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Elevated translocator protein in anterior cingulate in major depression and a role for inflammation in suicidal thinking: a PET study

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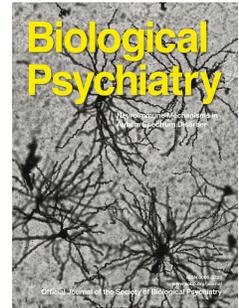
Title Page

Title

Elevated translocator protein in anterior cingulate in major depression and a role for inflammation in suicidal thinking: a PET study

Short title

PET imaging of translocator protein in major depression

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Holmes SE et al. PET imaging of translocator protein in major depression

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Abstract

Background: Major Depressive Disorder (MDD) is associated with raised peripheral inflammatory markers. Mounting evidence also suggests that inflammation is involved in suicidal behavior. However, the involvement of inflammation in the brains of depressed individuals, and its association with suicidal ideation, needs further clarification. Translocator protein (TSPO), which is upregulated in activated glia, predominantly microglia, can be measured as an indication of neuroinflammation *in-vivo* using Positron Emission Tomography (PET) and TSPO-specific radioligands.

Methods: We used $[^{11}C](R)$-PK11195 PET to compare TSPO availability in anterior cingulate cortex (ACC), prefrontal cortex (PFC) and insula between fourteen medication-free patients in a major depressive episode (MDE) of at least moderate severity and thirteen matched healthy controls. In a post-hoc analysis, we also compared TSPO availability between patients with and without suicidal thoughts.

Results: Multivariate analysis of variance indicated significantly higher TSPO in patients compared to controls (p=0.005). The elevation was of large effect size and significant in ACC (p=0.022; Cohen's d=0.95), with smaller, non-significant elevations in PFC (p=0.342; Cohen's d=0.38) and insula (p=0.466; Cohen's d=0.29). TSPO was not elevated in patients without suicidal thinking, but was significantly increased in those with suicidal thoughts compared to those without, most robustly in ACC (p=0.008) and insula (p=0.023).

Conclusions: We confirm evidence for increased TSPO availability, suggestive of predominantly microglial activation, in the ACC during a moderate to severe MDE. Our findings provide further incentive for evaluating anti-inflammatory therapies in MDD.

Introduction

Major Depressive Disorder (MDD) is one of the leading causes of disability worldwide (1, 2). However, approximately one third of patients fail to respond to conventional antidepressants (3) and there is a pressing need to develop more effective and better tolerated treatments. A promising avenue of research for new treatment strategies is inflammation (4-7), based on evidence that at least a subset of individuals with MDD have higher levels of peripheral pro-inflammatory cytokines (8-11); a high prevalence of depression in inflammatory medical disorders (12); an association of depression and its response to treatment with polymorphisms in inflammatory cytokine genes (13); the development of depression in patients administered therapeutic pro-inflammatory cytokines (14-16) and healthy volunteers given a peripheral immune challenge (17, 18); the association of inflammation with certain risk factors for depression (19-22); and evidence that inflammation may be associated with non-responsiveness to antidepressants (23-25).

Peripheral inflammation can lead directly to an inflammatory response in the human brain (26). In response to inflammation, the metabolism of tryptophan is diverted from the production of serotonin (5-HT) to kynurenine (KYN), which is subsequently converted into the neurotoxic quinolinic acid (QUIN) by activated microglia and infiltrating macrophages and monocytes (27, 28). That this mechanism may be involved in neuroinflammation-associated depression is supported by observations that activation of the KYN pathway is essential for depressive like behavior in rats (29), and that KYN and QUIN are increased in the cerebrospinal fluid of cancer patients who had undergone interferon (IFN)-alpha therapy, which correlated with depressive symptoms (30). Consistent with this, postmortem studies have found increased levels of QUIN in the anterior cingulate cortex (ACC) of depressed individuals who had committed suicide (31), and microglial and astrocytic activation in the ACC, thalamus and frontal cortex of depressed individuals (32-35). These studies have a number of potentially confounding factors including antemortem use of antidepressants,

which can have significant effects on inflammatory processes (36). A crucial question is therefore whether there is inflammation *in-vivo* in the brains of medication-free individuals currently experiencing a MDE.

