Decreased Frontal Serotonin$_{2A}$ Receptor Binding in Antipsychotic-Naive Patients With First-Episode Schizophrenia

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Context: Postmortem investigations and the receptor affinity profile of atypical antipsychotics have implicated the participation of serotonin$_{2A}$ receptors in the pathophysiology of schizophrenia. Most postmortem studies point toward lower cortical serotonin$_{2A}$ binding in schizophrenic patients. However, in vivo studies of serotonin$_{2A}$ binding report conflicting results, presumably because sample sizes have been small or because schizophrenic patients who were not antipsychotic-naive were included. Furthermore, the relationships between serotonin$_{2A}$ binding, psychopathology, and central neurocognitive deficits in schizophrenia are unclear.

Objectives: To assess in vivo brain serotonin$_{2A}$ binding potentials in a large sample of antipsychotic-naive schizophrenic patients and matched healthy controls, and to examine possible associations with psychopathology, memory, attention, and executive functions.

Design: Case-control study.

Setting: University hospital, Denmark.

Participants: A sample of 30 first-episode, antipsychotic-naive schizophrenic patients, 23 males and 7 females, and 30 matched healthy control subjects.

Interventions: Positron emission tomography with the serotonin$_{2A}$-specific radioligand fluorine 18–labeled altanserin and administration of a neuropsychological test battery.

Main Outcome Measures: Binding potential of specific tracer binding, scores on the Positive and Negative Syndrome Scale, and results of neuropsychological testing.

Results: Schizophrenic patients had significantly lower serotonin$_{2A}$ binding in the frontal cortex than did control subjects. A significant negative correlation was observed between frontal cortical serotonin$_{2A}$ binding and positive psychotic symptoms in the male patients. No correlations were found between cognitive functions and serotonin$_{2A}$ binding.

Conclusion: The results suggest that frontal cortical serotonin$_{2A}$ receptors are involved in the pathophysiology of schizophrenia.

Trial Registration: clinicaltrials.gov Identifier: NCT00207064

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A GROWING BODY OF EVIDENCE points toward impairment of the serotonin$_{2A}$ receptor function in schizophrenia. The initial serotonin hypothesis of schizophrenia was sparked by the observation that lysergic acid diethylamide (LSD), a drug with structural similarities to serotonin and a high affinity to serotonin$_{2A}$ receptors, has hallucinogenic properties similar to schizophrenic symptoms. This hypothesis is backed by 11[1-11] of 15[12-15] postmortem studies reporting decreased serotonin$_{2A/C}$ binding in cortical areas, especially in the frontal cortex. However, these reports were primarily based on chronically ill, medicated patients, and the techniques used to analyze postmortem tissue differed between studies.[16]

Indirect support for the involvement of the serotonin$_{2A}$ receptor in schizophrenia arises from the association between the receptor affinity profile and the clinical characteristics of new, atypical antipsychotic drugs. Atypical antipsychotic drugs have complex pharmacologic features. For example, clozapine has high affinity for a number of serotonin (serotonin$_{2A}$, serotonin$_{2C}$, serotonin$_6$, serotonin$_7$), dopamine (D_4), muscarinic (M_1, M_2, M_3, M_4, M_5), adrenergic (α_1- and α_2-subtypes), and other biogenic amine receptors.[17] However, compared with typical antipsychotic drugs, which primarily bind to the dopamine 2 (D_2) receptors, most atypical antipsychotics have higher affinity to cortical serotonin$_{2A}$ receptors than to striatal D_2 receptors.[18,19] This may account for the reduced extrapyra-

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midal side effects of atypical antipsychotics and their effect on negative symptoms.[20]

It is unclear how serotonin$_{2A}$ activity is associated with the most commonly found clinical cognitive deficits in schizophrenia,[21,22] for example, attention, executive functions, and spatial working memory. It has been proposed that working memory could be one of the central cognitive markers or endophenotypes of schizophrenia.[23-25] In general, the literature suggests that serotonin$_{2A}$ receptor antagonism improves cognition in schizophrenia.[26] Recent research has shown that the affinity of antipsychotic drugs to the serotonin$_{2A}$ receptor is associated with cognition in a subtle way. Spatial working memory has been suggested to improve by stimulation rather than blockade of serotonin$_{2A}$ receptors in both preclinical and clinical studies.[27-29] Conversely, blockade of serotonin$_{2A}$ receptors by the serotonin$_{2A}$ antagonist ketanserin in healthy control subjects impaired memory more than combined escitalopram oxalate–ketanserin treatment.[30] Atypical antipsychotic drugs with a high antagonistic action on serotonin$_{2A}$ may therefore benefit spatial working memory tasks less than low-affinity drugs.[27] These studies support a linkage between impaired working memory and decreased serotonin$_{2A}$ availability or function in the human brain.

