Original Investigation

Role of Translocator Protein Density, a Marker of Neuroinflammation, in the Brain During Major Depressive Episodes

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IMPORTANCE The neuroinflammatory hypothesis of major depressive disorder is supported by several main findings. First, in humans and animals, activation of the immune system causes sickness behaviors that present during a major depressive episode (MDE), such as low mood, anhedonia, anorexia, and weight loss. Second, peripheral markers of inflammation are frequently reported in major depressive disorder. Third, neuroinflammatory illnesses are associated with high rates of MDEs. However, a fundamental limitation of the neuroinflammatory hypothesis is a paucity of evidence of brain inflammation during MDE. Translocator protein density measured by distribution volume (TSPO \( V_T \)) is increased in activated microglia, an important aspect of neuroinflammation.

OBJECTIVE To determine whether TSPO \( V_T \) is elevated in the prefrontal cortex, anterior cingulate cortex (ACC), and insula in patients with MDE secondary to major depressive disorder.

DESIGN, SETTING, AND PARTICIPANTS Case-control study in a tertiary care psychiatric hospital from May 1, 2010, through February 1, 2014. Twenty patients with MDE secondary to major depressive disorder and 20 healthy control participants underwent positron emission tomography with fluorine F 18-labeled N-(2-(2-fluoroethoxy)benzyl)-N-(4-phenoxypyridin-3-yl)acetamide (\([18F]FEPPA\)). Patients with MDE were medication free for at least 6 weeks. All participants were otherwise healthy and nonsmokers.

MAIN OUTCOMES AND MEASURES Values of TSPO \( V_T \) in the prefrontal cortex, ACC, and insula.

RESULTS In MDE, TSPO \( V_T \) was significantly elevated in all brain regions examined (multivariate analysis of variance, \( F_{15,23} = 4.5 \) \( P = .001 \)). The magnitude of TSPO \( V_T \) elevation was 26% in the prefrontal cortex (mean [SD] TSPO \( V_T \), 12.5 [3.6] in patients with MDE and 10.0 [2.4] in controls), 32% in the ACC (mean [SD] TSPO \( V_T \), 12.3 [3.5] in patients with MDE and 9.3 [2.2] in controls), and 33% in the insula (mean [SD] TSPO \( V_T \), 12.9 [3.7] in patients with MDE and 9.7 [2.3] in controls). In MDE, greater TSPO \( V_T \) in the ACC correlated with greater depression severity (\( r = 0.63 \) \( P = .005 \)).

CONCLUSIONS AND RELEVANCE This finding provides the most compelling evidence to date of brain inflammation, and more specifically microglial activation, in MDE. This finding is important for improving treatment because it implies that therapeutics that reduce microglial activation should be promising for MDE. The correlation between higher ACC TSPO \( V_T \) and the severity of MDE is consistent with the concept that neuroinflammation in specific regions may contribute to sickness behaviors that overlap with the symptoms of MDE.

Published online January 28, 2015. Corrected on March 4, 2015.

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Major depressive disorder (MDD) is highly prevalent and has an important impact, with active symptoms present in 4% of the adult population. Although MDD exhibits multiple molecular phenotypes, accumulating evidence suggests a role of inflammation in generating the symptoms of a major depressive episode (MDE). For example, induction of inflammation is associated with sad mood in humans, and direct induction of the central immune system in rodents is associated with the sickness syndrome of anhedonia, weight loss, and anorexia, which overlap with the diagnostic criteria for MDE. Also in MDD, several markers of peripheral inflammation, including levels of C-reactive protein, interleukin 6 (IL-6), and tumor necrosis factor (TNF), are frequently increased. Conditions that create neuroinflammation, such as traumatic brain injury, systemic lupus erythematosus, and multiple sclerosis, are associated with prevalence rates of MDD as high as 50%, suggesting a link between brain inflammation and mood symptoms.