One index of neuroinflammation can be measured *in-vivo* using PET and radioligands specific for the 18kDa translocator protein (TSPO), a mitochondrial protein that is upregulated in activated glial cells, predominantly microglia, in a range of pathological conditions (37). To date, there have been two published PET studies investigating TSPO in MDD in working-age adults. The first found unaltered TSPO in a mild-to-moderate MDE (38). A second larger study found increased TSPO in medication-free patients in a moderate-to-severe MDE (39), most prominently in the prefrontal cortex (PFC), ACC and insula. There was, however, considerable overlap between patients and controls, with a subset of patients exhibiting higher levels of TSPO. This is consistent with the studies of peripheral inflammatory markers in depression, as well as a large survey showing CRP levels ≥5mg/L in around 30% of depressed individuals (40). This subpopulation of depressed individuals showing heightened inflammation may benefit from anti-inflammatory treatment strategies (23, 41, 42).

Mounting evidence also suggests that neuroinflammation may be particularly pronounced in suicidality (43). Robust increases have been found in interleukin-1β (IL-1β) and IL-6 in blood and postmortem samples of patients with suicidal thinking compared with patients without suicidal thinking and controls (44). Furthermore, a higher degree of suicidal ideation has been associated with an increased inflammatory index, independent of severity of depressive symptoms (45). Multiple postmortem studies have found evidence for inflammation in the brains of suicide victims (46, 47), with specific evidence for activated microglia in the ACC and PFC of depressed individuals who had committed suicide (32, 33), and significantly less microglial activation in the dorsal raphe nucleus in non-suicidal depressed patients who had died of other causes compared to suicidal depressed patients and controls (48). However, no

study to date has investigated the association between neuroinflammation and suicidality in depression *in-vivo.*

The aims of the current study were therefore to investigate brain TSPO availability in MDD and to explore factors that might be associated with heightened inflammation. We used the prototypical TSPO radioligand [^{11}C]*(R)*-PK11195 to measure brain TSPO availability in patients with moderate to severe depression who were non-smoking, medically healthy and antidepressant- naïve or antidepressant-free for at least 8 months. We hypothesized that TSPO availability would be higher in ACC, PFC and insula in depressed individuals compared to matched controls. These regions were chosen as they are the three regions hypothesised *a priori* in the study by Setiawan et al (39) and found to have significantly elevated TSPO; due to their role in mood regulation (49); and based on literature implicating the ACC in particular in the association between inflammation and depression (17, 31-33, 50, 51). Secondary aims were to explore associations between brain TSPO levels, symptom severity, suicidal ideation, exercise levels, childhood adversity and peripheral markers of inflammation.

Holmes SE et al. PET imaging of translocator protein in major depression

ACCEPTED MANUSCRIPT

Methods and Materials

Participants

~~Nineteen~~ patients with MDD were recruited from the Manchester region of the UK by self-referral following placement of advertisements in mental health services, voluntary organisations, doctors' surgeries and online. Three patients were excluded before data collection (two for not meeting criteria, one for possibility of pregnancy). Fourteen patients (7 males; mean ± SD age 31±12 yr) completed the study.

Diagnosis was confirmed using the Structured Clinical Interview for the DSM-IV (SCID-I) (52). All patients were in a moderate to severe MDE (mean±SD MADRS score 31±4; mean±SD HAM-D score 20±3) and had not taken antidepressants for at least eight months. Seven of the fourteen MDD patients had taken antidepressants in the past and seven were antidepressant-naïve (for details of past medication use see Supplementary Table S1). Patients were sex- and age-matched (±5 yr) with 13 healthy controls (7 males; age 33±11 yr) recruited as part of this and another recent study (53), and scanned using the same protocol. All participants ranged in age from 18-55, were medically healthy based on clinical history, physical examination, routine blood tests and negative urine toxicology, and were non-smoking. For demographic and clinical characteristics, see Table 1. Additional measures included body mass index (BMI); childhood adversity, measured by the Childhood Adversity Questionnaire (54); physical exercise, measured by the Godin Leisure-Time Exercise Questionnaire (55, 56); and the following markers of inflammation in plasma: TNF-α, IFN-γ, IL-6, IL-8, IL-1β and CRP. Exclusion criteria for all participants included substance misuse in the previous year, lifetime history of substance dependence, anti-inflammatory medications in the previous month, another Axis I disorder, pregnancy, and history of neurological or autoimmune disorder. The study was approved by the Greater Manchester East Research Ethics Committee and the United Kingdom Administration of Radioactive Substances Advisory Committee (ARSAC). All participants provided written informed consent.

