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Increased Translocator Protein Distribution Volume, A Marker of Neuroinflammation, in the Brain During Major Depressive Episodes

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Abstract

Importance—The neuroinflammatory hypothesis of major depressive disorder (MDD) 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 MDD. Third, neuroinflammatory illnesses are associated with high rates of MDE. However, a fundamental limitation of the neuroinflammatory hypothesis is a paucity of evidence for brain inflammation during MDE.

To investigate whether microglial activation, an important aspect of neuroinflammation, is present during MDE, [¹⁸F]FEPPA positron emission tomography (PET) was applied to measure translocator protein total distribution volume (TSPO V_T), an index of TSPO density. Translocator protein density is elevated in activated microglia.

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Author Contributions: JHM, AAW, SH, JLK, ES, and LM contributed to study design. JHM, SH, RM, PMR, and GR designed and validated region of interest analysis. JHM, ES, PVR, LM, PMR and IS recruited and assessed all participants, analyzed and interpreted data. JLK provided genotype data. ES and JHM drafted the manuscript. All authors contributed to critical revisions and final approval of the submitted manuscript.

Conflict of Interest Statement: JHM, AAW and SH have received operating grant funds for other studies from Eli-Lilly, GlaxoSmithKline, Bristol Myers Squibb, Lundbeck, and SK Life Sciences in the past 5 years. JHM has consulted to several of these companies as well as Takeda, Sepracor, Trius, Mylan and Teva. None of these companies participated in the design or execution of this study or in writing the manuscript. All authors declare no conflicts of interest.

Objective—To determine whether TSPO V_T is elevated in the prefrontal cortex, anterior cingulate cortex (ACC) and insula in MDE secondary to MDD.

Design—Case-control study.

Setting—Tertiary care psychiatric hospital.

Participants—20 subjects with MDE secondary to MDD and 20 healthy controls, underwent an [^{18}F]FEPPA PET scan. MDE subjects were medication-free for at least 6 weeks. All participants were otherwise healthy, and non-smoking.

Main Outcome Measure—TSPO V_T was measured 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, $F_{15,23}=4.46$, $P=0.001$). TSPO V_T was increased, on average, by 30% in the prefrontal cortex, ACC and insula. In MDE, greater TSPO V_T in the ACC correlated with greater depression severity (ACC: $r=0.628$, $P=0.005$).

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

Introduction

Major depressive disorder (MDD) is highly prevalent and impactful, with active symptoms present in 4% of the adult population.¹ Although MDD exhibits multiple molecular phenotypes²⁻⁵ there is accumulating evidence for a role of inflammation in generating symptoms of a major depressive episode (MDE). For example, induction of inflammation is associated with sad mood in humans⁶ 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 C-reactive protein, IL-6 and TNF- α are frequently increased.⁸ Interestingly, conditions which 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

Presently, it is not clear whether brain inflammation occurs during a current MDE because most postmortem investigations of neuroinflammation sampled either MDD with a history of MDE or suicide victims with varied diagnoses. Within such studies, the samples of subjects with current MDE were small. Van Otterloo et al.¹⁰ reported no difference in density of activated microglia, in the white matter of the orbitofrontal region in 10 MDD subjects. Dean et al. sampled 10 MDD subjects and found significantly increased levels of the transmembrane form of TNF in the dorsolateral prefrontal cortex but no difference in levels of this form of TNF in the anterior cingulate cortex and no difference in the soluble form of TNF in either region.¹¹ Steiner et al. reported increased density of quinolinic acid

