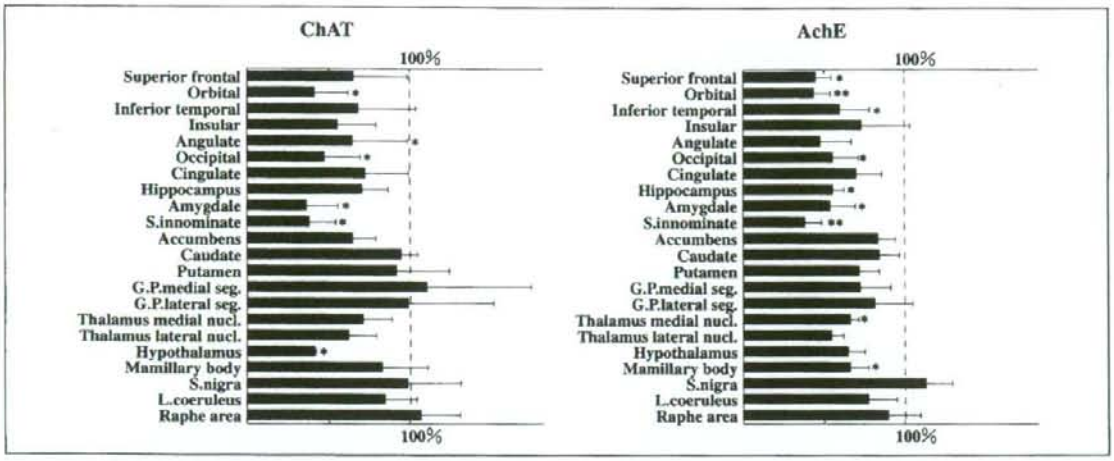


**図2 Ach合成酵素（ChAT）と分解酵素（AChE）の活性**[2]
健常者群の各部位の平均値を100%として表示した．G.P.:Globus pallidus.

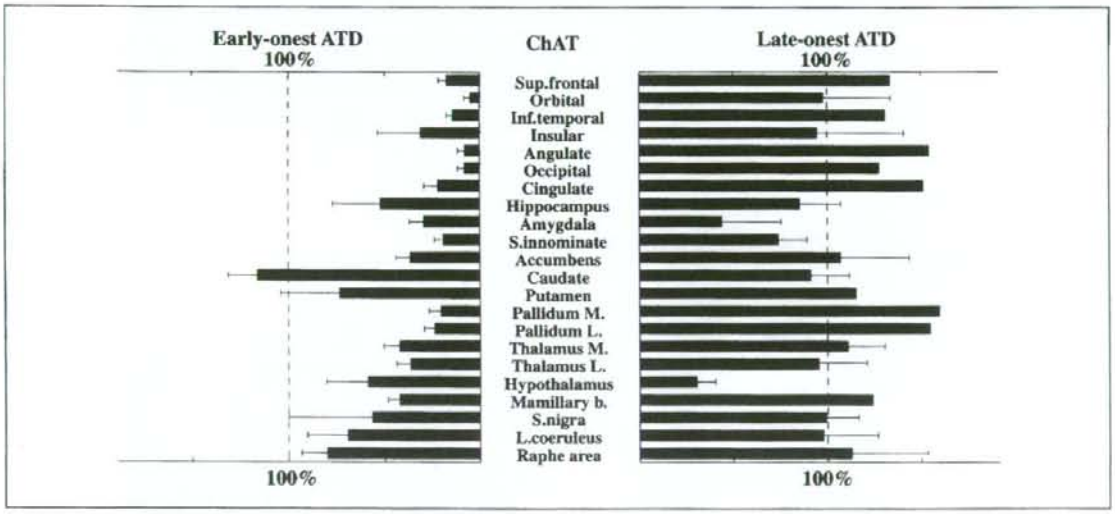


**図3 Ach合成酵素活性の初老期発症群と老年期発症群の違い**[4]
健常者群の各部位の平均値を100%として表示した．M:medial segment, L:lateral segment.

い．また，関連遺伝子領域の遺伝子変異検索や遺伝子発現も検討されたが，病因・病態に特につながる所見は得られていない．これらの中で注目されるのは，AchE関連のトレーサーを用いたPET研究であり，死後脳研究の成果を追試するとともに，ADの診断にも有用なことが示唆されてきている[3]．一方，病態解明に関連して，初老期発症ADでは老年期発症ADと比べて，Ach作動系の障害が著しいことが確認されている（図3）[4]．

## 生体アミン類作動系の障害

当初，Ach作動系障害がADの選択的障害であるように報告されたが，実際にははかの作動系にも障害が及び，ADは多系統変性疾患であることがわかった．まず，セロトニン作動系ではシナプス前マーカーであるセロトニンや代謝産物だけでなく，受容体結合能も低下していた．セロトニン作動系起始核である放線核では神経細胞数の減少も確認され，AD脳においてセロトニン作動系は著しく障

