

## ONLINE FIRST

# Brain Regional $\alpha$ -[ $^{11}\text{C}$ ]Methyl-L-Tryptophan Trapping in Medication-Free Patients With Obsessive-Compulsive Disorder

Alexandre Berney, MD; Marco Leyton, PhD; Paul Gravel, BIng; Igor Sibon, MD, PhD; Debbie Sookman, PhD; Pedro Rosa Neto, MD, PhD; Mirko Diksic, PhD; Akio Nakai, MD, PhD; Gilbert Pinard, MD; Christo Todorov, MD; Hidehiko Okazawa, MD, PhD; Pierre Blier, MD, PhD; Thomas Edward Nordahl, MD, PhD; Chawki Benkelfat, MD, DERBH

**Context:** The hypothesis of a serotonin (5-hydroxytryptamine [5-HT]) dysfunction in obsessive-compulsive disorder (OCD) stems largely from the clinical efficacy of 5-HT reuptake inhibitors. Serotonergic abnormalities in the unmedicated symptomatic state, however, remain to be fully characterized.

**Objective:** To investigate brain regional 5-HT synthesis, as indexed by positron emission tomography and the  $\alpha$ -[ $^{11}\text{C}$ ]methyl-L-tryptophan trapping constant ( $K^*$ ), in treatment-free adults meeting criteria for OCD.

**Design:** Between-group comparison.

**Setting:** Department of Psychiatry and Montreal Neurological Institute, McGill University, and Department of Psychology, McGill University Health Centre, Quebec, Canada.

**Participants:** Twenty-one medication-free patients with OCD (15 men with a mean [SD] age of 33.2 [9.3] years and 6 women with a mean [SD] age of 35.8 [7.1] years) and 21 healthy controls matched for age and sex (15 men with a mean [SD] age of 32.9 [10.1] years and 6 women with a mean [SD] age of 36.5 [8.6] years).

**Main Outcome Measure:** The  $\alpha$ -[ $^{11}\text{C}$ ]methyl-L-tryptophan brain trapping constant  $K^*$ , which was analyzed with Statistical Parametric Mapping (SPM8) and

with proportional normalization (extent threshold of 100 voxels with a peak threshold of  $P \leq .005$ ).

**Results:** Compared with healthy controls, the patients with OCD exhibited significantly greater  $\alpha$ -[ $^{11}\text{C}$ ]methyl-L-tryptophan trapping in the right hippocampus and left temporal gyrus (Brodmann area 20). In the larger subsample of all men, these same differences were also evident, as well as higher  $K^*$  values in the caudate nucleus. Individual differences in symptom severity correlated positively with  $K^*$  values sampled from the caudate and temporal lobe of the patients with OCD, respectively. There were no regions where the patients exhibited abnormally low  $K^*$  values. Volumetric analyses found no morphometric alterations that would account for the group differences.

**Conclusion:** The results support previous reports of greater striatal and temporal lobe activity in patients with OCD than in healthy controls and suggest that these disturbances include a serotonergic component. Previously reported glucose metabolic disturbances in OCD involving the orbitofrontal and cingulate cortices, in comparison, might reflect postsynaptic changes in the serotonergic system.

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**O**BSESSIVE-COMPULSIVE DISORDER (OCD) is a complex and often disabling disorder characterized by intrusive anxiogenic thoughts (obsessions) and repetitive stereotyped behaviors (compulsions).<sup>1</sup> Functional neuroimaging, neurosurgical lesion, and deep brain stimulation studies have implicated a limbic corticostriatal circuit that includes the orbitofrontal cortex, anterior cingulate, and caudate nucleus.<sup>2-5</sup>

The neurochemistry of OCD is less well understood. Primarily on the basis of the clinical efficacy of serotonin (5-hydroxytryptamine [5-HT]) reuptake inhibitors,<sup>6-10</sup> disturbance(s) of 5-HT neurotransmission in pathways mediating various components of the behavioral phenotype (repetitive behavior, behavioral control of fear, mental imagery, disinhibition, doubting, metamemory, and cognitive inflexibility) have been proposed, although direct tests of various individual hypothesis have been inconclusive. For example, m-

Author Affiliations are listed at the end of this article.

chlorophenylpiperazine (an agonist to 5-HT<sub>2C</sub>, 5HT<sub>1A</sub>, and 5-HT<sub>1D</sub>)<sup>11-14</sup> and sumatriptan (an agonist to 5-HT<sub>1D</sub>)<sup>15,16</sup> exacerbated symptoms of OCD in some studies, which suggests a 5-HT<sub>1D</sub> mediated effect.<sup>17</sup> However, these compounds failed to do so in other studies.<sup>18-20</sup> Moreover, neither obsessions nor compulsions were affected by the 5-HT releaser fenfluramine,<sup>21</sup> the 5-HT precursor L-tryptophan,<sup>22</sup> or acute tryptophan depletion,<sup>23,24</sup> suggesting that short-term changes in presynaptic 5-HT availability and release do not affect symptoms of OCD. Studies using indices of resting 5-HT function have also been equivocal. For example, cerebrospinal fluid levels of the primary 5-HT metabolite, 5-hydroxyindolacetic acid (5-HIAA), are elevated in some OCD studies<sup>25,26</sup> but not in others.<sup>27,28</sup> Preliminary functional neuroimaging studies have found brain regional densities of the 5-HT transporter that were higher,<sup>29</sup> lower,<sup>30-33</sup> or not different from controls.<sup>34,35</sup> Recently, a meta-analysis of 18 studies, involving 2283 patients with OCD, failed to demonstrate a significant association between 5-HT transporter polymorphism and susceptibility to OCD.<sup>36</sup>