The introduction of selective serotonin$_{2A}$ receptor radioligands for positron emission tomography (PET) made it possible to examine the serotonin$_{2A}$ receptor density in the living human brain. However, few PET studies on first-episode antipsychotic-naive schizophrenic patients have been performed so far, and the results are inconsistent. Three studies found no difference in serotonin$_{2A}$ binding between schizophrenic patients and healthy control subjects,[31-33] and 1 study found a decreased binding potential in the left lateral frontal cortex in 6 patients.[34] These studies are limited by small sample sizes and by their use of the radioligands setoperone labeled with fluorine 18 and *N*-methylspiperone labeled with carbon 11, which have a relatively low serotonin$_{2A}$ receptor selectivity.[35]

The radioligand altanserin labeled with fluorine 18 is highly selective for the serotonin$_{2A}$ receptor and allows measurements of serotonin$_{2A}$ receptor availability in both cortical and subcortical regions.[31,36,37] In a previous preliminary study, our group reported the use of this radioligand in 15 antipsychotic-naive schizophrenic patients.[35] We were unable to confirm our hypothesis of decreased frontal $5HT_{2A}$ binding. However, in a post hoc analysis[38] we found increased serotonin$_{2A}$ binding in the caudate nucleus. This result was considered a preliminary finding because of the modest receptor density of serotonin$_{2A}$ in subcortical brain regions. Larger sample sizes were deemed to be required to exclude type II errors.

The aim of the present PET study, therefore, is to use [^{18}F]altanserin-PET to investigate cortical and subcortical serotonin$_{2A}$ binding in an extended group of first-episode antipsychotic-naive schizophrenic patients and matched healthy control subjects. Fifteen of the patients were identical to the patients included in our previous preliminary study.[35]

A decrease in serotonin$_{2A}$ binding in the frontal cortex in these patients compared with matched healthy control subjects was expected a priori. We also expected to confirm our preliminary finding of serotonin$_{2A}$ receptor upregulation in the caudate nucleus. As an additional and new approach, we explored possible associations between serotonin$_{2A}$ binding, psychopathology, and central cognitive deficits, specifically spatial working memory, attention, and executive functions.

METHODS

The study was approved by the Ethics Committee of Copenhagen and Frederiksberg ([KF]11-061/03). The subjects participated after receiving a full explanation of the study and providing written informed consent according to the Declaration of Helsinki II.

PARTICIPANTS

Thirty-three patients (26 male and 7 female) were recruited after voluntary first-time referral to a psychiatric unit of one of the affiliated university hospitals in the Capital Region of Copenhagen (Bispebjerg Hospital, Rigshospitalet, Psychiatric University Center Glostrup, or Psychiatric University Center Gentofte).

Thirty of the 33 patients fulfilled the diagnostic criteria for schizophrenia according to both the *International Statistical Classification of Diseases, 10th Revision*, and *DSM-IV*. Three patients proved to have a diagnosis of schizotypal personality disorder at a later stage of the study and were therefore excluded. All included patients (mean [SD] age, 26.4 [5.5] years) were antipsychotic naive. The diagnoses of schizophrenia were verified by means of the Schedules for Clinical Assessment in Neuropsychiatry interview.[39]

Thirty healthy control subjects (mean [SD] age, 26.4 [5.7] years) matched for age, sex, and parental socioeconomic status were recruited from the community by advertisement. None of the healthy control subjects had present or previous psychiatric disorder or any history of psychotropic medication as determined by the Schedules for Clinical Assessment in Neuropsychiatry interviews.

Six patients were previous (n=4) or present (n=2) users of antidepressant medication (in all cases selective serotonin reuptake inhibitors [SSRIs]). Benzodiazepines were allowed, albeit not on the day of the PET scan. Eight patients fulfilled lifetime criteria for substance abuse. All abuse diagnoses were clearly secondary to the diagnosis of schizophrenia. Substance dependence was an exclusion criterion. The *DSM-IV* diagnoses of substance abuse were as follows: alcohol abuse, in sustained full remission (n=2); cannabis abuse, in a controlled environment (n=1); other abuse, sustained full remission (n=1); other abuse, moderate (n=1); other abuse, in a controlled environment (n=2); and other abuse, early partial remission (n=1). In 4 of the patients the diagnosis "other abuse" covered mixed cannabis and alcohol abuse, and in the remaining patient the diagnosis covered a history of amphetamine and cocaine use. Three patients had no history of abuse for the past year, and 4 patients had no abuse for the past month. All subjects had negative results of urine screening for substance intake before the PET scan.

