# Positron Emission Tomography Study of the Effects of Tryptophan Depletion on Brain Serotonin$_2$ Receptors in Subjects Recently Remitted From Major Depression

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**Context:** Decreased brain serotonin (5-hydroxytryptamine) levels are considered to mediate depressive relapse induced by the tryptophan depletion paradigm. However, in patients who recently achieved remission from a major depressive episode with antidepressant treatment, only about half become depressed following tryptophan depletion. We hypothesized that downregulation of brain serotonin$_2$ receptors might be a compensatory mechanism that prevents some patients from becoming depressed with tryptophan depletion.

**Objective:** To assess, with use of positron emission tomography, whether brain serotonin$_2$ receptor downregulation occurs in patients with recently remitted depression who do not have depressive relapse, but not in those who become depressed, following tryptophan depletion.

**Design:** Each patient underwent 2 fluorine 18–labeled–setoperone positron emission tomography scans, one following a tryptophan depletion session and another following a control session. The order of scanning was counterbalanced.

**Setting:** Academic university hospital with imaging facilities.

**Participants:** Seventeen patients in recent remission from a *DSM-IV* major depressive episode following treatment with selective serotonin reuptake inhibitors.

**Main Outcome Measures:** Changes in brain serotonin$_2$ receptor binding.

**Results:** Of the 17 patients, 8 (47%) became depressed during the tryptophan depletion session, and none developed depression during the control session. The depletion session was associated with a significant reduction in brain serotonin$_2$ receptor binding compared with the control session for all participants. A subgroup analysis revealed that the reduction in serotonin$_2$ receptor binding was significant only for the nondepressed group.

**Conclusion:** Reduction in brain serotonin$_2$ receptors might be a potential compensatory mechanism to prevent tryptophan depletion–induced depressive relapse.

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THE TRYPTOPHAN DEPLETION paradigm has been used extensively to investigate the neurobiology of depression and other psychiatric disorders.[1-5] Preclinical studies[5,6] in animals suggest that the tryptophan depletion challenge paradigm reduces plasma and brain free and total tryptophan levels, with a consequent reduction in brain serotonin (5-hydroxytryptamine) levels. Studies[2,7,8] in humans have shown that ingestion of an amino acid mixture containing 15 amino acids without tryptophan induces a marked reduction in plasma total and free tryptophan levels within 5 hours. Furthermore, a positron emission tomography (PET) study[9] has shown that tryptophan depletion leads to a reduction in brain serotonin synthesis.

The effects of tryptophan depletion on mood in healthy volunteers have been fairly consistent, with most studies reporting no changes.[10,11] In contrast, tryptophan depletion has been reported to induce significant depressive symptoms in patients who recently achieved remission from a major depressive episode following treatment with selective serotonin reuptake inhibitors (SSRIs).[7,12,13] This finding has been interpreted to suggest that adequate levels of brain serotonin are necessary to maintain the antidepressant effects of SSRIs. If such were to be the case, all patients with recent remission from depression resulting from SSRI treatment should become depressed with tryptophan depletion, as serotonin depletion likely occurs in those who ingest the amino acid mixture. However, studies[11] have in-

dicated that only approximately 50% of patients in remission become depressed following tryptophan depletion. This would suggest that those who do not become depressed may mount a neurobiological compensatory mechanism to counter the effects of tryptophan depletion and thus prevent depression. If so, what might that compensatory mechanism be?

Positron emission tomography studies suggest that several effective antidepressant treatments downregulate brain serotonin$_2$ receptors. These include agents with diverse neurobiological mechanisms, such as the norepinephrine reuptake inhibitor desipramine,[14] the serotonin reuptake inhibitor paroxetine,[15] and the serotonin reuptake inhibitor and serotonin$_2$ antagonist nefazodone,[16] as well as somatic intervention electroconvulsive therapy.[17] Furthermore, healthy volunteers who did not become depressed following tryptophan depletion have been reported to develop serotonin$_2$ receptor downregulation within 5 hours after ingestion of the tryptophan-deficient amino acid mixture.[18] Taken together, these data suggest that downregulation of serotonin$_2$ receptors may be associated with relief from or prevention of depressive symptoms. Hence, serotonin$_2$ receptor downregulation may be a potential compensatory mechanism that might prevent patients with recently remitted depression from becoming depressed following the acute tryptophan depletion paradigm.

In this study, we examined the effects of tryptophan depletion on mood and brain serotonin$_2$ receptors in patients who achieved remission from a major depressive episode with SSRI treatment within the past 3 months. We measured brain serotonin$_2$ receptors using PET with setoperone as a ligand in each participant 5 hours following the ingestion of a tryptophan-deficient amino acid mixture on one day and a balanced amino acid mixture (containing tryptophan) on another day. We hypothesized that brain serotonin$_2$ receptors would be downregulated in patients who did not become depressed during the tryptophan depletion session compared with the control session but not in those who became depressed.