Whether brain inflammation occurs during a current MDE remains unclear because most postmortem investigations of neuroinflammation sampled patients with MDD and a history of MDE or individuals who committed suicide (suicide completers) with varied diagnoses. Within such studies, the samples of patients with current MDD were small. Van Otterloo et al reported no difference in the density of activated microglia in the white matter of the orbitofrontal region in 10 patients with MDD. Dean et al sampled 10 patients with MDD and found significantly increased levels of the transmembrane form of TNF in the dorsolateral prefrontal cortex (PFC) but no difference in levels of this form of TNF in the anterior cingulate cortex (ACC) and no difference in the soluble form of TNF in either region. Steiner et al reported increased density of quinolinic acid–positive cells, a marker influenced by microglial activation, in the ACC of 7 patients with MDE. Microarray studies have had mixed results, with a positive finding by Shelton et al of increased proinflammatory and anti-inflammatory cytokine messenger RNA in Brodmann area 10 in 14 patients with MDD. In contrast, several other microarray studies, most of which sampled adjacent regions of the PFC, did not identify this result. Among investigations in suicide completers, a study reported greater HLA-DR staining, a marker of microglial activation, in the dorsolateral PFC and ACC, and a second study reported greater levels of IL-6, TNF, and IL-1β in Brodmann area 10. Neither study of suicide found a relationship to MDD (or MDE), but fewer than 10 patients with MDD were included in each study. The mixed results among postmortem investigations in MDD have been attributed to issues of variation in brain regions sampled, inclusion of patients with early- and late-onset MDD, comorbidity of other psychiatric disorders and addiction, and, with the exception of the microarray studies, small sample size, although lack of focus on sampling the state of MDE may be important for investigations of neuroinflammation.

To determine whether neuroinflammation occurs in MDE secondary to MDD, positron emission tomography (PET) may be applied to measure translocator protein (TSPO) binding in vivo. Translocator protein is an 18-kDa protein located on the outer mitochondrial membranes in microglia, and increased expression of TSPO occurs when microglia are activated during neuroinflammation. Recently, a new generation of PET radiotracers was developed with superior quantification of TSPO binding. Among these, fluorine F 18–labeled N-(2-fluoroethoxy)benzyl)-N-(4-phenoxypyridin-3-yl)acetamide (18F)FEPPA has excellent properties, including a high selective affinity for TSPO, increased binding during induced neuroinflammation, and a high ratio of specific binding relative to free and nonspecific binding.

To date, one neuroimaging study applied carbon 11–labeled N-(2-methoxybenzyl)-N-(4-phenoxypyridin-3-yl)acetamide (11C)PBR28 PET to investigate TSPO levels in MDD, with negative findings. This earlier study assessed whether TSPO levels were elevated in a sample of 10 patients with MDD who underwent scanning once under a variety of states (treated, untreated, symptomatic, or partially symptomatic); hence, results of that study cannot be considered definitive for determining whether the level of TSPO binding is elevated in MDE. Scores on the Montgomery-Åsberg Depression Rating Scale on the day of PET scanning ranged from 5 to 30, indicating that the severity ranged from almost asymptomatic to moderately symptomatic. Other issues limit interpretation of that study, including potential bias of ongoing antidepressant use, heterogeneity of combined sampling of early- and late-onset MDD, and incomplete information regarding a TSPO polymorphism (rs6971) known to influence binding of the new generation of TSPO PET radioligands, including [11C]PBR28 and [18F]FEPPA.

In the present study, [18F]FEPPA PET was applied to measure TSPO total distribution volume (V₂o), an index of TSPO density, during MDE in patients with MDD compared with healthy, age-matched control participants. The main hypothesis was that TSPO V₂o would be elevated in MDE in the PFC, ACC, and insula. The PFC and ACC were chosen because of their role in mood regulation circuitry and affect dysregulation in MDD. The insula is a strong candidate for mediating some of the sickness behaviors in MDD because it is activated in response to an immune challenge and may participate in homeostatic regulation and interoceptive signaling in MDD. The second hypothesis was that greater severity of symptom measures related to the sickness syndrome would be associated with greater elevation of TSPO V₂o in these regions.