害されていると考えられる．また，ノルアドレナリン作動系については，セロトニン作動系と同じように，シナプス前後のマーカーに変化がみられる一方で，ドパミン作動系は変化が少なく，パーキンソン病との対照的な所見であった．これらセロトニン作動系やノルアドレナリン作動系の障害は，ADでみられるうつ状態や睡眠障害との関連が想定されている．

## 神経ペプチド系の障害

神経ペプチドは，おもに大脳皮質の介在ニューロンのマーカーとして検討されている．なかでも，ソマトスタチンが多くの研究で一致してその濃度が低下していると報告され，注目を受けた．また，バソプレッシンやサブスタンスP濃度の低下も報告された，治療薬としての可能性も一時検討されたが，その後さらなる展開はみせていない．

## アミノ酸作動系の障害

アミノ酸についてはグリア細胞にも多く存在し，完全な神経細胞マーカーとはなりにくいことや死後変化の大きいことが問題にはなるが，死後時間が短いサンプルの検討で多くのアミノ酸のなかで，グルタミン酸濃度だけがAD群で低下した[5]との結果は興味深い．単に死後変化を受けやすいのか，それともグルタミン酸作動系神経細胞の変性を表しているのかは，まだ解明されていない．

## 伝達物質研究関連の病因論的課題

**1．逆行性変性か？**

伝達物質変化は減少する方向での変化であり，それは細胞変性と関連すると解釈されている．特に，Ach作動系と生体アミン類作動系は皮質下に起始核があり，そこの神経細胞が変性し数が減少している．特にマイネルト基底核の障害は，パーキンソン病における黒質と同じような意味合いで，ADの本質的病変であるとの解釈がなされた時期もある．一方で，これらの皮質下核の病変は大脳皮質の神経細胞障害に基づくいわゆる逆行性変性であるとの指摘もされている．病因・病態論的にどちらが正しいのかはいまだ解明されていない．

**2．作動系間での違い―選択性**

前述したように，ADは伝達物質に関して多系統に及ぶ変性疾患であることが明らかとなっているが，作動系障害の程度にはそれぞれの作動系間で異なる．この傾向は個人差によるものでなく，AD群としての1つの傾向であり，障害が強い作動系ではなぜそのような脆弱性があるのか，AD発症機序の解明に繋がる糸口でもあるが，いまだ解明はされていない．

## アルツハイマー病治療薬の開発

**1．現在までに開発された治療薬**

治療薬の詳細は別稿を参照されたいが，わが国で唯一のAD治療薬として承認されている塩酸ドネペジルは，Achを分解するアセチルコリンエステラーゼの阻害薬である．いうまでもなく，この治療薬はAD脳における神経伝達物質研究の一番の成果である．これにより，それまで専門治療薬がなく，脳代謝循環改善薬に頼っていた時代からは大きな発展であった（表2）．しかし，前述したAch作動系障害の報告から約20年を経過しないと新薬に結びつかないという現実も浮かび上がる．

一方，諸外国ではドネペジル以外にもガランタミンなど，ほかに3種類の薬剤がAD治療薬として承認されている（表3）．メマンチンはNMDA受容体関連薬剤であり，アミノ酸作動系のAD病態関与を示唆しているともいえる．わが国では，これらの3剤が現在まだ第III相の治験中であり，いかにわが国の新薬開発が遅れているかが理解できる．

表2 アルツハイマー病の治療薬の歴史

| 効果 | 抑制<br>精神症状・問題行動 | 維持<br>全般性脳機能 | 補充<br>認知機能 | 阻止<br>神経細胞変性 | 促進<br>神経伸張 | 置換<br>遺伝子異常 |
|---|---|---|---|---|---|---|
| 代表薬物 | 抗精神病薬剤 | アルカロイド | タクリン | 阻害薬 | 成長因子 | 置き換え |
| 時期 | 現在 | 現在 | 1995～2005 | 2010以降 | 2010以降 | 200?（限定） |

表3 AD治療薬の種類

| | |
|---|---|
| ドネペジル | アメリカ，ヨーロッパ，日本で承認 |
| リバスチグミン | アメリカ，ヨーロッパで承認 |
| ガランタミン | アメリカ，ヨーロッパで承認 |
| メマンチン | アメリカ，ヨーロッパで承認 |

### 2．開発中の薬剤

現在AD治療薬として開発されている薬剤の一部にはセロトニン作動系関連など神経伝達物質関連薬剤も残っているが，多くは神経細胞変性を引き起こす原因と想定されているアミロイドβ蛋白の沈着に介入しようとする薬剤である．これらは，より早期の段階から投与することによって病態の進行を阻止する効果が期待されており，現在の神経伝達物質関連治療薬が対症療法の範囲を超えないのと対照的に，根治的治療薬としての役割を担うものである．