## METHODS

The study was approved by the Clinical Research Ethics Board of the University of British Columbia. Eligible patients were recently treated for a major depressive episode with an SSRI (fluoxetine, paroxetine, sertraline, citalopram, or escitalopram), currently in remission from depression for a minimum of 1 week but less than 12 weeks, and able to provide informed consent. The diagnosis of major depressive disorder was based on all the clinical information, including a clinical interview and a Structured Clinical Interview for *DSM* Disorders. Remission was defined as a Hamilton Scale for Depression (HAM-D) score of 12 or less on the 29-item scale[19] for at least 1 week. Those taking other psychotropic medications, such as antipsychotics, lithium, valproate, lamotrigine, carbamazepine, or other antidepressants, were excluded. Individuals with substance abuse or other Axis I comorbidity within the past 6 months were also excluded, as were women who were pregnant and those not taking adequate contraceptive precautions. Each participant underwent magnetic resonance imaging to exclude cerebral abnormalities and for coregistration of PET images.

The protocol consisted of PET scanning on 2 days 5 hours after the ingestion of amino acid mixtures. The order of the sessions (control vs tryptophan depletion) was counterbalanced. Both the participants and raters who administered behavioral rating tests were blinded to the condition. The tryptophan depletion session involved ingesting an amino acid mixture consisting of 15 amino acids, without tryptophan. The control session included the same amino acid mixture with the addition of 2.3 g of L-tryptophan. The composition of the amino acid mixture was the same as that used by Delgado et al.[13] Chocolate syrup was added to the amino acid mixture drink to make it palatable, but amino acids with an unpleasant taste were administered in a capsule form.

Participants fasted from midnight and arrived at the mood disorders outpatient research program between 7 AM and 8 AM. Clinical assessment and behavioral ratings including a 20-item HAM-D scale (29-item HAM-D scale less 9 items that could not be rated within the same day, eg, sleep, eating, weight, and diurnal variation) were completed to confirm remission of depression. An intravenous cannula was then inserted and a blood sample was drawn to assay for total and free tryptophan. Participants then ingested the amino acid mixture and remained within a room for the next 5 hours reading newspapers or magazines of their choice. Approximately 5 hours later, the patients were escorted to the PET suite and a second blood sample was drawn. Behavioral ratings were completed at that time and PET was started immediately. Relapse of depression was defined as an increase in depression rating scores by 50% or more and a total score of 13 or higher.

Blood samples were immediately processed. Blood was centrifuged for 10 minutes at room temperature at 5000*g*. To obtain an ultrafiltrate of plasma, the sample was further centrifuged (2000*g*) at room temperature for 30 minutes through a cellulose ultrafiltration membrane system (Amicon Co). The ultrafiltrate samples were frozen at −70°C and were later assayed for free tryptophan levels using high-performance liquid chromatography with fluorometric detection.[20] In 2 patients, free tryptophan levels could not be estimated because the ultrafiltrate sample was too small.

The protocol for PET image acquisition was similar to that previously reported.[18] Briefly, after a transmission scan to correct PET images for attenuation, each participant was given 148 to 259 MBq of fluorine 18–labeled ($^{18}$F)–setoperone intravenously. A PET camera system (ECAT 953B/31; CTI/Siemens), which has a field of view of 10.8 cm, was used to measure radioactivity in the brain. The scanning began immediately after injection of $^{18}$F-setoperone and lasted for 110 minutes, during which 15 frame-dynamic emission scans were acquired (5 frames, each 2 minutes' duration; then 4 frames, each 5 minutes; then 4 frames, each 10 minutes; and then 2 frames, each 20 minutes). Positron emission tomography was repeated within 7 days so that each patient had 2 scans: one after the control session and the other after the tryptophan depletion session. At the end of each test session, patients were assessed clinically.

### IMAGE PROCESSING AND DATA ANALYSIS

Images from all frames of the first and second scan of each participant were realigned to the image obtained from the last 30 minutes of the data acquired during the first scanning session using the automated image realignment algorithm.[21,22] This procedure was done to correct for motion during a scanning sequence and to ensure that the brain structures were in the same position of the image space in the 2 scanning sequences to facilitate voxel-by-voxel comparison. The Logan tissue-input graphical analysis[23] was applied to the time activity curve of each voxel separately; the time activity curve of an elliptical re-

gion of interest ([ROI] area, 2590 $mm^2$) placed on the cerebellar image was used as an input function. The population-based tissue to plasma efflux constant ($k_2$) for the reference region, required for the Logan analysis, was derived for $^{18}$F-setoperone with a 3-tissue compartment model to be $k_2$=0.109/min.[24] The Logan slope was calculated from data acquired 30 minutes after injection. These data were applied to create a parametric setoperone distribution volume ratio (DVR) image for each scanning session. This resulted in a DVR (DVR=$BP_{ND}$ + 1, where $BP_{ND}$ indicates nondisplaceable binding potential)[25] parametric image for control and depletion sessions for each participant.

