表6　子どもに性教育をするケアワーカーのためのチェックリスト（塩田，2008）

□1．自分の性的指向を知っている（そのことで，罪悪感を持ったりしていない）。
□2．子どもの性的な悩み，疑問に援助したいという姿勢を見せることができる。子どもの性的指向を受け入れられる。
□3．性教育は，人の尊厳を守るための知識であるので，羞恥心なく肯定的に伝えられる。
□4．性の科学的な知識を大人と子どもとで共有できている。
□5．男らしさ女らしさにこだわらない声掛けをしている。
□6．性別は男と女だけで分けられない場合があると知っている。
□7．ケアワーカーが子どもにとって気になる異性の存在（刺激）にならない配慮をしている。服装などに十分な注意を払っている。
□8．死角はいつでもどこでもできると認識し，子どもがどこで何をしているのか常に把握できている。
□9．勝手に部屋（個室）に出入りしている子どもを止めている。
□10．子どもの年齢・性別・発達を考慮した上で，きれいに身体を洗うことを促している。自分で洗うべきプライベートパーツを教えている。
□11．幼児の頃から身体の細部の名称を教えている。
□12．子どもからの声に出せないサイン（性被害にあっている等）や言動の変化（性器いじり等）を受け止めようとしている。
□13．子ども同士の力関係を理解して介入している。
□14．子ども同士だけで入浴することを止めている。
□15．中高生のマスターベーションを肯定的に受け止めている。そのマナーについて教えている。
□16．子どもに，個でいられる力をつけることをよしとしている（常に集団行動を強制しない）。
□17．大人も子どもの境界内（身体と心）にむやみに配慮のない侵入をしないよう努力をしている（部屋に入るときはノックする・布団を勝手にめくらない・子どもの身体にむやみに触れない等）。また，子ども同士距離が取れるように支援している。
□18．施設で，男子同士の性被害の方が異性間の性被害より多いということを知っている。
□19．スキンシップ（身体接触）に頼らないケア技術を用いている。
□20．子どもたちだけでテレビをむやみに見せることをしない等，メディア・リテラシィー（情報を見極め選択する力）を獲得するための支援が行われている。
□21．中高生に幼児や低学年の子どもの世話を頼んでいない。
□22．レイプ被害や妊娠リスク発生時に，中容量ピルの処方など，対応を依頼できる婦人科医を知っている。
□23．性被害にあった子どもに対して嫌悪感を持たない。その子にも非がある等と責めない。
□24．性虐待の加害児に対し，嫌悪感を持たない。被害者であったかもしれないという視点を持つことができる。これからも支援していこうとする姿勢を持っている。
□25．年齢にあったさまざまな性教育の本（性の絵本）が施設内に適切に用意されており，子どもたちが読める，あるいはケアワーカーが適切なコメントとともに，読んであげられる工夫がなされている。
□26．性被害・加害から子どもを守らなくてはならないと，ケアワーカー同士，常に確認しあっている。
□27．大人と子どもの関係より子ども間の関係の方が強いという状況が生じていない。

ルノビデオを子どもたちを集めて見せながら，年少の男女を裸にしてビデオと同じことをさせるという加害を週に1回，数年間続けていたことが，その児童の開示によって明らかになった。このような状況を止めるものは，文化の変容以外にはない。したがって，性教育は非常に重要な意味を持つ。またメディア・リテラシーの問題も重要である。同じくケアテーカーとして長く働く高山由美子は，このマニュアルの中で，子どもたちの読んでいる雑誌や漫画を一緒に読み，子どもとドラマを一緒に見ることも重要な支援であることを強調している。子どもたちが手に入れる性についての情報の多くはこういったメディアからである。しかし多くの情報は間違ったものであることが多い。児童養護施設は子どもとケアワーカーが一緒にテレビを見ることのできる環境が整っている。子どもと一緒にテレビを見て，それをきっかけにコミュニケーションをしながら，正しい情報を選択できる力を育てていかなくてはならないのである。

2．子ども虐待のケアに関わる普遍的問題

子ども虐待によって生じる問題を発達精神病理

学的にまとめると，愛着の形成不全と慢性のトラウマである。この修復のためには，愛着を提供できる対象が存在することが必要不可欠であるが，この課題もまた，特に大舎制の児童養護施設においては，慢性的な人手不足の中で極端に難しい課題となっている。さらに愛着を形成する要素とは，感覚的で情動的な記憶である。虐待を受けた子どもにおいても親や養育者に愛着を持たずに生きることはできない。虐待した親の発する声，臭い，色，音，さらには叩かれた時の感触，恐怖と痺れなどが虐待した親との間の歪んだ愛着を形成する要素となってしまう。たとえ理想的なグループホームの小舎制養護施設に入所している子どもであっても，一度出来上がっているこういった歪んだ愛着（虐待的絆と呼ばれる）によるさまざまな歪んだ行動が，ケアの過程で問題行動として噴き出してくることが避けられない。被虐待児の対人関係はゼロからではなく，マイナスからの出発である。愛情が注がれればそれだけ，逆に問題が噴出するというパターンになるのである。性的虐待の場合には，虐待による侵襲性が非常に高く，また性を用いた力の支配という形になるため，虐待的絆がことさら形成されやすいことも知っておく必要があるだろう。特に性的な被害は嫌悪と同時に性感帯の興奮や快感が同時にもたらされるために，複雑な後遺症を作ってしまう。性的虐待順応症候群と呼ばれる現象がある。これは性的虐待の開示を行った子どもがすぐさま証言を翻し，加虐者の養護に走る行動である。性的虐待のケアとは，この絡み合った虐待的な絆を健康な愛着に塗り替えていく作業である。

またこのような愛着障害から始まり，解離や非行に至る過程が，情緒的な問題を越えて，脳の機能障害や器質的な変化まで生じるということについては，認識しておいた方が良いと思う（杉山，2007）。広汎性発達障害を精神療法だけで治療するという発想に大きな無理があると同じく，被虐待児に２週間に１時間の心理治療で治療ができるなどという幻想をケアする側が持つべきではない。

３．施設内性的虐待への個別的ケア

性的虐待では，性的な被害が嫌悪や苦痛と同時に，興奮や快感を引き起こすため，被害児は大きな混乱に陥ってしまう。また性器への性交や肛門性交，口腔性交などはまさに侵入される経験となるため，自分が汚れてしまったという汚辱感を伴うことが多い。この体験がそのままフラッシュバックの頻発につながり，解離による意識の断裂やスイッチング（突然に切れるなど，人柄がコロコロ変わる現象）に結びつきやすい。解離とは，辛く統合ができない体験に生じやすい。強いトラウマをきっかけに生じるこのような反応は，常識的に理解できる内容を突き抜けたものを含んでいるので，なぜそのような反応や行動が生じるのか，子ども自身に理解できないままに，さまざまな反応や攻撃的衝動的行動が噴出し，さらに各々の体験は解離が起きてしまって記憶が飛んでしまう状況になる。このため，性的なトラウマがどのような作用を人の心と対人関係に及ぼすのかという内容に関して，子どもに学んでもらう作業が必要となる。用いやすいのは「性的虐待を生きる力に変えて（明石書店）」のシリーズである。この本を，治療を担当する心理士と子どもが一緒に読む作業を通して，性的な被害が何をもたらすのか，子どもたちに学んでもらうことは重要な個別ケアである。

被虐待児は，他者の接近によって緊張と恐怖が生じ，場合によっては解離に入ってしまう。その一方で，ある距離を超えたら逆に接近した他者に抱きついてしまうこともある。このように他者の接近によって対人関係の病理が露呈される。意識が変容を起こさない，あるいは心臓がバクバクしない対人的な距離はどのあたりなのか治療者との間で学ぶ必要がある。常に人に抱きつきたくなる衝動に対しては，例えば「腕一本の距離を保つようにする」など具体的な設定を行い練習する。もう一つ重要な課題は衝動コントロールの技術である。生活の中でパニックになりそうなとき，じっと着席できなくなったとき，攻撃的な衝動や自己破壊的な行動が噴出しそうなときに，いかに自分

をクールダウンさせるのかという方法を，指導員とともに練習する。例えば次のような手順である。靴を脱ぎ裸足の足裏を床につける。深呼吸を3回繰り返す。見えるものを5つ挙げてみる。聞こえる音を同じく5つ数える。再度見えるものを5つ数える。それでも駄目なら水を飲む。アメをしゃぶる。さらには頓服を服用するなどなど。

これら一連の心理教育は，自己の感情への気づきが進む過程でもある。子どもたちに対して，自己の感情に絶えず注意するよう促し，対象化が可能となるよう努めることが重要である。感情の把握が非常に困難な子どもやその親には，一枚にいくつもの感情表出が絵で描かれた感情カードを用いて，自分にぴったりする気持ちの絵に指さしをすることで感情の把握が可能になるように計ることも必要である。グループで，あるいは個別に行う心理教育について詳細に紹介する紙数はない。これもマニュアルを参照してほしい。

性的虐待はネグレクトなどそれ以外の虐待と一緒に生じることが多く，先に述べたように解離性障害を引き起こしやすい。未治療の性的被虐待児がある年齢から先のことをごっそり覚えていないというのは珍しくない。自分の歴史を少しずつたどり，その時の事実と自分の感じたことを振り返る作業は，性的虐待という重症の心の傷を専門的にケアしてゆく時に次のステップとなる。この中で，トラウマが明確になってきた場合には，トラウマそのものに切り込まない限り，治療はなかなか進展しない。トラウマへの直面化がないと，治療が深まったと思った時に，フラッシュバックが体験を吹き飛ばし，堂々巡りに陥るという状態になる。トラウマへの治療の中で徐々に解離反応なしで，虐待場面への直面化が可能となってくる。ここで新たな性的被害の開示が続いて起きることも希ではない。このような過程を通して，トラウマが健康な愛着へと塗り替えられる作業が果たされていくのである（杉山ら，2008）。

被虐待児のケアは，薬物療法を併用した方が楽にできる。被虐待児に頻回に見られる問題行動の噴出の背後には，解離を伴った過覚醒がある。絶えず苛々し，些細な刺激からフラッシュバックを生じ，挑発と喧嘩を繰り返す。これらの症状は薬の服用である程度軽減させることができる。児童精神科医が近隣にいれば良いが，いない場合には成人の精神科医に依頼するしかない。一般に児童は，被虐待児といえどもごく少量の薬物から少しずつ適容量を決めるという作業をしなくてはならず，問題になるのは，しばしば成人精神科医が成人の統合失調症に用いるのと同じようにどんと大量を処方してしまう場合である。

V　おわりに

問題は，なぜわが国において社会的養護が旧態依然のまま取り残されたのかということである。子どもを社会でそだてることに対する，社会的な関心が乏しかったということに尽きるのではないか。人手がなくプライバシーも保障されず，家庭から保護された後に，さまざまな被害を保護された子どもたちが受けているこの状況は，国を挙げてのネグレクトという他はない。個々のケアワーカーの苦闘にも関わらず，今日の児童養護施設は先の資料に示すごとく，十全な子育てにははるかに遠い。この現状の中で，ケアを実践していこうとすれば，一人一人を大切にする施設文化の復活以外に方法はないと思う。

子ども虐待の報道は絶え間なく続いているのに，児童養護施設のこのような危機的な状況を知るものは，本当に限られている。日本の精神医学の開祖呉秀三は，1917年，精神病者が自宅に軟禁されている当時の日本の状況を「我が邦十何万の精神病者は実にこの病を受けたる不幸の他に，この邦に産まれたるの不幸を重ねるものと言うべし」と述べた。この全く同じことを，ネグレクト状態におかれている社会的養護の中の児童に言わざるをえない。「我が邦数万の被虐待児は，家庭に恵まれなかったという不幸の他に，この邦に生まれた不幸を重ねるものと言わざるべからざるなり」と。

この研究は厚生労働科学研究費補助金「子どもの心の診療に関する診療体制確保，専門的人材育成に関する研究」（主任研究者奥山眞紀子）の分担研究として行われた。

文　献

Hodges, J. & Tizard, B. (1989a) IQ and behavioural adjustment of ex-institutional adolescents. Journal of Child Psychology and Psychiatry, 30 ; 53-75.

Hodges, J. & Tizard, B. (1989b) Social and family relationships of ex-institutional adolescents. Journal of Child Psychology and Psychiatry, 30 ; 77-97.

久保田まり（2006）愛着研究はどのように進んできたか．そだちの科学，7 ; 2-10.

森田ゆり（2008）子どもの性的虐待．岩波新書．

Roy, P., Rutter, M. & Pickles, A. (2000) Institutional care : Risk from family background or pattern of rearing? Journal of Child Psychology and Psychiatry, 41 ; 139-149.