Methods

All participants provided written informed consent after all procedures were fully explained. The protocol and informed consent forms were approved by the Research Ethics Board of the Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Participants

Twenty patients with a current MDE secondary to MDD (hereinafter termed patients with MDE) and 20 age-matched healthy controls completed the study. Participants were recruited from the Toronto-area community and a tertiary care psychiatric hospital (Centre for Addiction and Mental Health) from May 1, 2010,
Population of participants was derived from a clinic-based sample of patients aged 18 to 72 years diagnosed with MDE according to the DSM-IV (Table 1). Healthy controls were age-matched within 4 years to the patients with MDE. Exclusion criteria for all participants included pregnancy; the use of any herb, drug, or medical condition; and any history of neurologic illness or injury. All participants underwent urinedrugscreening,andwomenreceivedanurinepregnancytestonthePETscanningday.

Participants with MDE were administered the 17-item Hamilton Depression Rating Scale (17-item HDRS) on enrollment and on the PET scanning day. For enrollment, a minimum score of 17 on the 17-item HDRS was required. All patients with MDE were medication free for at least 6 weeks before the PET scan (9 patients had completed ≥1 previous antidepressant trial). Other exclusion criteria consisted of concurrent active Axis I disorders, including current alcohol or substance dependence, MDE with psychotic symptoms, bipolar I or II disorder, and borderline or antisocial personality disorder. Depression severity was measured as the total score on the 17-item HDRS, which is also strongly correlated with sickness behaviors of low mood and anhedonia.22 Additional measures included body mass index (BMI) and levels of several peripheral inflammatory markers in serum (IL-1β, IL-6, TNF, and C-reactive protein) (eAppendix in the Supplement).

Table 1. Demographic Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients With MDE (n = 20)</th>
<th>Healthy Controls (n = 20)</th>
</tr>
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<tbody>
<tr>
<td>Female sex, No. (%)</td>
<td>12 (60)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>34.0 (11.3)</td>
<td>33.6 (12.8)</td>
</tr>
<tr>
<td>TSPO genotype, No. of participantsa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAB</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>MAB</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>23.4 (5.4)</td>
<td>24.8 (2.9)</td>
</tr>
<tr>
<td>17-Item HDRS score, mean (SD)b</td>
<td>20.0 (3.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Age at first MDE, mean (SD), y</td>
<td>15.7 (5.2)</td>
<td>NA</td>
</tr>
<tr>
<td>Previous MDE, No. (%)</td>
<td>6 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>Previous antidepress trial, No. (%)</td>
<td>9 (45)</td>
<td>NA</td>
</tr>
<tr>
<td>No previous antidepress trial, No. (%)</td>
<td>11 (55)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HAB, high-affinity binding; HDRS, Hamilton Depression Rating Scale; MAB, mixed-affinity binding; MDE, major depressive episode; NA, not applicable; TSPO, translocator protein.

DNA Extraction and Polymorphism Genotyping

The binding affinity of the second generation of radiotracers for TSPO, including [18F]FEPPA, is known to be affected by a codominantly expressed single-nucleotide polymorphism (rs6971; C→T) in exon 4 of the TSPO gene (NCBI Entrez Gene 706).24,25 Individuals with high-affinity binding (Ala147) and mixed-affinity binding (Ala147/Thr147) account for more than 90% of the population in North America.24 The polymorphism rs6971 was genotyped as described previously.26 One patient with MDE had low-affinity binding (Ala147/Thr147) and was not included in the analysis.

Statistical Analysis

For the primary hypothesis, we analyzed PET data by multivariate analysis of variance (MANOVA), with TSPO VT in the PFC, ACC, and insula as the dependent variables and diagnosis and genotype as the fixed factors. Main effects were considered significant at the conventional $P \leq .05$. Effects in each region, analyzed by univariate ANOVA, were considered significant after Bonferroni correction ($P \leq .017$).

