

The Effect of Paroxetine on 5-HT_{2A} Receptors in Depression: An [¹⁸F]Setoperone PET Imaging Study

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Objective: In the cortex of animals, serotonin (5-HT) levels increase after several weeks of treatment with selective serotonin reuptake inhibitors (SSRIs). Studies using an intrasubject design to examine the effects of SSRI treatment on 5-HT_{2A} receptors in the cortex of drug-free depressed patients are needed. In theory, agonist stimulation of 5-HT_{2A} receptors could be relevant to SSRI treatment by promoting neuronal growth and survival as well as direct elevation of mood. The objective of this study was to evaluate the effect of 6 weeks of paroxetine treatment on 5-HT_{2A} receptors in depressed patients.

Method: After a medication-free period of at least 3 months, 19 depressed patients were treated for 6 weeks with paroxetine, 20 mg/day. The authors used [¹⁸F]setoperone and positron emission tomography to assess 5-HT_{2A} receptor binding potential in the patients before and

after treatment and in 19 age-matched healthy subjects.

Results: 5-HT_{2A} binding potential declined with age in all cortical regions in the depressed and healthy subjects. There was a significant interaction between age and treatment effect on 5-HT_{2A} binding potential in all cortical regions. Subjects aged 20 to 30 years had a 10% decrease in 5-HT_{2A} binding potential after treatment, whereas subjects aged 30 to 40 had no change. No regional differences in 5-HT_{2A} binding potential between depressed and healthy subjects were found.

Conclusions: 5-HT_{2A} receptors downregulate in young depressed subjects after treatment with paroxetine, but this down-regulation attenuates with age. This suggests that over 6 weeks paroxetine treatment increases 5-HT agonism on 5-HT_{2A} receptors in the cortex of young patients with depression.

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While the specific mechanisms of antidepressant treatment are not known, serotonergic neurons may be important because they are targeted by selective serotonin reuptake inhibitors (SSRIs). In animal models, long-term SSRI administration raises intrasynaptic serotonin (5-HT) levels in the cortex (1). This could affect different types of 5-HT receptors, but there are several reasons why 5-HT_{2A} receptors could contribute to SSRI antidepressant effects. First, 5-HT_{2A} receptors have the potential to activate cAMP-response element-binding protein and/or brain-derived neurotrophic factor through Ca²⁺-dependent protein kinases in the cortex (2, 3). It is possible that elevated 5-HT concentrations after SSRI treatment could elevate mood by means of 5-HT_{2A} receptors because drugs with 5-HT_{2A} agonist properties may have euphoriant effects (4–8). The euphoria can occur after administration of low doses of 2,5-dimethoxy-4-methyl-amphetamine when hallucinations are not present (5, 7, 8).

While in theory some SSRI effects could be mediated by 5-HT_{2A} receptors, it is not known whether SSRIs at clinical doses have any effect at all on 5-HT_{2A} receptors in humans. Preclinical data with long-term SSRI treatment are not consistent, although there is a tendency toward upregulation or no change at lower doses (9–11) and down-

regulation at higher doses (12, 13). Rats have much faster drug metabolism than humans, and it may be that intraperitoneal dosing of SSRIs in rats differs from oral dosing in humans. We are aware of one published study of humans. Using [¹⁸F]setoperone positron emission tomography (PET), Massou et al. (14) found that a group of six depressed patients responding to SSRI treatment had higher 5-HT_{2A} receptor binding potential than a separate group of eight depressed patients who were untreated. This study did not use an intrasubject design, and five of the six treated patients were also taking benzodiazepines. It is reported that benzodiazepines lower serotonin release in animals, and this presents a potential confound (15–17).

To our knowledge, the relationship between 5-HT_{2A} receptors during depression and subsequent response to treatment has not been investigated. Postmortem methods cannot be used, and we know of no imaging study of 5-HT_{2A} receptors that has examined this question. Platelet 5-HT_{2A} receptor B_{max} (receptor density) is another in vivo measurement. Platelet and frontal cortex 5-HT_{2A} receptors have identical structures; hence, platelet measures may be used as a peripheral index of cortex 5-HT_{2A} receptors (18). One such study (19) showed no association be-

tween platelet 5-HT_{2A} B_{max} before treatment and the eventual response to SSRIs.