Failure to identify a consistent 5-HT phenotype in OCD may in part be due to the limited resolution of the available methods of investigation to assess 5-HT function in vivo, the small sample size of most studies, and the inherent complexity and multiple behavioral phenotypes linked to a diagnosis of OCD, as well as the widely distributed and ubiquitous innervation of serotonergic neurons across the brain. Indeed, a parsimonious interpretation of most biological studies to date would argue against the presence of specific regional alterations of 5-HT neurotransmission having pathophysiological relevance for the disorder. Yet, this view is not shared by many and is still a matter of significant controversy.<sup>2,24,37</sup> Instead, a model advocating a complex pattern of local facilitatory and inhibitory influences, modulating distinct 5-HT pathways underlying the different components of an OCD phenotype, is preferred. For example, coping or resistance appears a more attractive and plausible alternative; in particular, higher serotonergic input in amygdala is often associated with anxiety-like behaviors,<sup>38</sup> whereas lower serotonin function in the orbital frontal cortex is reportedly associated with behavioral disinhibition and impulsivity.<sup>39,40</sup>

In the past decade, we have developed and validated a method for estimating in vivo brain regional 5-HT synthesis capacity, using positron emission tomography (PET) in combination with a synthetic analog of the 5-HT precursor L-tryptophan,  $\alpha$ -[<sup>11</sup>C]methyl-L-tryptophan ( $\alpha$ -[<sup>11</sup>C]MTrp).<sup>41,42</sup> Unlike L-tryptophan,  $\alpha$ -[<sup>11</sup>C]MTrp is not incorporated into protein.<sup>43</sup> Like the 5-HT precursor, though,  $\alpha$ -[<sup>11</sup>C]MTrp is carried across the blood-brain barrier by a transport system that is active for large neutral amino acids.<sup>44</sup> Once inside the brain,  $\alpha$ -[<sup>11</sup>C]MTrp is taken up into 5-HT neurons where it enters the precursor pool and, eventually, is metabolized into  $\alpha$ M-5-HT. With a 2-tissue compartment model, we then use the tracer's net blood-to-brain clearance ( $K^*$ , in milliliters per gram per minute) as a proxy to estimate regional rates of 5-HT synthesis.<sup>41</sup> This method has been used to study 5-HT synthesis capacity in the brains of healthy adults and children,<sup>45-48</sup> as well as in the brains

of patients with a history of mood and personality disorders, migraines, autism, alternating hemiplegia during childhood, and serious suicide attempts.<sup>40,49-53</sup>

In our study, the  $\alpha$ -[<sup>11</sup>C]MTrp/PET method was used to measure in vivo brain regional 5-HT synthesis capacity rates in medication-free patients with OCD compared with age- and sex-matched healthy controls. We specifically focused on the cortical, subcortical, and limbic areas (orbitofrontal cortex, anterior cingulate gyrus, and caudate nucleus), which were reported to be of pathophysiological significance in the various anatomical models derived from functional neuroimaging studies in OCD.<sup>54-63</sup> Although specific predictions could be entertained as to the direction and/or location of change, if any, between patients with OCD and healthy controls (eg, greater 5-HT-mediated inhibitory inputs in circuits mediating impulsivity, as an attempt to regain behavioral control, or greater 5-HT-mediated facilitator effects in brain pathways mediating stress-related repetitive behaviors), our study was deemed, in many ways, exploratory rather than hypothesis testing.

## METHODS

### STUDY POPULATION

The primary entry criteria for the subjects with OCD were as follows: (1) right-handed man or woman, aged 18 to 65 years; (2) current diagnosis of OCD, per the Structured Clinical Interview for DSM-IV Axis I Disorders<sup>64</sup>; (3) a Yale-Brown Obsessive Compulsive Scale (Y-BOCS)<sup>65</sup> score of 19 or higher; (4) a Clinical Global Impressions rating of 3 or higher; (5) a Beck Depression Inventory<sup>66</sup> score lower than 16; (6) medication-free for at least 3 weeks or for more than 5 elimination half-lives of the drug, whichever was more; (7) no personal or family history of Tourette syndrome; (8) no history of other Axis I disorders, except for depression secondary to OCD; (9) no current or past substance dependence; and (10) never having used the putative 5-HT neurotoxins 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA). Twenty-one patients diagnosed with OCD and meeting the entry criteria were referred by psychiatric sites in Montreal and by the OCD clinic (D.S.) at the Department of Psychology, McGill University Health Centre.

The comparison group consisted of 21 healthy subjects matched for age and sex. These participants were recruited via newspaper advertisements and were interviewed using the Structured Clinical Interview for DSM-IV Axis I Disorders. All were physically healthy, as determined by a physical examination and standard laboratory tests. Exclusion criteria included a personal history of past or current DSM-IV Axis I psychiatric disorder, a DSM-IV Axis I psychiatric disorder in a first-degree relative, and past use of MDMA and/or MDA. On the day of the PET study, all participants tested negative on a urine drug screen (Triage Panel for Drugs of Abuse; Biosite Diagnostics Inc, San Diego, California) that is sensitive to cocaine, opiates, phenylcyclohexyl piperidine, tetrahydrocannabinol, barbiturates, benzodiazepines, and amphetamines. All women of fertile age were scanned during their follicular phase. Thus, the findings do not generalize to other phases, such as the luteal phase.

Written informed consent was obtained from all participants. Our study was conducted in accordance with the Declaration of Helsinki (18th World Medical Association General Assembly) and was approved by the Research Ethics Committee of the Montreal Neurological Institute (MNI).