As a secondary analysis, we performed a MANOVA that included every brain region sampled (eg, all cortical and subcortical regions) to assess the effect of diagnosis on TSPO VT. A partial correlation controlling for the rs6971 genotype was used in a secondary analysis to quantify the relationship between TSPO VT in the primary regions of interest and the severity of symptoms of MDE measured by the total 17-item HDRS score. The HDRS score was missing in 1 patient with MDE who...
Secondary to Major Depressive Disorder (MDD) diagnosis,
patients with MDE compared with the controls (main effect of
global brain effect of diagnosis with elevated TSPO VT in the
and several other cortical and subcortical regions indicated a
Table 2
(F)genotype (Figure 1) (effect of diagnosis in the MANOVA,
pared with healthy controls after controlling for the effect of
binding had greater TSPO VT compared with individuals with
mixed-affinity binding. Means scores on the 17-item HDRS in-
by region: prefrontal cortex, F1,37 = 8.1 (P = .007); anterior cingulate cortex,
F1,37 = 12.2 (P = .001); insula, F1,37 = 12.3 (P = .001); dorsal putamen,
F1,37 = 14.1 (P = .001); ventral striatum, F1,37 = 6.9 (P = .01); thalamus, F1,37 = 13.6
(P = .001); and hippocampus, F1,37 = 7.5 (P = .009). All second-generation TSPO
radioligands, such as fluorine F18–labeled N-(2-(2-fluoroethoxy)benzyl)-N-(4-
phenoxyphenyl-3-yl)acetamide ([18F]FEPPA), show differential binding
according to the single-nucleotide polymorphism rs6971 of the TSPO gene,
resulting in HAB and MAB. Horizontal bars indicate means in each group.

Figure 1. Elevated Translocator Protein Density Measured by Distribution Volume (TSPO VT) During a Major Depressive Episode (MDE)
Secondary to Major Depressive Disorder (MDD)

TSPO VT was significantly greater in the 20 patients with MDE (15 with
high-affinity binding [HAB] and 5 with mixed-affinity binding [MAB]) compared
with the 20 healthy control participants (14 with HAB and 6 with MAB).
Calculation of analysis of variance resulted in the following effects of diagnosis
by region: prefrontal cortex, F1,37 = 8.1 (P = .007); anterior cingulate cortex,
F1,37 = 12.2 (P = .001); insula, F1,37 = 12.3 (P = .001); dorsal putamen, F1,37 = 14.1
(P = .001); ventral striatum, F1,37 = 6.9 (P = .01); thalamus, F1,37 = 13.6
(P = .001); and hippocampus, F1,37 = 7.5 (P = .009). All second-generation TSPO
radioligands, such as fluorine F18–labeled N-(2-(2-fluoroethoxy)benzyl)-N-(4-
phenoxyphenyl-3-yl)acetamide ([18F]FEPPA), show differential binding
according to the single-nucleotide polymorphism rs6971 of the TSPO gene,
resulting in HAB and MAB. Horizontal bars indicate means in each group.

Results

We observed a global effect of diagnosis on TSPO VT (Figure 1 and
Table 2). A MANOVA including all subregions of the PFC
and several other cortical and subcortical regions indicated a
global brain effect of diagnosis with elevated TSPO VT in the
patients with MDE compared with the controls (main effect of
diagnosis, F15,23 = 4.5 (P = .001). We also evaluated the re-
gions selected in our hypothesis. Using the effect of diagno-
sis in the ANOVA by region, patients with MDE had signifi-
cantly greater TSPO VT in the PFC (r = .57 (P = .001)), ACC
(r = .55 (P = .001), and insula (r = .57 (P = .001)) com-
pared with healthy controls after controlling for the effect of
genotype (Figure 1) (effect of diagnosis in the MANOVA,
F3,35 = 4.7 (P = .007), elevations in magnitude of 26%, 32%, and
33%, respectively). In both groups, the effect of the rs6971 poly-
morphism was significant (effect of genotype in the MANOVA,
F3,35 = 4.5 (P = .009)) in which individuals with high-affinity
binding had greater TSPO VT compared with individuals with
mixed-affinity binding. Mean scores on the 17-item HDRS in-
dicated moderate to severe MDE (Table 1). Differences in TSPO
VT between the patients with MDE and healthy controls re-
mained significant if age was applied as a covariate (eAppen-
dix in the Supplement). The frequency of mixed- and high-
affinity–binding rs6971 genotype expression was not signifi-
cantly different between healthy controls and patients
with MDE.

The total 17-item HDRS score was positively correlated with
TSPO VT in the ACC after correcting for the rs6971 genotype
(r = 0.63 (P = .005)) (Figure 2). Similar correlations were found
in the insula and PFC, but these did not survive Bonferroni cor-
rection (insula, r = 0.57 (P = .01); PFC, r = 0.46 (P = .06)).