Eighteen of the patients (60%) and 6 of the control subjects (20%) were smokers. None of the participants had smoked 2 hours before the PET investigations. Smoking status was not a matching criterion because, in a recent study on 136 healthy subjects study, our group found no effect of smoking on serotonin$_{2A}$ binding.[40]

No subjects had a history of significant head injury or nonpsychiatric disorder. All subjects had normal results of a neurologic interview and examination, and structural magnetic resonance (MR) images of the brain were without clinical pathological findings as evaluated by a neuroradiologist.

PSYCHOPATHOLOGICAL RATINGS

Symptom severity was assessed by trained raters using the Positive and Negative Syndrome Scale (PANSS).[41] All interviews were recorded on DVD for validation purposes. A subsample of 10 randomly selected PANSS ratings showed an intraclass correlation coefficient of 0.85 between the raters in a 2-way fixed-effect model.[42]

NEUROCOGNITIVE TESTING

Memory, executive functions, and attention were assessed with the following subtests from the Cambridge Neuropsychological Test Automated Battery[43]: Spatial Working Memory, Stockings of Cambridge, Intra-Extradimensional Set Shifting, and Rapid Visual Information Processing.

MR IMAGING

High-resolution 3-dimensional, T1-weighted, sagittal, magnetization-prepared rapid gradient-echo images of the whole head (inversion time, 800 milliseconds; echo time, 3.93 milliseconds; repetition time, 1540 milliseconds; flip angle, 9°; matrix, 256×256; 192 sections) using an 8-channel head array coil were acquired in all subjects on a 3-T imager (TRIO; Siemens, Erlangen, Germany) at the MR department of the Copenhagen University Hospital, Hvidovre, Denmark.

[^{18}F]ALTANSERIN RADIOSYNTHESIS AND ADMINISTRATION

The radiosynthesis of [^{18}F]altanserin has been described previously.[44] Quality control was performed by means of thin-layer chromatography and high-performance liquid chromatography. The absence of residual solvents (methanol, tetrahydrofuran, and dimethyl sulfoxide) in the final formulation was confirmed by proton nuclear MR. For each PET study, 0.3 to 3.5 GBq of [^{18}F]altanserin was produced with a radiochemical yield exceeding 95%. (To convert [^{18}F]altanserin to curies, multiply by 2.7×10^{-3}.) Catheters were inserted in both cubital veins for tracer infusion and blood sampling, respectively. The [^{18}F]altanserin was administrated as a bolus to injection followed by continuous infusion to obtain a steady state of the tracer in blood and tissue. The bolus infusion ratio was 1.75 hours, as previously described.[45] Subjects received a maximum dose of 3.7 MBq of [^{18}F]altanserin per kilogram of body weight.

PET SCANNING

The PET scans were acquired in tracer steady-state conditions with an 18-ring scanner (GE-Advance; General Electric Co, Milwaukee, Wisconsin), operating in 3-dimensional acquisition mode, producing 35 image sections with an intersection distance of 4.25 mm. The total axial field of view was 15.2 cm, with an approximate in-plane resolution down to 5 mm. During steady state, the fraction of unmetabolized tracer in venous plasma was determined at 5 time points by means of high-performance liquid chromatography analysis. Reconstruction, attenuation, and scatter correction procedures were conducted as previously described.[45]

The subjects were placed in the scanner 90 minutes after the bolus injection of [^{18}F]altanserin. The subjects were aligned in the scanner by means of a laser system so that the detectors were parallel to the orbitomeatal line and positioned to include the cerebellum in the field of view with the use of a short 2-minute transmission scan. An individual head holder was made to ensure relative immobility. All subjects were scanned in a resting state. A 10-minute transmission scan was obtained for correction of tissue attenuation with retractable germanium Ge 68/gallium Ga 68 pin sources. The transmission scans were corrected for tracer activity by a 5-minute emission scan performed in 2-dimensional mode. Dynamic 3-dimensional emission scans (5 frames of 8 minutes) were started 120 minutes after tracer administration.

Data were reconstructed into a sequence of 128×128×35 voxel matrixes, each voxel measuring 2.0×2.0×4.25 mm, with software provided by the manufacturer. A 3-dimensional reprojection algorithm with a 6-mm transaxial Hann filter and an 8.5-mm axial ramp filter was applied. Corrections for dead time, attenuation, and scatter were performed.