### STATISTICAL PARAMETRIC MAPPING ANALYSIS

The Statistical Parametric Mapping (SPM5) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm5) was used to coregister each participant's DVR images for control and depletion sessions with that person's magnetic resonance image. Each magnetic resonance image was normalized to the standard Montreal Neurological Institute (MNI) T1 template in SPM5, and these normalization parameters were applied to DVR images to bring them to the standard MNI template space. The normalized DVR images were smoothed using an isotropic gaussian kernel of 10 mm, full width at half maximum.

The gray matter threshold was set at 1.0 times the mean global cerebral image intensity to exclude most nongray matter voxels in the analysis. A full factorial model with 2 sessions and 2 groups as implemented in SPM5 was used to determine the effect of session, group, and group × session interaction. Post hoc pairwise contrasts included assessing differences in DVRs within responders and nonresponders between the control session and depletion session. Because previous studies[18] indicated that any changes were likely to be spatially extensive, the primary analysis used a criterion of cluster significance to provide the greatest statistical power for detecting spatially extensive effects.[26] The corrected cluster significance was set at $P<.005$, and the statistical significance for height threshold for inclusion of contiguous voxels in a cluster was set at $P<.005$ uncorrected. We also assessed the significance of differences in DVRs in each voxel between the sessions, and a stringent familywise error (FWE) corrected significance was set at $P<.05$.

### ROI ANALYSIS

Previous studies[4,27,28] that examined the effects of tryptophan depletion on cerebral blood or glucose metabolism have consistently shown changes in the medial orbitofrontal cortex and anterior cingulate regions. Hence, these 2 regions were selected for ROI analysis of changes in DVRs of setoperone binding. The Pick_Atlas program (Wake Forest University School of Medicine; http://www.ansir.wfubmc.edu) was used to create masks for anterior cingulate cortex and medial orbitofrontal cortex regions, and these masks were applied to DVR images (tryptophan and depletion scans) to extract DVRs of setoperone binding. We performed repeated-measures analysis of variance to examine the effects of session, group, and group × session interaction. Furthermore, we performed paired *t* tests to determine whether DVRs were different in depressed or nondepressed groups between the 2 sessions.

### BEHAVIORAL AND PLASMA MEASURES

A repeated-measures multivariate analysis of variance was used to assess the effects of order (ie, those who had the control session first vs those who had the tryptophan depletion session first) as a grouping variable and time (baseline vs postsession) and session (control vs depletion session) as repeated measures. Data are presented as mean (SD) for behavioral measures and mean (SE) for plasma tryptophan levels, and all tests were 2-tailed, with significance set at $P<.05$.

## RESULTS

### CLINICAL DEMOGRAPHICS

Of the 20 patients recruited, 3 dropped out after the first PET scan. All 17 patients included in the analysis were in recent remission from a *DSM-IV* major depressive episode following treatment with SSRIs. The clinical characteristics of the patients are displayed in the **Table**. The mean age of the 17 (5 men, 12 women) patients who had both control and depletion scans was 41.8 (10.7) years. The patients had a mean of 3.5 (5.3) previous depressive episodes, and the duration of treatment for the current episode was 16.8 (8.6) weeks. The mean duration of remission from the most recent depressive episode was 5.4 (3.6) weeks.

### PLASMA TRYPTOPHAN LEVELS AND BEHAVIORAL DATA

The mean (SE) plasma tryptophan concentrations, both total and free, during the tryptophan and depletion sessions are displayed in **Figure 1**. There were no significant differences in baseline total ($t_{16}$=1.5; $P$=.16) and free ($t_{14}$=1.0; $P$=.34) tryptophan levels between the control and depletion sessions. As expected, there was no main effect for order ($F_{1,15}$=0.9; $P$=.35) or time ($F_{1,15}$=3.4; $P$=.08), but there was a significant session × time interaction effect for plasma total tryptophan levels ($F_{1,15}$=33.4; $P<.001$). Similarly, there was no order ($F_{1,13}$=0.03; $P$=.85) or time ($F_{1,13}$=2.4; $P$=.14) effect, but a significant session × time interaction ($F_{1,13}$=23.6; $P<.001$) was observed for free tryptophan levels. Plasma total and free tryptophan levels were significantly lower 5 hours after ingestion of amino acids in the depletion session (total tryptophan reduced by 75%; free tryptophan reduced by 66%), and the levels were significantly higher in the control session (total tryptophan increased by 46%; free tryptophan increased by 99%) (Figure 1).

There were no significant differences in 29-item HAM-D scores at baseline between control (5.5 [4.1]) and depletion (4.2 [3.1]) ($t_{16}$=1.1; $P$=.30) sessions. The changes in HAM-D scores for participants during each session are depicted in **Figure 2**. There was no main effect for order ($F_{1,15}$=0.1; $P$=.91) or session ($F_{1,15}$=2.2; $P$=.15), but there was a significant effect for time ($F_{1,15}$=7.5; $P$=.01) and for session × time interaction ($F_{1,15}$=14.0; $P$=.002). Paired *t* tests showed that the HAM-D scores were significantly higher in the depletion session relative to baseline (10.8 [7.8] vs 4.24 [3.1]; $t_{16}$=3.9; $P$=.001) but not in the control session (5.9 [4.9] vs 5.5 [4.1]; $t_{16}$=0.4; $P$=.73). Eight patients met HAM-D criteria for relapse during the depletion session (the depressed group), and 9 patients did not experience relapse (the nondepressed group). All participants who experienced relapse were women (8 of