### 3．伝達物質関連薬剤の今後の展開

まず重要なことは，今後いかに根治的治療薬が開発されようとも，ADの発症を皆無にすることは難しいと思われるので，神経伝達物質関連の薬剤は今後も必要とされるということである．言い換えれば，症状を対象とした治療効果を出現させるには，疾病の成因論的に最後の段階への介入のほうがより有効であるともいえる．したがって，何らかの形でAch作動系のみならず伝達物質関連薬剤は今後も開発される可能性はある．このような観点から今までに検討されていない領域として，後シナプス段階でのセカンドメッセンジャーレベルやエネルギー代謝関連に介入する薬剤も検討される可能性があろう．これらは細胞活性やアポトーシスに介入することによって，より直接的に認知機能を高めることも期待される．

## おわりに

以上，ADにおける伝達物質研究の歴史的な流れや意義をなるべく理解しやすいように記載したつもりである．AD研究の主体は神経伝達物質からより本質的な領域へと移行しているが，ADが高齢社会のなかで大きな社会問題となっている現代において，そしてまた将来においても，伝達物質関連治療薬剤の役割は続いていくものと思われる．

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# Trauma exposure and posttraumatic stress disorder in delinquent female adolescents

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**Background:** Although juveniles within the justice system have high psychiatric morbidity, few comprehensive investigations have shown posttraumatic stress disorder (PTSD) in female delinquents. Here, we aim to describe the nature and extent of PTSD and trauma exposure and to clarify the relationships among comorbidity and psychosocial factors in juvenile female offenders. **Method:** Sixty-four girls were randomly interviewed using structured tools. Self-report measures were used to assess depression, eating behaviour, impulsivity and parental attitude. **Results:** The PTSD prevalence was 33%, and 77% of the female juvenile offenders had been exposed to trauma. The offenders with PTSD showed a significantly high psychiatric comorbidity. Depression and adverse parenting were associated with PTSD development, and abnormal eating was also correlated with PTSD symptoms. Marked differences in the frequency and intensity of PTSD evaluation depending on the type of comorbidity and trauma were observed. **Conclusions:** Incarcerated young females in Japan have serious trauma-related problems, and the degree of depression is a strong predictor of PTSD development and symptoms. This study highlights the importance of adequate diagnosis and treatment of PTSD in delinquent female adolescents. **Keywords:** Trauma, female, delinquency, comorbidity, depression, eating disorder, posttraumatic stress disorder. **Abbreviations:** CAPS: Clinician-Administered PTSD Scale for DSM-IV; MINI-kid: Mini-International Neuropsychiatric Interview for Children and Adolescents; DSD: DSM Scale for Depression; BIS-11: Barratt Impulsiveness Scale 11th version; EAT-26: Eating Attitudes Test-26; PBI: Parental Bonding Instrument; IES-R: Impact of Event Scale-Revised.

Juvenile female offenders have high rates of trauma exposure. For instance, Cauffman, Feldman, Waterman, and Steiner (1998) showed that most incarcerated females are exposed to multiple types of trauma. Recent studies have revealed that witnessing a violent crime and being confronted with traumatic news are the most frequently reported sources of trauma in female juvenile offenders (Dixon, Howie, & Starling, 2005). In particular, a high lifetime PTSD incidence (67%) has been observed among young women in custody (Cauffman et al., 1998) compared with the general population's incidence range of 1–14% (American Psychiatric Association, 1994). It has been documented that chronic exposure to violence results in the numbing of feelings or substance use and increased risk-taking behaviours, including violent activities, in an attempt to cope with or adapt to the feeling of being unsafe (Crimmins et al., 2000). Additionally, Giaconia et al. (1995) found that those with any history of PTSD symptomatology (14.5%) were more likely than those without to have behavioural or emotional problems, interpersonal problems, academic failure, suicidal behaviour, and health problems. Based on the previous studies (Ruchkin, Schwab-Stone, Koposov, Vermeiren, & Steiner, 2002; Dixon et al., 2005), there is evidence that juvenile offenders with PTSD experience higher rates of comorbid psychiatric disorders than those without PTSD. In particular, evidence suggests that young female offenders with PTSD have more comorbidity than those without PTSD, with depression, substance abuse/dependence, psychoses and eating disorders occurring significantly and more frequently. Reasonably, it could be speculated that there is a mutual relevancy among juvenile offences including illicit drug use or delinquency, trauma exposures including adverse parenting, and psychological behavioural problems including mood liability, abnormal eating behaviours, or impulsivity.