BLOOD SAMPLES

Five venous blood samples were drawn at midscan times 4, 12, 20, 28, and 36 minutes after the start of the dynamic scanning sequence. The samples were immediately centrifuged, and 0.5 mL of plasma was counted in a well counter for determination of radioactivity. Three of the 5 blood samples drawn at 4, 20, and 36 minutes were also analyzed for percentage of parent compound ([^{18}F]altanserin) by means of reverse-phase high-performance liquid chromatography following a previously described method.[46]

In addition, the free fraction of [^{18}F]altanserin in plasma, f_P, was estimated by equilibrium dialysis, following a modified procedure.[47] The dialysis was performed with Teflon-coated dialysis chambers (Amika; Harvard Bioscience, Holliston, Massachusetts) with a cellulose membrane that retains proteins larger than 10 000 Da. A small amount of [^{18}F]altanserin (approximately 1 MBq) was added to 10-mL plasma samples drawn from the subjects. A 500-L portion of plasma was then dialyzed at 37°C for 3 hours against an equal volume of buffer because pilot studies had shown that a 3-hour equilibration time yielded stable values. The buffer consisted of 135mM sodium chloride, 3.0mM potassium chloride, 1.2nM calcium chloride, 1.0mM magnesium chloride, and 2.0mM phosphate (pH, 7.4). After the dialysis, 400 L of plasma and buffer was counted in a well counter, and the f_P of [^{18}F]altanserin was calculated as the ratio of disintegrations per minute in buffer vs plasma.

MR/PET CO-REGISTRATION

The PET images and 3-dimensional T1-weighted MR images were co-registered by means of a MATLAB-based program[48] (MathWorks Inc, Natick, Massachusetts), in which PET and MR images are brought to fit through manual translation and rotation of the PET image with subsequent visual inspection in 3 planes.[46]

VOLUMES OF INTEREST AND PARTIAL VOLUME CORRECTION

Volumes of interest (VOIs) were automatically delineated on each individual's transaxial MR imaging sections in a strictly user-independent manner.[49] This approach allowed automatic co-registration of a template set of 10 MR images to a new subject's MR image. The identified transformation parameters were used to define VOIs in the new subject MR imaging space, and through the co-registration these VOIs were transferred onto the PET images.

A frontal cortex region was defined for each subject and served as the primary VOI. The frontal cortex VOI consisted

Table 1. Mean Binding Potentials of Specific [^{18}F]Altanserin Binding in Frontal Cortex and Subregions of Interest in Patients and Controls

Region	Mean (SEM) Patients (n=30)	Mean (SEM) Controls (n=30)	P Value
Frontal cortex	2.91 (0.12)	3.37 (0.14)	.007
Orbitofrontal cortex	2.89 (0.13)	3.42 (0.15)	.004
Medial inferior frontal cortex	3.07 (0.12)	3.50 (0.13)	.07
Superior frontal cortex	3.34 (0.14)	3.85 (0.15)	.008
Anterior cingulate cortex	2.34 (0.09)	2.68 (0.13)	.02
Other regions			
Amygdala	0.68 (0.04)	0.77 (0.05)	.15[a]
Caudate nucleus	0.60 (0.04)	0.65 (0.04)	.34[a]
Entorhinal cortex	1.11 (0.05)	1.21 (0.06)	.20[a]
Hippocampus	0.74 (0.04)	0.81 (0.05)	.30[a]
Hypothalamus	0.34 (0.04)	0.38 (0.04)	.50[a]
Insula	1.82 (0.08)	2.10 (0.09)	.04
Medial inferior temporal cortex	2.66 (0.11)	3.08 (0.13)	.01
Occipital cortex	2.56 (0.11)	2.97 (0.12)	.01
Parietal cortex	3.26 (0.13)	3.70 (0.14)	.01
Posterior cingulate cortex	2.57 (0.11)	2.93 (0.12)	.03
Putamen	0.41 (0.05)	0.48 (0.03)	.08[a]
Sensorimotor cortex	2.72 (0.11)	3.13 (0.11)	.01
Superior temporal cortex	2.68 (0.11)	3.03 (0.12)	.004
Thalamus	0.48 (0.03)	0.52 (0.03)	.39[a]

[a] Not significant.

of a volume-weighted average of left and right cortical regions and included the orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, and anterior cingulate cortex.[49]

Other regions included were the amygdala, caudate nucleus, entorhinal cortex, hippocampus, hypothalamus, insula, occipital cortex, parietal cortex, posterior cingulate cortex, putamen, sensorimotor cortex, superior temporal cortex, and thalamus. The cerebellum was used for estimation of nonspecific binding.

