

# 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 serotonergic 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 ( $^{11}\text{C}$ )-labeled *trans*-1,2,3,5,6,10- $\beta$ -hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-*a*]isoquinoline ( $^{11}\text{C}$ )(+) McN-5652 and 2 $\beta$ -carbomethoxy-3- $\beta$ -(4-fluorophenyl) tropane ( $^{11}\text{C}$ )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.

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**A**UTISM IS A PERVERSIVE DEVELOPMENTAL disorder that is characterized by the behavioral traits of impaired social cognition and communication, and repetitive and/or obsessive behavior and interests.<sup>1</sup> 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.<sup>2</sup> In addition, genome-wide linkage scans and copy-number analyses have revealed "hot spots" on several chromosomes.<sup>3-5</sup> To clarify the pathophysi-

ologic 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 serotonergic system. Prior studies consistently found elevated serotonin levels in the whole blood cells and platelets of patients with autism<sup>6-10</sup> and their relatives.<sup>11-13</sup> 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 <sup>a</sup>	35.5 (2.9)	31-40	24.6 (6.6) <sup>b</sup>	8-34
Y-BOCS score <sup>c</sup>	NA	NA	10.5 (4.3)	2-20
17-Item HAM-A score <sup>c</sup>	NA	NA	3.7 (2.6)	0-8
17-Item HAM-D score <sup>c</sup>	NA	NA	2.1 (2.2)	0-6
AQ score <sup>c</sup>	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.

<sup>a</sup>Lower scores correspond to a poorer social cognitive ability.

<sup>b</sup> $P < .001$  (unpaired, 2-tailed  $t$  test).

<sup>c</sup>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.<sup>14</sup> 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.<sup>15</sup> Genetic studies have yielded evidence of a critical role for the serotonin transporter gene (*SLC6A4*; OMIM 182138), which is located on chromosome 17q11.<sup>5,16</sup> Several *SLC6A4* polymorphisms have been found to be associated with autism.<sup>17,18</sup> Furthermore, *SLC6A4* promoter polymorphisms may influence the gray matter volume of cerebral cortical structures in young male autistic individuals.<sup>19</sup> It has also been shown that *SLC6A4* modulates the function of social brain systems when healthy control subjects process facial emotions.<sup>20</sup> 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.<sup>21,22</sup> 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.<sup>23</sup>

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.<sup>24,25</sup> In addition, some direct evidence suggests that levels of the principal dopamine metabolite homovanillic acid are elevated in the cerebrospinal fluid of autistic individuals,<sup>26</sup> although this has not been consistently reported.<sup>27</sup> Previous genetic studies have demonstrated that the prevalence of the *A1* allele of the dopamine D<sub>2</sub> receptor is significantly increased in autism,<sup>28</sup> whereas the dopamine D<sub>1</sub> receptor gene may be a risk gene for core symptoms of autism in male-only affected sibling-pair families.<sup>29</sup> 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.<sup>30</sup> In a PET study of autistic children, low levels of medial prefrontal dopaminergic activity were observed under anesthesia,<sup>31</sup> whereas increased dopa-