**Table. Clinical Characteristics of Study Participants**

| Patient No./ Sex/Age, y | Response to Depletion | Medication Used, Daily Dose, mg | Duration of Medication Use, wk | No. of Previous Depressive Episodes | Duration of Remission, wk |
|---|---|---|---|---|---|
| 1/M/30 | No | Citalopram, 30 | 19 | 10 | 10 |
| 2/M/49 | No | Citalopram, 30 | 15 | 1 | 5 |
| 3/M/55 | No | Paroxetine, 40 | 16 | 5 | 3 |
| 4/F/43 | No | Paroxetine, 20 | 8 | 2 | 4 |
| 5/F/56 | Yes | Paroxetine, 40 | 44 | 0 | 4 |
| 6/F/43 | No | Citalopram, 20 | 19 | 0 | 12 |
| 7/F/19 | Yes | Citalopram, 30 | 4 | 3 | 1 |
| 8/M/38 | No | Citalopram, 40 | 17 | 20 | 3 |
| 9/M/40 | No | Citalopram, 30 | 17 | 0 | 4 |
| 10/F/44 | No | Citalopram, 20 | 14 | 10 | 2 |
| 11/F/37 | No | Citalopram, 30 | 17 | 4 | 2 |
| 12/F/55 | Yes | Sertraline, 300 | 17 | 3 | 6 |
| 13/F/24 | Yes | Citalopram 20 | 10 | 0 | 4 |
| 14/F/41 | Yes | Citalopram, 20 | 23 | 2 | 11 |
| 15/F/40 | Yes | Sertraline, 75 | 22 | 0 | 12 |
| 16/F/41 | Yes | Paroxetine CR, 25 | 14 | 0 | 6 |
| 17/F/56 | Yes | Citalopram, 40 | 9 | 0 | 3 |
| 5 M, 12 F/41.8 (10.7)[a] | 8 Yes<br>9 No | | 16.8 (8.6)[a] | 3.5 (5.3)[a] | 5.4 (3.6)[a] |

Abbreviation: CR, controlled-release.
[a]Given as mean (SD).

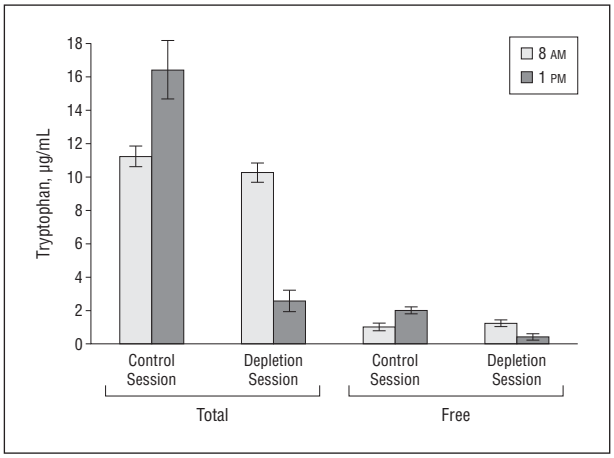


**Figure 1.** Mean plasma total and free tryptophan levels during the control and depletion sessions. Limit lines indicate standard error.

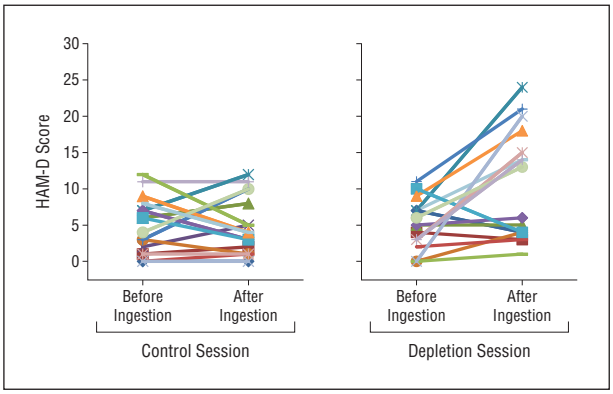


**Figure 2.** Hamilton Scale for Depression (HAM-D) score changes during the control and depletion sessions.

12); none of the 5 men relapsed, and this difference was significant ($P=.03$, Fisher exact test). However, none of the women experienced relapse during the control session. There were no significant differences in any clinical characteristics (Table) or total or free plasma tryptophan levels between those who experienced relapse and those who did not.

## SPM ANALYSIS

Statistical Parametric Mapping analysis of DVRs of setoperone binding revealed a significant session effect but no group effect or group × session interaction. The DVRs were significantly lower in the depletion session compared with the control session as revealed by an extensive cluster of voxels embracing right frontal, left medial frontal, right temporal, parietal, and occipital regions, as well as left medial parietal cortical regions (**Figure 3**). The cluster included 13 571 voxels, and the reduction in DVRs in this cluster was highly significant, even after correction for multiple comparisons ($P<.001$). The mean reduction in DVR for the cluster was 13%. There were 2 voxels in the cluster that survived the FWE correction, and these were located in the right superior temporal gyrus and right uncus. There were no significant differences in DVRs between the first and second scans, indicating that scanning order had no systematic effect on DVRs.