## PET AND MAGNETIC RESONANCE IMAGING

The  $\alpha$ - $^{11}\text{C}$ MTTrp was prepared as described previously.<sup>67</sup> Prior to the PET study, all subjects observed an overnight fast (water allowed ad libitum) preceded by a low-protein diet, the day before the PET study, to reduce interindividual variability in plasma amino acid concentrations.<sup>45</sup> The PET studies were conducted in the late morning or early afternoon (between 11 AM and 2 PM) using a whole-body scanner (ECAT HR+; CTI Molecular Imaging, Inc/Siemens, Knoxville, Tennessee). All images were collected and reconstructed in 3-dimensional mode with an intrinsic resolution of  $5 \times 5 \times 5$ -mm full width at half maximum. Before tracer injections, transmission scans for attenuation correction were performed using a  $^{68}\text{Ge}/\text{Ga}$  source. After the intravenous injection of 10 to 15 mCi (185-740 MBq) of  $\alpha$ - $^{11}\text{C}$ MTTrp (dose not scaled to body weight) administered as a 2-minute slow infusion, 60-minute dynamic PET data were acquired. During each PET scan, thirteen 2-mL blood samples were obtained from the antecubital vein of a heated arm to compute the  $\alpha$ - $^{11}\text{C}$ MTTrp input function. The input function was derived from intracranial venous sinus radioactivity (0-20 minutes) and "arterialized" venous plasma (20-60 minutes), as described previously.<sup>48,68</sup> Three 2-mL blood samples were centrifuged, and ultrafiltrates were stored at  $-80^\circ\text{C}$  for measurement of free plasma tryptophan concentrations using high-performance liquid chromatography.<sup>45,69</sup> Two additional plasma samples were treated with trichloroacetic acid (2:1) for determination by high-performance liquid chromatography of total plasma tryptophan concentrations.

All participants underwent high-resolution magnetic resonance imaging using a 1.5-T superconducting magnet system (Philips Gyroscan; Philips Medical Systems, Eindhoven, the Netherlands). Images were collected using 3-dimensional volume acquisition, T1-weighted (3-dimensional fast-field echo scan: repetition time, 18 milliseconds; echo time, 10 milliseconds; flip angle,  $30^\circ$ ) over the whole brain. Magnetic resonance imaging data were stored as a  $256 \times 256 \times 160$ -mm matrix with 1-mm<sup>3</sup> isotropic voxels.

### CALCULATION OF $\alpha$ - $^{11}\text{C}$ MTTrp TRAPPING ( $K^*$ )

The Patlak graphic method<sup>70</sup> was used to calculate  $K^*$  (in milliliters per gram per minute) using 40 minutes of dynamic PET data collected between 20 and 60 minutes after tracer injection.<sup>48,68</sup> Comparisons of regional normalized  $K^*$  values between controls and patients with OCD were performed using Statistical Parametric Mapping (SPM8; Wellcome Functional Imaging Laboratory, London, England) and separately via a magnetic resonance image-based region-of-interest method.

### SPM8 ANALYSIS

Comparisons of differences in regional normalized  $K^*$  values between patients with OCD and healthy controls were first performed using a brain-wide voxelwise approach with SPM8.  $K^*$  images were resampled to the MNI-305 template, 2-mm isotropic stereotaxic space (spatial normalization) using a standard linear automatic algorithm.<sup>71</sup> Each functional image in stereotaxic space was smoothed with a gaussian filter (14-mm full width at half maximum) to reduce the effect of individual variability in cortical gyral anatomy. Proportional scaling was applied to remove the effect of global differences on regional values among subjects. Functional images were normalized by setting for each subject the mean global  $K^*$  value of their gray matter to 100. SPM8 comparisons were restricted to voxels found in the gray matter. The  $t$  test was applied voxel-by-voxel to determine regional differences between groups. Statistically sig-

nificant regional differences were identified using dual criteria. First, the height threshold used to interpret the  $t$  test in terms of probability level was set at  $P = .005$  (uncorrected). Second, the extent threshold was set at 100 voxels, suitable for the 14-mm full width at half maximum filter and sufficient to remove small noisy clusters, which may reach significance by chance. SPM8 was also used to identify regions where individual differences in  $\alpha$ - $^{11}\text{C}$ MTTrp trapping correlated with Y-BOCS scores using the same criteria for statistical significance.

### MAGNETIC RESONANCE IMAGING-BASED REGION-OF-INTEREST ANALYSIS

Comparisons of regional normalized  $K^*$  values between healthy controls and patients with OCD were also performed using a magnetic resonance image-based region-of-interest approach. Individual magnetic resonance imaging data were corrected for field inhomogeneities<sup>72</sup> and resampled in a standard stereotaxic space (MNI-305 template).<sup>71</sup> Tissue classification into gray and white matter and cerebrospinal fluid was performed using the series of algorithms known as INSECT (Intensity Normalized Stereotaxic Environment for the Classification of Tissue).<sup>73</sup> These data were subsequently submitted to the algorithm known as ANIMAL (Automatic Nonlinear Imaging Matching and Anatomical Labeling)<sup>74</sup> for segmentation into 48 anatomical volumes. The bilateral medial and lateral orbitofrontal cortices, the cingulate complex, and the caudate constituted the a priori selected regions of interest. These regions of interest were convolved with a 7-mm full width at half maximum gaussian kernel filter and then resampled into PET acquisition space. Volumes of interest were then applied to dynamic native PET space to extract time-activity curves. Global  $K^*$  values were compared between subject groups. Subsequently, to minimize the effect of individual global differences on regional values, all regional  $K^*$  values were normalized by setting the mean global  $K^*$  values of the gray matter to 100. In addition to comparing functional differences, volumetric comparisons of the aforementioned anatomical volumes in stereotaxic space (MNI-305 template) were performed between healthy controls and patients with OCD.<sup>75</sup>