positive cells, a marker influenced by microglial activation, in the anterior cingulate cortex of 7 MDE subjects.¹² Microarray studies have had mixed results, with a positive finding by Shelton et al. of increased pro- and anti-inflammatory cytokine mRNA in Brodmann Area 10 (BA10) in 14 MDD subjects,¹³ but several other microarray studies, most of which sampled adjacent regions of the prefrontal cortex, did not identify this result.^{14, 15} Amongst investigations in suicide victims one study reported greater HLA-DR staining, a marker of microglial activation, in the dorsolateral prefrontal and anterior cingulate cortex¹⁶ and a second study reported greater levels of IL-6, TNF- α , and IL-1 β in BA10.¹⁷ Neither study of suicide found a relationship to MDD (or MDE) but there were less than 10 subjects with MDD in each study. The mixed results among postmortem investigations in MDD have been attributed to issues of variation in brain regions sampled, inclusion of early and late onset MDD, comorbidity of other psychiatric disorders and addiction and, with the exception of the microarray studies, small sample size, although it is plausible that 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 may be applied to measure translocator protein (TSPO) binding *in vivo*. TSPO is an 18 kDa protein located on outer mitochondrial membranes in microglia and increased expression of TSPO occurs when microglia are activated during neuroinflammation.¹⁸ Recently, a new generation of positron emission tomography (PET) radiotracers were developed with superior quantification of TSPO binding and among these, [¹⁸F]FEPPA has excellent properties including high, selective affinity for TSPO¹⁹, increased binding during induced neuroinflammation,²⁰ and a high ratio of specific binding relative to free and non-specific binding.²¹

To date, one neuroimaging study applied [¹¹C]PBR28 PET to investigate TSPO levels in MDD, which was negative.²² This earlier study assessed whether TSPO levels were elevated in a sample of 10 MDD subjects (scanned once) under a variety of states (treated, untreated, symptomatic, partially symptomatic) hence, this study cannot be considered definitive for determining whether TSPO binding is elevated in MDE. Scores on the Montgomery-Asberg Depression Rating Scale on the PET scan day ranged from 5 to 30, indicating that the severity ranged from almost asymptomatic to moderately symptomatic. Other issues which limit interpretation of this study include 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.^{23, 24}

In the current study, [¹⁸F]FEPPA PET was applied to measure TSPO V_T , an index of TSPO density, during MDE of MDD compared to healthy, age-matched controls. The main hypothesis was that TSPO V_T would be elevated in MDE in the prefrontal cortex (PFC), anterior cingulate cortex (ACC) and insula. The PFC and ACC were chosen because of their role in mood regulation circuitry and affect dysregulation of MDD.²⁵ The insula is a strong candidate for mediating some of the sickness behaviors in MDD as it is activated in response to an immune challenge²⁶ and may participate in homeostatic regulation and interoceptive signaling in MDD.^{27, 28} 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.

Materials and Methods

Participants

Twenty subjects with a current major depressive episode secondary to major depressive disorder (hereafter termed MDE subjects) and 20 age-matched healthy participants completed the study. Participants were recruited from the Toronto area community and a tertiary care psychiatric hospital (Centre for Addiction and Mental Health, Toronto, Canada) between May 1, 2010 and February 1, 2014. All were aged 18-70, non-smoking and in good physical health. None of the subjects had a history of autoimmune disease nor reported any recent illness. MDE subjects had early onset MDD (first MDE prior to age 45). Health or MDE was confirmed using the Structured Clinical Interview for DSM-IV. Healthy participants were age-matched within 4 years to depressed patients. Exclusion criteria for all subjects included: being pregnant, any herbal, drug or medication use within six weeks, except for oral contraceptives, and any history of neurological illness or injury. All participants underwent urine drug screening and women received a urine pregnancy test on the PET scan day. All subjects provided written informed consent after all procedures were fully explained. The protocol and informed consent forms were approved by the Center for Addiction and Mental Health Research Ethics Board, Toronto, Canada.

Participants with MDE were administered the 17-item Hamilton Depression Rating Scale (HDRS) at enrollment and on the PET scan day. For enrollment, a minimum score of 17 on the 17-item HDRS was required. All MDE subjects were medication-free for at least 6 weeks prior to the PET scan day (9 subjects had completed one or more previous antidepressant trials). Other exclusion criteria included concurrent active axis I disorders including current alcohol or substance dependence, MDE with psychotic symptoms, bipolar disorder (type I or II) 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.²⁹ Additional measures taken were body mass index (BMI) and levels of several peripheral inflammatory markers in serum (interleukin-1 β , interleukin-6, tumor necrosis factor α and C-reactive protein, see Supplementary Methods).