Rutter, M., Kreppner, J., Croft, C. et al. (2007) Early adolescent outcomes of institutionally deprived and non-deprived adoptees : III. Quasi-autism. Journal of Child Psychology and Psychiatry, 48 ; 1200-1207.

杉山登志郎（2007）子ども虐待という第四の発達障害．学研．

杉山登志郎，海野千畝子（2007）子ども虐待による解離性障害への治療．精神療法，33 ; 157-163.

杉山登志郎，海野千畝子，藤澤陽子，他（2008）児童養護施設における性虐待対応マニュアル．児童虐待等の子どもの被害及び子どもの問題行動の予防・介入・ケアに関する研究（主任研究者奥山眞紀子）H17-19年度包括報告書．

杉山登志郎（2009）子ども虐待への包括的ケア――医療機関を核とした子どもと親への治療――．子どもの虐待とネグレクト，11 ; 6-18.

Tizard, B. & Hodges, J. (1978) The effect of early institutional rearing on the development of eight year old children. Journal of Child Psychology and Psychiatry, 19 ; 99-118.

海野千畝子，杉山登志郎（2007）児童養護施設の施設内性的虐待への対応．小児の精神と神経，47 ; 273-279.

Vorria, P., Rutter, M., Pickles, A. et al. (1998a) A comparative study of Greek children in long-term residential group care and in two-parent families : I. Social, emotional, and behavioural differences. Journal of Child Psychology and Psychiatry, 39 ; 225-236.

Vorria, P., Rutter, M., Pickles, A. et al. (1998b) A comparative study of Greek children in long-term residential group care and in two-parent families : II. Possible mediating mechanisms. Journal of Child Psychology and Psychiatry, 39 ; 237-245.

Yang, M., Ullrich, S., Roberts, A. et al. (2007) Childhood institutional care and personality disorder traits in adulthood : Findings from the British national surveys of psychiatric morbidity. American Journal of Orthopsychiatry, 77 ; 67-75.

ORIGINAL ARTICLE

Brain Serotonin and Dopamine Transporter Bindings in Adults With High-Functioning Autism

Kazuhiko Nakamura, MD, PhD; Yoshimoto Sekine, MD, PhD; Yasuomi Ouchi, MD, PhD; Masatsugu Tsujii, MA; Etsuji Yoshikawa, BS; Masami Futatsubashi, BS; Kenji J. Tsuchiya, MD, PhD; Genichi Sugihara, MD, PhD; Yasuhide Iwata, MD, PhD; Katsuaki Suzuki, MD, PhD; Hideo Matsuzaki, MD, PhD; Shiro Suda, MD, PhD; Toshiro Sugiyama, MD, PhD; Nori Takei, MD, PhD; Norio Mori, MD, PhD

Context: Autism is a neurodevelopmental disorder that is characterized by repetitive and/or obsessive interests and behavior and by deficits in sociability and communication. Although its neurobiological underpinnings are postulated to lie in abnormalities of the serotoninergic and dopaminergic systems, the details remain unknown.

Objective: To determine the occurrence of changes in the binding of serotonin and dopamine transporters, which are highly selective markers for their respective neuronal systems.

Design: Using positron emission tomography, we measured the binding of brain serotonin and dopamine transporters in each individual with the radioligands carbon 11 (^{11}C)–labeled *trans*-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+) McN-5652) and 2β-carbomethoxy-3-β-(4-fluorophenyl) tropane ([^{11}C]WIN-35,428), respectively. Statistical parametric mapping was used for between-subject analysis and within-subject correlation analysis with respect to clinical variables.

Setting: Participants recruited from the community.

Participants: Twenty men (age range, 18-26 years; mean [SD] IQ, 99.3 [18.1]) with autism and 20 age- and IQ-matched control subjects.

Results: Serotonin transporter binding was significantly lower throughout the brain in autistic individuals compared with controls ($P<.05$, corrected). Specifically, the reduction in the anterior and posterior cingulate cortices was associated with the impairment of social cognition in the autistic subjects ($P<.05$, corrected). A significant correlation was also found between repetitive and/or obsessive behavior and interests and the reduction of serotonin transporter binding in the thalamus ($P<.05$, corrected). In contrast, the dopamine transporter binding was significantly higher in the orbitofrontal cortex of the autistic group ($P<.05$, corrected in voxelwise analysis). In the orbitofrontal cortex, the dopamine transporter binding was significantly inversely correlated with serotonin transporter binding ($r=-0.61$; $P=.004$).

Conclusions: The brains of autistic individuals have abnormalities in both serotonin transporter and dopamine transporter binding. The present findings indicate that the gross abnormalities in these neurotransmitter systems may underpin the neurophysiologic mechanism of autism. Our sample was not characteristic or representative of a typical sample of adults with autism in the community.

Arch Gen Psychiatry. 2010;67(1):59-68

AUTISM IS A PERVASIVE DEVELopmental disorder that is characterized by the behavioral traits of impaired social cognition and communication, and repetitive and/or obsessive behavior and interests.[1] There is no established treatment or cure for the disorder. Recent population-based surveys showing that autism is more common than previously believed have aroused serious public concern worldwide.[2] In addition, genome-wide linkage scans and copy-number analyses have revealed "hot spots" on several chromosomes.[3-5] To clarify the pathophysiologic mechanism of autism, the neuroimaging approach is a fruitful method. In this study, we used positron emission tomography (PET) to focus on neurotransmitter alterations in the autistic brain.

A wide array of transmitter systems has also been studied with respect to autism. Initial studies on the pathophysiologic mechanism of autism have focused on the serotoninergic system. Prior studies consistently found elevated serotonin levels in the whole blood cells and platelets of patients with autism[6-10] and their relatives.[11-13] Short-term dietary depletion of tryptophan (ie, the serotonin precursor) has

Author Affiliations are listed at the end of this article.

Table 1. Clinical Characteristics

Characteristics	Controls (n=20)		Autistic Participants (n=20)	
	Mean (SD)	Range	Mean (SD)	Range
Age, y	21.9 (2.0)	18-26	21.2 (2.0)	18-26
WAIS-R score	104.6 (15.2)	80-136	99.3 (18.1)	71-140
Faux Pas Test score[a]	35.5 (2.9)	31-40	24.6 (6.6)[b]	8-34
Y-BOCS score[c]	NA	NA	10.5 (4.3)	2-20
17-Item HAM-A score[c]	NA	NA	3.7 (2.6)	0-8
17-Item HAM-D score[c]	NA	NA	2.1 (2.2)	0-6
AQ score[c]	NA	NA	50.2 (12.5)	34-69

Abbreviations: AQ, Aggression Questionnaire; HAM-A, Hamilton Anxiety Scale; HAM-D, Hamilton Scale for Depression; NA, not applicable; WAIS-R, Wechsler Adult Intelligence Scale–Revised; Y-BOCS, Yale-Brown Obsessive Compulsive Scale.

[a] Lower scores correspond to a poorer social cognitive ability.

[b] $P<.001$ (unpaired, 2-tailed t test).

[c] Higher scores represent severe symptoms. The range of scores for the Y-BOCS is 0 to 40.

been shown to exacerbate repetitive behavior and to elevate anxiety and feelings of unhappiness in autistic adults.[14] Conversely, treatment with selective serotonin reuptake inhibitors—commonly used antidepressants—has been shown to be effective in ameliorating the repetitive and/or obsessive behavior and interests in some but not all autistic individuals.[15] Genetic studies have yielded evidence of a critical role for the serotonin transporter gene (*SLC6A4*; OMIM 182138), which is located on chromosome 17q11.[5,16] Several *SLC6A4* polymorphisms have been found to be associated with autism.[17,18] Furthermore, *SLC6A4* promoter polymorphisms may influence the gray matter volume of cerebral cortical structures in young male autistic individuals.[19] It has also been shown that *SLC6A4* modulates the function of social brain systems when healthy control subjects process facial emotions.[20] Neuroimaging studies with PET have provided further evidence that the levels of serotonin synthesis in autistic children aged 2 to 5 years are significantly lower than those in control children.[21,22] A recent single-photon emission computed tomography study has shown that autistic children, under light sedation, have a reduction in serotonin transporter binding in the medial frontal cortex, midbrain, and temporal lobe areas.[23]

Interest in the role of dopamine has been stimulated by the observations that dopamine blockers (ie, antipsychotics) are effective in treating some aspects of autism, such as hyperactivity, aggression, and self-injury.[24,25] In addition, some direct evidence suggests that levels of the principal dopamine metabolite homovanillic acid are elevated in the cerebrospinal fluid of autistic individuals,[26] although this has not been consistently reported.[27] Previous genetic studies have demonstrated that the prevalence of the *A1* allele of the dopamine D_2 receptor is significantly increased in autism,[28] whereas the dopamine D_1 receptor gene may be a risk gene for core symptoms of autism in male-only affected sibling-pair families.[29] Furthermore, it has been suggested that the 9- and 10-repeat alleles of the dopamine transporter may be associated with hyperactivity, impulsivity, social anxiety, and tic symptoms in autistic children.[30] In a PET study of autistic children, low levels of medial prefrontal dopaminergic activity were observed under anesthesia,[31] whereas increased dopamine D_2 receptor binding in the whole caudate and putamen has also been demonstrated.[32] These findings suggest that the alteration of both the serotonin and the dopamine systems is a feature of autism, although these findings remain equivocal and inconclusive.

Taking these results together, we hypothesized that alterations in both the serotoninergic and the dopaminergic systems exist in the brain of autistic individuals, and that the changes are associated with the clinical features of autism. To examine this hypothesis, we used PET to measure the binding of the serotonin and dopamine transporters, which are highly selective markers for their respective neuronal systems, in adults with high-functioning autism. We also examined the relationships between some of the clinical symptoms of autism and the binding levels of both transporters.

METHODS

SUBJECTS

Twenty men with autism (mean [SD] age, 21.2 [2.0] years; age range, 18-26 years) and 20 healthy male controls (mean [SD] age, 21.9 [2.0] years; age range, 18-26 years) participated in this study. All participants were right-handed and had an IQ of greater than 70 (estimated using the Wechsler Adult Intelligence Scale–Revised). The IQ did not differ significantly between the 2 groups (mean [SD], 99.3 [18.1] for the autistic group and 104.6 [15.2] for the control group; $P=.30$) (**Table 1**). An autism diagnosis was based on the following: the *DSM-IV-TR*[1]; the Autism Diagnostic Interview–Revised[33]; and the Autism Diagnostic Observation Schedule–Generic.[34] All of the autistic individuals and controls underwent screening to exclude comorbid psychiatric illnesses (ie, schizophrenia, affective disorders, mental retardation, and personality or behavioral disorders) by means of the Structured Clinical Interview for the *DSM-IV*.[35] Individuals with a history of neurological disorders (eg, epilepsy or head injury) or genetic disorders (eg, fragile X syndrome or tuberous sclerosis) were also excluded. In addition, controls were excluded if they had a family history of psychiatric illness, measured using the Family History Research Diagnostic Criteria.[36] All autistic participants were drug naive. The present study was approved by the local ethics committees. Written informed consent was obtained from each of the participants.

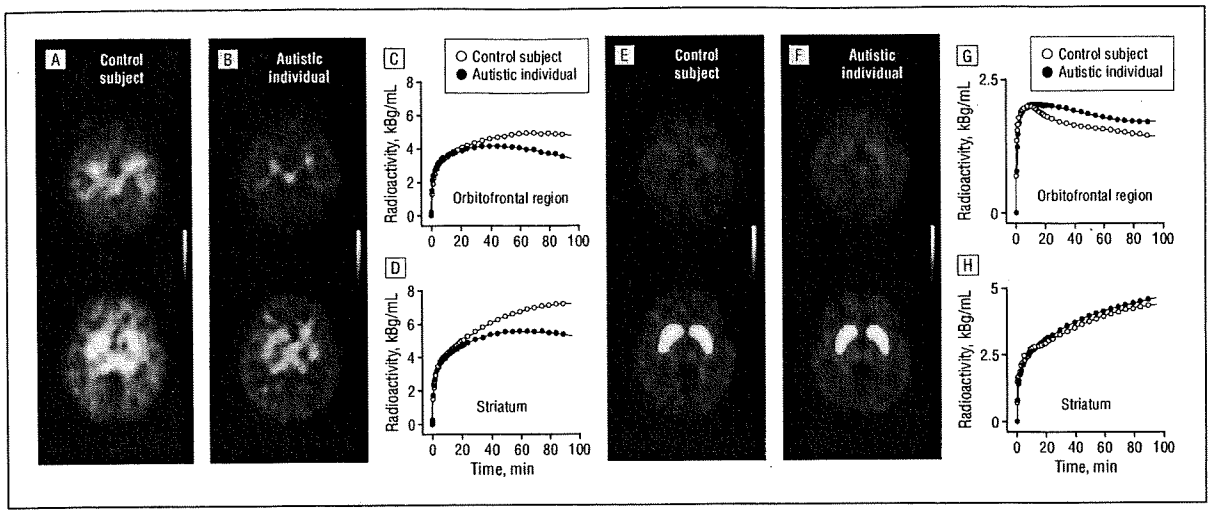

Figure 1. Positron emission tomography images of radioactive carbon (^{11}C)–labeled *trans*-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl[pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652) and 2β-carbomethoxy-3-β-(4-fluorophenyl)tropane ([^{11}C]WIN-35,428) binding in a healthy control subject and an individual with autism. A and B, Images of the [^{11}C](+)McN-5652 distribution volume with a color scale ranging from 0 to 60 mL/g show a control brain and a global reduction in [^{11}C](+)McN-5652 distribution in an autistic individual. C and D, Radioactivity produced by [^{11}C](+)McN-5652 in the orbitofrontal region and the striatum of a representative control and an autistic subject. E and F, Images of the [^{11}C]WIN-35,428 ratio index reflect the binding potential of [^{11}C]WIN-35,428 with a color scale ranging from 0 to 10 compared with a control and the elevation of its value in the orbitofrontal cortex in an autistic subject. G and H, Radioactivity caused by [^{11}C]WIN-35,428 in the orbitofrontal region and the striatum of a representative control and an autistic subject. To convert radioactivity to curies per milliliter, multiply by 2.7 $\times 10^{-8}$.