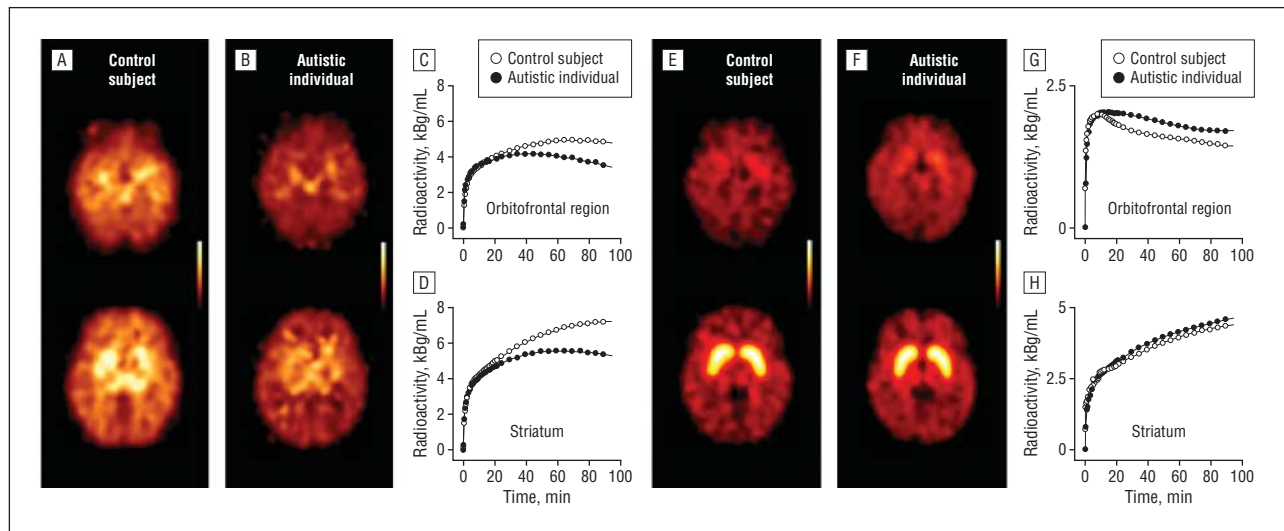
mine D<sub>2</sub> receptor binding in the whole caudate and putamen has also been demonstrated.<sup>32</sup> These findings suggest that the alteration of both the serotonin and the dopamine systems is a feature of autism, although these findings remain equivocal and inconclusive.

Taking these results together, we hypothesized that alterations in both the serotonergic 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*<sup>1</sup>; the Autism Diagnostic Interview-Revised<sup>33</sup>; and the Autism Diagnostic Observation Schedule-Generic.<sup>34</sup> 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*.<sup>35</sup> 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.<sup>36</sup> 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 ( $^{11}\text{C}$ )–labeled *trans*-1,2,3,5,6,10- $\beta$ -hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ( $[^{11}\text{C}(+)\text{McN-5652}]$ ) and 2 $\beta$ -carbomethoxy-3- $\beta$ -(4-fluorophenyl)tropane ( $[^{11}\text{C}]\text{WIN-35,428}$ ) binding in a healthy control subject and an individual with autism. A and B, Images of the  $[^{11}\text{C}(+)\text{McN-5652}]$  distribution volume with a color scale ranging from 0 to 60 mL/g show a control brain and a global reduction in  $[^{11}\text{C}(+)\text{McN-5652}]$  distribution in an autistic individual. C and D, Radioactivity produced by  $[^{11}\text{C}(+)\text{McN-5652}]$  in the orbitofrontal region and the striatum of a representative control and an autistic subject. E and F, Images of the  $[^{11}\text{C}]\text{WIN-35,428}$  ratio index reflect the binding potential of  $[^{11}\text{C}]\text{WIN-35,428}$  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  $[^{11}\text{C}]\text{WIN-35,428}$  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.<sup>37-39</sup> 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).<sup>40,41</sup> We also assessed anxiety and depressive symptoms using the 17-item Hamilton Anxiety Scale (HAM-A)<sup>42</sup> and the 17-item Hamilton Scale for Depression (HAM-D),<sup>43</sup> respectively. Aggression was evaluated using the Aggression Questionnaire (AQ).<sup>44</sup> These evaluations were performed on the day of the PET examination with radioactive carbon ( $^{11}\text{C}$ )–labeled *trans*-1,2,3,5,6,10- $\beta$ -hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-a]isoquinoline ( $[^{11}\text{C}(+)\text{McN-5652}]$ ).