Image Acquisition and Analysis

Each participant underwent one [^{18}F]FEPPA PET scan conducted at the Research Imaging Centre at the Centre for Addiction and Mental Health, Toronto, Canada. For this, intravenous [^{18}F]FEPPA²¹ was administered as a bolus (mean \pm SD, 180.5 \pm 14.5 MBq or 4.88 \pm 0.4 mCi). [^{18}F]FEPPA was of high radiochemical purity (>96%) and high specific activity (119 \pm 125 TBq/mmol). Manual and automatic (ABSS, Model #PBS-101 from Veenstra Instruments, Joure, The Netherlands) arterial blood samples were obtained 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.³⁰ The scan duration was 125 minutes following the injection of [^{18}F]FEPPA. The

PET images were obtained using 3D HRRT brain tomography (CPS/Siemens, Knoxville, TN, USA). All PET images were corrected for attenuation using a single photon point source, ^{137}Cs ($T_{1/2} = 30.2$ years, $E_g = 662$ keV) and were reconstructed by filtered back projection algorithm, with a HANN filter at Nyquist cutoff frequency.²³

Each subject underwent a 2D axial proton density magnetic resonance scan acquired with a General Electric (Milwaukee, WI, USA) Signa 1.5 T magnetic resonance image scanner (slice thickness = 2mm, repetition time > 5 300 ms, echo time = 13 ms, flip angle = 90 degree, number of excitations = 2, acquisition matrix = 256×256 , and field of view = 22cm). Regions of interest were automatically generated using the in-house software, ROMI, as previously described.³¹ Time activity curves were used to estimate TSPO V_T using a two-tissue compartment model, which has been shown previously to be an optimal model to quantitate TSPO V_T with [^{18}F]FEPPA PET.³⁰

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 co-dominantly expressed single nucleotide polymorphism (rs6971, C \rightarrow T) in exon 4 of the TSPO gene.^{23, 24} High affinity binders (HAB, Ala147/Ala147) and mixed affinity binders (MAB, Ala147/Thr147) account for >90% of the population in North America.²³ The polymorphism rs6971 was genotyped as described previously.²³ One MDE subject was a low affinity binder (LB, Ala147/Thr147) and was not included in the analysis.

Statistical Analysis

For the primary hypothesis, PET data were analyzed by multivariate ANOVA with TSPO V_T in PFC, ACC and insula as the dependent variables and diagnosis and genotype as fixed factors. Main effects were considered significant at the conventional P 0.05. Effects in each region, analyzed by univariate ANOVA, were considered significant after Bonferroni correction (P 0.017).

As a secondary analysis, a MANOVA including every brain region sampled (including all cortical and subcortical regions) was performed to assess the effect of diagnosis on TSPO V_T . A partial correlation, controlling for rs6971 genotype, was used in a secondary analysis to quantitate the relationship between TSPO V_T in the primary regions of interest and severity of symptoms of MDE measured by total HDRS score. HDRS score was missing in one MDE participant and was not included in this analysis. Partial correlations were considered significant at the Bonferroni corrected threshold of P 0.008.

Results

A global effect of diagnosis on TSPO V_T was observed (Figure 1, Table 2). A MANOVA including all subregions of the prefrontal cortex as well as several other cortical and subcortical regions indicated a global brain effect of diagnosis with elevated TSPO V_T in MDE compared to health (main effect of diagnosis, $F_{15,23} = 4.46$, $P = 0.001$). We also evaluated the regions selected in our hypothesis. Individuals in a MDE had significantly greater TSPO V_T in prefrontal cortex (PFC), anterior cingulate cortex (ACC) and insula

compared to healthy controls, after controlling for the effect of genotype (Figure 1. Effect of diagnosis, MANOVA, $F_{3,35} = 4.73$, $P = 0.007$. Effect of diagnosis, ANOVA by region: PFC, $F_{1,37} = 8.07$, $P = 0.007$; ACC, $F_{1,37} = 12.24$, $P = 0.001$; insula, $F_{1,37} = 12.34$, $P = 0.001$; magnitude increases 26%, 32%, 33%, respectively). In both groups, the effect of the rs6971 polymorphism was significant (MANOVA, effect of genotype: $F_{3,35} = 4.5$, $P = 0.009$) where HAB had higher TSPO V_T compared to MAB. Scores on the HDRS indicated, on average, moderate to severe MDE (Table 1). Differences in TSPO V_T between MDE and healthy subjects remained significant if age is applied as a covariate (see Supplemental Results). The frequency of MAB and HAB rs6971 genotype expression was not significantly different between healthy subjects and those with MDE.