To enable partial volume correction of the PET data, MR images corrected for radiofrequency inhomogeneities by means of the N3 software[50] were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes with Statistical Parametric Mapping software (Wellcome Department of Cognitive Neurology, London, England). Partial volume correction was performed according to the Müller-Gärtner method.[51,52] The white matter value was extracted from the uncorrected PET scan as the mean voxel value from a brain region containing predominantly white matter (centrum semiovale).

QUANTIFICATION OF SEROTONIN$_{2A}$ RECEPTOR BINDING

The outcome measure was the binding potential of specific tracer binding (BP_P). The cerebellum was used as a reference region, since it represents nonspecific binding only. In the steady state, BP_P is defined as follows:

$$BP_P = [(C_{VOI} - C_{Reference})/C_{Plasma}] = f_p(B_{max}/K_d),$$

where C_{VOI} and $C_{Reference}$ are the steady-state mean count densities in the VOI and in the reference region, respectively; C_{Plasma} is the steady-state activity of nonmetabolized tracer in plasma; f_p is the free fraction of radiotracer; B_{max} is the density of receptor sites available for tracer binding; and K_d is the affinity constant of the radiotracer to the receptor.

STATISTICS

All analyses were performed with SPSS software (SPSS Inc, Chicago, Illinois). Between-group (patient or control) comparisons of all reported outcome measures were performed by parametric analysis after verifying that the data were normally distributed according to the Kolmogorov-Smirnov test. Potential outliers were detected with the Grubbs outlier test[53] and subsequently excluded from analysis. The planned comparison in frontal serotonin$_{2A}$ binding between patients and controls was performed with an independent-sample *t* test (1-tailed, because of our directional hypothesis). In addition, an analysis of variance (ANOVA) was performed with between-factor group (patient or control) and within-factor region (frontal or other), to test whether a potential effect of group was more a global effect across all regions than a regional effect principally affecting frontal cortex. Furthermore, to test for additional regional group differences in binding, an ANOVA was performed with between-factor group (patient or control) and within-factor region (the regions specified in **Table 1**). Independent-sample *t* tests (2-tailed) were performed only when these ANOVAs indicated statistically significant results.

Independent-sample *t* tests were further used to test for differences between patients and controls with regard to neurocognitive and psychopathological measures (2-tailed). Correlation analyses were performed with the Pearson product-moment correlation coefficient. The potential effect on the results of antidepressant medication, benzodiazepines, and substance abuse was examined by including these parameters in a multiple analysis of covariance as covariates.

RESULTS

SEROTONIN$_{2A}$ BINDING

The planned comparison of frontal cortical binding showed reduced serotonin$_{2A}$ binding in patients compared with controls (t_{58}=2.54, P=.01).

The ANOVA on region and group disclosed significant main effects of group ($F_{1,58}$=5.58, P=.02) and region ($F_{17,42}$=82.19, P<.001), and a significant region × group interaction effect ($F_{17,986}$=5.77, P<.001). Further analysis of these results indicated that serotonin$_{2A}$ binding in patients was significantly reduced not only in the frontal cortex but also in a number of other cortical—but not subcortical—regions (Table 1). Therefore, to test whether the frontal cortical region showed an even lower serotonin$_{2A}$ receptor binding than the other cortical regions, a post hoc ANOVA was performed with within-factor region (frontal cortex or other regions, Table 1) and between-factor group. This ANOVA demonstrated main effects of region ($F_{1,58}$=1109, P<.001) and group ($F_{1,58}$=6.00, P=.02) as well as a first-order interaction between region and group ($F_{1,58}$=7.78, P=.007), indicating a more pronounced reduction in serotonin$_{2A}$ receptor binding in the frontal cortical region than in the other cortical regions (**Figure 1**).

In the control group, the Grubbs test indicated 1 significant outlier with an increased binding in the frontal cortex. After exclusion of this outlier, the differences in serotonin$_{2A}$ binding remained significant. None of the results changed when the subjects with previous (n=4) or current (n=2) antidepressant treatment or cocaine and amphetamine abuse (n=1) were excluded from the analyses. Data related to antidepressant medication is described in detail in **Table 2**. Furthermore, use of benzodiazepines did not covary significantly. The 2 groups did not differ significantly with regard to body mass index, injected radioactive dose, plasma free fraction, and specific radioactivity of [^{18}F]altanserin (**Table 3**). The patients had significantly lower nonspecific binding than did the healthy control subjects.