CLINICAL ASSESSMENTS

To assess social cognitive ability, we used the Faux Pas Test.[37-39] A low score on this test indicates poor social cognition. This test is appropriate for the measurement of theory-of-mind impairment at a higher level. To evaluate the degree of repetitive and/or obsessive behavior and interests, we used the Yale-Brown Obsessive Compulsive Scale (Y-BOCS).[40,41] We also assessed anxiety and depressive symptoms using the 17-item Hamilton Anxiety Scale (HAM-A)[42] and the 17-item Hamilton Scale for Depression (HAM-D),[43] respectively. Aggression was evaluated using the Aggression Questionnaire (AQ).[44] These evaluations were performed on the day of the PET examination with radioactive carbon (^{11}C)–labeled *trans*-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ([^{11}C](+)McN-5652).

IMAGING PROCEDURES AND DATA ANALYSIS

All participants underwent 3-dimensional magnetic resonance imaging (MRI) with a static magnet (MRP7000AD; Hitachi, Tokyo) just before the PET measurement. The MRI and PET examinations were performed under sedation-free conditions. The PET scans were conducted with a high-resolution brain-purpose unit (SHR12000; Hamamatsu Photonics K.K.). The MRI measurements and a mobile PET gantry allowed us to reconstruct PET images parallel to the anterior-posterior intercommissural line without resectioning. Using this approach, we were able to allocate a region of interest (ROI) to the target area of the original PET image. In quantitative PET brain imaging, the partial volume effect is an important degrading factor.[45,46] To reduce the partial volume effect, we set ROIs on the MRIs and transferred them onto PET images as described elsewhere.[47,48] Participants in both groups underwent 38 serial PET scans during a period of 92 minutes with periodic arterial blood sampling after an intravenous injection of [^{11}C](+)McN-5652 to determine their serotonin transporter binding.[49,50] The reproducibility of PET images with [^{11}C](+)McN-5652 was reported in *Papio anubis* baboons[51]; when the primates underwent scanning with [^{11}C](+)McN-5652 at 3- to 4-week intervals, good test-retest reliability was obtained. Accordingly, within 4 weeks of the initial PET scan, a second PET measurement with [^{11}C]-labeled 2β-carbomethoxy-3-β-(4-fluorophenyl)tropane ([^{11}C]WIN-35,428) was performed under the same protocol as in the [^{11}C](+)McN-5652 study to measure dopamine transporter binding.[52-54] As described previously,[49] we estimated [^{11}C](+)McN-5652 binding on the basis of a single-tissue–compartment 3-parameter model. Because the distribution volume of [^{11}C](+)McN-5652 estimated by this model correlated with the binding of the serotonin transporter in the brain,[49] we constructed parametric images of the [^{11}C](+)McN-5652 distribution volume for all participants with the use of biomedical imaging software (PMOD, version 2.5; PMOD Technologies Ltd, Zurich, Switzerland) (**Figure 1** A and B). Similarly, applying a 3-compartment 4-parameter model to the [^{11}C]WIN-35,428 data allowed us to estimate the binding potential of the tracer[47,53] to evaluate the dopamine transporter binding. This curve-fitting model cannot generate the distribution volume directly. In our voxelwise imaging analyses, we instead calculated the ratio index for subsequent use with statistical parametric mapping (SPM) software (SPM99; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, England). Because this binding potential has been shown to correlate well with the reference tissue-derived ratio index (ie, the ratio of the PET binding value in the target region to the PET binding value in the cerebellum in the late integrated image),[53] we constructed parametric images of the [^{11}C]WIN-35,428 ratio index (Figure 1E and F) for subsequent voxelwise analysis. These voxelwise image analyses of the serotonin and dopamine transporter binding were conducted using the SPM software.[49,53]

STATISTICAL ANALYSIS

Demographic and clinical variables were compared between the autistic and control groups using the *t* test, in which a 2-tailed α level of .05 was set as the level of significance (SPSS software, version 11.0J; SPSS Japan Inc, Tokyo). In the SPM analysis, voxel-

Table 2. Results of the Whole-Brain Voxel-Based Statistical Parametric Mapping Analyses of [^{11}C](+)McN-5652 and [^{11}C]WIN-35,428 Binding Parameters[a]

Brain Area	Coordinates			Voxel Level	
	x	y	z	Corrected *P* Value	*z* Score
	[^{11}C](+)McN-5652 Binding				
Decrease in binding in the autistic vs control groups					
Frontal region					
Left middle frontal cortex, BA 6	−52	10	48	<.001	6.86
Left superior frontal cortex, BA 10	−16	54	−4	<.001	5.93
Left medial frontal cortex, BA 10	−12	46	10	<.001	5.82
Left middle frontal cortex, BA 11	−30	38	−18	<.001	5.63
Right superior frontal cortex, BA 10	18	70	−2	.001	5.40
Right medial frontal cortex, BA 25	6	26	−18	.002	5.23
Left inferior frontal cortex; BA 47	−34	32	−6	.002	5.19
Right superior frontal cortex, BA 11	20	52	−14	.004	5.11
Left subcallosal cortex, BA 25	−4	8	−12	.005	5.05
Temporal region					
Left superior temporal cortex, BA 22	−68	−6	6	<.001	6.54
Right inferior temporal cortex, BA 20	38	−8	−44	<.001	6.53
Left middle temporal cortices, BA 21	−70	−12	−8	<.001	6.20
Right inferior temporal cortex, BA 20	48	−22	−32	<.001	6.01
Left inferior temporal cortex, BA 37	−58	−56	−8	.001	5.48
Left fusiform cortex, BA 37	−34	−54	−12	.001	5.39
Right superior temporal cortex, BA 38	42	18	−20	.001	5.34
Left orbitofrontal cortex, BA 11	−6	40	−22	.002	5.21
Right orbitofrontal cortex, BA 11	8	44	−20	.003	5.19
Right superior temporal cortex, BA 22	50	4	2	.004	5.11
Right middle temporal cortex, BA 21	56	0	−12	.006	5.01
Right postcentral cortex, BA 5	44	−56	64	.006	5.00
Limbic region					
Left hippocampus	−28	−36	−6	<.001	6.02
Left parahippocampal cortex, BA 19	−40	−48	−4	<.001	5.70
Right anterior cingulate cortex, BA 24	2	26	8	.001	5.34
Right cingulate cortex, BA 23	6	−20	24	.004	5.11
Parietal region					
Left inferior parietal cortex, BA 40	−62	−46	−52	<.001	6.82
Right inferior parietal cortex, BA 39	46	−66	42	<.001	6.18
Right precuneus cortex, BA 19	10	−88	44	<.001	5.72
Left superior parietal cortex, BA 7	−30	−56	46	.002	5.22

(continued)

wise between-group comparisons were performed to investigate regional differences in the binding levels of [^{11}C](+)McN-5652 and [^{11}C]WIN-35,428. Correlation analyses were conducted between the 5 clinical behavior scores (Faux Pas Test, Y-BOCS, HAM-A, HAM-D, and AQ) and the total voxel analysis of the whole brain by using SPM analysis within the autistic group. To avert the risk of a type I error, the levels of statistical significance for the voxel and cluster analyses were set at $P<.05$ after allowing for multiple comparisons. In addition, we performed ROI analysis to examine whether regional serotonin and dopamine binding covaried in autistic individuals. Based on the results of the SPM analysis, we restricted the ROI analysis to the orbitofrontal area, where pronounced disturbances were present in the binding of serotonin and dopamine transporters (**Table 2**). In this analysis, the Pearson product moment correlation coefficient was computed. $P<.05$ was considered statistically significant.

RESULTS

The demographic and clinical variables of the participants are shown in Table 1. The mean Faux Pas Test score was significantly lower in the autistic participants than in the controls ($P<.001$).

COMPARISON OF SEROTONIN TRANSPORTER BINDING BETWEEN GROUPS

The SPM results showed significant reductions in the [^{11}C](+)McN-5652 distribution volume throughout the global brain in the autistic group compared with the control group ($P<.05$, corrected), with the reductions being most pronounced in the frontal, temporal, parietal, and occipital lobes; in the limbic and subcortical regions; and in the cerebellum (Table 2 and **Figure 2**A).

CORRELATES OF SEROTONIN TRANSPORTER WITH CLINICAL CHARACTERISTICS IN AUTISTIC PARTICIPANTS

The [^{11}C](+)McN-5652 distribution volume in the anterior cingulate cortex, the cingulate cortex, and the posterior cingulate cortex extending to the precuneus had a significantly positive correlation with the scores of the Faux Pas Test ($P<.05$, corrected) (Table 2 and Figure 2B).

Table 2. Results of the Whole-Brain Voxel-Based Statistical Parametric Mapping Analyses of [^{11}C](+)McN-5652 and [^{11}C]WIN-35,428 Binding Parameters[a] (continued)

Brain Area	Coordinates			Voxel Level	
	x	y	z	Corrected *P* Value	*z* Score
	[^{11}C](+)McN-5652 Binding				
Decrease in binding in the autistic vs control groups					
Occipital region					
Right lingual cortex, BA 18	30	−74	−6	<.001	6.02
Left inferior occipital cortex, BA 19	−32	−76	0	<.001	5.64
Left lingual cortex, BA 18	0	−74	−4	.001	5.50
Right occipital cortex, BA 19	50	−82	10	.006	4.98
Subcortical region					
Left claustrum	−28	10	−4	<.001	5.79
Right lentiform nucleus/putamen	23	3	7	<.001	5.59
Left midbrain	−2	−20	−8	<.001	5.52
Right thalamus	6	−18	0	<.001	5.46
Right midbrain	6	−18	−9	.001	5.44
Right caudate	16	−2	18	.001	5.43
Left lentiform nucleus/putamen	−26	−24	−2	.001	5.42
Left thalamus	−12	−16	2	.002	5.20
Left pons	−4	−12	−26	.002	5.18
Left midbrain	−12	−18	−12	.003	5.14
Left insula, BA 13	−30	20	4	.006	5.00
Cerebellum					
Left dentate nucleus	−20	−50	−34	<.001	5.81
Left lobule VIII	−24	−56	−42	<.001	5.62
Right lobule VIII	10	−68	−34	.001	5.51
Right lobule VI	18	−56	−30	.001	5.45
Right lobule VI	28	−52	−36	.003	5.18
Brain regions correlated with reduction in the Faux Pas Test score in autistic participants					
Right posterior cingulate cortex, BA 30	18	−50	16	<.001	7.61
Left posterior cingulate cortex, BA 30	−16	−54	12	<.001	6.91
Anterior cingulate cortex, BA 32	−8	40	16	<.001	6.61
Cingulate cortex, BA 24	−4	−19	40	<.001	6.44
Brain region correlated with an increase in the Y-BOCS score in autistic participants					
Thalamus	4	−25	3	<.001	6.96
	[^{11}C]WIN-35,428 Binding				
Increase in binding in the autistic vs control groups					
Orbitofrontal cortex, BA 11	−2	30	−10	.02	4.28

Abbreviations: BA, Brodmann area; [^{11}C](+)McN-5652, radioactive carbon (^{11}C)–labeled *trans*-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl[pyrrolo-[2,1-a]isoquinoline; [^{11}C]WIN-35,428, ^{11}C-labeled 2β-carbomethoxy-3-β-(4-fluorophenyl)tropane; Y-BOCS, Yale-Brown Obsessive Compulsive Scale.