[¹⁸F]Setoperone has several properties favorable for PET imaging of 5-HT_{2A} receptors in the human cortex (20). Setoperone is an antagonist with high affinity and specificity for 5-HT_{2A} receptors in the cortex (6). The affinity of setoperone is 100 times as great for 5-HT_{2A} receptors as for 5-HT_{2C} receptors (6), and cortex 5-HT_{2C} receptor density is relatively low (21, 22). The affinity of setoperone for 5-HT_{2A} receptors is 10 to 50 times as great as its affinity for D₂ receptors, but D₂ receptors have a low density in the cortex (23). Both 5-HT_{2A,C} and D₂ antagonists displace [¹⁸F]setoperone in the striatum, but only 5-HT_{2A,C} antagonists displace [¹⁸F]setoperone in the cortex; thus, specific binding of [¹⁸F]setoperone in the cortex reflects binding to 5-HT_{2A} receptors. 5-HT_{2A} antagonists do not displace [¹⁸F]setoperone in the cerebellum, and the cerebellum is almost devoid of 5-HT_{2A} receptors, hence it may be used as a reference region (24, 25). Metabolites of [¹⁸F]setoperone do not show significant brain uptake (26). Other properties have been previously reviewed (27).

The main purpose of this study was to use [¹⁸F]setoperone PET to investigate the effect of 6 weeks of treatment with clinical doses of paroxetine on 5-HT_{2A} receptors in depressed patients. A secondary aim was to assess whether 5-HT_{2A} binding potential is abnormal in treatment-responsive patients before treatment.

Since SSRIs raise intrasynaptic 5-HT levels in animals (1) and 5-HT_{2A} agonists down-regulate 5-HT_{2A} receptors (28), it was hypothesized that with clinical doses of paroxetine, down-regulation of 5-HT_{2A} receptors would occur. We also hypothesized that 5-HT_{2A} receptor binding potential would not differ in treatment responders given the report that platelet 5-HT_{2A} B_{max} in drug-free depressed patients showed no relationship with later SSRI response (19).

Method

Subjects

This study was approved by the University of Toronto Human Subjects Review Committee. Nineteen depressed subjects aged 18 to 41 years and 19 age-matched healthy subjects aged 18 to 41 years were recruited by advertisement. All subjects were free of psychotropic drug use for more than 3 months. Data on the prefrontal cortex from baseline (pretreatment) scans of 14 depressed subjects and 19 healthy subjects have been previously reported (27). All of the subjects were physically healthy and right-handed. For each subject, written consent was obtained after the procedures had been fully explained.

The healthy subjects were screened by using the Structured Clinical Interview for DSM-IV—Non-Patient Edition (29). A diagnosis of a major depressive episode secondary to major depressive disorder was confirmed by the Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition (SCID-P) (30), which was administered by a trained research assistant. Each patient received a psychiatric consultation (by J.H.M. or S.H.K.) to verify the SCID-P diagnosis. A score of greater than 17 on the 17-item Hamilton Depression Rating Scale (31) was required for entry into the study. Patients with psychotic symptoms, bipolar disorder

(type I or II), or comorbid axis I diagnoses were excluded, as were subjects with a history of alcohol or drug abuse or dependence. In addition, all past drug use was recorded. Subjects with a history of substance use that did not meet the criteria for the SCID-P diagnosis of substance abuse received a urine drug screen and then were included in the study only if the result was negative. Six subjects had previously completed an antidepressant trial longer than 6 weeks, but none had received any antidepressant treatment within the last 6 months. Two had a history of past suicide attempts. Each depressed subject had routine tests to rule out common medical causes of depression (thyroid function, electrolyte levels, complete blood cell count).

Imaging and Treatment Protocol

For the depressed subjects, [¹⁸F]setoperone scanning occurred before and after treatment. The treatment was paroxetine, 20 mg/day in the evening for 6 weeks. Before treatment, the patients were informed that their serum paroxetine levels would be sampled on the day of the second [¹⁸F]setoperone PET scan. The serum sample and second [¹⁸F]setoperone PET scan took place 11 to 15 hours after the last paroxetine dose, when the intersubject variability of serum paroxetine levels is low. The samples taken were frozen at -20°C and subsequently assayed by means of high-performance liquid chromatography with fluorescence detection (Medical Toxicology Unit, Guy's & St. Thomas' Hospital Trust, London, U.K.) (32).