## RESULTS

### DEMOGRAPHICS

Twenty-one patients with OCD (15 men with a mean [SD] age of 33.2 [9.3] years and 6 women with a mean [SD] age of 35.8 [7.1] years) and 21 healthy controls (15 men with a mean [SD] age of 32.9 [10.1] years and 6 women with a mean [SD] age of 36.5.5 [8.6] years) participated in our study (**Table**). Age did not differ between groups when analyzed as a whole or according to sex. The patients with OCD had significantly higher scores on the Beck Depression Inventory (mean [SD], 13.3 [9.3]) than did the healthy controls (mean [SD], 2.1 [2.8]) ( $t_{23} = 3.07$ ,  $P \leq .005$ ), but their scores were still within the subclinical range, and no patient met diagnostic criteria for major depression. Six patients had a lifetime history of major depression that had occurred secondarily to their OCD symptoms. Of the 21 patients with OCD, 11 had childhood-onset OCD (age,  $<10$  years), 11 had checking behaviors as their predominant compulsion, and 10 had predominantly washing compulsions. None of the subjects included in our study ever had a substance abuse prob-



lem, except for 1 patient with OCD who had a lifetime history of cocaine dependence that ended 11 years prior to the study. There were no differences in plasma concentrations of total or free tryptophan between patients with OCD and healthy controls or between men and women.

#### GLOBAL AND REGIONAL $\alpha$ -[<sup>11</sup>C]MTrp TRAPPING

There was no significant group effect in the sample as a whole ( $t_{40}=1.37$ ,  $P=.18$ ), or in either sex analyzed separately for global  $K^*$  values (men:  $t_{28}=0.095$ ,  $P=.92$ ; women:  $t_{10}=1.94$ ,  $P=.08$ ). In comparison, normalized  $\alpha$ -[<sup>11</sup>C]MTrp trapping was significantly higher for patients with OCD than for healthy controls in the right hippocampus ( $t_{40}=3.37$ ,  $k=151$  voxels, coordinates x, y, z, respectively: 30, -38, 4 mm) and the left temporal gyrus (Brodmann area 20;  $t_{40}=3.10$ ,  $k=157$  voxels, coordinates x, y, z, respectively: -62, -20, -24 mm). There were no regions with significant lower values in patients with OCD compared with healthy controls (**Figure 1**).

When analyzed separately by sex, in the men (15 patients with OCD vs 15 healthy controls), greater  $\alpha$ -[<sup>11</sup>C]MTrp trapping in patients with OCD compared with controls was present in the same regions as for the whole group (right hippocampus:  $t_{28}=4.70$ ,  $k=419$  voxels, coordinates x, y, z, respectively: 32, -40, 2 mm; left inferior temporal gyrus [Brodmann area 20]:  $t_{28}=4.70$ ,  $k=101$  voxels, coordinates x, y, z, respectively: -64, -22, -26 mm), with no regions in the patients with OCD demonstrating values lower than those in healthy controls. In the smaller subgroup of women (6 patients with OCD vs 6 healthy controls),  $\alpha$ -[<sup>11</sup>C]MTrp trapping was significantly higher for the subjects with OCD in the right parahippocampal uncus (Brodmann areas 20 and 36;  $t_{10}=4.89$ ,  $k=679$  voxels, coordinates x, y, z, respectively: 28, -4, -38 mm) only. Significant decreases in  $\alpha$ -[<sup>11</sup>C]MTrp trapping were also seen in the left cuneus/precuneus (Brodmann area 7;  $t_{10}=8.89$ ,  $k=104$  voxels, coordinates x, y, z, respectively: -10, -72, 32 mm) and uncus (Brodmann areas 28 and 34;  $t_{10}=4.40$ ,  $k=102$  voxels, coordinates x, y, z, respectively: -18, 8, -26 mm) for women with OCD.

Region-of-interest analyses confirmed the SPM8-based findings. A group  $\times$  hemisphere analysis of variance yielded a significant interaction for  $K^*$  values in the hippocampus ( $F_{1,40}=14.75$ ,  $P\leq .001$ ), reflecting greater regional trapping in the patient's right hemisphere, compared with healthy controls. Region-of-interest analysis also confirmed a significant main effect of group in both the left and right inferior temporal gyrus, with greater tracer trapping in patients with OCD than in healthy controls ( $F_{1,40}=7.97$ ,  $P\leq .007$ ). Finally, a group  $\times$  hemisphere interaction approached significance in the group as a whole, reflecting higher  $K^*$  values in the right caudate of patients ( $F_{1,40}=3.83$ ,  $P\leq .06$ ). This effect was larger in the men, and a region-of-interest analysis that was restricted to men confirmed the main effect of group ( $F_{2,39}=9.06$ ,  $P\leq .001$ ), reflecting higher  $K^*$  values in the patients with OCD than in healthy controls for both the left ( $P\leq .03$ ) and right caudate nucleus ( $P\leq .001$ ). No volumetric differences were found for any of the regions of interest between patients with OCD and healthy controls.