SEROTONIN$_{2A}$ BINDING AND NEUROCOGNITION

The cognitive data represent a subsample of a larger unpublished data set (R.A., B.O., Birgitte Fagerlund, PhD, Anders Gade, PhD, H.R., B.H.E., and B.G., unpublished data, 2009). Patients had significantly lower neurocognitive scores than healthy control subjects on the following tests: Spatial Working Memory strategy, Spatial Working Memory total errors, Spatial Working Memory between errors, Intra-Extradimensional Set Shifting total errors, and Intra-Extradimensional Set Shifting total number of trials on all stages attempted. There were no significant differences in Stockings of Cambridge or Rapid Visual Information Processing (**Table 4**). In the frontal cortex, no significant correlations were detected between serotonin$_{2A}$ binding and the neurocognitive measures. No significant correlations were found between the other VOIs and neurocognitive performance.

SEROTONIN$_{2A}$ BINDING AND PSYCHOPATHOLOGY

In the patients, the PANSS mean (SEM) scores were as follows: positive, 20.0 (0.93); negative, 22.0 (1.20); general, 38.5 (1.30); and total, 80.0 (2.60). A significant negative correlation ($r = -0.57$, $P < .01$) was found between serotonin$_{2A}$ binding in the frontal cortex and positive symptoms in the larger group of male patients. An explorative post hoc analysis showed significant negative correlations between frontal serotonin$_{2A}$ binding and the following subitems of the positive PANSS scale: P1 delusions ($r = -0.47$, $P = .03$) and P6 suspiciousness ($r = -0.53$, $P = .01$) (**Figure 2**). No significant differences were found between the other VOIs and psychopathology. There was no sex effect on symptom severity or serotonin$_{2A}$ binding.

COMMENT

In this study of serotonin$_{2A}$ binding in antipsychotic-naive, first-episode schizophrenic patients, we confirmed our hypothesis of lower frontal cortical serotonin$_{2A}$ binding in patients than in matched healthy control subjects. The serotonin$_{2A}$ binding was also reduced in a number of other cortical regions, but the reduction in the frontal cortical region was more pronounced. This is in agreement with the many postmortem studies suggesting decreased cortical serotonin$_{2A}$ receptor binding in schizophrenic patients. Moreover, the data demonstrated a significant negative correlation between frontal cortical serotonin$_{2A}$ binding and positive psychotic symptoms in male patients. We were not, however, able to confirm correlations between cognitive functions and serotonin$_{2A}$ binding, even though the patients performed significantly worse in spatial working memory and aspects of executive function than did the healthy controls.

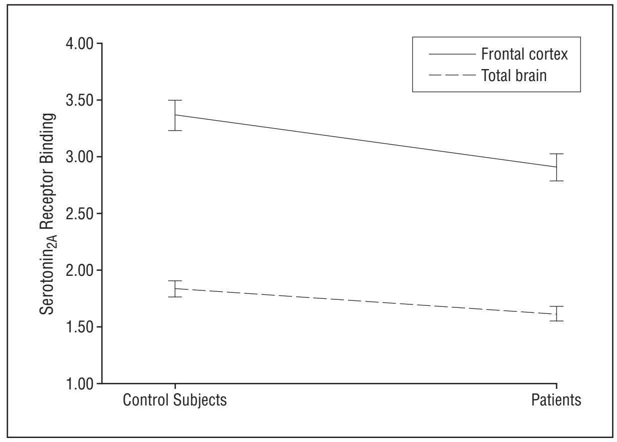

Figure 1. Mean frontal cortical and total serotonin$_{2A}$ receptor binding in 30 antipsychotic-naive patients with first-episode schizophrenia and 30 matched healthy controls.

Our results are based on what we believe to be the largest sample studied with PET, because earlier PET studies have reported results based on 6 to 15 patients.[31-35] The majority of these studies, including our own,[54] were unable to identify differences in cortical serotonin$_{2A}$ binding between schizophrenic patients and healthy control subjects. In our previous study[38] we found increased serotonin$_{2A}$ receptor binding in the caudate nucleus. This nucleus is a region with a relatively low serotonin$_{2A}$ receptor density; hence, the post hoc analyses were more prone to type II errors. The present study does not confirm our preliminary finding of increased binding in the caudate nucleus, but it does support the study by Ngan and colleagues,[34] who reported reduced serotonin$_{2A}$ binding in the frontal cortex of 6 neuroleptic-naive schizophrenic subjects. Similarly, Hurlemann and colleagues[55,56] reported decreased cortical serotonin$_{2A}$ binding in subjects at high risk of developing schizophrenia.