Post hoc contrasts assessed whether DVRs were different in the depletion session compared with the control session in the nondepressed and depressed groups. The DVRs were significantly lower in the depletion session in the nondepressed group but not in the depressed group. The SPM analysis revealed a cluster of 14 097 voxels in which DVRs were significantly lower (corrected $P<.001$) in the nondepressed group, and this cluster embraced right frontal, left medial frontal, right

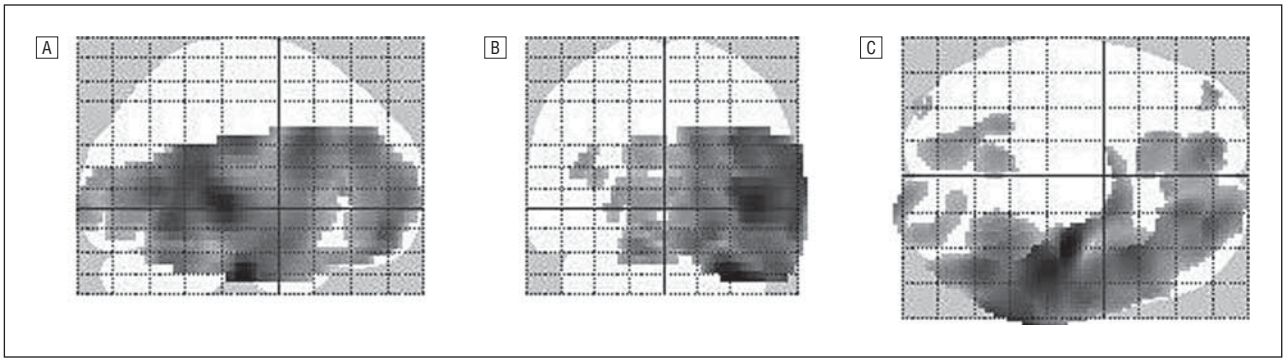


**Figure 3.** Brain areas showing significant reduction in setoperone binding during the depletion session compared with the control session. The cluster inclusion threshold was set at $P<.005$ uncorrected; the cluster of 13 571 voxels was significant after correction for multiple comparisons ($P<.001$). A, Sagittal view. B, Coronal view. C, Transverse view.

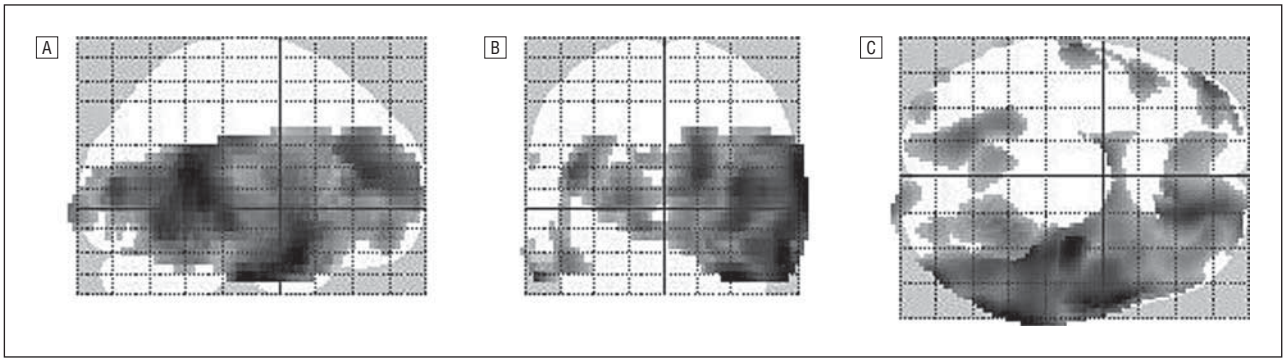


**Figure 4.** Brain areas showing significant reduction in setoperone binding in the nondepressed group during the depletion session compared with the control session. The cluster inclusion threshold was set at $P<.005$ uncorrected; the cluster of 14 097 voxels was significant after correction for multiple comparisons ($P<.001$). A, Sagittal view. B, Coronal view. C, Transverse view.