Treatment response was determined by the Hamilton depression score at the end of 6 weeks of treatment. A final score of 16 or greater indicated nonresponse. A score of 9 to 15 indicated a partial response, and a score of 8 or less was considered a full response.

[¹⁸F]Setoperone was prepared by [¹⁸F]fluoride substitution on the nitro-derivative precursor of setoperone (33). The [¹⁸F]setoperone was of high radiochemical purity (>99%) and high specific activity (mean=51 GBq/ μ mol, SD=36, at the time of injection). Imaging was based on the approach described by Blin et al. (34). An intravenous bolus of 185 MBq of [¹⁸F]setoperone was injected. PET images were obtained by using a GEMS 2048-15B camera. Images were obtained in five 1-minute frames, followed by 17 5-minute frames. The images were corrected for attenuation by using a ⁶⁸Ge transmission scan and reconstructed by filtered back projection (Hanning filter, 5 mm full width at half maximum).

Data Analysis

To obtain a measure of 5-HT_{2A} binding potential, we used the pseudoequilibrium cortex-to-cerebellum ratio minus one (35). In a two-tissue compartment model, one compartment is composed of free and nonspecifically bound ligand, and the second compartment represents setoperone specifically bound to 5-HT_{2A} receptors. Given that the cerebellum has no displaceable [¹⁸F]setoperone binding, the data for the time activity curve within this region represent the first tissue compartment of free and nonspecifically bound ligand (20, 24). With the pseudoequilibrium ratio method, if the free and nonspecifically bound ligand within the cerebellum is representative of the free and nonspecifically bound ligand in the cortex, then the ratio (cortex-cerebellum)/cerebellum is proportional to k₃/k₄ as well as B_{max}/K_d. (k₃ and k₄ are rate constants for ligand transfer between the two tissue compartments. B_{max} represents the receptor density, and K_d is the dissociation constant for the ligand at the receptor. Pseudoequilibrium refers to a period of time in which the k₃/k₄ ratio is stable.) Between 65 and 90 minutes, pseudoequilibrium is present, as there is no significant change in the cortex-to-cerebellum ratio (35). Petit-Taboue et al. (36) empirically demonstrated that the pseudoequilibrium cortex-to-cerebellar ratio is highly correlated with the kinetically obtained k₃/k₄ ratio ($r=0.91-0.97$). Between 0 and 90 minutes, under the condition of high doses of

TABLE 1. Cortical Regions of Interest Selected for Measurement of 5-HT_{2A} Receptor Binding in Depressed and Healthy Subjects

Region ^a	Brodmann's Areas	Number of Voxels ^b
Medial frontal gyrus	9, part of 10, 46	200
Lateral orbitofrontal cortex	47, 11	200
Parahippocampal gyrus	28, 35	75
Posteromedial temporal cortex	20, 21	300
Rostral anterior cingulate	24, part of 32	150

^a Each region was measured bilaterally except the rostral anterior cingulate.

^b The size of each voxel was 2.0×2.0×6.5 mm in the x, y, z axes.

5-HT_{2A} antagonists, it has been demonstrated that the parameters describing the first tissue compartment (free and nonspecific) are similar in the cerebellum and cortex (24). By using the cortex-to-cerebellum ratio, the need for an arterial puncture is avoided, making this approach less invasive.

Traditionally, binding potential values are determined within regions of interest. There are a couple of limitations to region-of-interest analyses. They do not describe the spatial extent of a finding. If the region of interest does not overlap the spatial extent of a true difference, power may decline. Voxel-based analyses may offer greater sensitivity under such circumstances.

In the present study, two methods were used; one was a voxel-by-voxel analysis with statistical parametric mapping (37–39), and the second was a region-of-interest approach. For the first method, parametric binding potential images were created by using the pseudoequilibrium cortex-to-cerebellum ratio. Then these individual images were transformed and deformed to obtain a common brain shape within Montreal Neurological Institute space by using the ligand-specific template method developed for SPM 96 (40). Then the images were spatially smoothed with a Gaussian filter (12 mm full width at half maximum).

