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 ([^{18}F]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.

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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.[1] Although MDD exhibits multiple molecular phenotypes,[2-5] 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,[6] 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.[7] 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.[8] Conditions that create neuroinflammation, such as traumatic brain injury, systemic lupus erythematosus, and multiple sclerosis, are associated with prevalence rates of MDE as high as 50%, suggesting a link between brain inflammation and mood symptoms.[9]

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 MDE were small. Van Otterloo et al[10] 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[11] 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[12] 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[13] of increased proinflammatory and anti-inflammatory cytokine messenger RNA in Brodmann area 10 in 14 patients with MDD. In contrast, several other microarray studies,[14,15] most of which sampled adjacent regions of the PFC, did not identify this result. Among investigations in suicide completers, 1 study[16] reported greater HLA-DR staining, a marker of microglial activation, in the dorsolateral PFC and ACC, and a second study[17] 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.[18] Recently, a new generation of PET radiotracers was developed with superior quantification of TSPO binding. Among these, fluorine F 18–labeled *N*-(2-(2-fluoroethoxy)benzyl)-*N*-(4-phenoxypyridin-3-yl)acetamide ([^{18}F]FEPPA) has excellent properties, including a high selective affinity for TSPO,[19] increased binding during induced neuroinflammation,[20] and a high ratio of specific binding relative to free and nonspecific binding.[21]

To date, one neuroimaging study[22] applied carbon 11–labeled *N*-(2-methoxybenzyl)-*N*-(4-phenoxypyridin-3-yl) acetamide ([^{11}C]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[23] 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 [^{11}C]PBR28 and [^{18}F]FEPPA.[24,25]

In the present study, [^{18}F]FEPPA PET was applied to measure TSPO total distribution volume (V_T), an index of TSPO density, during MDE in patients with MDD compared with healthy, age-matched control participants. The main hypothesis was that TSPO V_T 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.[26] 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[27] and may participate in homeostatic regulation and interoceptive signaling in MDD.[28,29] The second hypothesis was that greater severity of symptom measures related to the sickness syndrome would be associated with greater elevation of TSPO V_T 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 Center 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,

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Table 1. Demographic Characteristics of Study Participants

Characteristic	Patients With MDE (n = 20)	Healthy Controls (n = 20)
Female sex, No. (%)	12 (60)	11 (55)
Age, mean (SD), y	34.0 (11.3)	33.6 (12.8)
TSPO genotype, No. of participants[a]		
HAB	15	14
MAB	5	6
BMI, mean (SD)	23.4 (5.4)	24.8 (2.9)
17-Item HDRS score, mean (SD)[b]	20.0 (3.8)	NA
Age at first MDE, mean (SD), y	15.7 (5.2)	NA
Previous MDE, No. (%)	6 (3)	NA
Previous antidepressant trial, No. (%)	9 (45)	NA
No previous antidepressant trial, No. (%)	11 (55)	NA

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.

[a] Indicates single-nucleotide polymorphism rs6971 of the *TSPO* gene known to influence fluorine F 18–labeled *N*-(2-(2-fluoroethoxy)benzyl)-*N*-(4-phenoxypyridin-3-yl)acetamide binding.

[b] Scores were derived on the day of scanning, with data missing for 1 patient with MDE.

through February 1, 2014. All participants ranged in age from 18 to 72 years and were nonsmokers in good physical health. None of the participants had a history of autoimmune disease, and all were free of illness for at least 2 weeks. Patients with MDE had early-onset MDD (first MDE prior to age 45 years). Health or MDE was confirmed using the Structured Clinical Interview for *DSM-IV*[30] (**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 medication within the past 6 weeks, except for oral contraceptives; and any history of neurologic illness or injury. All participants underwent urine drug screening, and women received a urine pregnancy test on the PET scanning day.

Patients with MDE were administered the 17-item Hamilton Depression Rating Scale (17-item HDRS)[31] at 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.[32] 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).