Decreased frontal serotonin$_{2A}$ binding and the relationship with positive psychotic symptoms may reflect either a primary pathophysiologic disturbance in schizophrenia or a compensatory downregulation of receptors in response to altered endogenous serotonin levels. Alternatively, the finding could indicate a downregulation compensating for hyperactive second messenger systems or hyperactivity in other systems on which the serotonin$_{2A}$ receptors have a modifying effect. Finally, the finding could imply that frontal serotonin$_{2A}$ receptors are important targets for antipsychotic drugs.

The correlation between serotonin$_{2A}$ binding and symptoms was present only in the male subjects. Various aspects of schizophrenia, including age at onset, pathophysiology, symptomatology, course of illness, and treatment response, have previously been shown to be related to sex. These sex differences supply evidence for a potential role of gonadal hormones and for an interaction of these hormones with neurotransmitters (for a re-

Table 2. Patients Receiving Antidepressant Medication

Patient No./Sex	Antidepressant	Mean Daily Dose, mg	Treatment Period	Discontinuation Before PET Scan
1/M	Citalopram hydrobromide	NA	14 d	2 y
2/F	Citalopram hydrobromide	20	1 d	13 d
3/F	Citalopram hydrobromide	40	60 d	Current
4/M	Citalopram hydrobromide	10	12 d	5 d
5/M	Sertraline hydrochloride	40	28 d	14 d
6/F	Fluoxetine hydrochloride	40	6 y	Current

Abbreviations: NA, not available; PET, positron emission tomography.

Table 3. PET-Related Data

	Mean (SEM)		
	Patients	Controls	P Value
BMI	24.17 (0.69)	23.40 (0.48)	.37[a]
Injected dose, GBq	0.2721 (0.0115)	0.2819 (0.0892)	.50[a]
Free fraction, %	0.34 (0.03)	0.51 (0.10)	.10[a]
Specific radioactivity, GBq/μmol	62.40 (9.01)	51.05 (4.93)	.28[a]
Nonspecific binding	1.47 (0.06)	1.78 (0.06)	.01

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); PET, positron emission tomography.
SI conversion factor: To convert injected dose to curies and radioactivity to curies per micromoles, multiply by 2.7×10^{-3}.
[a] Not significant.

view, see Halbreich and Kahn[57]). Indeed, we have previously reported sex differences in drug-naive schizophrenic patients with regard to the dopamine system, namely, a correlation between D_2 receptor binding in the frontal cortex and positive psychotic symptoms in male patients only.[58]

As expected, patients showed significantly poorer performance in spatial working memory and aspects of executive functions than did healthy control subjects. This is in agreement with previous studies that have shown that spatial working memory and executive functions are centrally impaired neurocognitive domains in schizophrenia.[21,22] However, we detected no correlations between the cognitive measures and $serotonin_{2A}$ binding in any of the VOIs. Hence, our data do not support previous findings relating $serotonin_{2A}$ receptor to cognition in general[26] and spatial working memory in particular.[27,28]

The interaction between serotonin and cognition is complex. Indeed, the interactions between serotonin, dopamine, norepinephrine, and the cholinergic system have been suggested to mediate cognitive behavior.[28] Moreover, studies differ in design with regard to subjects (rodents, healthy controls, or patients), type of serotonin manipulation (global, specific depletion, or stimulation), serotonin receptor subtype (currently 15 serotonin receptor subtypes have been identified), and the cognitive tests being used.[27]

There are some methodologic issues in the study that need to be addressed.

Six patients had previously received (n=4) or were currently receiving (n=2) SSRI treatment, which is known to affect serotonergic innervation. However, we have previously found that [^{18}F]altanserin binding to $serotonin_{2A}$

Table 4. Neurocognitive Performance of Memory, Executive Functions, and Attention in Schizophrenic Patients Compared With Healthy Controls

	Mean (SEM)		
Cognitive Domain	Patient	Control	P Value
Memory			
SWM strategy	30.57 (1.13)	26.48 (1.00)	.009
SWM total errors	19.07 (3.15)	10.10 (1.83)	.02
SWM between errors	18.71 (3.11)	9.73 (1.80)	.02
Executive functions			
SOC problems solved in minimum moves	9.32 (0.32)	9.20 (0.38)	.80[a]
SOC mean number of moves	4.15 (0.09)	4.13 (0.09)	.91[a]
IED total errors (adjusted for stages not completed, ie, 25/stage)	14.52 (2.16)	9.86 (0.50)	.045
IED completed stage errors (errors at stages successfully completed)	12.14 (1.35)	9.86 (0.49)	.12[a]
IED EDS errors (errors made at the EDS stage, ie, stage 8)	6.04 (1.35)	2.07 (0.23)	.007
IED total No. of trials on all stages attempted (adjusted for stages not completed, ie, 50/stage)	77.44 (3.63)	68.55 (1.19)	.03
Attention			
RVP A′ signal detection measure of sensitivity to errors test 3-5-7	0.9847 (0.0026)	0.9890 (0.0017)	.15[a]
RVP total hits 3-5-7	69.89 (0.76)	71.16 (0.46)	.15[a]
RVP total misses 3-5-7	4.07 (0.73)	2.83 (0.46)	.16[a]