The statistical analysis was done with SPM 99. With the transformed and deformed PET images of 5-HT_{2A} binding potential, statistical parametric mapping generates an analysis at each voxel that assigns a p value to each voxel, resulting in an image of probability values. In this study the threshold for display within the probability image was set at $p<0.05$. The voxels displayed do not account for multiple comparisons. Given the known distributions of random probability values in three-dimensional space, statistical parametric mapping can correct for multiple comparisons by assessing the number of adjacent suprathreshold voxels (cluster significance) or by setting extremely low individual voxel value thresholds (peak significance) (39). To avoid false positives, a conservative approach was used: the regions displayed were considered only if the change was significant for corrected cluster significance or corrected peak significance ($p<0.05$). All peak height and cluster thresholds reported are corrected for multiple-voxel comparisons and are not based on any a priori hypotheses with respect to the regional location.

For the region-of-interest method, each subject had a magnetic resonance imaging (MRI) scan (GE Signa 1.5-T scanner, spin-echo sequence proton-density-weighted image; x, y, z voxel dimensions 0.78, 0.78, 3.00 mm, respectively). The MRI scans were coregistered to each PET image (41). For region-of-interest analysis, the cerebellum regions of interest were drawn on summated [¹⁸F]setoperone scans with reference to the coregistered MRI scan by a rater blinded to the identity of the scans. The nine cortex regions of interest were found as follows: points in Montreal Neurological Institute template space were found in the center of the medial frontal gyrus (Brodmann's area 9), lateral orbitofrontal cortex, parahippocampal gyrus, posteromedial temporal gyrus, and rostral anterior cingulate. Each point was found bilaterally except the rostral anterior cingulate. These points were trans-

formed onto the original PET summated image by using the inverse of the 12-parameter component of the statistical parametric mapping spatial normalization. For each point now on the original image, the nearest 75 to 300 voxels were chosen above an edge threshold (depending on region). See Table 1 for the region locations and sizes. Then the regions of interest were automatically superimposed on the PET image and individually visually verified to be accurate by means of the coregistered MRI. Decay-corrected time activity curves were obtained for each of these regions of interest.

The technique just described is well standardized in our laboratory. To test the reliability of this measure, seven normal subjects were scanned twice, and their data showed a test-retest standard deviation of 8% and a mean scan-rescan difference of 6%. We have found that this technique is sensitive to age-related changes in 5-HT_{2A} receptors (27, 35, 42).

Results

Nineteen patients (12 male, seven female; mean age=30.8 years, SD=6.1) were scanned before and after treatment. The mean baseline score on the Hamilton Depression Rating Scale of these patients was 21.8 (SD=3.8). All patients scanned before and after treatment had detectable serum paroxetine levels and were included in the analysis (mean=30 µg/liter, SD=24).

Nineteen age-matched healthy subjects were recruited (eight male, 11 female; mean age=31.8, SD=6.9); 18 were matched within 1 year of age, and one was matched within 2 years. There was no significant difference in gender between groups (Fisher's exact test, $p=0.33$).

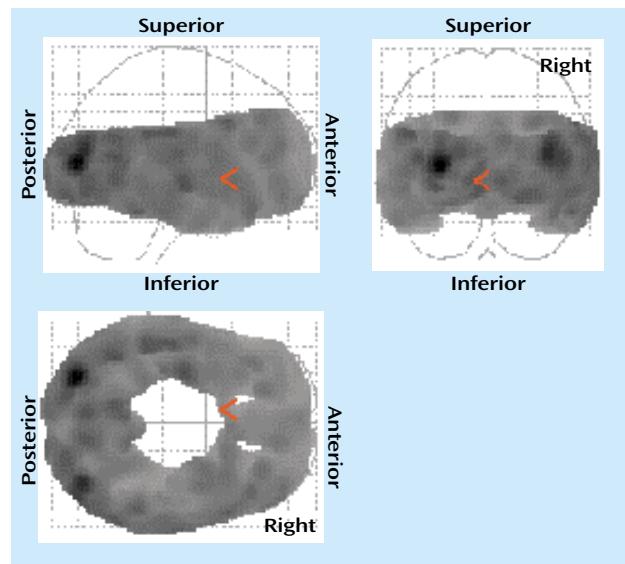
In the following sections we will first report results of our reassessment, in a larger study group, of previous findings: the age-associated decline in 5-HT_{2A} binding potential (27, 43–45) and the lack of diagnosis effect on 5-HT_{2A} binding potential (27). Then the primary question of a treatment effect will be addressed by examining the effect of treatment on cortical 5-HT_{2A} binding potential and the effect of the interaction between age and treatment on cortical 5-HT_{2A} binding potential. Finally, we will examine whether initial cortical 5-HT_{2A} binding potential has any association with eventual treatment response.