In the patients with MDE but not in the healthy controls
(eAppendix in the Supplement), BMI was significantly and
negatively correlated with TSPO VT in the insula after correct-
ning for rs6971 genotype (r = −0.61 (P = .006)). The relation-
ship between BMI and TSPO VT was also present in the ACC
(r = −0.55 (P = .02)) and the PFC (r = −0.49 (P = .03)), but nei-
ther survived Bonferroni correction (for further details on the
relationship to clinical characteristics, see the eTable in the
Supplement). In the patients with MDE, none of the serum
markers of inflammation had a significant positive correla-
tion with TSPO VT in the primary regions of interest (Table 3).

Discussion

This study is the first, to our knowledge, to detect microglial
activation, as indicated by increased TSPO VT, in a substan-
tial sample of patients with MDE. Although the finding was
prominent in the a priori regions of the PFC, ACC, and insula,
it was also present throughout all the regions assayed. The high-
est levels of TSPO VT occurred in patients with MDE with the
Table 2. ANOVA of Regional TSPOVT by Diagnosis and TSPO Genotype

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>Total (N = 20)</th>
<th>HAB (n = 15)</th>
<th>MAB (n = 5)</th>
<th>Total (N = 20)</th>
<th>HAB (n = 14)</th>
<th>MAB (n = 6)</th>
<th>Total (N = 20)</th>
<th>F1,19 Effect Value</th>
<th>P Value</th>
<th>F1,18 Effect Value</th>
<th>P Value</th>
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<td></td>
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<tr>
<td>MPFC</td>
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<td>8.4 (2.0)</td>
<td>12.3 (3.5)</td>
<td>9.8 (2.0)</td>
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<td>14.2 .001</td>
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<tr>
<td>VLPGC</td>
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<td>10.5 (2.4)</td>
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<td>8.8 (2.5)</td>
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<td>11.6 .002</td>
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<td>7.8 .008</td>
<td>9.4 .004</td>
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<td>8.9 (2.0)</td>
<td>12.2 (3.3)</td>
<td>10.2 (2.1)</td>
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<td>11.8 .002</td>
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<tr>
<td>ACC</td>
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<td>Insula</td>
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<td>12.6 .001</td>
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<td>Temporal cortex</td>
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<td>8.7 (2.1)</td>
<td>12.9 (3.6)</td>
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<td>13.8 .001</td>
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<td>Occipital cortex</td>
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<td>12.9 (3.9)</td>
<td>11.0 (2.1)</td>
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<tr>
<td>Hippocampus</td>
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<td>7.9 (2.7)</td>
<td>11.5 (3.3)</td>
<td>9.4 (2.3)</td>
<td>8.6 (2.3)</td>
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<td>7.5 .009</td>
<td>9.4 .004</td>
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<td>Thalamus</td>
<td>16.9 (3.6)</td>
<td>10.2 (2.2)</td>
<td>15.2 (4.4)</td>
<td>11.8 (2.2)</td>
<td>10.4 (2.9)</td>
<td>11.4 (2.4)</td>
<td>13.6 .001</td>
<td>12.5 .001</td>
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<td>Dorsal putamen</td>
<td>12.3 (2.6)</td>
<td>7.3 (1.5)</td>
<td>11.1 (3.2)</td>
<td>8.5 (1.6)</td>
<td>7.5 (2.3)</td>
<td>8.2 (1.8)</td>
<td>14.1 .001</td>
<td>12.4 .001</td>
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<tr>
<td>Dorsal caudate</td>
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<td>6.4 (1.8)</td>
<td>9.8 (3.1)</td>
<td>8.2 (1.9)</td>
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<td>13.4 .001</td>
<td></td>
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<tr>
<td>Ventral striatum</td>
<td>12.2 (3.2)</td>
<td>7.4 (2.3)</td>
<td>11.0 (3.7)</td>
<td>9.0 (1.8)</td>
<td>7.9 (2.1)</td>
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<td>6.9 .01</td>
<td>9.2 .004</td>
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</table>

Abbreviations: ACC, anterior cingulate cortex; ANOVA, analysis of variance; DLPFC, dorsolateral prefrontal cortex; HAB, high-affinity binding; MAB, mixed-affinity binding; MDD, major depressive episode; MPFC, medial PFC; OFC, orbitofrontal cortex; TSPOVT, translocator protein density measured by distribution volume; VLPGC, ventrolateral PFC.