## 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.<sup>45,46</sup> To reduce the partial volume effect, we set ROIs on the MRIs and transferred them onto PET images as described elsewhere.<sup>47,48</sup> 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  $[^{11}\text{C}(+)\text{McN-5652}]$  to determine their serotonin transporter binding.<sup>49,50</sup> The reproducibility of PET images with  $[^{11}\text{C}(+)\text{McN-5652}]$  was reported in *Papio anubis* baboons<sup>51</sup>; when

the primates underwent scanning with  $[^{11}\text{C}(+)\text{McN-5652}]$  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  $[^{11}\text{C}]$ -labeled 2 $\beta$ -carbomethoxy-3- $\beta$ -(4-fluorophenyl)tropane ( $[^{11}\text{C}]\text{WIN-35,428}$ ) was performed under the same protocol as in the  $[^{11}\text{C}(+)\text{McN-5652}]$  study to measure dopamine transporter binding.<sup>52-54</sup> As described previously,<sup>49</sup> we estimated  $[^{11}\text{C}(+)\text{McN-5652}]$  binding on the basis of a single-tissue-compartment 3-parameter model. Because the distribution volume of  $[^{11}\text{C}(+)\text{McN-5652}]$  estimated by this model correlated with the binding of the serotonin transporter in the brain,<sup>49</sup> we constructed parametric images of the  $[^{11}\text{C}(+)\text{McN-5652}]$  distribution volume for all participants with the use of biomedical imaging software (PMOD, version 2.5; PMOD Technologies Ltd, Zurich, Switzerland) (Figure 1A and B). Similarly, applying a 3-compartment 4-parameter model to the  $[^{11}\text{C}]\text{WIN-35,428}$  data allowed us to estimate the binding potential of the tracer<sup>47,53</sup> 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),<sup>53</sup> we constructed parametric images of the  $[^{11}\text{C}]\text{WIN-35,428}$  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.<sup>49,53</sup>

## STATISTICAL ANALYSIS

Demographic and clinical variables were compared between the autistic and control groups using the *t* test, in which a 2-tailed  $\alpha$  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 [<sup>11</sup>C](+)McN-5652 and [<sup>11</sup>C]WIN-35,428 Binding Parameters<sup>a</sup>**

Brain Area	Coordinates			Voxel Level	
	x	y	z	Corrected P Value	z Score
[ <sup>11</sup> 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 [<sup>11</sup>C](+)McN-5652 and [<sup>11</sup>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 [<sup>11</sup>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 2A).

### CORRELATES OF SEROTONIN TRANSPORTER WITH CLINICAL CHARACTERISTICS IN AUTISTIC PARTICIPANTS

The [<sup>11</sup>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 [<sup>11</sup>C](+)McN-5652 and [<sup>11</sup>C]WIN-35,428 Binding Parameters<sup>a</sup> (continued)**

Brain Area	Coordinates			Voxel Level	
	x	y	z	Corrected P Value	z Score
<b>[<sup>11</sup>C](+)McN-5652 Binding</b>					
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
<b>[<sup>11</sup>C]WIN-35,428 Binding</b>					
Increase in binding in the autistic vs control groups					
Orbitofrontal cortex, BA 11	-2	30	-10	.02	4.28

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

<sup>a</sup>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 [<sup>11</sup>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 [<sup>11</sup>C](+)McN-5652 distribution volume and the symptom profiles of the HAM-A, HAM-D, or AQ.