**Table. Demographic Characteristics of the Study Population**

Characteristic	Patients With OCD (n = 21)	Healthy Controls (n = 21)
Age, y		
Mean (SD)	33.95 (8.64)	33.95 (9.59)
Range	18-53	20-56
Y-BOCS score, mean (SD)	23.57 (5.33)	NA
BDI score, <sup>a</sup> mean (SD)	13.2 (9.27)	1.40 (2.19)
Disease duration, mean (SD), y	21.28 (8.73)	NA
Childhood-onset OCD (age, <10 y), No.	11	NA
Predominant compulsion, No.		NA
Washing	10	NA
Checking	11	NA
Lifetime history of MDE, No.	6	0
Past SSRI treatment, No.	2	0
Past substance abuse, No.	1	0
Intravenously injected, mean (SD), mCi	10.16 (3.14)	9.74 (2.11)
Plasma free tryptophan, mean (SD), nmol/L	8.37 (3.69)	8.91 (3.76)
Global $K^*$ , mean (SD), mL/g/min	5.07 (1.49)	5.81 (1.96)

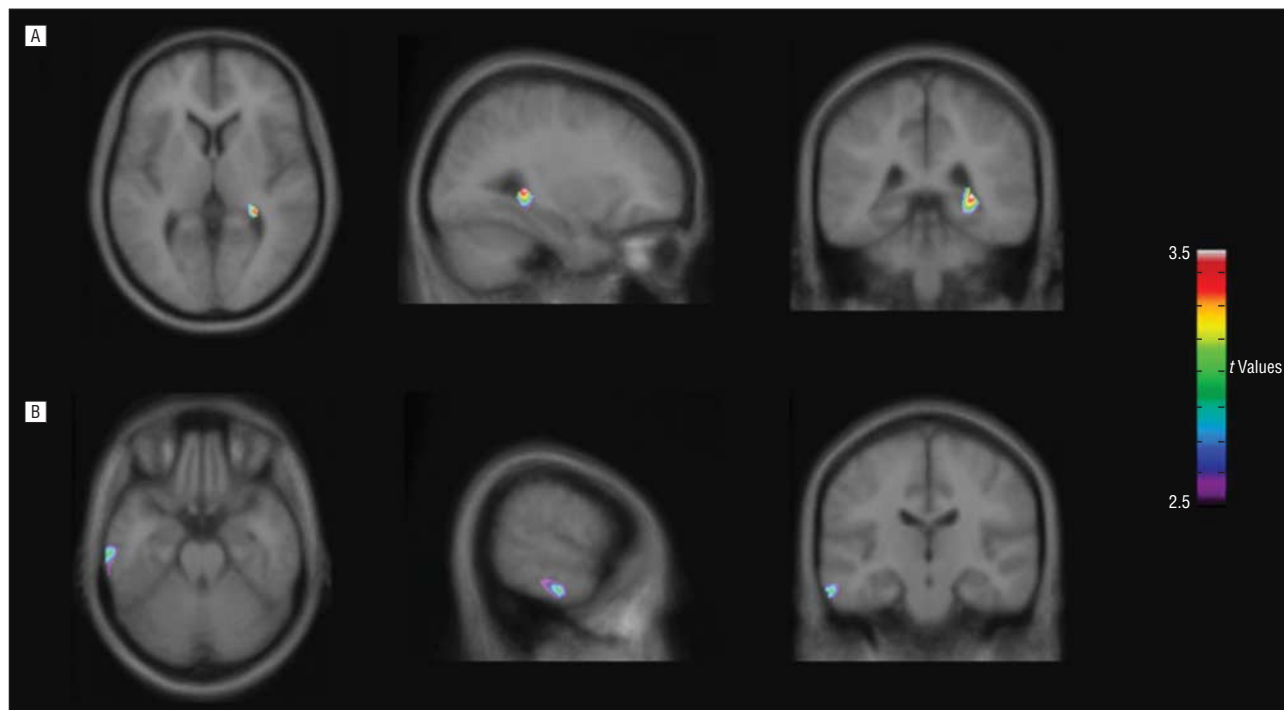
Abbreviations: BDI, Beck Depression Inventory; MDE, major depressive episode; NA, not applicable; OCD, obsessive-compulsive disorder; SSRI, selective serotonin reuptake inhibitor; Y-BOCS, Yale-Brown Obsessive-Compulsive Scale.

<sup>a</sup>Statistically significant difference between patients with OCD and healthy controls at  $P < .001$ .

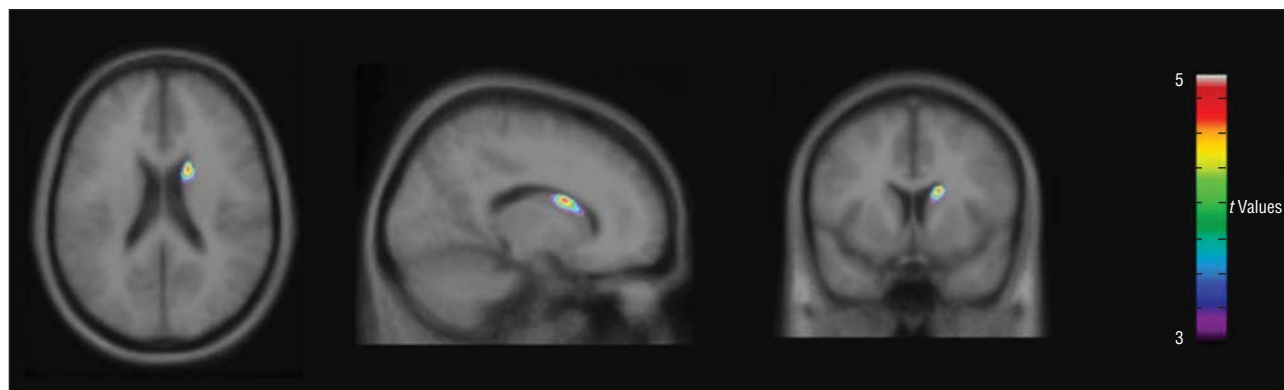
#### CORRELATIONS BETWEEN $\alpha$ -[<sup>11</sup>C]MTrp TRAPPING AND CLINICAL SCORES