[a]The significance thresholds at the voxel cluster levels were $P < .05$ after correction for multiple comparisons. Coordinates are given in millimeters based on the Talairach stereotaxic brain atlas. Each location is a peak within a cluster (defined as the voxel with the highest *z* score).

We also evaluated the degree of repetitive and/or obsessive behavior and interests, which are additional clinical features of autism, using the Y-BOCS. A higher Y-BOCS score signifies more severe symptoms. There was a significant negative correlation between the Y-BOCS scores and the distribution volume of [^{11}C] (+)McN-5652 in the thalamus extending to the parahippocampal region ($P < .05$, corrected) (Table 2 and Figure 2C).

No significant correlation was found between the [^{11}C] (+)McN-5652 distribution volume and the symptom profiles of the HAM-A, HAM-D, or AQ.

COMPARISON OF DOPAMINE TRANSPORTER DISTRIBUTION BETWEEN GROUPS

The SPM analysis revealed a significant increase in [^{11}C]-WIN-35,428 binding in the medial frontal region covering the orbitofrontal cortex in the autistic group compared with the control group ($P < .05$, corrected in voxel-level analysis) (Table 2 and Figure 2D).

No significant correlation was found between [^{11}C]-WIN-35,428 binding and the symptom profiles of the Faux Pas Test, Y-BOCS, HAM-A, HAM-D, or AQ.

CORRELATION BETWEEN SEROTONIN AND DOPAMINE TRANSPORTER BINDINGS

In the ROI analysis of the orbitofrontal cortex, which showed disturbances in [^{11}C](+)McN-5652 and [^{11}C]WIN-35,428 binding in the autistic group (Figure 1C, D, G, and H), the [^{11}C](+)McN-5652 distribution volumes were significantly negatively correlated with the [^{11}C]WIN-35,428 binding potentials of the autistic group (**Figure 3**) ($r = -0.61$; $P = .004$, according to Pearson product moment correlation coefficient).

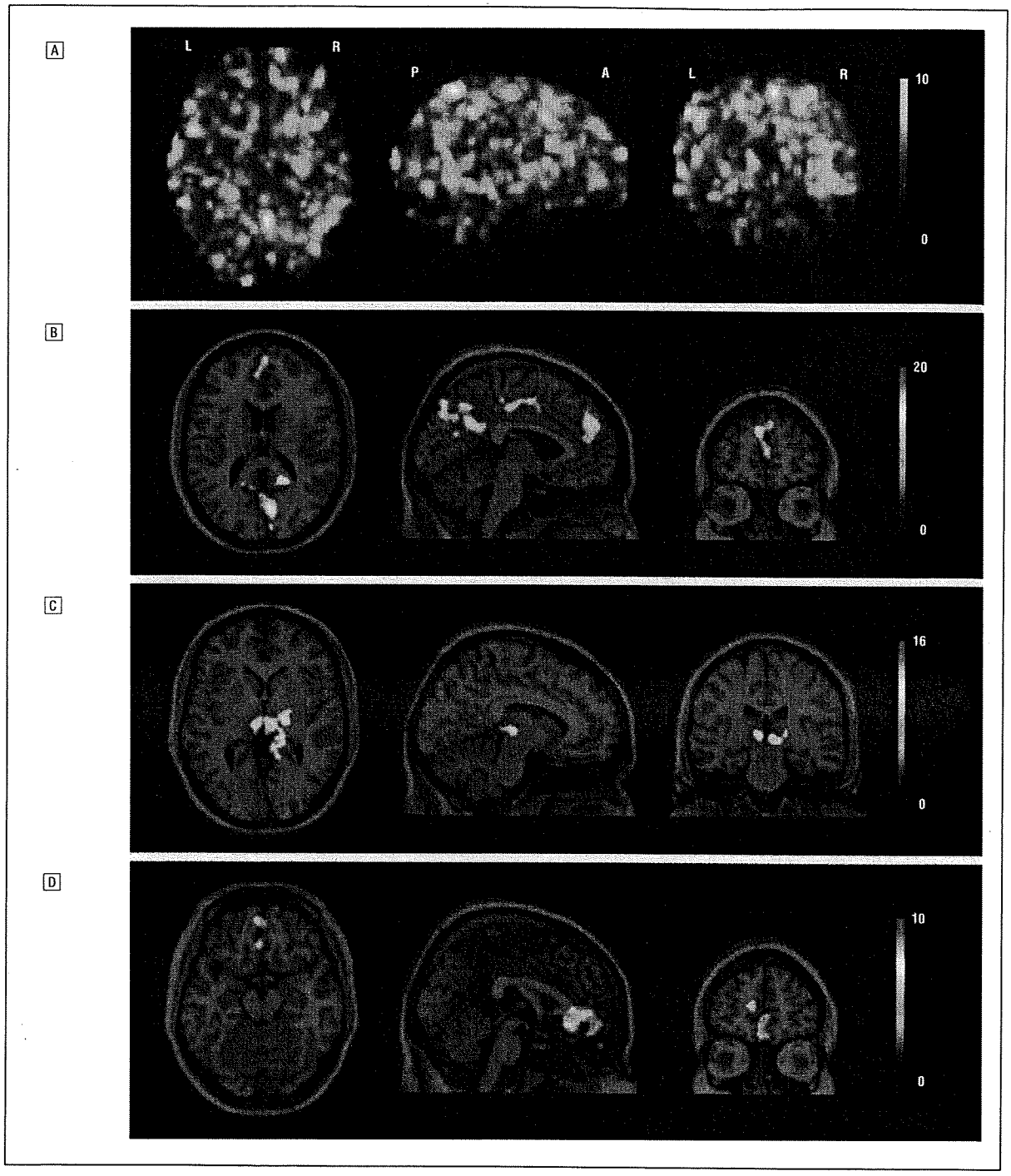

Figure 2. Statistical parametric mapping results for [^{11}C](+)McN-5652 and [^{11}C]WIN-35,428 binding. A, Glass brain images indicate extensive reduction in the [^{11}C](+)McN-5652 distribution volume in the autistic group ($P<.05$, corrected). B and C, Statistical parametric maps show brain regions in which the [^{11}C](+)McN-5652 distribution volume correlates positively with the Faux Pas Test score and negatively with the Yale-Brown Obsessive Compulsive Scale score, respectively, in autism ($P<.05$, corrected). D, A statistical parametric map showing a brain region in which the [^{11}C]WIN-35,428 ratio index is significantly higher in the autistic group than in the control group ($P<.05$, corrected). Color bars indicate T values. A indicates anterior; L, left; P, posterior; and R, right. See the legend to Figure 1 for expansion of other abbreviations.

COMMENT

The autistic participants had a significantly decreased [^{11}C](+)McN-5652 distribution volume throughout the brain, whereas they had a significantly increased [^{11}C]WIN-35,428 distribution volume in the medial region of the orbitofrontal cortex, compared with those of the controls. These results suggest the impairment of the function of the serotoninergic systems throughout the brain and the overfunctioning of the dopaminergic systems in the orbi-

tofrontal cortex of the autistic adults. However, the autistic participants studied herein are not a representative or a typical sample of the population of autistic individuals. We opted for autistic individuals with an IQ of greater than 70 in this study (ie, high-functioning individuals), although about 65% of autistic individuals are known to have an IQ of less than 70.[55] In addition, approximately 20% to 38% of autistic individuals are reported to have epilepsy.[56,57] However, in the present study, our autistic participants had no comorbidity, including epilepsy. Furthermore, our autistic participants were all drug naive. Therefore, our findings cannot be generalized to the entire population of autistic adults.

In the anterior and posterior cingulate cortices, where reduced serotonin transporter binding was noted in the autistic group, the magnitude of reduction was correlated with poor performance on the Faux Pas Test, which assesses social cognition ability. Our finding is in line with those of previous PET studies, which showed that reduced metabolism or blood flow in the cingulate cortices is associated with impairment of social cognition in autistic individuals.[58,59] Our finding is also supported by a study that used single-photon emission computed tomography and demonstrated that adults with Asperger syndrome, a clinical entity that is part of a spectrum of pervasive developmental disorders, exhibit a reduction in serotonin 2A receptor binding in the cingulate cortices and that this binding reduction is related to impaired social interaction.[60]

We also found that, in the autistic participants studied, the reduction in the serotonin transporter binding in the thalamus correlated with repetitive and/or obsessive behavior and interests as assessed by the Y-BOCS. This finding is compatible with previous studies that showed that the thalamus is the principal site for the accumulation of selective serotonin reuptake inhibitors,[61] which in turn ameliorate repetitive behaviors in some but not all autistic individuals.[15] In the present study, there was, however, no correlation in any of the other regions that have been implicated as responsible for repetitive behavior in individuals with obsessive-compulsive disorder (eg, the basal ganglia, frontal regions, and hippocampus). A prior hydrogen 1–labeled magnetic resonance spectroscopy study has shown that, in adults with Asperger syndrome, increased prefrontal *N*-acetylaspartate levels are positively correlated with obsessional behavior.[62] Furthermore, MRI studies of autistic adults have demonstrated enlargement of the caudate and putamen volumes, which is positively correlated with repetitive behaviors.[63] Repetitive behaviors have also been shown to be related to the hippocampus volume in obsessive-compulsive disorder.[64] In addition, individuals with autistic spectrum disorders were reported to have significantly higher concentrations of glutamate/glutamine and creatine/phosphocreatine in the amygdale-hippocampal region.[65] One possible explanation for the lack of correlations found in these regions (the basal ganglia, frontal regions, and hippocampus) is that impairments in the regions other than the thalamus, if any, could be accounted for by altered dysfunctions that are not related to disturbed serotonin transporter bindings per se. Nevertheless, further work is needed to determine whether the localized reduction in serotonin transporter binding in the thalamus is specific to repetitive and/or obsessive behavior and interests seen in adults with high-functioning autism.

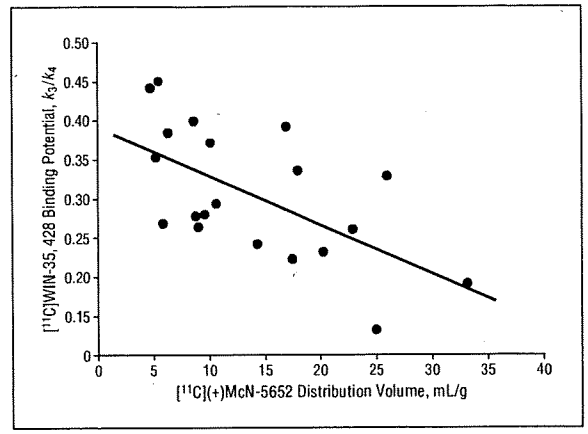

Figure 3. Correlation between [^{11}C](+)McN-5652 and [^{11}C]WIN-35,428 binding. Pearson product moment correlation analysis shows a significantly negative correlation between the [^{11}C](+)McN-5652 distribution volume and the [^{11}C]WIN-35,428 binding potential in the orbitofrontal cortex in autistic subjects (r=−0.61; P=.004; y=−0.006x+0.39). The k values represent the binding potential. See the legend to Figure 1 for other abbreviations.

Increases in peripheral serotonin levels have been the most consistent finding in autistic children.[6-10] High levels of peripheral serotonin are known to cause a loss of serotonin terminals during development, when serotonin transporters are located,[66-69] and this may happen in the brain as well. Therefore, we speculate that the reduction of serotonin transporter binding found in the brain of autistic adults in this study may stem from altered serotoninergic systems at the developmental stage. The *SLC6A* gene polymorphism has been associated with autism,[17,18] although other reports have not replicated these findings.[70,71] Because the gene polymorphism could modulate the neurodevelopment and function of the brain[19,20] and influence *SLC6A4* expression,[72,73] it may be responsible for the reduction of serotonin transporter binding that we observed in the present study.

Several limitations of our study bear mention. We repeated the SMP analysis separately for each of 5 clinical behaviors within the autistic participants, which may have led to a type I error. However, we found that 2 of the 5 clinical behaviors were correlated with the serotonin transporter bindings in particular brain regions, and, as discussed in the preceding paragraphs, these regions are considered to be critical and biologically plausible areas for involvement in these behaviors. Therefore, our results may not be attributable merely to type I error. Serotoninergic activity of the prefrontal cortical regions has been shown to correlate with aggressive behavior in humans.[74] Some autistic individuals were reported to have aggression.[75] In this context, we anticipated that our sample of autistic adults would show the relationship between reduced serotonin transporter binding and the degree of aggression. However, SPM analysis did not reveal any brain regions in which the reduced binding correlated with aggression as assessed by the AQ. This negative finding in the present study may have been because we recruited adults with high-functioning autism who were coopera-

tive with the imaging procedures. We showed correlates of alterations in the serotonin transporter binding with clinical features. Causative inference cannot be based merely on such correlations. Therefore, our findings cannot be considered conclusive. To elucidate the direct causal relationship between altered serotonin transporter binding and autism, further studies will be needed. Finally, the present study was limited by its small sample size and lack of female participants.