Total HDRS score, was positively correlated with TSPO V_T in the ACC, after correcting for rs6971 genotype ($r = 0.628$, $P = 0.005$, Figure 2). Similar correlations were found in the insula and PFC but these did not survive Bonferroni correction (insula, $r = 0.574$, $P = 0.013$; PFC, $r = 0.457$, $P = 0.057$).

In MDE subjects, but not healthy (see Supplementary Results), BMI was significantly, negatively correlated with TSPO V_T in the insula, after correcting for rs6971 genotype ($r = -0.605$, $P = 0.006$). The relationship between BMI and TSPO V_T was also present in ACC ($r = -0.547$, $P = 0.015$) and PFC ($r = -0.488$, $P = 0.034$) but neither survived Bonferroni correction. In MDE subjects, none of the serum markers of inflammation had a significant positive correlation with TSPO V_T in the primary regions of interest (see Table 3).

Discussion

This is the first study to detect microglial activation, as indicated by increased TSPO V_T , in a substantial sample of MDE subjects. While the finding was prominent in the *a priori* regions of the PFC, ACC and insula, it was also present throughout all the regions assayed. Interestingly the highest levels of TSPO V_T occurred in MDE subjects with the highest depression severity scores. These findings have important implications for the pathophysiology of MDE, identifying mechanisms contributing to symptom severity in MDE, and clinical targeting of treatment.

Since 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 macrophage-like state, responding to infections or insults by phagocytosing 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 towards repair oriented functions by activating purinergic receptors may hold promise.³² Reducing microglial activation itself might also have therapeutic utility and consistent with this viewpoint, minocycline, a second generation tetracycline antibiotic known to reduce microglial activation and TSPO expression in rodents,^{33, 34} can attenuate depressive behaviors in

rodents.³⁵ 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 was associated with elevated TSPO V_T across all brain regions examined and regional TSPO V_T was inter-correlated, although the relationships between TSPO V_T with severity of MDE were most pronounced in the ACC. We propose that while 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 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 higher TSPO V_T and greater HDRS score in the ACC, can be interpreted as an epiphenomenon secondary to a common origin or that one phenomenon predisposes to the other. We favor a causal mechanism of neuroinflammation contributing towards symptoms because induction of inflammation in humans is associated with depressed mood^{26, 36} and 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 this interpretation: The ACC participates in regulating and processing negative emotional responses.²⁵ In MDD, active MDE symptoms are associated with higher metabolic function in the ACC and direct stimulation of the subgenual ACC results in reduction of MDE symptoms.²⁵ The negative relationship between TSPO V_T and BMI may be consistent with anorexia following induction of central inflammation.⁷ The insula is important in this relationship as it integrates interoceptive and affective signaling, is involved in homeostatically driven responses to food cues.²⁷ Future studies in preclinical models to induce microglial activation in combinations of regions that include the ACC and insula would be important to clarify the role of this pathology in relation to depressive behavior.

The lack of correlation between central and peripheral inflammatory markers is consistent with previous reports. Bromander et al., found no correlation between serum and cerebrospinal fluid TNF- α in neurosurgical patients.³⁷ Similarly, dissociation between central and peripheral cytokines in preclinical data have been reported following peripheral^{38, 39} or central inflammatory stimuli.⁴⁰ It has been proposed that peripheral cytokines cross the blood brain barrier in severe medical illness to induce neuroinflammation and symptoms of depression.⁴¹ However, our results suggest that central inflammation may be present during MDE even when peripheral inflammation is absent.

There are several limitations in this study, most of which are related to the interpretation of TSPO density 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 outer to inner mitochondrial membranes for steroid hormone synthesis and participating in the mitochondrial permeability transition pore heterooligomer, which influences predisposition towards apoptosis.¹⁸ Hence, other explanations for elevated TSPO binding might be found in future study. Also, the resolution of the scanner does not allow for identification of the cell type involved. While elevated TSPO binding is most convincingly related to microglial activation,¹⁸ there is a minority in the field who suggest that it may also reflect astrocyte

activation, at least under conditions, such as after exposure to high concentrations of cytokine neurotrophic factor.⁴²