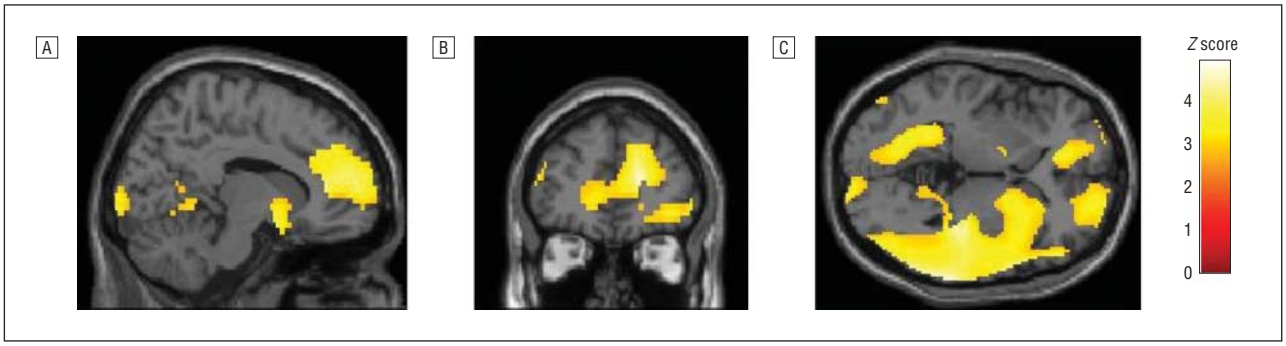


**Figure 5.** Sagittal (A), coronal (B), and transverse (C) renderings of the brain in the nondepressed group, illustrating significant decreases in setoperone binding in the anterior cingulate, right and left medial prefrontal, and right lateral temporal regions during the depletion session in comparison with the control session.

temporal and parietal, and right and left occipital regions (**Figures 4**, **5**, and **6**). The mean reduction in DVRs in the cluster was 13%.

There were also several independent voxels that met criteria for an FWE-corrected significance, and these were located in the right superior and inferior temporal gyri, right medial frontal gyrus, right middle occipital gyrus, and left middle frontal gyrus. The reduction in binding in these voxels ranged from 8.4% to 13.2%. In the group who became depressed, decrease in DVR was observed in a cluster of only 4 voxels (uncorrected $P=.09$). A cluster of at least this size would be very likely to occur under the null hypothesis. Furthermore, the peak $z$ value within this cluster was only 2.62 ($P=.56$; FWE).

## ROI ANALYSIS

Consistent with the SPM analysis, the ROI analysis revealed a significant effect for session for both anterior cingulate ($F_{1,15}=14.2$; $P=.002$) and medial orbitofrontal cortex ($F_{1,15}=8.8$; $P=.01$) but no effect for group (anterior cingulate, $F_{1,15}=3.3$; $P=.09$; medial orbitofrontal cortex, $F_{1,15}=3.5$; $P=.08$) or group × session interaction (anterior cingulate, $F_{1,15}=0.01$; $P=.99$; medial orbitofrontal cortex, $F_{1,15}=0.1$; $P=.75$) . The percentage change in setoperone binding in the anterior cingulate region for the depressed and nondepressed groups is displayed in **Figure 7**. Post hoc paired $t$ tests showed that setoperone binding was significantly decreased in the deple-

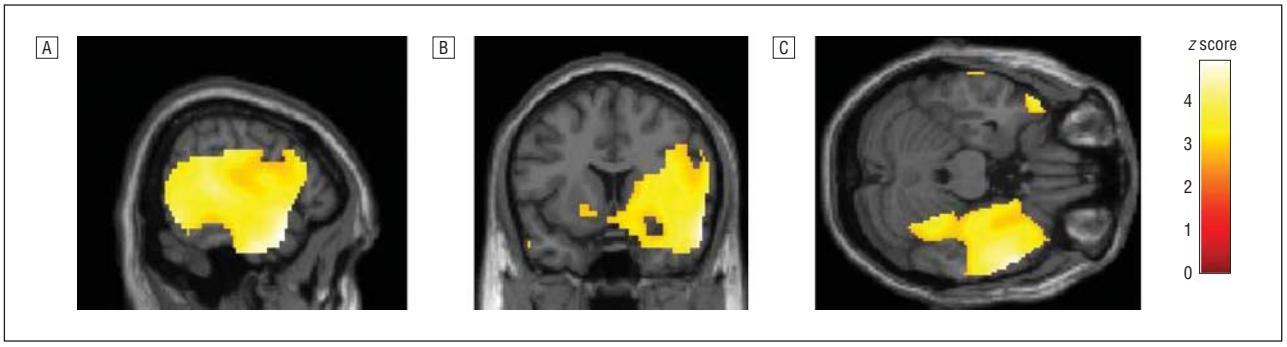


**Figure 6.** Sagittal (A), coronal (B), and transverse (C) renderings of the brain displaying significant decreases in setoperone binding in the right temporal region in the nondepressed group during the depletion session vs control session.