Effects of Age

Since age has been previously associated with a decline in both 5-HT_{2A} B_{max} and 5-HT_{2A} binding potential (27, 43–45), we assessed the effect of age on 5-HT_{2A} binding potential. In the healthy subjects, age was a significant covariate globally in the cortex in both type of analyses. In the voxel-wise analysis, analysis of covariance (ANCOVA) at each voxel (86,821 voxels) yielded corrected probability levels of $p<0.001$ (corrected peak) and $p<0.001$ (corrected cluster). Similar levels resulted from the ANCOVAs for the regions of interest ($F=19–30$, $df=1, 17$, $p<0.001$).

Within the pretreatment scans, all cortical regions showed an association with age (Figure 1); for the ANCOVAs at each voxel (80,688 voxels), $p<0.001$ (corrected peak) and $p<0.001$ (corrected cluster). Within the posttreatment scans, all cortical regions also showed an association with

FIGURE 1. Global Cortical Decline in 5-HT_{2A} Binding Potential With Age in 19 Depressed Patients^a



^a p<0.05 for each voxel. Results of analysis of covariance were converted into z scores and then probability values. Degrees of freedom for individual voxels=1, 17, the number of voxels was 80,688, and p<0.001 was the corrected cluster significance. Age negatively covaried with 5-HT_{2A} binding potential in all regions.

age; for the ANCOVAs at each voxel (80,477 voxels), p<0.001 (corrected peak) and p<0.001 (corrected cluster). Then the relationship between age and 5-HT_{2A} binding potential was examined in each region within the pretreatment scans and then within the posttreatment scans. Every region showed a significant association with age (ANCOVA, F=15–30, df=1, 17, p<0.001).

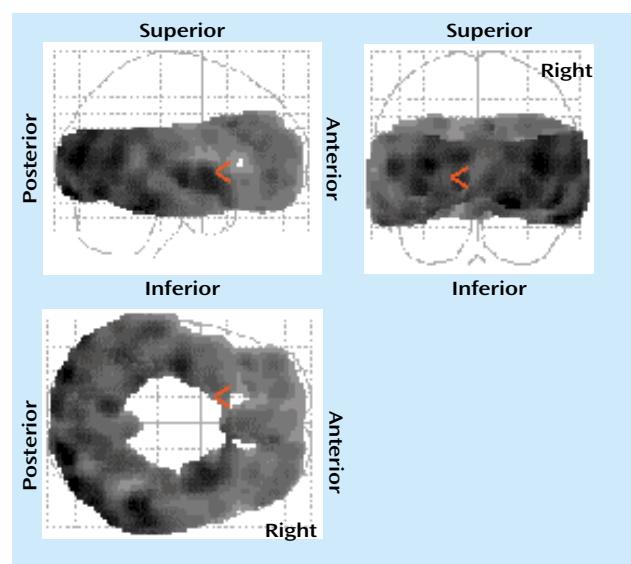
Effects of Diagnosis

While the main aim of this study was to examine the effects of paroxetine treatment in depression, the treatment effect is better appreciated in the context of how 5-HT_{2A} binding potential may differ between depressed and healthy subjects. The scans of the patients before treatment were compared to the scans of the healthy subjects by using ANCOVA with age as a covariate; a similar comparison was conducted with the patients' posttreatment scans. With the voxel-by-voxel approach, no differences in 5-HT_{2A} binding potential between the healthy subjects and depressed patients (pretreatment or posttreatment scans) were found. Similarly, no differences in 5-HT_{2A} binding potential were found between the depressed and healthy subjects in the region-of-interest analyses. Furthermore, differences in 5-HT_{2A} binding potential between the two groups did not interact with age in the voxel-wise or region-of-interest analyses.