Dopamine transporter binding was significantly and locally increased in the medial region of the orbitofrontal cortex in our autistic participants. Our finding of overfunctioning in the dopaminergic system is compatible with previous PET studies, which showed increased striatal dopamine D_2 receptor binding in autistic children[32] and elevated dopamine synthesis and storage in the striatum and frontal cortex of adults with Asperger syndrome.[76] The orbitofrontal cortex is a key structure in the network underlying emotional regulation; dysfunction in the orbitofrontal-limbic circuit may be associated with behaviors in autism,[77] such as impulsive and aggressive behaviors.[75,78] However, the increased dopamine transporter binding was not correlated with aggression as assessed by the AQ in the present study. As mentioned in the preceding paragraphs, this may have been due to a bias arising from the selection of individuals with high-functioning autism in the present study, who are more cooperative with the PET imaging procedures than are autistic individuals as a whole. Thus, more work is needed in this regard.

When the relationship between dopamine and serotonin transporter binding was examined in our autistic participants, the dopamine transporter binding was significantly negatively correlated with that of the serotonin transporter. The mechanism underlying the interaction between the 2 transporters in the orbitofrontal region in autism is still unknown. However, some animal studies have illustrated that the number of dopaminergic neuron fibers increases in response to disruption of the serotoninergic system by a lesion in the nucleus raphe[79] and that the uptake of serotonin into dopamine neurons takes place by means of dopamine transporters.[80]

With respect to our use of [^{11}C]WIN-35,428 to evaluate dopamine transporter binding in the orbitofrontal cortex, a methodological issue should be addressed. The capability of the tracer for measuring low levels of dopamine transporter binding in the extrastriatal region is disputable. In the present study, we conducted 2 types of analytic procedures (ie, ROI method and SPM analysis) to estimate quantitative values of the orbitofrontal binding and to detect brain regions with significant changes. The difference in the shape of the time-activity curve of the orbitofrontal cortex between the groups (Figure 1G and H) and a series of our previous studies that have reported significant changes in the extrastriatal dopamine transporter binding[48,81-83] indicate the validity for the use of [^{11}C]WIN-35,428 for the purpose of the present study. This contention is also supported by our findings that the level of the orbitofrontal binding potential is higher in autistic individuals (0.27, based on our present data) than in their normal counterparts (0.19)[48] and that the magnitude of this increase (58%) is greater than the reported level of within-subject test-retest variability (9.3%).[84] Despite these accounts, a PET tracer with a much higher affinity to the extrastriatal dopamine transporter may be desirable.

Submitted for Publication: November 8, 2008; final revision received March 26, 2009; accepted April 27, 2009.
Author Affiliations: Department of Psychiatry and Neurology (Drs Nakamura, Sekine, Iwata, Suzuki, Suda, and Mori), Laboratory of Human Imaging Research, Molecular Imaging Frontier Research Center (Dr Ouchi), and Osaka-Hamamatsu Joint Research Center for Child Mental Development (Mr Tsujii and Drs Tsuchiya, Sugihara, Matsuzaki, Takei, and Mori), Hamamatsu University School of Medicine, Positron Medical Center, Hamamatsu Medical Center (Dr Ouchi), and Central Research Laboratory, Hamamatsu Photonics K.K. (Messrs Yoshikawa and Futatsubashi), Hamamatsu, Japan; Faculty of Sociology, Chukyo University, Toyota, Japan (Mr Tsujii); and Aichi Children's Health and Medical Center, Obu, Japan (Dr Sugiyama).
Correspondence: Norio Mori, MD, PhD, Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, Shizuoka 431-3192, Japan (morin@hama-med.ac.jp).
Financial Disclosure: None reported.
Funding/Support: This study was supported by Special Expenses for Educational Research to Osaka-Hamamatsu Joint Research Center for Child Mental Development (Osaka University and Hamamatsu University School of Medicine) and a Grant-in-Aid for Scientific Research (B) (Dr Nakamura) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; the Research on Brain Science Fund (Dr Mori) from the Ministry of Health, Labor, and Welfare, Japan; by Takeda Science Foundation (Dr Nakamura); and by the Kato Memorial Trust For Nambyo Research (Dr Nakamura).
Additional Contributions: Toshihiko Kanno, BS, Yutaka Naito, MS, Katsuhiko Nishimura, MD, PhD, Kiyokazu Takebayashi, MD, PhD, and Yoshifumi Takai, MA, provided excellent technical support. Masayoshi Kawai, MD, PhD, and Shigeyuki Yamamoto, PhD, recruited the participants. Kaori Matsumoto, MA, conducted clinical assessments, including the Autism Diagnostic Interview–Revised and Autism Diagnostic Observation Schedule.

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed, text revision. Washington, DC: American Psychiatric Association; 2000.
2. Baird G, Simonoff E, Pickles A, Chandler S, Loucas T, Meldrum D, Charman T. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *Lancet*. 2006;368(9531):210-215.
3. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, Vincent JB, Skaug JL, Thompson AP, Senman L, Feuk L, Qian C, Bryson SE, Jones MB, Marshall CR, Scherer SW, Vieland VJ, Bartlett C, Mangin LV, Goedken R, Segre A, Pericak-Vance MA, Cuccaro ML, Gilbert JR, Wright HH, Abramson RK, Betancur C, Bourgeron T, Gillberg C, Leboyer M, Buxbaum JD, Davis KL, Hollander E, Silverman JM, Hallmayer J, Lotspeich L, Sutcliffe JS, Haines JL, Folstein SE, Piven J, Wassink TH, Sheffield V, Geschwind DH, Bucan M, Brown WT, Cantor RM, Constantino JN, Gilliam TC, Herbert M, Lajonchere C, Ledbetter DH, Lese-Martin C, Miller J, Nelson S, Samango-

Sprouse CA, Spence S, State M, Tanzi RE, Coon H, Dawson G, Devlin B, Estes A, Flodman P, Klei L, McMahon WM, Minshew N, Munson J, Korvatska E, Rodier PM, Schellenberg GD, Smith M, Spence MA, Stodgell C, Tepper PG, Wijsman EM, Yu CE, Rogé B, Mantoulan C, Wittemeyer K, Poustka A, Felder B, Klauck SM, Schuster C, Poustka F, Bölte S, Feineis-Matthews S, Herbrecht E, Schmötzer G, Tsiantis J, Papanikolaou K, Maestrini E, Bacchelli E, Blasi F, Carone S, Toma C, Van Engeland H, de Jonge M, Kemner C, Koop F, Langemeijer M, Hijimans C, Staal WG, Baird G, Bolton PF, Rutter ML, Weisblatt E, Green J, Aldred C, Wilkinson JA, Pickles A, Le Couteur A, Berney T, McConachie H, Bailey AJ, Francis K, Honeyman G, Hutchinson A, Parr JR, Wallace S, Monaco AP, Barnby G, Kobayashi K, Lamb JA, Sousa I, Sykes N, Cook EH, Guter SJ, Leventhal BL, Salt J, Lord C, Corsello C, Hus V, Weeks DE, Volkmar F, Tauber M, Fombonne E, Shih A, Meyer KJ; Autism Genome Project Consortium. Mapping autism risk loci using genetic linkage and chromosomal rearrangements [published correction appears in *Nat Genet.* 2007;39(10):1285]. *Nat Genet.* 2007;39(3):319-328.

4. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimäki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M. Strong association of de novo copy number mutations with autism. *Science.* 2007;316(5823):445-449.
5. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ; Autism Consortium. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med.* 2008;358(7):667-675.
6. Schain RJ, Freedman DX. Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. *J Pediatr.* 1961;58:315-320.
7. Hanley HG, Stahl SM, Freedman DX. Hyperserotonemia and amine metabolites in autistic and retarded children. *Arch Gen Psychiatry.* 1977;34(5):521-531.
8. Ciaranello RD. Hyperserotonemia and early infantile autism. *N Engl J Med.* 1982; 307(3):181-183.
9. Anderson GM, Freedman DX, Cohen DJ, Volkmar FR, Hoder EL, McPhedran P, Minderaa RB, Hansen CR, Young JG. Whole blood serotonin in autistic and normal subjects. *J Child Psychol Psychiatry.* 1987;28(6):885-900.
10. Cook EH Jr, Leventhal BL, Freedman DX. Serotonin and measured intelligence. *J Autism Dev Disord.* 1988;18(4):553-559.
11. Abramson RK, Wright HH, Carpenter R, Brennan W, Lumpuy O, Cole E, Young SR. Elevated blood serotonin in autistic probands and their first-degree relatives. *J Autism Dev Disord.* 1989;19(3):397-407.
12. Cook EH Jr, Leventhal BL, Heller W, Metz J, Wainwright M, Freedman DX. Autistic children and their first-degree relatives: relationships between serotonin and norepinephrine levels and intelligence. *J Neuropsychiatry Clin Neurosci.* 1990;2(3): 268-274.
13. Cross S, Kim SJ, Weiss LA, Delahanty RJ, Sutcliffe JS, Leventhal BL, Cook EH Jr, Veenstra-Vanderweele J. Molecular genetics of the platelet serotonin system in first-degree relatives of patients with autism. *Neuropsychopharmacology.* 2008; 33(2):353-360.
14. McDougle CJ, Naylor ST, Cohen DJ, Aghajanian GK, Heninger GR, Price LH. Effects of tryptophan depletion in drug-free adults with autistic disorder. *Arch Gen Psychiatry.* 1996;53(11):993-1000.
15. Kolevzon A, Mathewson KA, Hollander E. Selective serotonin reuptake inhibitors in autism: a review of efficacy and tolerability. *J Clin Psychiatry.* 2006;67(3): 407-414.
16. Yonan AL, Alarcón M, Cheng R, Magnusson PK, Spence SJ, Palmer AA, Grunn A, Juo SH, Terwilliger JD, Liu J, Cantor RM, Geschwind DH, Gilliam TC. A genome-wide screen of 345 families for autism-susceptibility loci. *Am J Hum Genet.* 2003; 73(4):886-897.
17. Cook EH Jr, Courchesne R, Lord C, Cox NJ, Yan S, Lincoln A, Haas R, Courchesne E, Leventhal BL. Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatry.* 1997;2(3):247-250.
18. Klauck SM, Poustka F, Benner A, Lesch KP, Poustka A. Serotonin transporter (5-HTT) gene variants associated with autism? *Hum Mol Genet.* 1997;6(13): 2233-2238.
19. Wassink TH, Hazlett HC, Epping EA, Arndt S, Dager SR, Schellenberg GD, Dawson G, Piven J. Cerebral cortical gray matter overgrowth and functional variation of the serotonin transporter gene in autism. *Arch Gen Psychiatry.* 2007;64(6):709-717.
20. Surguladze SA, Elkin A, Ecker C, Kalidindi S, Corsico A, Giampietro V, Lawrence N, Deeley Q, Murphy DG, Kucharska-Pietura K, Russell TA, McGuffin P, Murray R, Phillips ML. Genetic variation in the serotonin transporter modulates neural system-wide response to fearful faces. *Genes Brain Behav.* 2008;7(5):543-551.
21. Chugani DC, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, Chugani HT. Developmental changes in brain serotonin synthesis capacity in autistic and non-autistic children. *Ann Neurol.* 1999;45(3):287-295.
22. Chandana SR, Behen ME, Juhász C, Muzik O, Rothermel RD, Mangner TJ, Chakraborty PK, Chugani HT, Chugani DC. Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. *Int J Dev Neurosci.* 2005;23(2-3):171-182.
23. Makkonen I, Riikonen R, Kokki H, Airaksinen MM, Kuikka JT. Serotonin and dopamine transporter binding in children with autism determined by SPECT. *Dev Med Child Neurol.* 2008;50(8):593-597.
24. Anderson LT, Campbell M, Grega DM, Perry R, Small AM, Green WH. Haloperidol in the treatment of infantile autism: effects on learning and behavioral symptoms. *Am J Psychiatry.* 1984;141(10):1195-1202.
25. Anderson LT, Campbell M, Adams P, Small AM, Perry R, Shell J. The effects of haloperidol on discrimination learning and behavioral symptoms in autistic children. *J Autism Dev Disord.* 1989;19(2):227-239.
26. Gillberg C, Svennerholm L. CSF monoamines in autistic syndromes and other pervasive developmental disorders of early childhood. *Br J Psychiatry.* 1987; 151:89-94.
27. Narayan M, Srinath S, Anderson GM, Meundi DB. Cerebrospinal fluid levels of homovanillic acid and 5-hydroxyindoleacetic acid in autism. *Biol Psychiatry.* 1993; 33(8-9):630-635.
28. Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E, Kocsis P, Baumgarten R, Kovacs BW, Levy DL, Smith M, Borison RL, Evans DD, Klein DN, MacMurray J, Tosk JM, Sverd J, Gysin R, Flanagan SD. The dopamine D_2 receptor locus as a modifying gene in neuropsychiatric disorders. *JAMA.* 1991; 266(13):1793-1800.
29. Hettinger JA, Liu X, Schwartz CE, Michaelis RC, Holden JJA. DRD1 haplotype is associated with risk for autism spectrum disorders in male-only affected sib-pair families. *Am J Med Genet B Neuropsychiatr Genet.* 2008;147B(5):628-636.
30. Gadow KD, Roohi J, DeVincent CJ, Hatchwell E. Association of ADHD, tics, and anxiety with dopamine transporter (DAT1) genotype in autism spectrum disorder. *J Child Psychol Psychiatry.* 2008;49(12):1331-1338.
31. Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Cohen RM. Low medial prefrontal dopaminergic activity in autistic children [letter] [published correction appears in *Lancet.* 1998;351(9100):454]. *Lancet.* 1997;350(9078):638.
32. Fernell E, Watanabe Y, Adolfsson I, Tani Y, Bergström M, Hartvig P, Lilja A, von Knorring AL, Gillberg C, Långström B. Possible effects of tetrahydrobiopterin treatment in six children with autism-clinical and positron emission tomography data: a pilot study. *Dev Med Child Neurol.* 1997;39(5):313-318.
33. Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview–Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord.* 1994;24(5):659-685.
34. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, Pickles A, Rutter M. The Autism Diagnostic Observation Schedule–Generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* 2000;30(3):205-223.
35. American Psychiatric Association. *User's Guide for the Structured Clinical Interview for* DSM-IV *Axis I Disorders SCID-I: Clinician Version.* Washington, DC: American Psychiatric Press; 1997.
36. Andreasen NC, Endicott J, Spitzer RL, Winokur G. The family history method using diagnostic criteria: reliability and validity. *Arch Gen Psychiatry.* 1977;34(10): 1229-1235.
37. Stone VE, Baron-Cohen S, Knight RT. Frontal lobe contributions to theory of mind. *J Cogn Neurosci.* 1998;10(5):640-656.
38. Baron-Cohen S, O'Riordan M, Stone V, Jones R, Plaisted K. Recognition of faux pas by normally developing children and children with Asperger syndrome or high-functioning autism. *J Autism Dev Disord.* 1999;29(5):407-418.
39. Stone VE, Baron-Cohen S, Calder A, Keane J, Young A. Acquired theory of mind impairments in individuals with bilateral amygdala lesions. *Neuropsychologia.* 2003;41(2):209-220.
40. Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, Heninger GR, Charney DS. The Yale-Brown Obsessive Compulsive Scale, I: development, use, and reliability. *Arch Gen Psychiatry.* 1989;46(11):1006-1011.
41. Goodman WK, Price LH, Rasmussen SA, Mazure C, Delgado P, Heninger GR, Charney DS. The Yale-Brown Obsessive Compulsive Scale, II: validity. *Arch Gen Psychiatry.* 1989;46(11):1012-1016.
42. Hamilton M. Diagnosis and rating of anxiety. In: Lader MH, ed. *Studies of Anxiety: Papers Read at the World Psychiatric Association Symposium, "Aspects of Anxiety," London, November, 1967.* Ashford, England: Headley Brothers Ltd for Royal Medico-Psychological Association; 1969:76-79. Third special publication of *The British Journal of Psychiatry.*
43. Hamilton M. The assessment of anxiety states by rating. *Br J Med Psychol.* 1959; 32(1):50-55.
44. Buss AH, Perry M. The Aggression Questionnaire. *J Pers Soc Psychol.* 1992;63 (3):452-459.
45. Rousset OG, Ma Y, Evans AC. Correction for partial volume effects in PET: principle and validation. *J Nucl Med.* 1998;39(5):904-911.
46. Aston JA, Cunningham VJ, Asselin MC, Hammers A, Evans AC, Gunn RN. Posi-