In the sample of patients with OCD as a whole ( $n=21$ ), significant positive correlations between Y-BOCS scores and  $\alpha$ -[<sup>11</sup>C]MTrp trapping were present in the right middle (Brodmann area 21;  $t_{19}=4.24$ ,  $k=345$  voxels, coordinates x, y, z, respectively: 70, -26, -14 mm) and superior temporal gyrus (Brodmann area 38;  $t_{19}=4.40$ ,  $k=551$  voxels, coordinates x, y, z, respectively: 46, 8, -28 mm) and in the left inferior and middle temporal gyrus (Brodmann areas 20 and 21;  $t_{19}=3.81$ ,  $k=183$  voxels, coordinates x, y, z, respectively: -60, -6, -26 mm). In the male patients with OCD, a significant positive correlation between Y-BOCS scores and  $\alpha$ -[<sup>11</sup>C]MTrp trapping was seen in the right caudate ( $t_{13}=4.82$ ,  $k=145$  voxels, coordinates x, y, z, respectively: 16, 8, 20 mm) (**Figure 2**), whereas in the female patients with OCD, a significant positive correlation was seen in the left precuneus (Brodmann area 19;  $t_4=18.32$ ,  $k=143$  voxels, coordinates x, y, z, respectively: -24, -74, 32 mm), the right inferior and middle temporal gyrus (Brodmann areas 20 and 21;  $t_4=11.04$ ,  $k=155$  voxels, coordinates x, y, z, respectively: 60, -12, -16 mm), and the right inferior and middle frontal gyrus (Brodmann area 47;  $t_4=7.60$ ,  $k=126$  voxels, coordinates x, y, z, respectively: 52, 42, -6 mm).

The Beck Depression Inventory did not correlate with  $\alpha$ -[<sup>11</sup>C]MTrp uptake and trapping. In particular, it did not correlate with any of the brain regions distinguishing patients with OCD from controls, thus ruling out the possibility that current subclinical secondary depression may have confounded the results.



**Figure 1.** Statistical parametric maps (SPM8; Wellcome Functional Imaging Laboratory, London, England) showing brain regions where  $K^*$  values were significantly higher in patients with obsessive-compulsive disorder ( $n=21$ ) than in healthy controls ( $n=21$ ). The statistical  $t$ -map threshold was 2.70, with  $P=.005$  and an extent threshold of 100 voxels. Significant clusters were found in (A) the right hippocampus ( $t_{40}=3.37$ ,  $k=151$  voxels, coordinates  $x, y, z$ , respectively: 30, -38, 4 mm) and (B) the left inferior temporal gyrus ( $t_{40}=3.10$ ,  $k=157$ , coordinates  $x, y, z$ , respectively: -62, -20, -24 mm).



**Figure 2.** Statistical parametric maps (SPM8; Wellcome Functional Imaging Laboratory, London, England) showing brain regions where  $K^*$  values correlated positively with Yale-Brown Obsessive-Compulsive Scale scores in male patients with obsessive-compulsive disorder ( $n=15$ ). The statistical  $t$ -map threshold was 3.01, with  $P=.005$  and an extent threshold of 100 voxels. A significant cluster was found in the right caudate ( $t_{13}=4.82$ ,  $k=145$  voxels, coordinates  $x, y, z$ , respectively: 16, 8, 20 mm).

#### COMMENT

Largely on the basis of the clinical efficacy of 5-HT reuptake inhibitors, it was proposed, more than 20 years ago, that perturbed serotonergic neurotransmission may contribute to the development and expression of OCD.<sup>11,25,76</sup> Our study provides some evidence that these putative disturbances may include regionally specific alterations in brain 5-HT synthesis capacity, with medication-free patients with OCD exhibiting, relative to age- and sex-matched controls, significantly elevated normalized  $\alpha$ - $^{11}\text{C}$ ]MTrp trapping in the right hippocampus and left inferior temporal gyrus (Brodmann area 20). These observations were more robust when the analysis was restricted to male patients

with OCD ( $n=15$ ), who also demonstrated increased  $\alpha$ - $^{11}\text{C}$ ]MTrp trapping bilaterally in the caudate nucleus. Individual differences in  $\alpha$ - $^{11}\text{C}$ ]MTrp trapping in the right caudate and, bilaterally, in the temporal cortex correlated positively with OCD symptom severity in the whole group of patients with OCD. There were no regions of interest where the patients with OCD exhibited significantly lower  $\alpha$ - $^{11}\text{C}$ ]MTrp trapping values than did controls. The lack of robustness for differences in normalized  $\alpha$ - $^{11}\text{C}$ ]MTrp trapping in female patients with OCD might be related to the near significance reported (trend) for global  $K^*$ , between the female patients with OCD and the female healthy controls, as well as to the small number of women studied.

During the past 2 decades, considerable progress has been made in the dissection of the functional neuroanatomy of OCD, with compelling evidence in support of hyperactivity in parts of a brain circuit loop linking the orbital frontal cortex, the caudate nucleus, and the anterior cingulate gyrus.<sup>3</sup> Much less consensus exists, however, as to its significance, whether primary (correlating with symptom severity) or secondary (reflecting resistance and attempts to regain thought and/or behavioral control), or both. A common belief among OCD researchers is that hyperactivity of the frontostriatal loop underscores the motor and cognitive habits progressively evolving toward inflexibility and rigid ritualistic behavior, whereas hyperactivity in the orbitofrontal cortex reflects unsuccessful resistance (ie, failure to inhibit and failed attempts at stress control).<sup>3,77</sup> However, primary deficits in orbitofrontal cortex function have also been reported.