Image Acquisition and Analysis

Each participant underwent 1 [^{18}F]FEPPA PET scan conducted at the Research Imaging Centre at the Centre for Addiction and Mental Health. For this scan, intravenous [^{18}F]FEPPA[21] was administered as a bolus (mean [SD], 180.5 [14.5] MBq [to convert to millicuries, multiply by 0.02703]). The [^{18}F]FEPPA was of high radiochemical purity (>96%) and high specific activity (mean [SD], 119 [125] TBq/mmol). Manual and automatic arterial blood sampling (programmable blood sampler PBS-101; Veenstra Instruments) was performed to determine the ratio of radioactivity in whole blood to radioactivity in plasma and the unmetabolized radioligand in plasma needed to create the input function for the kinetic analysis.[33] The scan duration was 125 minutes after the injection of [^{18}F]FEPPA. The PET images were obtained using a 3-dimensional brain scanner (HRRT; CPS/Siemens). All PET images were corrected for attenuation using a single photon point source, cesium 137 (half-life, 30.2 years; energy, 662 keV) and were reconstructed using a filtered back-projection algorithm, with a Hann filter at Nyquist cutoff frequency.[24]

Each participant underwent 2-dimensional axial proton-density magnetic resonance imaging acquired with a 1.5-T scanner (Signa; General Electric) (section thickness, 2 mm; repetition time, >5300 milliseconds; echo time, 13 milliseconds; flip angle, 90°; number of excitations, 2; acquisition matrix, 256 × 256; and field of view, 22 cm). Regions of interest were automatically generated using the in-house software (ROMI) as previously described.[34] Time activity curves were used to estimate TSPO V_T using a 2-tissue compartment model that has been shown previously to be an optimal model to quantitate TSPO V_T with [^{18}F]FEPPA PET.[33]

DNA Extraction and Polymorphism Genotyping

The binding affinity of the second generation of radiotracers for TSPO, including [^{18}F]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/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.[24] 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 V_T 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 V_T. A partial correlation controlling for the rs6971 genotype was used in a secondary analysis to quantitate the relationship between TSPO V_T 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

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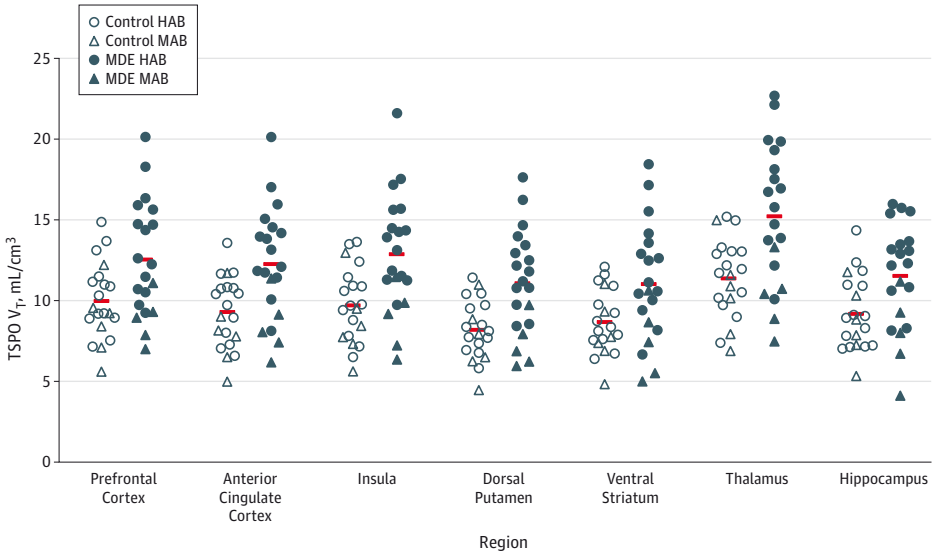
Figure 1. Elevated Translocator Protein Density Measured by Distribution Volume (TSPO V_T) During a Major Depressive Episode (MDE) Secondary to Major Depressive Disorder (MDD)