tron emission tomography partial volume correction: estimation and algorithms. *J Cereb Blood Flow Metab.* 2002;22(8):1019-1034.
47. Sekine Y, Iyo M, Ouchi Y, Matsunaga T, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Takei N, Mori N. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. *Am J Psychiatry.* 2001;158(8):1206-1214.
48. Ouchi Y, Yoshikawa E, Okada H, Futatsubashi M, Sekine Y, Iyo M, Sakamoto M. Alterations in binding site density of dopamine transporter in the striatum, orbitofrontal cortex, and amygdala in early Parkinson's disease: compartment analysis for β-CFT binding with positron emission tomography. *Ann Neurol.* 1999;45(5):601-610.
49. Sekine Y, Ouchi Y, Takei N, Yoshikawa E, Nakamura K, Futatsubashi M, Okada H, Minabe Y, Suzuki K, Iwata Y, Tsuchiya KJ, Tsukada H, Iyo M, Mori N. Brain serotonin transporter density and aggression in abstinent methamphetamine abusers. *Arch Gen Psychiatry.* 2006;63(1):90-100.
50. Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, Goulding V, Kennedy J, Wilson AA. Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry.* 2004;61(12):1271-1279.
51. Szabo Z, McCann UD, Wilson AA, Scheffel U, Owonikoko T, Mathews WB, Ravert HT, Hilton J, Dannals RF, Ricaurte GA. Comparison of (+)-^{11}C-McN5652 and ^{11}C-DASB as serotonin transporter radioligands under various experimental conditions. *J Nucl Med.* 2002;43(5):678-692.
52. Wong DF, Yung B, Dannals RF, Shaya EK, Ravert HT, Chen CA, Chan B, Folio T, Scheffel U, Ricaurte GA, et al. In vivo imaging of baboon and human dopamine transporters by positron emission tomography using [^{11}C]WIN 35,428. *Synapse.* 1993;15(2):130-142.
53. Ouchi Y, Kanno T, Okada H, Yoshikawa E, Futatsubashi M, Nobezawa S, Torizuka T, Tanaka K. Changes in dopamine availability in the nigrostriatal and mesocortical dopaminergic systems by gait in Parkinson's disease. *Brain.* 2001;124(pt 4):784-792.
54. Meyer JH, McNeely HE, Sagrati S, Boovariwala A, Martin K, Verhoeff NP, Wilson AA, Houle S. Elevated putamen D_2 receptor binding potential in major depression with motor retardation: an [^{11}C]raclopride positron emission tomography study. *Am J Psychiatry.* 2006;163(9):1594-1602.
55. Ritvo ER, Jorde LB, Mason-Brothers A, Freeman BJ, Pingree C, Jones MB, McMahon WM, Petersen PB, Jenson WR, Mo A. The UCLA–University of Utah epidemiologic survey of autism: recurrence risk estimates and genetic counseling. *Am J Psychiatry.* 1989;146(8):1032-1036.
56. Tsakanikos E, Costello H, Holt G, Bouras N, Sturmey P, Newton T. Psychopathology in adults with autism and intellectual disability. *J Autism Dev Disord.* 2006;36(8):1123-1129.
57. Danielsson S, Gillberg IC, Billstedt E, Gillberg C, Olsson I. Epilepsy in young adults with autism: a prospective population-based follow-up study of 120 individuals diagnosed in childhood. *Epilepsia.* 2005;46(6):918-923.
58. Haznedar MM, Buchsbaum MS, Metzger M, Solimando A, Spiegel-Cohen J, Hollander E. Anterior cingulate gyrus volume and glucose metabolism in autistic disorder. *Am J Psychiatry.* 1997;154(8):1047-1050.
59. Ohnishi T, Matsuda H, Hashimoto T, Kunihiro T, Nishikawa M, Uema T, Sasaki M. Abnormal regional cerebral blood flow in childhood autism. *Brain.* 2000;123(pt 9):1838-1844.
60. Murphy DG, Daly E, Schmitz N, Toal F, Murphy K, Curran S, Erlandsson K, Eersels J, Kerwin R, Ell P, Travis M. Cortical serotonin 5-HT2A receptor binding and social communication in adults with Asperger's syndrome: an in vivo SPECT study. *Am J Psychiatry.* 2006;163(5):934-936.
61. Smith DF. Neuroimaging of serotonin uptake sites and antidepressant binding sites in the thalamus of humans and "higher" animals. *Eur Neuropsychopharmacol.* 1999;9(6):537-544.
62. Murphy DG, Critchley HD, Schmitz N, McAlonan G, Van Amelsvoort T, Robertson D, Daly E, Rowe A, Russell A, Simmons A, Murphy KC, Howlin P. Asperger syndrome: a proton magnetic resonance spectroscopy study of brain. *Arch Gen Psychiatry.* 2002;59(10):885-891.
63. Hollander E, Anagnostou E, Chaplin W, Esposito K, Haznedar MM, Licalzi E, Wasserman S, Soorya L, Buchsbaum M. Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biol Psychiatry.* 2005;58(3):226-232.
64. Atmaca M, Yildirim H, Ozdemir H, Ozler S, Kara B, Ozler Z, Kanmaz E, Mermi O, Tezcan E. Hippocampus and amygdalar volumes in patients with refractory obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry.* 2008;32(5):1283-1286.
65. Page LA, Daly E, Schmitz N, Simmons A, Toal F, Deeley Q, Ambery F, McAlonan GM, Murphy KC, Murphy DG. In vivo ^{1}H-magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. *Am J Psychiatry.* 2006;163(12):2189-2192.
66. Cook EH Jr. Brief report: pathophysiology of autism: neurochemistry. *J Autism Dev Disord.* 1996;26(2):221-225.
67. Whitaker-Azmitia PM. Behavioral and cellular consequences of increasing serotonergic activity during brain development: a role in autism? *Int J Dev Neurosci.* 2005;23(1):75-83.
68. Janusonis S, Anderson GM, Shifrovich I, Rakic P. Ontogeny of brain and blood serotonin levels in 5-HT receptor knockout mice: potential relevance to the neurobiology of autism. *J Neurochem.* 2006;99(3):1019-1031.
69. McNamara IM, Borella AW, Bialowas LA, Whitaker-Azmitia PM. Further studies in the developmental hyperserotonemia model (DHS) of autism: social, behavioral and peptide changes. *Brain Res.* 2008;1189:203-214.
70. Maestrini E, Lai C, Marlow A, Matthews N, Wallace S, Bailey A, Cook EH, Weeks DE, Monaco AP; International Molecular Genetic Study of Autism Consortium. Serotonin transporter (5-HTT) and γ-aminobutyric acid receptor subunit β3 (*GABRB3*) gene polymorphisms are not associated with autism in the IMGSA families. *Am J Med Genet.* 1999;88(5):492-496.
71. Persico AM, Militerni R, Bravaccio C, Schneider C, Melmed R, Conciatori M, Damiani V, Baldi A, Keller F. Lack of association between serotonin transporter gene promoter variants and autistic disorder in two ethnically distinct samples. *Am J Med Genet.* 2000;96(1):123-127.
72. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science.* 1996;274(5292):1527-1531.
73. Bradley SL, Dodelzon K, Sandhu HK, Philibert RA. Relationship of serotonin transporter gene polymorphisms and haplotypes to mRNA transcription. *Am J Med Genet B Neuropsychiatr Genet.* 2005;136B(1):58-61.
74. Siever LJ. Neurobiology of aggression and violence. *Am J Psychiatry.* 2008;165(4):429-442.
75. Matson JL, Nebel-Schwalm MS. Comorbid psychopathology with autism spectrum disorder in children: an overview. *Res Dev Disabil.* 2007;28(4):341-352.
76. Nieminen-von Wendt TS, Metsähonkala L, Kulomaki TA, Aalto S, Autti TH, Vanhala R, Eskola O, Bergman J, Hietala JA, von Wendt LO. Increased presynaptic dopamine function in Asperger syndrome. *Neuroreport.* 2004;15(5):757-760.
77. Bachevalier J, Loveland KA. The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism. *Neurosci Biobehav Rev.* 2006;30(1):97-117.
78. Davidson RJ, Putnam KM, Larson CL. Dysfunction in the neural circuitry of emotion regulation: a possible prelude to violence. *Science.* 2000;289(5479):591-594.
79. Bolte Taylor J, Cunningham MC, Benes FM. Neonatal raphe lesions increase dopamine fibers in prefrontal cortex of adult rats. *Neuroreport.* 1998;9(8):1811-1815.
80. Zhou FC, Lesch KP, Murphy DL. Serotonin uptake into dopamine neurons via dopamine transporters: a compensatory alternative. *Brain Res.* 2002;942(1-2):109-119.
81. Ouchi Y, Kanno T, Okada H, Yoshikawa E, Futatsubashi M, Nobezawa S, Torizuka T, Tanaka K. Changes in dopamine availability in the nigrostriatal and mesocortical dopaminergic systems by gait in Parkinson's disease. *Brain.* 2001;124(pt 4):784-792.
82. Sekine Y, Iyo M, Ouchi Y, Matsunaga T, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Takei N, Mori N. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. *Am J Psychiatry.* 2001;158(8):1206-1214.
83. Sekine Y, Minabe Y, Ouchi Y, Takei N, Iyo M, Nakamura K, Suzuki K, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Mori N. Association of dopamine transporter loss in the orbitofrontal and dorsolateral prefrontal cortices with methamphetamine-related psychiatric symptoms. *Am J Psychiatry.* 2003;160(9):1699-1701.
84. Villemagne V, Yuan J, Wong DF, Dannals RF, Hatzidimitriou G, Mathews WB, Ravert HT, Musachio J, McCann UD, Ricaurte GA. Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: evidence from [^{11}C]WIN-35,428 position emission tomography studies and direct *in vitro* determinations. *J Neurosci.* 1998;18(1):419-427.