The finding of greater  $\alpha$ -[<sup>11</sup>C]MTrp trapping in the caudate nucleus for male patients with OCD than for male healthy controls might reflect serotonergic modulation of a structure known to be hyperactive in patients with current OCD, as noted earlier. Within the striatum, 5-HT exerts primarily, though not exclusively, inhibitory effects.<sup>78,79</sup> The greater 5-HT neurotransmission in the striatum of patients with OCD, therefore, could be a contributing factor to the disorder's pathophysiology and symptom profile and/or locus that might be helped by treatment with selective serotonin reuptake inhibitors.<sup>80</sup>

The present results do not provide support for the hypothesis of altered 5-HT neurotransmission in the orbitofrontal cortex or other aspects of the frontal lobe via a presynaptic mechanism. Rather, the enhanced glucose metabolic and regional cerebral blood flow changes previously reported in these regions may be the result of postsynaptic 5HT alteration(s). Indeed, the decreased binding potential of the 5-HT<sub>2a</sub> ligand [C-11]MDL100.907 in several cortical areas, including the orbitofrontal cortex, represents a specific postsynaptic downregulation of a 5-HT receptor subtype, which mediates an inhibitory response in the rodent brain region equivalent to the human orbitofrontal cortex.<sup>81,82</sup> Physiologically, this would translate into a dampened inhibitory tone and thus increased metabolic activity of the orbitofrontal cortex.

There are certainly other possibilities to account for the lack of orbital frontal findings. In particular, increased  $\alpha$ -[<sup>11</sup>C]MTrp trapping was observed in the hippocampus and the rostral parahippocampal uncus, which covers the amygdala's dorsal surface. These regions have been identified in at least some previous functional neuroimaging studies of OCD,<sup>3</sup> and they influence activity in the orbitofrontal cortex via both direct and indirect projections.<sup>83,84</sup>

The hippocampus and caudate have been proposed to play important roles in 2 independent forms of memory, context-dependent cognitive processes and stimulus-response habit behaviors, respectively.<sup>85-87</sup> As part of a serotonergic septohippocampal behavioral inhibition system,<sup>88</sup> the hippocampus is also thought to influence anxiety-related behaviors, increasing the salience of negatively valenced affective stimuli and interacting with the amygdala and neocortex to facilitate choices between con-

flicting alternatives.<sup>89,90</sup> Excessive hippocampal output to the striatum might perturb memory-guided coping<sup>91,92</sup> and, in the presence of high dopamine levels, promote the perseveration of inflexible, context-dependent habits.<sup>87,93,94</sup> This revised model suggests that hippocampal, amygdaloid, and striatal hyperfunction could reflect a tendency to resolve conflicts in terms of a negative bias, potentially contributing to an OCD-susceptible phenotype characterized by danger overestimation, reduced confidence that an act has been completed adequately, and compulsive stereotypies.

To our knowledge, our study describes the only psychiatric population reported to date to exhibit exclusively elevated  $\alpha$ -[<sup>11</sup>C]MTrp trapping values. In comparison, patients with major depression,<sup>33</sup> borderline personality disorder,<sup>49</sup> and/or a history of serious suicide attempts<sup>40</sup> are all reported to have abnormally low  $\alpha$ -[<sup>11</sup>C]MTrp trapping values in limbic regions of the frontal lobe. One potential implication is that patients with OCD who have abnormally low 5-HT activity in these regions might become susceptible to comorbid disorders. The patients in our study were carefully screened to rule out the presence of significant comorbidities.

Subjects with borderline personality disorder are also reported to exhibit lower values in  $\alpha$ -[<sup>11</sup>C]MTrp trapping in the basal ganglia, and individual differences in these K\* values correlated negatively with impulsivity scores.<sup>49</sup> In comparison, patients with OCD exhibited abnormally high striatal K\* values, with individual differences correlating positively with compulsivity. In the presence of other risk factors (eg, hypo- vs hyperfrontality), a continuum of 5-HT activity within the basal ganglia might contribute to a proposed impulsivity-compulsivity dimension.<sup>95</sup>

The interpretation of our results rests on the following methodological considerations: (1) The significance of the  $\alpha$ -[<sup>11</sup>C]MTrp/PET method has been questioned, and, in particular, Shoaf et al<sup>96</sup> have suggested that it might measure blood brain barrier transport of tryptophan rather than synthesis of 5-HT. However, subsequent autoradiography studies on rodents,<sup>97</sup> along with analyses of tracer kinetics and the effects of manipulations that selectively increase<sup>44,98</sup> vs decrease 5-HT synthesis,<sup>99,100</sup> support the general consensus that brain regional  $\alpha$ -[<sup>11</sup>C]MTrp trapping provides an acceptable proxy for 5-HT synthesis.<sup>41,42,45,48,101-104</sup> (2) Medication-free patients with OCD did not differ significantly from healthy controls in total and free plasma tryptophan concentrations or in the free plasma tryptophan fraction, making it unlikely that increased normalized K\* values reflect group differences in circulating tryptophan concentrations, which otherwise could affect brain 5-HT synthesis.<sup>45,105-108</sup> (3) As in other functional neuroimaging studies of psychiatric populations, the sample size is relatively small (21 patients with OCD vs 21 healthy controls), such that general conclusions cannot be drawn until there is an independent replication. A larger sample would also allow one to test whether specific abnormalities are associated with early- vs late-onset OCD or phenotypical subtypes, such as predominantly "checkers" or "washers," or more recently proposed symptom subtypes such as "symmetry" or "hoarding."<sup>109,110</sup> Other potential confounders, such as body mass index or seasonal variations, could also be