In Japan, although there has been extensive research on the frequency of PTSD in incarcerated juvenile delinquents (Yoshinaga, Kadomoto, Otani, Sasaki, & Kato, 2004), there is little comprehensive and structured research on PTSD development, including several psychosocial measurements in female juvenile delinquents. The aims of this study are (1) to describe the nature and extent of trauma exposure and PTSD, (2) to clarify the point prevalence of PTSD, (3) to examine the relationship between psychiatric comorbidity and PTSD (traumatic exposure), (4) to analyse the associations between PTSD diagnosis and socio-demographic factors, depressive symptoms, impulsivity, abnormal eating behaviour and parenting attitude, (5) to determine the risks and factors that can be used to predict PTSD development, and whether PTSD

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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

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 re-experience 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 $\chi^2$ 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 $\alpha$ 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

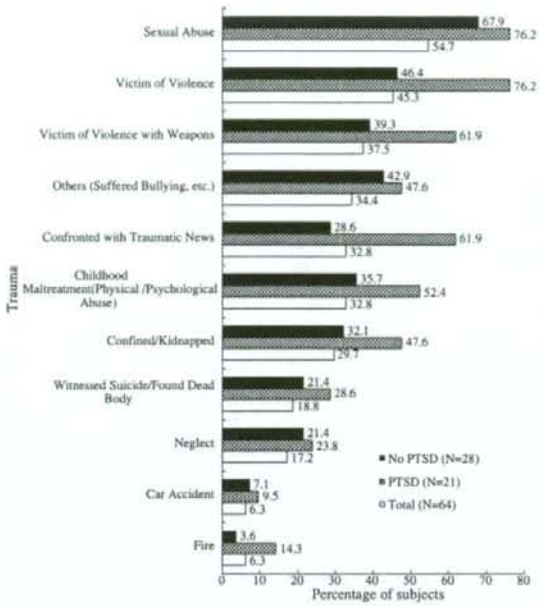


**Figure 1** Trauma exposure of female offenders with and without PTSD for each trauma type. Overall, 76.5% of the subjects experienced a traumatic event. Most of the participants were exposed to multiple types of trauma, with sexual abuse being the most frequently reported type of trauma (54.7%)

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

**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

**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: $\chi^2$ = 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

**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

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.

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Original Article

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

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**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 postmortem 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.

## 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.

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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.

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 $\chi^2$ 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 $\chi^2$ test) (Table 1), these variables were not included in the analysis as covariates.

### Neurons and glia

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

### CBP-IR neurons

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

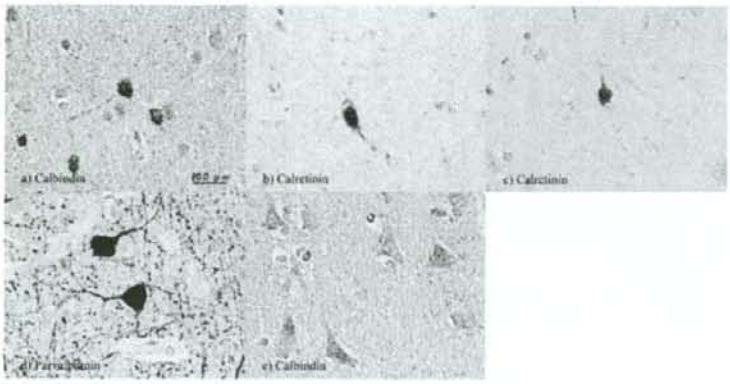


**Fig. 1** Cells labeled by immunoreactivity to calbindin in layer 2 (a), calretinin in layer 1 (b) and layer 3 (c), parvalbumin in layer 3 (d), and labeled by low immunoreactivity to calbindin in layer 3 (e) of control subjects (bar = 20 μm).

**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

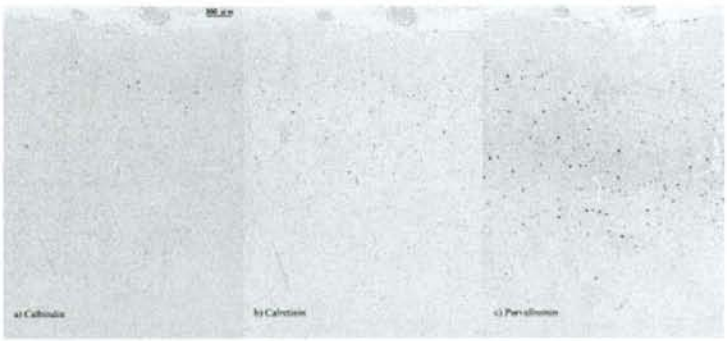

**Fig. 2** Composite image showing calcium-binding protein-immunoreactive neurons. The composite images were made up of a series of contiguous images obtained individually at ×4 magnification, that were merged to form a single large image. (bar = 200 μm)

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