International Journal of Neuropsychopharmacology, Page 1 of 6. Copyright © CINP 2009
doi:10.1017/S1461145709990952

BRIEF REPORT

CINP

Metabolite alterations in the hippocampus of high-functioning adult subjects with autism

Katsuaki Suzuki[1], Katsuhiko Nishimura[2], Genichi Sugihara[1], Kazuhiko Nakamura[2], Kenji J. Tsuchiya[1], Kaori Matsumoto[1], Kiyokazu Takebayashi[2], Haruo Isoda[3], Harumi Sakahara[3], Toshiro Sugiyama[4], Masatsugu Tsujii[5], Nori Takei[1] and Norio Mori[2]

[1] *Osaka-Hamamatsu Joint Research Center for Child Mental Development, Hamamatsu University School of Medicine, Hamamatsu, Japan*
[2] *Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Japan*
[3] *Department of Radiology, Hamamatsu University School of Medicine, Hamamatsu, Japan*
[4] *Aichi Children's Health and Medical Center, Obu, Japan*
[5] *Faculty of Sociology, Chukyo University, Toyota, Japan*

Abstract

The aim of the present study was to investigate metabolite alterations in the hippocampal formation as they relate to aggression in high-functioning adults with autism. We measured concentrations of *N*-acetylaspartate (NAA), choline-containing compounds (Cho), and creatine plus phosphocreatine (Cr + PCr) in the hippocampal formation by proton magnetic resonance spectroscopy in 12 non-medicated male subjects with autism and 12 age- and sex-matched controls. Aggression was scored in the autistic subjects using the Buss–Perry Aggression Questionnaire. The concentrations of Cho and Cr + PCr in the hippocampal formation in autistic subjects were significantly higher than the corresponding values in control subjects, and a significant positive correlation was observed between the concentrations of these metabolites in the hippocampal formation and scores on the Buss–Perry Aggression Questionnaire in autistic subjects. Results suggest that high-functioning adult subjects with autism have abnormal metabolite concentrations in the hippocampal formation, which may in part account for their aggression.

Received 12 April 2009; Reviewed 2 June 2009; Revised 13 June 2009; Accepted 8 October 2009

Key words: Aggression, autism, hippocampus, magnetic resonance spectroscopy.

Introduction

Autism is a neurodevelopmental disorder characterized by qualitative impairment of social interaction and communication, as well as by restricted repetitive and stereotyped patterns of behaviours, interests, and activities. In addition to the core features of autism, there are common comorbid psychiatric symptoms such as anxiety, depression, and aggression (Matson & Nebel-Schwalm, 2007). The presence of aggressive behaviour can reduce the effectiveness of treatment interventions, and many individuals with autism remain significantly impaired. Therefore, from a treatment perspective, it would be advantageous to clarify the underlying mechanism for the development of aggression in individuals with autism. The brain structures that are involved in the control of aggression include limbic structures such as the amygdala, hippocampus (Gregg & Siegel, 2001), and the cerebellum (Berman, 1997). Interestingly, the most significant findings from post-mortem studies of autism have been confined to regions of the limbic system and the cerebellum, e.g. reduced neuronal cell size and increased cell packing density in the hippocampus (Bauman & Kemper, 2005), shrinkage of nuclei of the amygdala (Schumann & Amaral, 2006), and reduced Purkinje cell density in the cerebellum (Bailey *et al.* 1998; Bauman & Kemper, 2005; Ritvo *et al.* 1986). However, the correlation between structural abnormalities associated with dysfunction in these structures and aggression in autism has not yet been clearly established.

Address for correspondence: Dr K. Suzuki, Osaka-Hamamatsu Joint Research Center for Child Mental Development, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi, Hamamatsu 431-3192, Japan.
Tel.: +81 (53) 435-2295 *Fax*: +81 (53) 435-2295
Email: k-suzuki@hama-med.ac.jp

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive tool for evaluating neurochemical changes related to the clinical characteristics of psychiatric disorders. ^{1}H-MRS produces spectra that represent concentrations of *N*-acetylaspartate (NAA), a marker of neuronal integrity (Clark, 1998); choline-containing compounds (Cho), markers of cell number and/or membrane turnover (Miller *et al.* 1996); and creatine plus phosphocreatine (Cr + PCr), a marker of overall (i.e. neuronal plus glial) cellular density (Sartorius *et al.* 2008). Of the 35 post-mortem cases of autism reported in the previous studies mentioned above, 26 had died aged $\geqslant$19 yr. It is expected that ^{1}H-MRS study of the brain of autistic adults will be a helpful initial step in clarifying the involvement of the limbic system and cerebellum in the expression of aggression by individuals with autism. However, previous ^{1}H-MRS studies of the limbic system and cerebellum of autistic patients have primarily focused on children (i.e. patients aged $<$20 yr) and have been confounded by the inclusion of learning-disabled subjects and subjects with seizure disorders. In this regard, the only exception was a study by Page *et al.* (2006), in which the authors examined high-functioning adults with autism and reported a significant increase in Cr + PCr levels in the amygdala–hippocampal region. However, in that study, the relationship between neurobiological changes and clinical features was not examined. Furthermore, the putative roles of the amygdala and hippocampus in the expression of aggression appeared to differ (Gregg & Siegel, 2001), although these two regions are closely interconnected. To date, there has been no report of a ^{1}H-MRS evaluation of concentrations of metabolites in the cerebellum of adult subjects with autism.

In the present study, we hypothesized that alterations of metabolites may be present in the hippocampus and cerebellum of high-functioning adults with autism, and such metabolite alterations could be correlated with the aggression exhibited in a subset of these patients. To test these hypotheses, we recruited non-medicated, high-functioning male adults with autism in order to measure metabolite concentrations in the hippocampal formation (not including the amygdala) and cerebellum of these subjects using ^{1}H-MRS. In addition, we examined the correlation between metabolite levels and aggression in autistic adults.

Methods

The Ethics Committee of the Hamamatsu University School of Medicine approved the study. Each participant gave written informed consent after being given a complete description of the study. We conducted the Wechsler Adult Intelligence Scale – Revised (WAIS-R) to assess the intelligence quotient (IQ) of all participants. The diagnosis of autism was made according to the Japanese version of the Autism Diagnostic Interview – Revised (ADI-R; Lord *et al.* 1994) and the Autism Diagnostic Observation Schedule (ADOS; Lord *et al.* 2000). Control subjects were recruited from the community by advertisement. All autistic and control subjects were screened to exclude psychiatric illnesses (i.e. schizophrenia, affective disorders, mental retardation, and personality or behavioural disorders) by means of the Structured Clinical Interview for DSM-IV (SCID). We excluded individuals with epilepsy, with psychotropic medication, and those with mental retardation as defined by a full-scale IQ of $<$70. Twelve male subjects with autism and 12 age-matched healthy male controls were included in the present study. All participants were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). We evaluated aggression in the participants using the Japanese version of the Aggression Questionnaire (Ando *et al.* 1999). The original version of the Aggression Questionnaire (Buss & Perry, 1992) is a 29-item, self-administered test designed to measure aggression (i.e. physical aggression, verbal aggression, anger, and hostility) as a personality trait. The validity and reliability of the Japanese version of the Aggression Questionnaire has previously been established (Ando *et al.* 1999).

Participants were scanned using a GE 1.5 T magnetic resonance scanner at the Hamamatsu University Hospital. We selected two volumes of interest (VOIs) for each subject, i.e. the left hippocampal region and the right cerebellar hemisphere (Fig. 1*a–c*). The rationale for the selection of two VOIs was as follows: (1) since all the participants were right-handed, the left hippocampus and the right cerebellum were regarded as dominant (Jansen, 2005); (2) although there is no known direct projection from the hippocampus to the cerebellum, most of the efferents from the temporolimbic region send information to the contralateral cerebellar cortex via the cortico-ponto-cerebellar pathway (Schmahmann, 2000); and (3) left-sided abnormalities have been associated with aggression in humans (Tebartz van Elst *et al.* 2000; Zetzsche *et al.* 2007). To establish these VOIs, whole-brain images were acquired with a 3D fast-spoiled gradient-echo imaging protocol. A sagittal scout image was used to select oblique coronal slices perpendicular to the long axis of the hippocampal formation (Fig. 1*a*). A slanted rectangular VOI ($2 \times 2 \times 1.5$ cm^3) was selected such

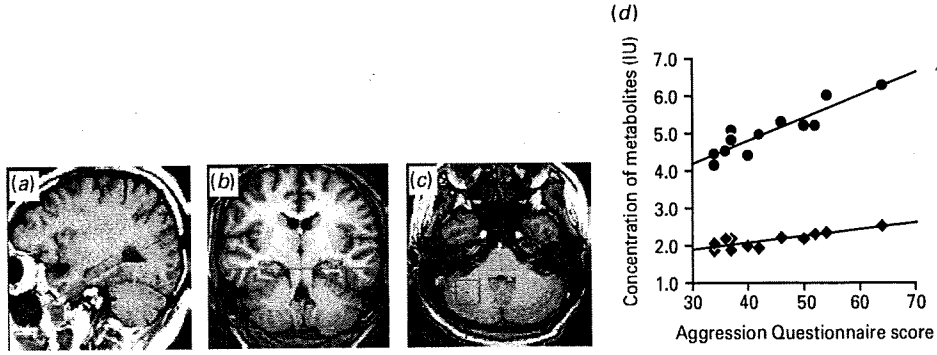

Fig. 1. (*a–c*) Locations of a 6-ml volume of interest (VOI) in the left hippocampus (*a*, *b*) and of an 8-ml VOI in the right cerebellum (*c*). (*d*) Choline-containing compounds (◆; Cho) and creatine plus phosphocreatine (●; Cr+PCr) concentrations in the hippocampus of autistic subjects. Both values showed a significant and positive correlation with the Aggression Questionnaire score (Cho: $\rho = 0.719$, $p = 0.008$; Cr+PCr: $r = 0.884$, $p < 0.001$).

that it was aligned along the long axis of the hippocampus, starting just posterior to the amygdala (Fig. 1*a*, *b*). The centre of the VOI was at the cornu ammonis of the left hippocampus; as a result, the VOI contained the hippocampus proper and a portion of the parahippocampal gyrus. A cerebellar VOI ($2 \times 2 \times 2$ cm^3) was placed on one axial image, which showed the full length of the middle cerebellar peduncle (Fig. 1*c*). To explore metabolite levels in the cerebellar cortex, we excluded the dentate nucleus from each VOI; the medial side of the VOI was just adjacent to the lateral margin of the dentate nucleus, which was determined by axial T_2-weighted images. A point-resolved spectroscopy (PRESS) spectrum [repetition time (TR) = 1500 ms, echo time (TE) = 144 ms, 256 averages] was obtained after chemical shift selective water suppression. To determine the tissue composition of the VOI, fast-spoiled gradient-echo images were segmented into white matter, gray matter, and cerebrospinal fluid (CSF) using the software package Dr View/Linux. We measured the concentrations of NAA, Cho, and Cr+PCr using the LC model algorithm and corrected the concentrations for the proportion of CSF within the VOI.

Statistical analyses were performed using two-tailed Student's *t* test, Spearman's ρ correlation coefficient, and Bonferroni's correction. The level of significance was set at $p < 0.05$.

