ORIGINAL ARTICLE

Positron Emission Tomography Study of the Effects of Tryptophan Depletion on Brain Serotonin₂ 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 serotonin₂ 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 serotonin₂ 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 serotonin₂ 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 serotonin₂ receptor binding compared with the control session for all participants. A subgroup analysis revealed that the reduction in serotonin₂ receptor binding was significant only for the nondepressed group.

Conclusion: Reduction in brain serotonin₂ 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. Preclinical studies 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 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 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. 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). 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 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 serotonin2 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 serotonin2 antagonist nefazodone16 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 receptor downregulation within 5 hours after ingestion of the tryptophan-deficient amino acid mixture.18 Taken together, these data suggest that downregulation of serotonin2 receptors may be associated with relief from or prevention of depressive symptoms. Hence, serotonin2 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 serotonin2 receptors in patients who achieved remission from a major depressive episode with SSRI treatment within the past 3 months. We measured brain serotonin2 receptors using PET with setoperone as a ligand in each participant 5 months. We hypothesized that brain serotonin2 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 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.15 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 3 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 5000g. To obtain an ultrafiltrate of plasma, the sample was further centrifuged (2000g) 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 (18F)—setoperone intravenously. A PET camera system (ECAT 933B/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 18F—setoperone and lasted for 110 minutes, during which 15 frames 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 analysis23 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 mm²) placed on the cerebel-
lar image was used as an input function. The population-
based tissue to plasma efflux constant (kₑ) for the reference
region, required for the Logan analysis, was derived for ¹⁸F-
setoperone with a 3-tissue compartment model to be kₑ = 0.109/
min.26 The Logan slope was calculated from data acquired 30
minutes after injection. These data were applied to create a para-
metric setoperone distribution volume ratio (DVR) image for
each scanning session. This resulted in a DVR (DVR = BPND +
1, where BPND 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 coreg-
ister each participant’s DVR images for control and depletion
sessions with that person’s magnetic resonance image. Each mag-
netic resonance image was normalized to the standard Mon-
tréal 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 nor-
malized DVR images were smoothed using an isotropic gaus-
sian 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 vox-
ells in the analysis. A full factorial model with 2 sessions and 2
groups as implemented in SPM5 was used to determine the ef-
eft of session, group, and group × session interaction. Post
hoc pairwise contrasts included assessing differences in DVRs
during responders and nonresponders between the control ses-
tion and depletion session. Because previous studies18 indi-
cated that any changes were likely to be spatially extensive, the
primary analysis used a criterion of cluster significance to pro-
gide the greatest statistical power for detecting spatially exten-
sive 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 studies4-7,18 that examined the effects of tryptophan
depression on cerebral blood or glucose metabolism have con-
sistently shown changes in the medial orbitofrontal cortex and
anterior cingulate regions. Hence, these 2 regions were se-
lected for ROI analysis of changes in DVRs of setoperone bind-
ing. The Pick_Atlas program (Wake Forest University School
of Medicine; http://www.mrisr.wfubmc.edu) was used to cre-
ate masks for anterior cingulate cortex and medial orbitofron-
tal cortex regions, and these masks were applied to DVR im-
ages (tryptophan and depletion scans) to extract DVRs of
setoperone binding. We performed repeated-measures analy-
sis 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 non-
depressed 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 ses-
tion first vs those who had the tryptophan depletion session first) as a grouping variable and time (baseline vs possession)
and session (control vs depletion session) as repeated
measures. Data are presented as mean (SD) for behavioral mea-
sures 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 epi-
sode following treatment with SSRIs. The clinical char-
acteristics 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 ses-
tions are displayed in Figure 1. There were no signi-
cant differences in baseline total (t₁,₁₅ = 1.5; P = .16) and free
(t₁,₁₅ = 1.0; P = .34) tryptophan levels between the control
and depletion sessions. As expected, there was no main
effect for order (F₁,₁₅ = 0.9; P = .35) or time (F₁,₁₅ = 3.4;
P = .08), but there was a significant session × time interac-
tion effect for plasma total tryptophan levels (F₁,₁₅ = 33.4;
P < .001). Similarly, there was no order (F₁,₁₅ = 0.03; P = .85)
or time (F₁,₁₅ = 2.4; P = .14) effect, but a significant ses-
tion × time interaction (F₁,₁₅ = 23.6; P < .001) was ob-
served 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 con-
trol session (total tryptophan increased by 46%; free tryp-
tophan 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]) (t₁₆ = 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 (F₁,₁₅ = 0.1; P = .91) or session (F₁,₁₅ = 2.2;
P = .15), but there was a significant effect for time (F₁,₁₅ = 7.5;
P = .01) and for session × time interaction (F₁,₁₅ = 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 4.2 [3.1];
t₁₆ = 3.9; P = .001) but not in the control session
(5.9 [4.9] vs 5.5 [4.1]; t₁₆ = 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 par-
ticipants who experienced relapse were women (8 of...
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.

**Table. Clinical Characteristics of Study Participants**

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Response to Depletion</th>
<th>Medication Used, Daily Dose, mg</th>
<th>Duration of Medication Use, wk</th>
<th>No. of Previous Depressive Episodes</th>
<th>Duration of Remission, wk</th>
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<tr>
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<td>5 M, 12 F/41.8 (10.7)</td>
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<td></td>
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<td>3.5 (5.3)</td>
<td>5.4 (3.6)</td>
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<tr>
<td></td>
<td>9 No</td>
<td></td>
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</table>


*Given as mean (SD).*

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**](#fig3)). 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 temporal, and occipital regions (Table). 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.

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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.
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 \( \times \) 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-
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. The reduction in serotonin_2 binding during the tryptophan depletion session compared with the control session in the nondepressed group (anterior cingulate, _t_ = 3.8, _P_ = .003; medial orbitofrontal cortex, _t_ = 3.1; _P_ = .02) but not in the depressed group (anterior cingulate, _t_ = 2.1; _P_ = .08; medial orbitofrontal cortex, _t_ = 1.7; _P_ = .13). There were no correlations between changes in depression scores and reduction in setoperone binding in the anterior cingulate (Pearson _r_ = .11; _P_ = .64) or medial orbitofrontal cortex (Pearson _r_ = .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 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 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 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 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. 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 non-depressed 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, ventrolateral prefrontal cortex, and ventral anterior cingulate cortical regions. However, another study 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, 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 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 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. 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 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 serotonin₂ receptors.

Notwithstanding the limitations, the findings of the present study suggest that reduction in brain serotonin₂ 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 serotonin₂ receptors. The findings of this study have important clinical implications because they raise the possibility that SSRIs given in conjunction with agents that block serotonin₂ 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 the neurobiology factors of depression and its treatment.

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