*Indicates binding to the single-nucleotide polymorphism rs6971 of the TSPO gene known to influence fluorine F18-labeled N-(2-(2-fluoroethoxy)benzyl)-N-(4-phenoxypyridin-3-yl)acetamide binding. A more detailed description of the subregions of the PFC is given in the Appendix in the Supplement.

bIndicates main effect of univariate ANOVA.

Because TSPO is upregulated in activated microglia, elevated TSPOVT implies that greater microglial activation, a potentially targetable process of neuroinflammation, is present during MDE. During activation, microglia transform from a monitoring role into a macrophagelike state, responding to infections or insults by phagocytosis of pathogens and dying cells and recruiting immune cells via cytokine secretion. However, active microglia during MDE may represent a maladaptive response. Identifying greater microglial activation in MDE suggests that selective therapeutic strategies, such as stimulating microglial targets like CX3CR1 to promote a more quiescent state, suppressing the effects of cytokines in the central nervous system, or promoting a shift in microglial activity toward repair-oriented functions by activating purinergic receptors, may hold promise. Reducing microglial activation itself might also have therapeutic utility. Consistent with this viewpoint, minocycline hydrochloride, a second-generation tetracycline antibiotic known to reduce microglial activation and TSPO expression in rodents, can attenuate depressive behaviors in rodents. The present study also suggests that the ability of such interventions to reduce microglial activation may be monitored by techniques such as [18F]FEPPA PET.

We found MDE to be associated with elevated TSPOVT across all brain regions examined, and regional TSPOVT was intercorrelated, although the relationships between TSPOVT and the severity of MDE were most pronounced in the ACC. We propose that although global mechanisms may account for elevated TSPOVT in multiple brain regions in MDD, greater TSPOVT in specific regions and/or their associated circuitry...
may be influential for the expression of particular symptoms within this complex disorder. As with any association between symptoms and a central biomarker, the correlation found between greater TSPO VT and greater 17-item HDRS scores in the ACC can be interpreted as an epiphenomenon secondary to a common origin or as one phenomenon predisposing to the other. We favor a causal mechanism of neuroinflammation contributing toward symptoms because induction of inflammation in humans is associated with depressed mood\(^2^7\,^3^9\) and because direct induction of central inflammation in rodents is associated with anhedonia.\(^7\) The function of this region in relation to symptoms of MDE is consistent with the interpretation that the ACC participates in regulating and processing negative emotional responses.\(^2^6\) In MDD, active MDE symptoms are associated with higher metabolic function in the ACC and direct stimulation of the subgenual ACC results in the reduction of MDE symptoms.\(^2^6\) The negative relationship between TSPO VT and BMI may be consistent with anorexia after induction of central inflammation.\(^7\) The insula is important in this relationship because it integrates interoceptive and affective signaling and is involved in homeostatically driven responses to food cues.\(^2^8\) Future studies in preclinical models to induce microglial activation in combinations of regions that include the ACC and insula might clarify the role of this abnormality in relation to depressive behavior.

The lack of correlation between the central and peripheral inflammatory markers is consistent with previous reports. Bromander et al\(^4^0\) found no correlation between serum and cerebrospinal fluid TNF in patients undergoing knee surgery. Similarly, dissociation between central and peripheral cytokines in preclinical data has been reported after peripheral\(^4^1\,^4^2\) or central inflammatory stimuli.\(^4^3\) Peripheral cytokines have been proposed to cross the blood-brain barrier in severe medical illness to induce neuroinflammation and symptoms of depression.\(^4^4\) However, our results suggest that central inflammation may be present during MDE even when peripheral inflammation is absent.