Abbreviations: EDS, extradimensional shift; IED, Intra-Extradimensional Set Shifting; RVP, Rapid Visual Information Processing; SOC, Stockings of Cambridge; SWM, Spatial Working Memory.
[a] Not significant.

receptors is insensitive to a citalopram hydrobromide challenge increasing extracellular serotonin.[59] Furthermore, the effect of long-term SSRI treatment on $serotonin_{2A}$ density is unclear because SSRIs have different effects on $serotonin_{2A}$ receptors. Fluoxetine hydrochloride has been reported to have no effect on $serotonin_{2A}$ receptor number or to actually increase receptor number. Similarly, paroxetine hydrochloride has been shown to increase or have no effect on $serotonin_{2A}$ receptor density. In contrast, long-term citalopram treatment has been shown to downregulate $serotonin_{2A}$ receptors.[60]

For the foregoing reasons, we initially chose to include patients taking previous or current antidepres-

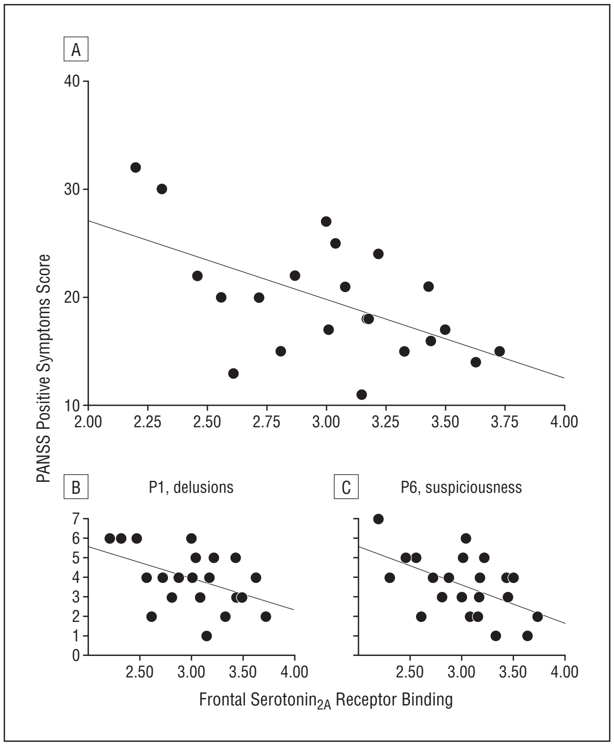

Figure 2. Correlation in male schizophrenic patients between mean frontal cortical serotonin$_{2A}$ receptor binding, positive symptoms on the Positive and Negative Syndrome Scale (PANSS), and 2 subitems. Negative correlations are evident for the PANSS positive scale ($r = -0.57$, $P = .007$) (A), as well as for subitems P1, delusions ($r = -0.47$, $P = .03$) (B), and P6, suspiciousness ($r = -0.53$, $P = .01$) (C).

sants but controlled for the potential effect in a post hoc analysis in which these patients were removed from the analyses. This did not change the results.

Similarly, 1 patient had a history of amphetamine and cocaine abuse. These substances are known to affect serotonergic innervation in the brain; however, the patient did not differ in serotonin$_{2A}$ receptor binding, and exclusion of this patient from the analyses did not alter the results.

Finally, there was a significantly lower binding in the cerebellum in patients than in control subjects. We have no explanation for this finding; the fraction of [^{18}F]altanserin metabolites in venous blood was the same in both groups. However, lower nonspecific cerebellar binding in the patients is not likely to bias the results because a relative underestimation of nonspecific binding in the patients would lead to an overestimation of the composite measure BP_P (see the equation in the "Methods" section).

In conclusion, this study of serotonin$_{2A}$ receptor binding in first-episode, antipsychotic-naive schizophrenic patients shows decreased binding in the frontal cortex and a negative correlation with positive symptoms in male patients. The results suggest that frontal cortical serotonin$_{2A}$ receptors are involved in the pathophysiology of schizophrenia. Because no correlations were found between binding and cognition, this study does not support the involvement of serotonin$_{2A}$ receptors in cognitive deficits in this early stage of the disease.

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