This is the first study to find a significant elevation of brain TSPO density *in vivo*, a marker of microglial activation and neuroinflammation, during MDE. Though MDD has often been associated with increased peripheral inflammatory markers, the current study provides the first important compelling evidence for a neuroinflammatory process of microglial activation during MDE in a substantial group of subjects unbiased by other psychiatric illnesses or recent medication. Correlations found between greater regional TSPO density in the anterior cingulate cortex 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 MDE and healthy subjects, 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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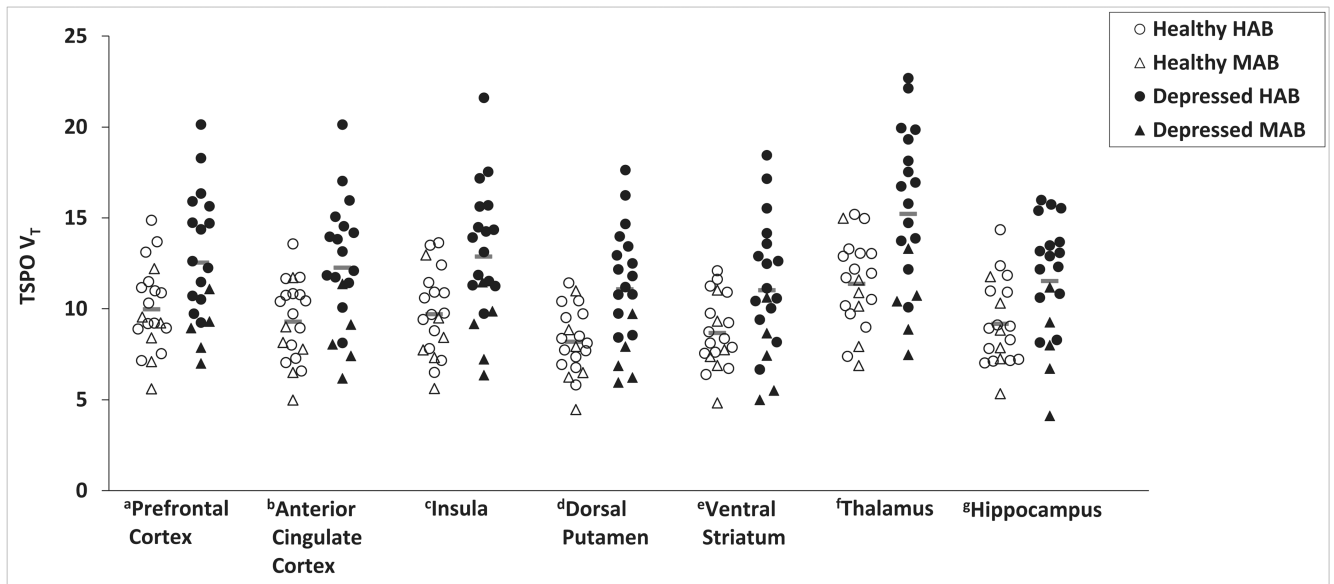


Figure 1. Elevated translocator protein density (TSPO V_T) during a major depressive episode (MDE) secondary to major depressive disorder (MDD)

TSPO V_T was significantly greater in MDE of MDD (Depressed, N=20, 15 HAB, 5 MAB) compared to controls (Healthy, N=20, 14 HAB, 6 MAB): ANOVAs, ^aprefrontal cortex, $F_{1,37} = 8.07$, $P = 0.007$; ^banterior cingulate cortex, $F_{1,37} = 12.24$, $P = 0.001$; ^cinsula, $F_{1,37} = 12.34$, $P = 0.001$; ^ddorsal putamen, $F_{1,37} = 14.1$, $P = 0.001$; ^eventral striatum, $F_{1,37} = 6.9$, $P = 0.013$; ^fthalamus, $F_{1,37} = 13.6$, $P = 0.001$; ^ghippocampus, $F_{1,37} = 7.5$, $P = 0.009$. All second generation TSPO radioligands, such as [¹⁸F]FEPPA, show differential binding according to the SNP rs6971 of the TSPO gene resulting in high affinity binders (HAB) and mixed affinity binders (MAB). Red bars indicate means in each group.