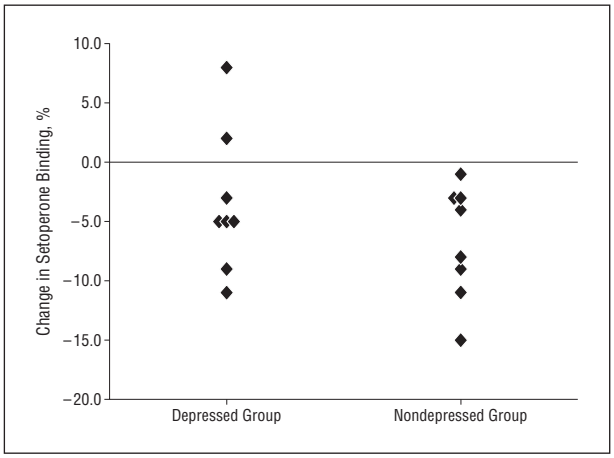


**Figure 7.** Percentage changes in setoperone binding during the depletion session compared with the tryptophan session in the anterior cingulate cortex in depressed and nondepressed patients.

tion session compared with the control session in the nondepressed group (anterior cingulate, $t_8=3.8$; $P=.005$; medial orbitofrontal cortex, $t_8=3.1$; $P=.02$) but not in the depressed group (anterior cingulate, $t_7,=2.1$; $P=.08$; medial orbitofrontal cortex, $t_7=1.7$; $P=.13$). There were no correlations between changes in depression scores and reduction in setoperone binding in the anterior cingulate (Pearson $r=0.11$; $P=.64$) or medial orbitofrontal cortex (Pearson $r=0.40$; $P=.10$). There was also no correlation between duration of remission and changes in setoperone binding in the anterior cingulate ($r=0.29$; $P=.91$) or medial orbitofrontal cortex ($r=0.02$; $P=.93$).

## COMMENT

To our knowledge, this is the first study to examine the effects of tryptophan depletion on brain serotonin$_2$ receptors in subjects recently remitted from major depression following treatment with SSRIs. The major findings are (1) 47% of patients experienced relapse of depressive symptoms in the depletion session compared with none in the control session; (2) a significant reduction in brain serotonin$_2$ receptors, as indicated by a reduction in DVRs, was observed in the depletion session compared with the control session for the entire sample, notably by a large cluster of voxels that occupied right frontal, temporal, parietal, occipital, and left medial frontal and parietal cortical regions; and (3) in contrast to patients who became depressed, those who did not become depressed during tryptophan depletion showed a robust reduction in the similar brain regions in serotonin$_2$ binding compared with the control session.

Previous studies[3,7,11] of tryptophan depletion in patients with recently remitted depression achieved with SSRI treatment reported that approximately half the patients experienced transient relapse of depressive symptoms. The finding in the present study of 47% of patients experiencing a depressive relapse is thus consistent with findings of previous tryptophan depletion studies. Furthermore, the fact that depressive relapse occurred in 8 of 12 women, but in none of 5 men, is also consistent with previous studies[29] in that women are more vulnerable to tryptophan depletion–induced depressive relapses than are men. The tryptophan depletion led to reduction in both total and free plasma tryptophan levels in all participants, with no significant differences in those who became depressed compared with those who did not. Hence, changes in plasma tryptophan levels cannot explain why some patients became depressed during tryptophan depletion while others did not.

Reduction in plasma tryptophan levels is expected to reduce brain tryptophan levels, with a consequent reduction in brain serotonin synthesis. The receptor regulation theory predicts an increase in brain postsynaptic serotonin receptors as a consequence of a reduction in brain serotonin levels. Thus, the finding of a reduction in brain serotonin$_2$ receptor binding in this study after tryptophan depletion is at odds with the classic concepts of receptor pharmacologic action. However, a reduction in brain serotonin$_2$ receptors as a consequence of lower brain serotonin levels is consistent with the well-documented paradoxical regulation of brain serotonin$_2$ receptors, since previous studies[30] reported that both agonists and some antagonists downregulate these receptors. Furthermore, the finding of a reduction in serotonin$_2$ receptor binding in the present study following tryptophan depletion is also consistent with the results of the 2 previous studies[18,31] of tryptophan depletion in healthy volunteers.

The reduction in brain serotonin$_2$ receptors was observed in an extensive cluster of voxels that embraced bilateral frontal, temporal, parietal, and occipital regions in healthy volunteers who did not become depressed following tryptophan depletion.[18] The reduction in serotonin$_2$ binding during the tryptophan depletion

session was also observed for the entire sample in the present study. However, the reduction in binding was less extensive bilaterally and was more prominent in the right cortical regions. The spatial extent of reduction in serotonin$_2$ receptors observed in SPM analysis depends on the threshold used for inclusion of voxels. Therefore, it is not possible to draw any confident conclusion regarding the lesser spatial extent of the reduction in serotonin$_2$ binding observed in patients with remitted depression compared with that observed in healthy individuals, especially in light of the fact that this study did not include a healthy comparison group. Notwithstanding these limitations, the results of this study may suggest that healthy individuals are more efficient at mounting compensatory mechanisms to reduce brain serotonin$_2$ receptors than are patients with recently remitted depression. If such were the case, the less-extensive reduction of brain serotonin$_2$ receptors in the patient group may explain why only about half of them became depressed during tryptophan depletion. Indeed, post hoc contrasts revealed a significant reduction in brain serotonin$_2$ receptors in patients who did not become depressed with tryptophan depletion but not in those who became depressed. Furthermore, the reduction in serotonin$_2$ binding in the nondepressed group was more extensive than the reduction in the right cortical region observed for the entire sample, since it additionally included the left medial frontal, lateral frontal, orbitofrontal, and left temporal regions. These data suggest that the more extensive reduction in brain serotonin$_2$ receptors in both the right and left cortical regions is more effective in preventing tryptophan depletion–induced depressive relapse.