This study has several limitations, most of which are related to the interpretation of TSPO VT and the use of PET imaging. To the best of our knowledge, the most supported explanation for greater TSPO binding with PET is microglial activation, although TSPO has other roles, such as translocating cholesterol from the outer to the inner mitochondrial membranes for steroid hormone synthesis and participating in the mitochondrial permeability transition pore hetero-oligomer, which influences predisposition toward apoptosis.\(^1^8\) Hence, other explanations for elevated TSPO binding might be found in future studies. Also, the resolution of the scanner does not allow for identification of the cell type involved. Although greater TSPO binding is most convincingly related to microglial activation,\(^1^8\) some investigators suggest that it may also reflect astrocyte activation, at least under specific conditions, such as after exposure to high concentrations of cytokine neurotrophic factor.\(^4^5\)

### Conclusions

To our knowledge, this study is the first to find evidence of a significant elevation of brain TSPO density, a marker of mi-
crogial activation and neuroinflammation, during MDE. Although MDD often has been associated with increased peripheral inflammatory markers, the present study provides the first important compelling evidence of a neuroinflammatory process of microglial activation during MDE in a substantial group of patients unbiased by other psychiatric illnesses or recent medication. Correlations found between greater regional TSPO \text{V}_T in the ACC and insula with severity of MDE and BMI, respectively, may be explained by microglial activation leading to abnormal function in these regions contributing to symptoms. Given the magnitude of difference in TSPO \text{V}_T between the patients with MDE and healthy controls, replication should be possible in future studies, particularly if the MDE sample is focused on those with higher overall severity. Finally, the current results support further investigation of brain-penetrant therapeutics that reduce microglial activation to treat MDE.

**ARTICLE INFORMATION**

Submitted for Publication: April 28, 2014; final revision received August 29, 2014; accepted September 2, 2014.

Published Online: January 28, 2015.


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**Conflict of Interest Disclosures:** Drs Wilson, Houle, and Meyer have received operating grant funds for other studies from Bristol-Myers Squibb, Eli Lilly and Company, GlaxoSmithKline, Lundbeck, and SKF Life Sciences in the past 5 years. Dr Meyer has been a consultant to Mylan, Sepracor, Takeda, Teva, and Trius. None of these companies participated in the design or execution of this study or in the writing of the manuscript. No other disclosures were reported.

**Funding/Support:** This study was supported by an operating grant (principal investigator, Dr Meyer), a fellowship (Dr Setiawan), and a Canada Research Chair (Dr Meyer) from the Canadian Institutes of Health Research, by a National Alliance for Research in Schizophrenia and Affective Disorders Young Investigator Grant from the Brain and Behavior Research Foundation (Dr Setiawan), by grant R30 GM103328 from the National Institutes of Health (Dr Rajkowska), and by the Canadian Foundation for Innovation and the Ontario Ministry for Research and Innovation (infrastructure).

**Role of the Funder/Sponsor:** The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Previous Presentations:** This paper was presented in part at the 52nd Annual Meeting of the American College of Neuropsychopharmacology; December 9, 2013, Hollywood, Florida; at the 69th Annual Meeting of the Society of Biological Psychiatry; May 8, 2014, New York, New York; and at the 53rd Annual Meeting of the American College of Neuropsychopharmacology, December 8, 2014; Phoenix, Arizona.

**Additional Contributions:** Cynthia Xu, MD, Research Imaging Centre, Centre for Addiction and Mental Health, coordinated research for this study. Ian Fan, BSc, Research Imaging Centre, Centre for Addiction and Mental Health, provided research assistance. Nathan Kolla, MD, Research Imaging Centre, Centre for Addiction and Mental Health, Institute of Medical Science, University of Toronto assisted with recruitment and medical coverage. Andrea Tyrer, BSc, Research Imaging Centre, Centre for Addiction and Mental Health, and Department of Pharmacology, University of Toronto assisted with recruitment. Alving Ng, BSc, and Laura Nguyen, BSc, worked as study technicians. Jun Parkes, MSc, Armando Garcia, BSc, Winston Stableford, BSc, and Min Wong, BSc, Research Imaging Centre, served as chemistry staff. Terry Bell, BSc, and Ted Harris-Brandts, BSc, Research Imaging Centre, provided engineering support. Dr. Kalla was funded from the Canadian Institutes of Health research and Ms. Tyrer was funded by Brain Canada; all other contributors are paid employees of the Centre for Addiction and Mental Health.

**Correction:** This article was corrected on March 4, 2015, to fix Table 2.

**REFERENCES**


Translocator Protein Density in MDE