more appropriately controlled for in a larger sample. Nevertheless, the sample size is in the upper range of similar neuroimaging studies and is one of the largest in a PET study of patients with OCD reported to date that used a 5-HT system tracer. Healthy controls and patients with OCD were also enrolled and observed in a parallel manner over time. Moreover, precautions included rigorous matching for age and sex, a negative toxicological screen on the day of the scan, and patient selection restricted to medication-free individuals without current comorbid conditions. (4) As frequently seen, 6 of 21 patients had a past history of depression that had developed secondarily to OCD and was in remission at the time of scanning. This, of course, could potentially bias our results because depression is linked to state abnormalities, and perhaps trait 5-HT abnormalities, as well as morphological and structural changes, in particular in the hippocampus. The latter, however, seems to develop with recurrent chronic major depressive disorder,<sup>111</sup> which does not correspond to our sample phenotype, in which only some individuals suffered at some point a minor form of depression. Moreover, complementary analyses using Beck Depression Inventory scores did not reveal any significant correlations with K\*. In addition, an analysis contrasting OCD patients with and without a history of depression did not reveal any significant differences in regional K\*, and removing patients with a history of depression from the analysis did not change the results. (5) In contrast to our results, previous functional neuroimaging studies of the 5-HT transporter have not identified consistent changes in patients with OCD vs controls, either in the midbrain or terminal regions. A recent study,<sup>112</sup> though, has measured [<sup>18</sup>F] altanserin binding values and found evidence of increases in 5-HT<sub>2A</sub> densities specifically within the caudate nucleus. (6) Under certain pathological conditions, such as inflammatory neurological diseases,<sup>113</sup> intractable epilepsy in childhood,<sup>114</sup> or brain tumors,<sup>115</sup> an increased tryptophan metabolism might reflect the activation of the initial and rate-limiting enzyme of the kynurenine pathway, indoleamine 2,3 dioxygenase. Indeed, cases of pediatric autoimmune neuropsychiatric disorders,<sup>116</sup> including a subgroup of childhood-onset OCD with or without tics, have been associated with streptococcal infections. Moreover, abnormally increased serotonin synthesis was recently reported in Tourette syndrome.<sup>117</sup> Whether neuroinflammatory processes prompted activation of the kynurenine pathway in some OCD cases, thus resulting in the increased rate of uptake and clearance of  $\alpha$ -[<sup>11</sup>C]MTrp reported here, is unknown. (7) Finally, although volumetric differences in patients with OCD have been reported previously, these findings have been inconsistent; for example, the orbitofrontal cortex and striatal tissue volumes have been reported to be abnormally high,<sup>118,119</sup> abnormally low,<sup>119,120</sup> or to not differ from those in controls.<sup>121</sup> Moreover, recent meta-analyses indicate possible structural alterations in parietofrontal areas, the anterior cingulate cortex, the thalamus, lenticular/caudate nuclei, and the putamen, but they do not support the presence of structural alterations in the hippocampus, where we found functional differences.<sup>122-124</sup> In our study, careful segmentation (blind to the study group)

did not reveal volumetric differences between the OCD and control groups.

In conclusion, our study revealed elevated normalized  $\alpha$ -[<sup>11</sup>C]MTrp trapping in the right hippocampus, the left inferior temporal gyrus, and the bilateral caudate nucleus in medication-free patients with OCD, relative to age- and sex-matched controls. These findings add to the evidence supporting a serotonergic dysfunction in OCD and, more specifically, point to the critical role played by serotonergic innervation of limbic structures, such as the hippocampus, closely connected to previously identified regions (caudate and orbitofrontal cortex) believed to mediate OCD symptoms. The abnormally high 5-HT synthesis, suggested by the elevated  $\alpha$ -[<sup>11</sup>C]MTrp uptake in the unmedicated symptomatic state, might represent a compensatory mechanism, which could be engaged further in the course of effective anti-OCD treatments.

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**Author Affiliations:** Department of Psychiatry, McGill University (Drs Berney, Leyton, Sibon, Sookman, Rosa Neto, Pinard, Blier, and Benkelfat and Mr Gravel), Department of Neurology and Neurosurgery, McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University (Drs Berney, Leyton, Sibon, Rosa Neto, Diksic, Nakai, Okazawa, and Benkelfat and Mr Gravel), Obsessive-Compulsive Disorder Clinic, Department of Psychology, McGill University Health Centre (Dr Sookman), and Department of Psychiatry, University of Montreal, L. H. Lafontaine Hospital (Dr Todorov), Quebec, Canada; and Department of Psychiatry and Behavioral Science, UC Davis Medical Center, Sacramento, California (Dr Nordah). Dr Berney is now with the Department of Psychiatry, Consultation Liaison Psychiatry Unit, Lausanne University Hospital, Lausanne, Switzerland. Dr Sibon is now with the Department of Neurology, Victor Segalen University (Bordeaux 2), France. Dr Nakai is now with the Department of Pediatrics, Faculty of Medical Sciences, University of Fukui, Japan. Dr Okazawa is now with the Biomedical Imaging Research Center, University of Fukui, Japan. Dr Blier is now with the Mood Disorders Research Unit, University of Ottawa Institute of Mental Health Research, Ontario, Canada.

**Correspondence:** Chawki Benkelfat, MD, DERBH, Department of Psychiatry, McGill University, 1033 Pine Ave West, Montreal, QC H3A 1A1, Canada (chawki.benkelfat@mcgill.ca).

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