Effects of Treatment

5-HT_{2A} binding potential within a large region composed of bilateral occipital and medial temporal cortex decreased with treatment, but this was not significant

FIGURE 2. Age-Related Attenuation of Decrease in Cortical 5-HT_{2A} Binding Potential After Paroxetine Treatment in 19 Depressed Patients^a



^a p<0.05 for each voxel. Results of repeated measures analysis of covariance examining interaction of treatment effect with age were converted into z scores and then probability values. Degrees of freedom for individual voxels=1, 34, the number of voxels was 56,928, and p<0.001 was the corrected cluster significance. At each voxel the decrease after treatment interacted with age such that the decrease after treatment became less with age.

when the cluster threshold was applied; for the repeated measures ANCOVA with age as a covariate (3,377 voxels), p=0.48 (corrected peak) and p=0.20 (corrected cluster). The results of region-of-interest analyses of 5-HT_{2A} binding potential using repeated measures (within subject) ANCOVA were not significant. Having a full response (N=10) versus having a partial response or nonresponse (N=9) had no association with change in 5-HT_{2A} binding potential with treatment in voxel-wise or regional analyses. Using the region-of-interest data, we examined a plot of the residual values versus age, and an obvious decrease with treatment was present in younger individuals.

Interaction Between Treatment Effect and Age

The effect of the interaction between treatment and age on 5-HT_{2A} binding potential was found globally in the voxel-by-voxel analysis; for the repeated measures ANCOVA of the treatment-by-age interaction (56,928 voxels), p<0.05 (corrected peak) and p<0.001 (corrected cluster) (Figure 2). The decrease in 5-HT_{2A} binding potential after treatment became less pronounced as age increased. This finding was also examined with the region-of-interest approach. Using multivariate repeated measures analysis of covariance (MANCOVA), we found the effect of treatment, the treatment-by-age interaction, and the effect of region were significant with respect to 5-HT_{2A} binding potential (treatment: F=7.71, df=1, 17, p=0.01; treatment by age: F=6.33, df=1, 17, p=0.02; region: F=2.92, df=8, 10, p=

TABLE 2. Effects of Paroxetine Treatment on 5-HT_{2A} Receptor Binding in Cortical Regions in 19 Depressed Patients

Region and Side	Decrease in Patients Aged 20–30 (N=9) ^a		Decrease in Patients Aged 31–40 (N=10) ^a		Treatment-by-Age Interaction (ANCOVA) ^b	
	Mean	SD	Mean	SD	F (df=1, 17)	p
Lateral orbitofrontal cortex						
Left	0.10	0.17	-0.02	0.14	5.34	0.03
Right	0.14	0.16	0.02	0.10	11.33	0.004
Rostral anterior cingulate, bilaterally						
Medial frontal gyrus	0.10	0.18	0.02	0.13	4.76	0.04
Left	0.09	0.18	0.00	0.13	3.67	0.07
Right	0.11	0.15	-0.04	0.14	6.09	0.03
Parahippocampal gyrus						
Left	0.07	0.22	-0.15	0.24	5.67	0.03
Right	0.09	0.24	-0.04	0.11	6.55	0.02
Posteromedial temporal cortex						
Left	0.09	0.15	-0.02	0.11	5.04	0.04
Right	0.13	0.14	0.00	0.10	8.92	0.008

^a Decrease in 5-HT_{2A} binding potential was calculated as follows: (pretreatment – posttreatment) / pretreatment.

^b Analysis of covariance with age as a covariate.

0.05). The multiple variables refer to the different regions, and the repeated measure is the within-subject value before and after treatment. Repeated measures ANCOVA with age as a covariate for each region showed that the treatment-by-age interaction was significant for almost every region (Table 2). The within-subject repeated measure is the region-of-interest 5-HT_{2A} binding potential. In patients aged 20 to 30 years, the mean decrease was 10% across all regions, whereas in patients aged 30 to 40 the mean change was 0%. For the region-of-interest analysis, there were no outliers, as the Cook's distances were less than 1 for all observations. Pearson correlation indicated that there was no correlation between age and serum paroxetine level ($r=-0.02$, $N=19$, $p=0.93$).