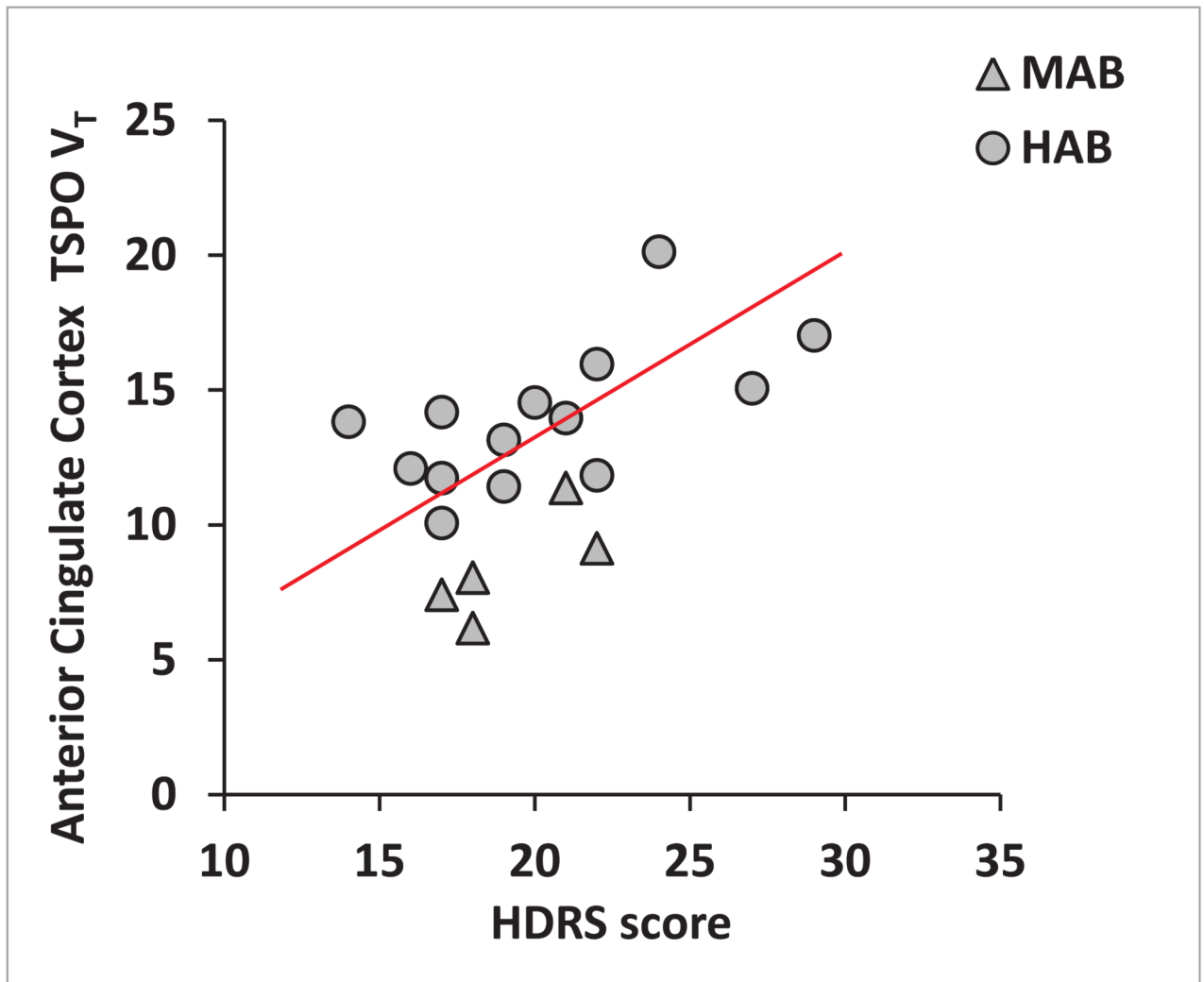


Figure 2. Relationship between regional translocator protein density (TSPO V_T) and symptoms of current major depressive episode

TSPO V_T in the anterior cingulate cortex was positively related to scores on the 17-item Hamilton Depression Rating Scale (HDRS), after correcting for rs6971 genotype ($r=0.628$, $P=0.005$). All second generation TSPO radioligands, such as [¹⁸F]FEPPA, show differential binding according to the SNP rs6971 of the TSPO gene resulting in high affinity binders (HAB) and mixed affinity binders (MAB).