Image acquisition and analysis

The methodology for image acquisition and analysis was recently published (57). In summary, following intravenous injection of $[^{11}C](R)$-PK11195, emission data were acquired for 60 minutes on a high-resolution research tomograph (HRRT; Siemens/CTI, Knoxville, Tennessee). A T_1-weighted MRI brain scan was also acquired to exclude significant abnormality, for identification of regions of interest (ROIs), and Voxel Based Morphometry (VBM) analysis. The hypothesized regions (ACC, PFC, insula) were identified using a maximum probability brain atlas (58, 59) in which ACC and insula are individual ROIs. Our PFC ROI is a composite of the following atlas ROIs: middle frontal, inferior frontal and superior frontal gyri.

There is no reference region devoid of TSPO for PET studies as TSPO expression is ubiquitous throughout the brain. An alternative is to use a tissue with relatively low TSPO expression as a pseudo-reference region. To optimise our choice, we compared the use of two pseudo-reference regions in our data: i) cerebellar grey matter (GM) (60); and ii) supervised cluster reference input function (SVC6), a data-driven method which extracts a cluster of GM voxels with kinetic behaviour closest to that of healthy GM (see Supplement for further details). Binding potential (BP_{ND}), representing the ratio at equilibrium of specifically bound radioligand to that of non-displaceable radioligand in tissue (61), was calculated using the simplified reference tissue model (SRTM) (62) and the two pseudo-reference regions. Parametric maps of BP_{ND} were generated with a basis-function implementation of the SRTM (63) and the individualised GM brain atlases were then projected onto these parametric maps to obtain mean BP_{ND} values for the ROIs. Overall, regional BP_{ND} values derived from SVC6 were modestly lower and had higher variance compared to using the cerebellar GM input function (data not shown). We therefore present the latter data, in concurrence with the superiority of the cerebellum over a data-driven approach in the literature (64) and our previous $[^{11}C](R)$-PK11195 studies on the HRRT (57, 65-68). TSPO availability in the cerebellum (BP_{ND} derived by SVC6) did not significantly differ (p=0.77) in our data between

Holmes SE et al. PET imaging of translocator protein in major depression

ACCEPTED MANUSCRIPT

healthy controls and MDD patients (see Supplementary text and Figure S1 and further

details).

Statistical analysis

Statistical analysis was performed in SPSS Statistics Version 22 (Armonk, NY: IBM Corp).

Independent-samples *t*-tests and univariate analysis of variance (ANOVA) were used to

assess differences between demographic, clinical and radiotracer characteristics across

groups. Sex differences were compared using Fisher's exact test (2-tailed). Group differences

in $[^{11}C](R)$-PK11195 BP_{ND} were determined using a multivariate ANOVA (MANOVA), with

BP_{ND} in ACC, PFC and insula as the dependent variables, and group (MDD or healthy

controls) as the fixed independent variable. The effect size of the group differences in the

three ROIs was calculated using partial eta-squared (η^2) and Cohen's *d* (mean difference

divided by the pooled standard deviation). In a further exploratory analysis of potential effects

of suicidal ideation on TSPO availability, patients were stratified into those with and without

current suicidal thinking and a MANOVA performed with regional BP_{ND} (ACC, PFC and

insula) as dependent variables, and trichotomous group (healthy controls, MDD with suicidal

thoughts, MDD without suicidal thoughts) as the fixed independent variable, with Bonferroni

correction for multiple comparisons across the three groups.

The normal distribution of BP_{ND} for each combination of the variables was confirmed by

Shapiro-Wilk's test (p >0.05) and Normal Q-Q Plot. Equality of covariance was confirmed by

Box's test. Homogeneity of variances was checked by Levine's test. Correlations (Pearson's *r*,

2-tailed) were used to determine the association between TSPO availability and symptom

severity, childhood adversity, exercise and peripheral inflammatory markers in the patient

group. Comparison of these measurements with healthy controls was not performed as these

data were not collected for all the controls. Findings were considered significant at the *p*<0.05

level.