Alternatively, it is conceivable that the reductions in brain serotonin$_2$ receptors in certain brain regions might be more critical than others for providing a compensatory protection against tryptophan depletion–induced depressive relapse. If such were the case, what might be those regions? Previous studies of cerebral blood and glucose metabolism have shown that tryptophan depletion is consistently associated with changes in neural activity in brain regions such as the orbitofrontal, lateral frontal, and cingulate cortices. For instance, tryptophan depletion–induced depressive relapse is associated with reduced neural activity in the orbitofrontal cortex,[4,27] ventrolateral prefrontal cortex, pregenual cingulate,[27] and ventral anterior cingulate cortical regions.[4] However, another study[28] found no significant differences in regional cerebral glucose metabolic rates during tryptophan depletion between patients who became depressed and those who did not but instead reported an increase in glucose metabolic rates in comparison with healthy individuals in the orbitofrontal cortex, anterior and posterior cingulate cortical regions, medial thalamus, and ventral striatum. Thus, these data suggest that changes in the orbitofrontal and cingulate regions appear to be consistent correlates of tryptophan depletion. Consistent with this, the ROI analysis performed in the present study indicated significant reductions in serotonin$_2$ receptor binding in the medial orbitofrontal cortex and anterior cingulate cortex, suggesting that the changes in serotonin$_2$ receptors in these regions might be more critical for prevention of depression.

The findings of this study are limited first by the fact that we did not find a significant effect for group and session interaction. This may be because all participants mounted a compensatory reduction in brain serotonin$_2$ receptors in response to tryptophan depletion, but in some the extent of reduction or magnitude of reduction in critical areas was not sufficient to prevent transient relapse of depressive symptoms. This is supported by the observation that the extent of reduction in binding was greater in the nondepressed group compared with the reduction observed for the entire sample. Furthermore, the reduction in binding in potential critical areas, such as the anterior cingulate and orbitofrontal regions, was slightly more than 6% in the nondepressed group and was less than 3% for the depressed group. The sample size of subgroups was small and hence may not have adequate statistical power to detect significant differences between the 2 groups. Second, because all participants who had depressive relapse in the present study were women and the magnitude of reduction in brain serotonin$_2$ receptors was smaller in this group, one could argue that this may be related to a sex effect in that women are less efficient at mounting a compensatory response. Given that the study included only 4 women who did not have a depressive relapse, this sample size does not permit a meaningful analysis of sex effect. However, a widespread and robust reduction in brain serotonin$_2$ receptors occurred in a PET study of healthy volunteers,[18] and all participants in that study were women, which makes the sex effect an unlikely explanation for the findings of the present study. Third, we did not use arterial sampling for input function to estimate DVR for setoperone binding. Instead, we used the cerebellum as a reference region, and there is a suggestion that nonspecific binding of setoperone is slightly different between the cerebellum and cortex. However, a previous study[32] showed that setoperone binding potential estimated using the cerebellum as a reference region has a high correlation with the estimate using arterial input function, thus validating this method. Furthermore, several studies[15,33,34] used the cerebellum as a reference region to estimate setoperone binding potential. Fourth, the binding of some PET ligands to receptors is affected by changes in the levels of endogenous neurotransmitter.[35,36] Therefore, one could argue that changes in brain serotonin levels during the tryptophan depletion session might have accounted for the reduction in serotonin$_2$ binding observed in the present study. However, such is not likely because tryptophan depletion is expected to reduce brain serotonin levels, which would leave more serotonin$_2$ receptors available for setoperone binding. In such circumstances, one would expect to see an increase rather than a reduction in setoperone binding observed in the participants in this study. Finally, we cannot ascertain whether the reduction in setoperone binding observed was due to changes in affinity or receptor internalization or density of receptors because the methods used in this study cannot provide an independent determination of these measures. However, previous studies[37,38] of the effects of pharmacologic treatments have shown that the changes in binding are

due to receptor density and not affinity; thus, it is likely that the reduction in setoperone binding observed in the present study is the result of either downregulation or internalization of brain serotonin$_2$ receptors.

Notwithstanding the limitations, the findings of the present study suggest that reduction in brain serotonin$_2$ receptors may be the compensatory mechanism that prevents tryptophan depletion–induced depressive relapse. In patients with recently remitted depression treated with SSRIs, reduction of brain serotonin levels is likely to lead to relapse of depression, particularly in women, unless they are able to mount a compensatory mechanism to sufficiently reduce brain serotonin$_2$ receptors. The findings of this study have important clinical implications because they raise the possibility that SSRIs given in conjunction with agents that block serotonin$_2$ receptors (eg, atypical antipsychotics) may be more effective in sustaining remission in depressed patients. If confirmed in further studies, the findings of this study also have important implications for understanding of the neurobiology factors of depression and its treatment.

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