Relation of Treatment Response to Pretreatment 5-HT_{2A} Binding Potential

The depressed patients were classified according to treatment response, resulting in 10 treatment responders, six partial responders, and three nonresponders. No differences in 5-HT_{2A} binding potential were found between the responders and the remaining patients (partial responders and nonresponders) in either the voxel-based or regional analyses using ANCOVA to examine the effect of being an eventual responder, with age as a covariate. No interaction between age and difference in 5-HT_{2A} binding potential between groups was present. The age of the treatment responders matched that of the non- and partial responders within 2 years, with the exception of an additional 21-year-old subject in the treatment-responding group.

5-HT_{2A} binding potential in the 10 treatment-responding subjects was compared to 5-HT_{2A} binding potential in the age-matched healthy subjects. The voxel-wise analysis showed globally higher 5-HT_{2A} binding potential in the treatment-responding patients; for the ANCOVA of the diagnosis effect in the responders (44,852 voxels), $p<0.18$ (corrected peak) and $p<0.001$ (corrected cluster). A region-of-interest MANCOVA showed somewhat greater 5-HT_{2A}

binding potential in the treatment-responding patients than in the healthy subjects (diagnosis: $F=2.62$, $df=9, 9$, $p=0.09$; age: $F=5.63$, $df=9, 9$, $p=0.009$). The multiple variables in the MANCOVA were the different regions. Within individual regions, there was no significant difference between the treatment-responding and healthy subjects.

5-HT_{2A} binding potential in the remaining nine subjects—the partial responders and nonresponding patients—was compared to that in a different set of nine age-matched healthy subjects. Using ANCOVA with voxel-based analyses and MANCOVA and ANCOVA for the region-of-interest data, we found no differences between these two groups (and no age interaction was present).

Discussion

To our knowledge, this is the first report of 5-HT_{2A} receptor changes during SSRI treatment shown by a within-subject design in previously drug-free depressed patients. In patients under age 30, we found decreases of 5-HT_{2A} binding potential in all brain regions; however, these decreases attenuated with increasing age.

There are two alternative explanations for the decrease in 5-HT_{2A} binding potential with paroxetine treatment. One is that paroxetine increased 5-HT secondary to 5-HT transporter blockade (1) and that 5-HT_{2A} down-regulation occurred. The second is that paroxetine directly antagonized 5-HT_{2A} receptors and decreased the detected 5-HT_{2A} receptor binding potential. This latter possibility can be ruled out. Using [¹⁸F]setoperone PET, we previously demonstrated no change in 5-HT_{2A} receptor binding potential after single-dose paroxetine administration, thus ruling out a short-term antagonist effect *in vivo* (46). Moreover, the affinity of paroxetine for 5-HT_{2A} receptors is extremely low, being in the nonspecific range (6). On the other hand, the first explanation is plausible since long-term SSRI exposure is associated with increased synaptic 5-HT (1).

The binding potential reflects B_{max}/K_d , and hence the down-regulation observed could be attributed to either decreased B_{max} (decreased receptor density) or increased K_d (decreased affinity). In ex vivo studies, changes in 5-HT_{2A} receptors after long-term agonist administration are characterized mostly by decreased 5-HT_{2A} B_{max} (47). Thus, it is likely that decreased 5-HT_{2A} B_{max} would account for the majority of the change in 5-HT_{2A} binding potential found in the current study.

The decrease in 5-HT_{2A} binding potential after treatment became less so with age. It could be that down-regulation of 5-HT_{2A} receptors decreases with age. This notion is consistent with the well-established finding that 5-HT_{2A} receptor density decreases with age (27, 43–45), since a floor effect would limit down-regulation with age. To our knowledge, the relationship between age and down-regulation of 5-HT_{2A} receptors has not been investigated in animal models, but some aspects of 5-HT_{2A} receptor regulation appear to change with age. One effector pathway of 5-HT_{2A} receptors includes activation of phospholipase C, production of inositol triphosphate, increased intracellular calcium, and activation of protein kinase C (48, 49). Activation of protein kinase C influences stability of messenger RNA for 5-HT_{2A} receptors, and the activation of calcium-dependent protein kinase C has been reported to change with age in rodent brains (50).