TSPO V_T 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, $F_{1,37}$ = 8.1 (P = .007); anterior cingulate cortex, $F_{1,37}$ = 12.2 (P = .001); insula, $F_{1,37}$ = 12.3 (P = .001); dorsal putamen, $F_{1,37}$ = 14.1 (P = .001); ventral striatum, $F_{1,37}$ = 6.9 (P = .01); thalamus, $F_{1,37}$ = 13.6 (P = .001); and hippocampus, $F_{1,37}$ = 7.5 (P = .009). All second-generation TSPO radioligands, such as fluorine F 18–labeled *N*-(2-(2-fluoroethoxy)benzyl)-*N*-(4-phenoxypyridin-3-yl)acetamide ([^{18}F]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.

was not included in this analysis. Partial correlations were considered significant at the Bonferroni-corrected threshold of $P \leq .008$.

Results

We observed a global effect of diagnosis on TSPO V_T (**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 V_T in the patients with MDE compared with the controls (main effect of diagnosis, $F_{15,23}$ = 4.5 [P = .001]). We also evaluated the regions selected in our hypothesis. Using the effect of diagnosis in the ANOVA by region, patients with MDE had significantly greater TSPO V_T in the PFC ($F_{1,37}$ = 8.1 [P = .007]), ACC ($F_{1,37}$ = 12.2 [P = .001]), and insula ($F_{1,37}$ = 12.3 [P = .001]) compared with healthy controls after controlling for the effect of genotype (Figure 1) (effect of diagnosis in the MANOVA, $F_{3,35}$ = 4.7 [P = .007]; elevations in magnitude of 26%, 32%, and 33%, respectively). In both groups, the effect of the rs6971 polymorphism was significant (effect of genotype in the MANOVA, $F_{3,35}$ = 4.5 [P = .009]) in which individuals with high-affinity binding had greater TSPO V_T compared with individuals with mixed-affinity binding. Mean scores on the 17-item HDRS indicated moderate to severe MDE (Table 1). Differences in TSPO V_T between the patients with MDE and healthy controls remained significant if age was applied as a covariate (eAppendix in the Supplement). The frequency of mixed- and high-affinity-binding rs6971 genotype expression was not significantly different between healthy controls and patients with MDE.

The total 17-item HDRS score was positively correlated with TSPO V_T 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 correction (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 V_T in the insula after correcting for rs6971 genotype (r = −0.61 [P = .006]). The relationship between BMI and TSPO V_T was also present in the ACC (r = −0.55 [P = .02]) and the PFC (r = −0.49 [P = .03]), but neither 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 correlation with TSPO V_T 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 V_T, in a substantial 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 highest levels of TSPO V_T occurred in patients with MDE with the

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Table 2. ANOVA of Regional TSPO V_T by Diagnosis and *TSPO* Genotype