Results

The demographic characteristics of the autistic and control subjects are shown in Table 1. There was no significant difference in the distribution of age or full-scale IQ between the two groups, indicating that subject matching was successful. As shown in Table 1, there were no significant inter-group differences in the compositions of gray matter, white matter, and CSF within either of the two VOIs. In the hippocampus, the autistic group had significantly higher concentrations of Cho ($p < 0.001$) and Cr+PCr ($p < 0.001$) compared to control subjects (Table 1). In the cerebellum, the concentration of NAA in the autistic group was significantly lower than that in the control group ($p < 0.001$, Table 1). We performed a Spearman's correlation analysis to determine whether the concentrations of Cho and Cr+PCr in the hippocampus and NAA in the cerebellum were correlated with autistic subjects' scores on the Aggression Questionnaire. Both Cho ($p = 0.008$) and Cr+PCr ($p < 0.001$) concentrations in the hippocampus, but not the NAA concentration in the cerebellum, were significantly positively correlated with the Aggression Questionnaire scores (Fig. 1*d*). There was no correlation between metabolite alterations (i.e. hippocampal Cho and Cr+PCr, and cerebellar NAA) and IQ or autistic symptoms as assessed by ADI-R (data not shown).

Discussion

In the present study, the observed increase in the Cr+PCr concentration in high-functioning subjects with autism was compatible with the results of a previous ^{1}H-MRS study by Page *et al.* (2006), who measured the concentration of this metabolite combination in the right amygdala–hippocampal complex in autistic adults of normal intelligence. We also found an elevation of Cho in the hippocampal formation (not including the amygdala) of high-functioning adults with autism, although it should be noted that the

Table 1. Demographic characteristics and ^{1}H-MRS measurements of the hippocampus and cerebellum in subjects with autism and controls

	Control ($n=12$)	Autism ($n=12$)	t value	p value
Age (yr)	22.3±1.8 (19–24)	22.0±2.2 (18–25)	0.384	0.705
Full-scale IQ	105.2±12.6 (85–125)	96.3±14.0 (71–117)	1.645	0.114
ADI-R (Diagnostic algorithm)				
(A) Social	n.a.	20.9±5.3 (12–28)		
(B) Communication				
Verbal	n.a.	14.2±5.4 (4–22)		
Non-verbal	n.a.	8.4±4.0 (2–14)		
(C) Stereotype	n.a.	5.5±2.0 (3–10)		
Aggression Questionnaire	n.a.	45.3±9.4 (34–64)		
Hippocampus				
NAA (IU)	7.79±1.57	8.14±0.84	−0.672	0.509
Cho (IU)	1.87±0.21	2.19±0.17	−4.176	<0.001*
Cr+PCr (IU)	3.80±0.97	5.16±0.59	−4.124	<0.001*
Gray matter (%)	64.5±6.9	65.6±7.3	0.394	0.866
White matter (%)	33.9±8.3	32.2±7.5	0.609	0.769
Cerebrospinal fluid (%)	1.6±2.0	2.2±1.9	0.215	0.880
Cerebellum				
NAA (IU)	9.01±0.50	8.29±0.71	3.949	<0.001*
Cho (IU)	2.12±0.18	2.07±0.22	0.505	0.618
Cr+PCr (IU)	6.08±0.56	5.85±0.63	0.909	0.373
Grey matter (%)	71.6±9.2	71.8±8.0	0.620	0.495
White matter (%)	28.2±9.2	27.7±8.9	0.154	0.448
Cerebrospinal fluid (%)	0.0±0.0	0.1±0.0	0.031	0.084

ADI-R, Autism Diagnostic Interview – Revised; n.a., not applicable. NAA, *N*-acetylaspartate; Cho, choline-containing compounds; Cr+PCr, creatine plus phosphocreatine; IU, institutional unit.
Values are expressed as means ± S.D. (range).
* Statistically significant difference, as determined by two-tailed Student's t test.

study by Page *et al.* (2006) did not demonstrate such changes in Cho level. The discrepancy between the two studies presumably may reflect a difference in VOI placement, i.e. the Page *et al.* study included the amygdala, whereas our study did not. The ^{1}H-MRS Cho peak is known to be caused by free choline, phosphocholine (components of membrane synthesis), and glycerophosphocholine (a product of the degradation of membrane phosphatidylcholine). Therefore, an increase in the Cho concentration has been interpreted as representing increased membrane synthesis and/or membrane disruption in processes including tumour growth, demyelinization, and gliosis (Miller *et al.* 1996). An elevation of Cr+PCr on ^{1}H-MRS reflects high-energy phosphate metabolism (Sartorius *et al.* 2008). Therefore, the elevation of Cho and Cr+PCr levels observed here suggests active, viable neuronal turnover in the hippocampal region of autistic adults. Previous structural MRI studies (Rojas *et al.* 2004; Schumann *et al.* 2004) have demonstrated enlarged hippocampal volume in autistic adults of normal intelligence. However, since in our sample no changes were observed in the NAA concentration in the hippocampal region, it remains unclear whether or not elevated Cho and Cr+PCr levels are associated with changes in hippocampal volume. Further studies are required to clarify this issue.

Both Cho and Cr+PCr concentrations in the left hippocampal region were significantly and positively correlated with trait aggression, as assessed by the Aggression Questionnaire administered to our adult subjects with autism. To the best of our knowledge, this is the first report to describe a link between metabolite alterations in the hippocampal formation and a clinical feature of autism. There is no available data to account for the mechanism by which the hippocampus affects the trait of aggression in autism. However, the accumulated evidence does suggest that the hippocampal formation modulates aggression in animals. For instance, electrical stimulation of the

dorsal or ventral hippocampus has been shown to inhibit or facilitate, respectively, aggressive behaviour in cats (Gregg & Siegel, 2001). Electrophysiological recordings have revealed that aggressive behaviours are associated with increased frequency in hippocampal discharge patterns in rabbits (Fontani & Vegni, 1990). Furthermore, the present findings agree with the results of previous clinical studies indicating that left hemispheric lesions may be associated with a higher risk for the development of aggression (Tebartz van Elst *et al.* 2000). Zetzsche *et al.* (2007) reported that increased lifetime aggression in patients with borderline personality disorder was significantly correlated with volume of the left, but not the right, hippocampus. Taken together, these findings suggest that the elevations in the Cho and Cr + PCr concentrations observed here reflect altered function in the hippocampal formation, which in turn was found to be associated with aggression in adult subjects with autism. Since it is still unclear whether the functional alterations in the hippocampal formation are causative of aggression in people with autism, further studies are needed.

As assessed by ^{1}H-MRS, the concentration of NAA in the cerebellum was significantly lower in autistic adults than controls. This finding is comparable with that of previous ^{1}H-MRS studies of autistic children, who exhibited a similar reduction in metabolite concentrations in the cerebellum (Chugani *et al.* 1999; Otsuka *et al.* 1999). Since post-mortem studies have repeatedly demonstrated decreased Purkinje cell density in the cerebellum (Bailey *et al.* 1998; Bauman & Kemper, 2005; Ritvo *et al.* 1986), and since a decrease in the ^{1}H-MRS NAA peak is a putative marker of neuronal loss (Clark, 1998), it is possible that the decreased NAA concentration observed here reflects a relative decrease in the number of Purkinje cells in the cerebellum of high-functioning subjects with autism.

There are some limitations of our study. The small sample size renders the data presented here preliminary, and a larger study with more subjects with autism will be necessary. However, recruitment for the present study was limited to a group of high-functioning subjects with autism, all of whom were given no psychotropic drugs, and all were able to complete magnetic resonance spectroscopy without sedation. Therefore, we believe that our data are free from possible confounding factors and thus may reflect a certain common pathology in people with autism. Other limitations of this study include the following: (1) the lack of an assessment of the right hippocampal formation; (2) inclusion of not only the hippocampus proper, but also the parahippocampal gyrus in the hippocampal VOI; and (3) a relatively short TR acquisition period in the ^{1}H-MRS method, which could have affected metabolite levels. Furthermore, we did not include autistic adults without aggression as a comparison group, which might have been useful to test our hypothesis. These limitations should be factored into any interpretation of the present results. Nevertheless, in the existing literature, few studies to date have combined results from neuropsychological tests with neurometabolic levels in specific brain areas, which is a main advantage of our study.

In conclusion, although the sample size was small, our findings suggest that high-functioning people with autism have metabolite alterations in the cerebellum and hippocampus, and the latter in particular may play an important role in the expression of aggression in individuals with autism.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research (B) and (C) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan to Dr K. Nakamura and Dr G. Sugihara, respectively.

Statement of Interest

None.

References

Ando A, Soga S, Yamasaki K, Shimai T, *et al.* (1999) Development of the Japanese version of the Buss–Perry Aggression Questionnaire (BAQ) [in Japanese]. *Japanese Journal of Psychology* **70**, 384–392.

Bailey A, Luthert P, Dean A, Harding B, *et al.* (1998). A clinicopathological study of autism. *Brain* **121**, 889–905.

Bauman M, Kemper TL (2005). Neuroanatomic observations of the brain in autism: a review and future directions. *International Journal of Developmental Neuroscience* **23**, 183–187.

Berman AJ (1997). Amelioration of aggression: response to selective cerebellar lesions in the rhesus monkey. *International Review of Neurobiology* **41**, 111–119.

Buss AH, Perry M (1992). The aggression questionnaire. *Journal of Personality and Social Psychology* **63**, 452–459.

Chugani DC, Sundram BS, Behen M, Lee ML, *et al.* (1999). Evidence of altered energy metabolism in autistic children. *Progress in Neuropsychopharmacology and Biological Psychiatry* **23**, 635–641.

Clark JB (1998). *N*-acetyl aspartate: a marker for neuronal loss or mitochondrial dysfunction. *Developmental Neuroscience* **20**, 271–276.

Fontani G, Vegni V (1990). Hippocampal electrical activity during social interactions in rabbits living in

a seminatural environment. *Physiology & Behavior* **47**, 175–183.

Gregg TR, Siegel A (2001). Brain structures and neurotransmitters regulating aggression in cats: implications for human aggression. *Progress in Neuropsychopharmacology and Biological Psychiatry* **25**, 91–140.

Jansen A, Floël A, Van Randenborgh J, Konrad C, *et al.* (2005) Crossed cerebro-cerebellar language dominance. *Human Brain Mapping* **24**, 165–172.

Lord C, Rutter M, Le Couteur A (1994). Autism diagnostic interview-revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders* **24**, 659–685.

Lord C, Risi S, Lambrecht L, Cook Jr. EH, *et al.* (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders* **30**, 205–223.

Matson JL, Nebel-Schwalm MS (2007). Comorbid psychopathology with autism spectrum disorder in children: an overview. *Research in Developmental Disabilities* **28**, 341–352.

Miller BL, Chang L, Booth R, Ernst T, *et al.* (1996) In vivo ^{1}H MRS choline: correlation with in vitro chemistry/histology. *Life Sciences* **58**, 1929–1935.

Oldfield RC (1971). The assessment and analysis of handedness: the Edinburgh Inventory. *British Journal of Psychology* **66**, 53–59.

Otsuka H, Harada M, Mori K, Hisaoka S, *et al.* (1999). Brain metabolites in the hippocampus–amygdala region and cerebellum in autism: a ^{1}H-MR spectroscopy study. *Neuroradiology* **41**, 517–519.

Page LA, Daly E, Schmitz N, Simmons A, *et al.* (2006). In vivo ^{1}H-magnetic resonance spectroscopy study of amygdala–hippocampal and parietal regions in autism. *American Journal of Psychiatry* **163**, 2189–2192.

Ritvo ER, Freeman BJ, Scheibel AB, Duong T, *et al.* (1986). Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC Autopsy Research Report. *American Journal of Psychiatry* **143**, 862–866.

Rojas DC, Smith JA, Benkers TL, Camou SL, *et al.* (2004). Hippocampus and amygdala volumes in parents of children with autistic disorder. *American Journal of Psychiatry* **161**, 2038–2044.

Sartorius A, Lugenbiel P, Mahlstedt MM, Ende G, *et al.* (2008). Proton magnetic resonance spectroscopic creatine correlates with creatine transporter protein density in rat brain. *Journal of Neuroscience Methods* **172**, 215–219.

Schmahmann JD (2000). The role of the cerebellum in affect and psychosis. *Journal of Neurolinguistics* **13**, 189–214.

Schumann CM, Amaral DG (2006). Stereological analysis of amygdala neuron number in autism. *Journal of Neuroscience* **26**, 7674–7679.

Schumann CM, Hamstra J, Goodlin-Jones BL, *et al.* (2004). The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *Journal of Neuroscience* **24**, 6392–6401.

Tebartz van Elst L, Woermann FG, Lemieux L, Thompson PJ, *et al.* (2000). Affective aggression in patients with temporal lobe epilepsy: a quantitative MRI study of the amygdala. *Brain* **123**, 234–243.

Zetzsche T, Preuss UW, Frodl T, Schmitt G, *et al.* (2007). Hippocampal volume reduction and history of aggressive behaviour in patients with borderline personality disorder. *Psychiatry Research* **154**, 157–170.