Holmes SE et al. PET imaging of translocator protein in major depression

ACCEPTED MANUSCRIPT

Results

Patients and healthy controls were well matched for age, sex, BMI, smoking status (all non-smoking) and injected mass of radiotracer (see Table 1). For all analyses, ANOVA assumptions were not violated. There was no significant main effect of age on BP_{ND} (MANOVA: $F_{3, 22}=0.85$, $p=0.479$).

Across the hypothesized regions (ACC, PFC and insula) TSPO availability ($[^{11}C](R)$-PK11195 BP_{ND}) was higher in the MDD patients than the controls by a mean of 39%, which was statistically significant (MANOVA, main effect of group: $F_{3, 23}=5.63$, $p=0.005$). The increase was highest in the ACC (67%), with smaller elevations seen in the PFC (29%) and insula (24%). Univariate tests on the individual regions indicated that the elevation in the ACC was of large effect size and statistically significant ($F_{1, 25}=5.99$, $p=0.022$; partial $\eta^2=0.193$; Cohen's $d=0.95$), but was of small effect size and failed to reach significance in PFC ($F_{1, 25}=0.94$, $p=0.342$; partial $\eta^2=0.036$; Cohen's $d=0.38$) or insula ($F_{1, 25}=0.549$, $p=0.466$; partial $\eta^2=0.021$; Cohen's $d=0.29$) (see Figure 1 and Table 2). The significance of these differences was not materially altered if age was applied as a covariate (see Supplement).

In the exploratory analysis of the effects of suicidal ideation on TSPO availability, patients were stratified into those with (n=9) and without (n=5) current suicidal thoughts. The presence of suicidal thoughts was defined as the disclosure of suicidal thoughts during the previous two weeks on direct enquiry and a score of 3 or higher on the 'Suicidal Thoughts' item of the MADRS. Their absence was defined as the denial of any suicidal thinking and a score of zero on the MADRS item. There were no significant differences in age, sex, BMI or injected mass of radiotracer between the two MDD subgroups. In addition, the two subgroups were well matched for overall MDE severity on the MADRS and HAM-D, mean scores being high in the moderate depression range for both subgroups (see Table 1). TSPO availability differed significantly between the three groups (controls, patients with suicidal thinking, and patients without suicidal thinking) across the three regions (MANOVA, main effect of group:

$F_{6, 46}=4.22$, $p=0.002$). Visual inspection (Figure 2) shows a pattern across all three regions whereby mean BP_{ND} in patients without suicidal thinking is very similar to, or slightly lower than, healthy controls; while BP_{ND} in patients with suicidal thinking is much higher than in both other groups. Univariate tests on the individual regions show that these differences were statistically significant and of large effect size in ACC ($F_{2, 24}=9.91$, $p=0.001$; partial $\eta^2=0.452$) and insula ($F_{2, 24}=4.59$, $p=0.021$; partial $\eta^2=0.277$), and reached trend significance in PFC ($F_{2, 24}=3.15$, $p=0.061$; partial $\eta^2=0.208$). Pairwise comparisons of BP_{ND} in each region between the three groups, with Bonferroni adjustment for multiple comparisons, showed that the patients with suicidal thinking had significantly higher TSPO availability than those without suicidal thinking in ACC (+118%; p=0.008) and insula (+245%; p=0.023), and trend higher TSPO availability in PFC (+129%; p=0.096). Patients with suicidal thinking also had significantly higher TSPO availability than healthy controls in ACC (+107%; p=0.001) (see Table 2 and Figure 2). Elevations compared to healthy controls in the PFC (+61%) and insula (+66%) were not statistically significance (Table 2). Nor were there any significant differences between healthy controls and patients without suicidal thinking in any of the regions.

There were no significant correlations between BP_{ND} in any of the regions and symptom severity (MADRS and HAM-D scores), duration of illness, BMI, childhood adversity, or any of the peripheral inflammatory markers. Nor did we find any differences in concentration of peripheral inflammatory markers between patients with and without suicidal thoughts. In the MDD patients (n=14), a negative correlation between BP_{ND} in the ACC and their degree of physical exercise reached trend significance (r=-0.47, $p=0.07$).