#### COMPARISON OF DOPAMINE TRANSPORTER DISTRIBUTION BETWEEN GROUPS

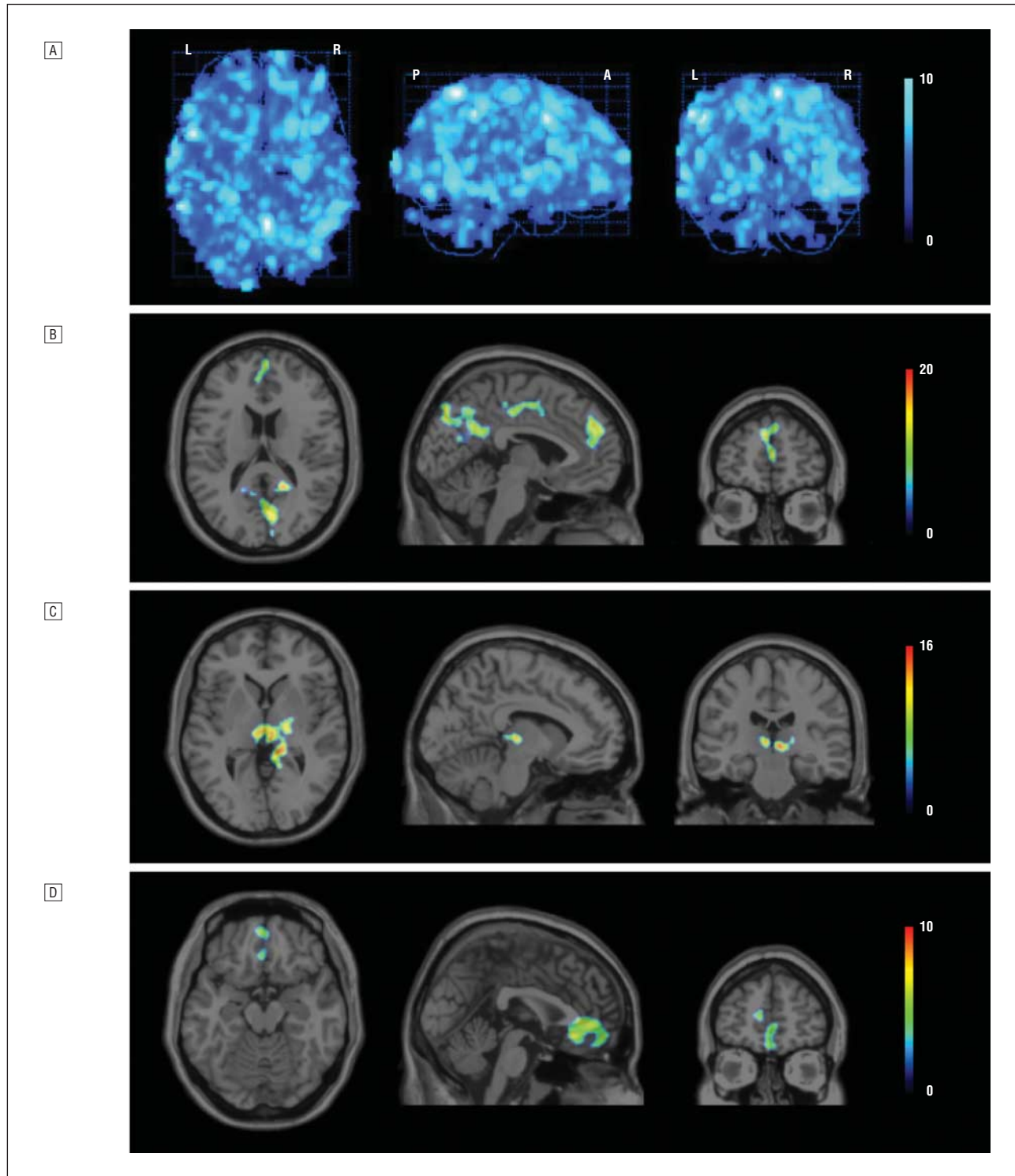
The SPM analysis revealed a significant increase in [<sup>11</sup>C]WIN-35,428 binding in the medial frontal region covering the orbitofrontal cortex in the autistic group com-

pared with the control group ( $P < .05$ , corrected in voxel-level analysis) (Table 2 and Figure 2D).

No significant correlation was found between [<sup>11</sup>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 [<sup>11</sup>C](+)McN-5652 and [<sup>11</sup>C]WIN-35,428 binding in the autistic group (Figure 1C, D, G, and H), the [<sup>11</sup>C](+)McN-5652 distribution volumes were significantly negatively correlated with the [<sup>11</sup>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  $[^{11}\text{C}](+)\text{McN-5652}$  and  $[^{11}\text{C}]\text{WIN-35,428}$  binding. A, Glass brain images indicate extensive reduction in the  $[^{11}\text{C}](+)\text{McN-5652}$  distribution volume in the autistic group ( $P < .05$ , corrected). B and C, Statistical parametric maps show brain regions in which the  $[^{11}\text{C}](+)\text{McN-5652}$  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  $[^{11}\text{C}]\text{WIN-35,428}$  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