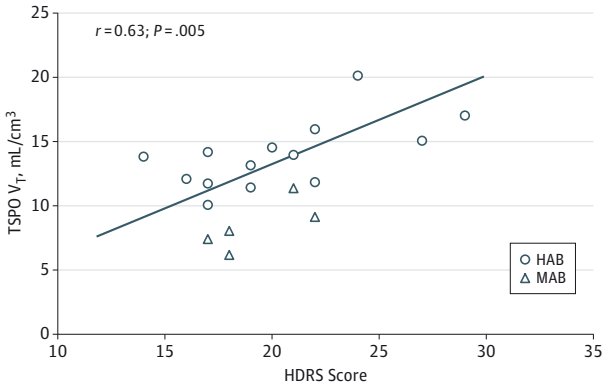
Region of Interest	TSPO V_T, Mean (SD), mL/cm^3						Effect[b]			
	Patients With MDE			Healthy Controls			Diagnosis		Genotype	
	HAB (n = 15)[a]	MAB (n = 5)[a]	Total (N = 20)	HAB (n = 14)[a]	MAB (n = 6)[a]	Total (N = 20)	$F_{1,37}$ Value	*P* Value	$F_{1,37}$ Value	*P* Value
MPFC	13.6 (3.1)	8.5 (2.0)	12.3 (3.6)	9.8 (2.1)	8.3 (2.4)	9.3 (2.2)	11.4	.002	11.2	.002
VLPFC	14.9 (2.9)	9.4 (1.7)	13.5 (3.6)	11.3 (2.4)	9.5 (2.6)	10.8 (2.5)	9.1	.005	13.5	.001
DLPFC	13.6 (3.2)	8.9 (1.3)	12.4 (3.5)	10.7 (2.3)	8.8 (2.5)	10.1 (2.5)	6.5	.02	11.6	.002
OFC	14.4 (2.9)	9.5 (2.7)	13.2 (3.6)	10.9 (2.4)	9.6 (2.8)	10.5 (2.5)	7.8	.008	9.4	.004
Frontal pole	13.3 (3.0)	8.9 (2.0)	12.2 (3.3)	10.2 (2.1)	8.3 (2.3)	9.6 (2.3)	9.1	.005	11.8	.002
ACC	13.5 (2.9)	8.4 (2.0)	12.3 (3.5)	9.8 (2.0)	8.0 (2.3)	9.3 (2.2)	12.2	.001	14.2	.001
Insula	14.2 (3.0)	8.8 (2.1)	12.9 (3.7)	10.2 (2.2)	8.6 (2.5)	9.7 (2.3)	12.3	.001	12.6	.001
Temporal cortex	14.4 (2.8)	8.7 (2.1)	12.9 (3.6)	10.9 (2.2)	9.0 (2.5)	10.3 (2.4)	8.7	.006	15.9	<.001
Parietal cortex	15.0 (3.1)	9.6 (2.0)	13.7 (3.7)	11.5 (2.2)	9.6 (2.5)	10.9 (2.4)	8.9	.005	13.8	.001
Occipital cortex	14.5 (3.0)	8.4 (2.2)	12.9 (3.9)	11.0 (2.1)	9.3 (2.7)	10.5 (2.4)	7.0	.01	14.8	<.001
Hippocampus	12.8 (2.5)	7.9 (2.7)	11.5 (3.3)	9.4 (2.3)	8.6 (2.3)	9.2 (2.3)	7.5	.009	9.4	.004
Thalamus	16.9 (3.6)	10.2 (2.2)	15.2 (4.4)	11.8 (2.2)	10.4 (2.9)	11.4 (2.4)	13.6	.001	12.5	.001
Dorsal putamen	12.3 (2.6)	7.3 (1.5)	11.1 (3.2)	8.5 (1.6)	7.5 (2.3)	8.2 (1.8)	14.1	.001	12.4	.001
Dorsal caudate	10.9 (2.6)	6.4 (1.8)	9.8 (3.1)	8.2 (1.9)	6.7 (2.1)	7.8 (2.0)	6.7	.01	13.4	.001
Ventral striatum	12.2 (3.2)	7.4 (2.3)	11.0 (3.7)	9.0 (1.8)	7.9 (2.1)	8.7 (2.0)	6.9	.01	9.2	.004

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

[a] Indicates binding to the single-nucleotide polymorphism rs6971 of the *TSPO* gene known to influence fluorine F 18–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 eAppendix in the Supplement.

[b] Indicates main effect of univariate ANOVA.

Figure 2. Relationship Between Anterior Cingulate Cortex Translocator Protein Density Measured by Distribution Volume (TSPO V_T) and Symptoms of Current Major Depressive Episode



TSPO V_T in the anterior cingulate cortex was positively related to scores on the 17-item Hamilton Depression Rating Scale (17-item HDRS) after correcting for the rs6971 genotype (0-7 indicates no depression; 8-15, mild symptom level; 16-20, substantial level of symptoms; and >20, moderate to severe depression). All second-generation TSPO radioligands, such as fluorine F 18–labeled *N*-(2-(2-fluoroethoxy)benzyl)-*N*-(4-phenoxypyridin-3-yl)acetamide ([^{18}F]FEPPA), show differential binding according to the single-nucleotide polymorphism rs6971 of the *TSPO* gene, resulting in high-affinity binding (HAB) and mixed-affinity binding (MAB).

highest 17-item HDRS scores. These findings have important implications for the pathophysiological features of MDE, identification of mechanisms contributing to symptom severity in MDE, and clinical targeting of treatment.