A post-hoc VBM analysis using SPM12 was carried out on the MRI scans to examine the potential contribution of differences in GM volume between MDD patients and controls to the significant between-group differences in BP_{ND}. For details of the VBM methodology, see Supplement. No significant between-group GM volume differences were found, suggesting

Holmes SE et al. PET imaging of translocator protein in major depression

ACCEPTED MANUSCRIPT

that the significant differences in BP_{ND} between groups are unlikely to be an artefact of differences in regional tissue volumes.

A secondary between-group comparison (independent-samples t-test) on nine further ROIs is presented in Supplementary Table S2 and Figure S2. TSPO was higher in MDD patients compared to healthy controls in the posterior cingulate cortex (PCC; $p=0.04$). However, this would not be considered significant after adjustment for multiple comparisons.

Holmes SE et al. PET imaging of translocator protein in major depression

ACCEPTED MANUSCRIPT

Discussion

Our study provides the first confirmatory evidence, to the best of our knowledge, for elevated TSPO in the ACC of drug-free, working-age adults with MDD in a moderate to severe MDE *in vivo*, following the earlier report of Setiawan et al (39). It also provides the first *in vivo* evidence in humans that elevated TSPO in MDD may be associated more with suicidality than the diagnosis of MDD itself.

Under pathological conditions TSPO expression increases in microglia, infiltrating macrophages, astrocytes, and vascular endothelial cells (69-72). However, TSPO ligand binding appears to represent principally microglial activation *in vivo* (37, 73-75). We therefore tentatively interpret our finding as support for the presence of microglial activation in a moderate to severe MDE, while acknowledging the need for caution in interpreting altered TSPO binding in mental disorders in the absence of more selective microglial PET markers (72) and that we cannot exclude a contribution from other cell types.

Although our study design was independent of the Setiawan study, the patients in both studies are comparable. All were antidepressant-free non-smokers with similar mean age, symptom severity, and normal BMI. Our MDD group size was smaller (n=14 vs 20) while our patients were drug-free for longer (>8 months vs >6 weeks). Given this clinical comparability, it is interesting that our increases in TSPO across the ACC (67%), PFC (29%) and insula (24%) (mean 39%) are similar to the increases of 32%, 26% and 33% (mean ~30%), respectively, seen in the Setiawan study. We also found the most robust increase in the ACC. Although TSPO was also elevated in the PFC and insula of MDD patients in our study, these group differences were of small effect size, were not statistically significant, and we were unable to replicate the findings of the Setiawan study in these regions. With $[^{11}C](R)$-PK11195 the signal to noise ratio is low, as reflected in the low BP_{ND} values, and this may contribute to lack of statistical power. Post-hoc calculation based on the observed effect sizes (Cohen's *d*) of the between-group differences in our data indicates that group sizes of at least 100 would

have been required to be fully powered to detect significant differences in PFC and insula (2-tailed, $\alpha=0.05$, power=0.8). This suggests that for these regions the combination of the size of the biological effect, the variance in the data and the sensitivity of the methodology limit its suitability for future studies in MDD in anything other than extremely large group sizes. For the ACC, our sample size can detect a significant difference with a power of 0.7, suggesting that our methodology is adequate for the ACC. However, in addition to reducing the chance of detecting a true effect, low power also reduces the probability that a statistically significant result reflects a true effect and increases the chance that the estimate of the effect size is exaggerated (76). The modest group sizes and lack of statistical power in our study therefore reduces the probability of our positive finding in the ACC and its effect size. On the other hand, the probability that elevated TSPO in the ACC is a false positive is controlled by our having limited our *a priori* hypothesis to the three regions which were themselves hypothesised *a priori* in the initial study (39) based on their biological association with MDD, and found to have significantly elevated TSPO. We would therefore have needed to be particularly fortunate to have obtained this positive finding in the ACC. Nevertheless, further replication studies will be important, ideally with group sizes even larger than the initial study, to arrive at a more accurate estimation of the effect size in these regions (76).