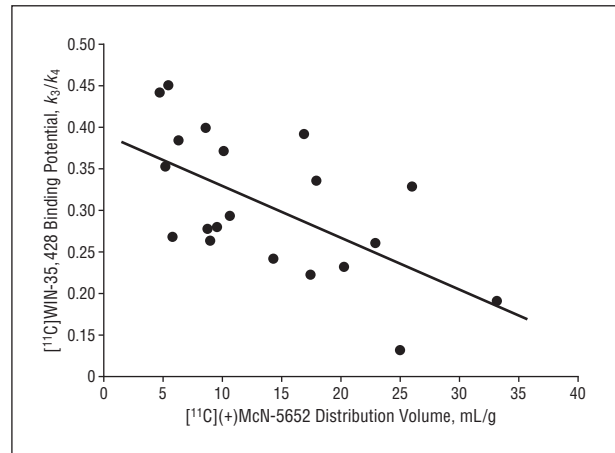
The autistic participants had a significantly decreased  $[^{11}\text{C}](+)\text{McN-5652}$  distribution volume throughout the brain, whereas they had a significantly increased  $[^{11}\text{C}]\text{WIN-}$

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 serotonergic 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.<sup>55</sup> In addition, approximately 20% to 38% of autistic individuals are reported to have epilepsy.<sup>56,57</sup> 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.<sup>58,59</sup> 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.<sup>60</sup>

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,<sup>61</sup> which in turn ameliorate repetitive behaviors in some but not all autistic individuals.<sup>15</sup> 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.<sup>62</sup> Furthermore, MRI studies of autistic adults have demonstrated enlargement of the caudate and putamen volumes, which is positively correlated with repetitive behaviors.<sup>63</sup> Repetitive behaviors have also been shown to be related to the hippocampus volume in obsessive-compulsive disorder.<sup>64</sup> 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.<sup>65</sup> 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



**Figure 3.** Correlation between [<sup>11</sup>C](+)McN-5652 and [<sup>11</sup>C]WIN-35,428 binding. Pearson product moment correlation analysis shows a significantly negative correlation between the [<sup>11</sup>C](+)McN-5652 distribution volume and the [<sup>11</sup>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.

serotonin transporter binding in the thalamus is specific to repetitive and/or obsessive behavior and interests seen in adults with high-functioning autism.

Increases in peripheral serotonin levels have been the most consistent finding in autistic children.<sup>6-10</sup> High levels of peripheral serotonin are known to cause a loss of serotonin terminals during development, when serotonin transporters are located,<sup>66-69</sup> 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 serotonergic systems at the developmental stage. The *SLC6A* gene polymorphism has been associated with autism,<sup>17,18</sup> although other reports have not replicated these findings.<sup>70,71</sup> Because the gene polymorphism could modulate the neurodevelopment and function of the brain<sup>19,20</sup> and influence *SLC6A4* expression,<sup>72,73</sup> 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. Serotonergic activity of the prefrontal cortical regions has been shown to correlate with aggressive behavior in humans.<sup>74</sup> Some autistic individuals were reported to have aggression.<sup>75</sup> 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<sub>2</sub> receptor binding in autistic children<sup>32</sup> and elevated dopamine synthesis and storage in the striatum and frontal cortex of adults with Asperger syndrome.<sup>76</sup> 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,<sup>77</sup> such as impulsive and aggressive behaviors.<sup>75,78</sup> 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 serotonergic system by a lesion in the nucleus raphe<sup>79</sup> and that the uptake of serotonin into dopamine neurons takes place by means of dopamine transporters.<sup>80</sup>

With respect to our use of [<sup>11</sup>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<sup>48,81-83</sup> indicate the validity for the use of [<sup>11</sup>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)<sup>48</sup> and that the magnitude of this increase (58%) is greater than the re-

ported level of within-subject test-retest variability (9.3%).<sup>84</sup> Despite these accounts, a PET tracer with a much higher affinity to the extrastriatal dopamine transporter may be desirable.

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**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).

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