Because TSPO is upregulated in activated microglia, elevated TSPO V_T 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.[35] 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,[36,37] can attenuate depressive behaviors in rodents.[38] The present study also suggests that the ability of such interventions to reduce microglial activation may be monitored by techniques such as [^{18}F]FEPPA PET.

We found MDE to be associated with elevated TSPO V_T across all brain regions examined, and regional TSPO V_T was intercorrelated, although the relationships between TSPO V_T and the severity of MDE were most pronounced in the ACC. We propose that although global mechanisms may account for elevated TSPO V_T in multiple brain regions in MDD, greater TSPO V_T in specific regions and/or their associated circuitry

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Table 3. Correlation Between Regional TSPO V_T and Peripheral Inflammatory Markers in Major Depressive Episodes

Inflammatory Marker	Region, *r* Value (*P* Value)[a]		
	Cortex		
	Prefrontal	Anterior Cingulate	Insula
Marker Only			
IL-1β	−0.35 (.13)[a]	−0.39 (.09)	−0.35 (.14)
IL-6	−0.20 (.39)	−0.04 (.88)	−0.09 (.70)
TNF	−0.29 (.21)	−0.34 (.14)	−0.36 (.12)
CRP	−0.27 (.25)	−0.16 (.51)	−0.26 (.27)
Controlled for rs6971 Genotype			
IL-1β	−0.39 (.10)	−0.45 (.05)	−0.40 (.09)
IL-6	−0.29 (.24)	−0.08 (.75)	−0.15 (.53)
TNF	−0.33 (.16)	−0.40 (.09)	−0.44 (.06)
CRP	−0.52 (.02)	−0.40 (.09)	−0.54 (.02)
Controlled for BMI			
IL-1β	−0.18 (.46)	−0.21 (.38)	−0.14 (.56)
IL-6	−0.12 (.62)	0.09 (.71)	0.03 (.89)
TNF	0.18 (.46)	0.16 (.52)	0.18 (.46)
CRP	−0.15 (.54)	0.01 (.98)	−0.12 (.63)
Controlled for rs6971 Genotype and BMI			
IL-1β	−0.25 (.31)	−0.31 (.21)	−0.23 (.36)
IL-6	−0.22 (.39)	0.04 (.87)	−0.04 (.89)
TNF	0.05 (.84)	0.01 (.97)	0.03 (.90)
CRP	−0.42 (.08)	−0.25 (.31)	−0.43 (.07)

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IL-1β, interleukin (IL) 1β; TNF, tumor necrosis factor; TSPO V_T, translocator protein density measured by distribution volume.

[a] The *r* values represent the correlation coefficient (or partial correlation coefficient) followed by the 2-tailed, uncorrected *P* value. Positive *r* values reflect greater TSPO V_T when a higher serum level of the peripheral marker is present. Body mass index was included in 2 analyses because all of these serum markers are also secreted by adipocytes.

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 V_T 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[27,39] 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.[26] 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.[26] The negative relationship between TSPO V_T 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.[28] 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[40] 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[41,42] or central inflammatory stimuli.[43] Peripheral cytokines have been proposed to cross the blood-brain barrier in severe medical illness to induce neuroinflammation and symptoms of depression.[44] 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 V_T 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 heterooligomer, which influences predisposition toward apoptosis.[18] 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,[18] 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.[45]

Conclusions

To our knowledge, this study is the first to find evidence of a significant elevation of brain TSPO density, a marker of mi-

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croglial 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 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 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.

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