Our finding contrasts with that of Massou et al. (14), who reported higher 5-HT_{2A} receptor binding potential in six treated depressed patients than in eight untreated depressed patients. There are several methodological differences between this study and the current one that could explain the discrepancy. The earlier study did not use an intrasubject design, the dose and type of SSRI varied, concomitant benzodiazepines were used, and the timing of the [¹⁸F]setoperone scan varied among subjects. Benzodiazepines may influence 5-HT release (15–17). The timing of the second scan relative to the onset of treatment could influence results because preclinical models of SSRI administration demonstrate that both the firing rate of cortical 5-HT neurons and the concentration of 5-HT may fluctuate markedly over the course of treatment (1, 51, 52).

It has been reported that 5-HT_{2A} binding potential declines after treatment with desipramine (53). The authors of that study proposed that decreases in 5-HT_{2A} binding potential might contribute to a mechanism of therapeutic effect for desipramine. With SSRI treatment it seems unlikely that down-regulation itself is directly therapeutic because in the current study there was no association between down-regulation and treatment response. On the other hand, the power to find this association may be very low for several reasons. The true difference could be small. Placebo responders add variance. The degree of down-regulation could vary among individuals of similar age because of genetic differences, adding more variance. 5-HT agonism on multiple receptors rather than one receptor may be important for therapeutic effect; thus, down-regu-

lation of a single receptor subtype may not associate strongly with treatment response.

Even if down-regulation itself is not a therapeutic effect of SSRIs, this does not rule out the interesting possibility that increased 5-HT agonism on 5-HT_{2A} receptors is therapeutic. While down-regulation of 5-HT_{2A} receptors may occur in the presence of increased 5-HT, down-regulation itself can reduce the agonist effect of 5-HT on 5-HT_{2A} receptors.

The decrease in 5-HT_{2A} binding potential with treatment in younger subjects was a global cortical change. Given that 5-HT transporter sites are widespread throughout the cortex, it is not surprising that paroxetine would have global effects on 5-HT_{2A} receptors (54).

We found no difference between depressed and healthy subjects in any cortical region. This is consistent with findings from studies of medication-free depressed subjects (27, 45).

It is likely that 5-HT reuptake inhibition resulted in down-regulation of the 5-HT_{2A} binding potential. In theory, indirect mechanisms, such as occupancy by endogenous 5-HT, could also contribute to a decrease in 5-HT_{2A} receptor binding potential. The concentration of endogenous 5-HT in the human cortex is about 0.5 to 1.5 nM (55), and the K_I of 5-HT for cortical 5-HT_{2A} receptors is 300 to 5000 nM (6) (K_I is the concentration at which one-half of the receptor sites are displaced by a drug or chemical and is inversely proportional to the affinity of the drug or chemical). If these cortical 5-HT concentrations reflect synaptic concentrations, about 1% of synaptic 5-HT_{2A} receptors could be occupied by 5-HT at baseline. This is consistent with a finding in our previous study (46), in which we found that single-dose paroxetine had no effect on the setoperone binding potential of healthy subjects.

Several methodological limitations are present in the study. The resolution of the PET camera does not allow one to distinguish the cell type or cellular location attributable to the change in 5-HT_{2A} receptors. Candidate cell types likely to account for a large proportion of 5-HT_{2A} receptors would include interneurons as well as pyramidal cell neurons (56). Another limitation is that the binding potential reflects B_{max}/K_d , and we were unable to discern between these two measures. However, down-regulation processes are the predominant responses to agonist stimulation in ex vivo models (47). With respect to the paroxetine treatment, we took many precautions to enhance compliance, such as informing patients that their serum levels were to be assayed at the time of the second PET scan as part of the study. We also took measurements and detected paroxetine levels in every patient. Even so, it is possible that some patients may have temporarily interrupted their treatment earlier, and this would not be detected in our protocol.

In conclusion, we found a down-regulation of 5-HT_{2A} receptor binding potential after paroxetine treatment in younger depressed patients. This suggests that long-term

paroxetine treatment increases 5-HT agonism on 5-HT_{2A} receptors in the cortex of young patients with depression.

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