Table 1
Demographic Characteristics of Study Participants^a

Characteristics	Depressed (n=20)	Healthy (n=20)
Female, No. (%)	12 (67)	11 (55)
Age, y	34.0 (11.3)	33.6 (12.8)
TSPO Genotype ^b	15 HAB, 5 MAB	14 HAB, 6 MAB
BMI	23.4 (5.4)	24.8 (2.9)
HDRS Score ^c	20.0 (3.8)	na
Age of first MDE, y	15.7 (5.2)	na
Previous MDEs, No.	6 (3)	na
Previous AD Trial, No. (%)	9 (45)	na
No Previous AD Trial, No. (%)	11 (55)	na

^aValues are expressed as mean (SD) except where indicated.

^bSingle nucleotide polymorphism rs6971 of the TSPO gene known to influence [¹⁸F]FEPPA binding: HAB, high affinity binders; MAB, mixed affinity binders.

^c17-item Hamilton Depression Rating Scale; scores derived on the day of scanning. Missing data in one subject.

Table 2
Analysis of Variance of Regional TSPO V_T by Diagnosis and TSPO Genotype^a

Region of Interest	Depressed (n=20)			Healthy (n=20)			Diagnosis Effect ^b		Genotype Effect ^b	
	HAB (n=15)	MAB (n=5)	Total	HAB (n=14)	MAB (n=6)	Total	F _{1,37}	P	F _{1,37}	P
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	.015	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	.000
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	.012	14.8	.000
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	.013	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	.013	9.2	.004

^aValues are expressed as mean (SD).

^bMain effect of univariate ANOVA. MPFC, medial prefrontal cortex; VLPFC, ventrolateral prefrontal cortex; DLPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex. HAB, high affinity binders and MAB, mixed affinity binders refer to the single nucleotide polymorphism rs6971 of the TSPO gene known to influence [¹⁸F]FEPPA binding. For a more detailed description of the subregions of the prefrontal cortex please refer to the supplemental section.

Table 3
Lack of Positive Correlation Between Regional TSPO V_T and Peripheral Inflammatory Markers in Major Depressive Episodes

Marker	Prefrontal Cortex	Anterior Cingulate Cortex	Insula
IL-1β	-0.35 (0.13) ^a	-0.39 (0.09)	-0.35 (0.14)
IL-6	-0.20 (0.39)	-0.04 (0.88)	-0.09 (0.70)
TNFα	-0.29 (0.21)	-0.34 (0.14)	-0.36 (0.12)
CRP	-0.27 (0.25)	-0.16 (0.51)	-0.26 (0.27)
Correlations Controlling for rs6971 Genotype			
IL-1β	-0.39 (0.10)	-0.45 (0.05)	-0.40 (0.09)
IL-6	-0.29 (0.24)	-0.08 (0.75)	-0.15 (0.53)
TNFα	-0.33 (0.16)	-0.40 (0.09)	-0.44 (0.06)
CRP	-0.52 (0.02)	-0.40 (0.09)	-0.54 (0.02)
Correlations Controlling for Body Mass Index			
IL-1β	-0.18 (0.46)	-0.21 (0.38)	-0.14 (0.56)
IL-6	-0.12 (0.62)	0.09 (0.71)	0.03 (0.89)
TNFα	0.18 (0.46)	0.16 (0.52)	0.18 (0.46)
CRP	-0.15 (0.54)	0.01 (0.98)	-0.12 (0.63)
Correlations Controlling for Both rs6971 Genotype and Body Mass Index			
IL-1β	-0.25 (0.31)	-0.31 (0.21)	-0.23 (0.36)
IL-6	-0.22 (0.39)	0.04 (0.87)	-0.04 (0.89)
TNFα	0.05 (0.84)	0.01 (0.97)	0.03 (0.90)
CRP	-0.42 (0.08)	-0.25 (0.31)	-0.43 (0.07)

^aValues represent the correlation coefficient (or partial correlation coefficient) followed by the two tailed, uncorrected p-value. Positive r values would reflect greater TSPO V_T when a higher serum level of the peripheral marker is present. Body mass index (BMI) was included in two analyses since all of these serum markers are also secreted by adipocytes.