We observed significantly greater TSPO in the ACC and insula of patients experiencing suicidal thoughts than patients without suicidal thoughts. This is consistent with mounting evidence for an association between inflammation and suicide (32, 33, 43, 46, 47, 77-79) and a higher specificity of inflammation for suicide than for diagnosis (33, 47, 77). Ours is the first study, to our knowledge, to show such an association *in-vivo*. However, because of the small subgroup sizes and post-hoc nature of the analysis, our results are preliminary and require replication. TSPO availability in the patients without suicidal thoughts was the same as, or slightly lower than healthy controls (Figure 2). We cannot necessarily conclude that neuroinflammation is absent in those with normal or lowered TSPO as increased levels of inflammatory cytokines can occur with a downregulation (rather than upregulation) of TSPO

(72). Nevertheless, the pattern of TSPO availability in our patients is consistent with recent postmortem findings of significantly decreased microglial activation in non-suicidal depressed patients compared to suicidal depressed patients and controls in the dorsal raphe nucleus, which provides the major serotonergic innervation to the ACC, PFC and insula (48). This is particularly interesting given recent evidence that abnormal 5-HT function measured using PET predicts higher suicidal ideation and more lethal suicidal behavior (80). A limitation for our study is that there was an overlap between patients experiencing suicidal thoughts and those who had taken antidepressants in the past, raising the possibility that there are other differences between the two MDD subgroups. Of the nine patients with suicidal thinking, BP_{ND} was lower in each of the ROIs in the three patients who were antidepressant-naïve than the six with prior medication use. The fact that these patients had been drug-free for at least eight months makes a residual direct effect of antidepressants unlikely. However, they reported stopping the antidepressants due to lack of efficacy, and there is some evidence for an association between inflammation and non-responsiveness to antidepressants (23-25). Although these are very small subgroups, we cannot exclude a potential role of treatment resistance in the TSPO increase seen in our patients with suicidal thinking.

Our results contribute to an emerging view that glial, principally microglial, activation during an MDE may be particularly prominent in the ACC. The ACC plays a key role in regulating normal cognitive and emotional processing (81) and in the pathophysiology of MDD (82-87). Postmortem studies find increased inflammatory markers in the ACC of depressed individuals (31-33), and levels of systemic cytokines are associated with increased activation in the ACC (17, 50, 51), suggesting that the ACC might be particularly sensitive to heightened peripheral inflammation and be central to inflammation-induced changes in mood. The trend-significant negative correlation between TSPO in the ACC and physical exercise in our data suggests that a potential association between brain inflammation and exercise levels warrants further investigation in a larger sample.

We found no significant correlations between central TSPO and peripheral inflammatory markers. The mechanisms of immune-to-brain communication remain to be fully elucidated. However, this lack of correlation is consistent with previous PET studies in humans reporting both central and peripheral measures in depression (39) and schizophrenia (57, 88), and preclinical models involving experimental induction of both local and systemic peripheral inflammation (89-92).

Our study has several additional limitations. Firstly, we used a pseudo-reference region (cerebellum). Although the presence of some specific binding in the cerebellum will cause an underestimation of the specific binding in the ROIs, this remains a reasonable approach as long as there is no significant systematic difference in cerebellar TSPO availability between healthy subjects and patients. To the best of our knowledge, there are no published postmortem data on TSPO or microglia in the cerebellum in MDD so we cannot exclude the possibility that a difference exists. Our finding of no difference in cerebellar BP_{ND} (SVC6) between patients and controls provides some reassurance that the study findings are not confounded by a systematic difference in cerebellar TSPO binding between controls and patients. However, this reassurance is to a limited degree and cerebellar BP_{ND} is not as strong as measurement of cerebellar total volume of distribution (V_T) would have been using a metabolite-corrected arterial input function. Secondly, a limitation common to all TSPO PET studies is that microglia have a range of pro- and anti-inflammatory chemical phenotypes including cytotoxic, repair and regeneration, and immunomodulatory (93), and at present PET is unable to distinguish between these. However, given the postmortem studies implicating a pro-inflammatory microglial phenotype in MDD and in suicide (31, 32), we propose that increased TSPO binding in MDD represents a cytotoxic phenotype.

In conclusion, we have replicated the first PET findings of increased TSPO availability, suggestive of microglial activation, in the ACC of medication-free patients in a MDE. Our findings add support for the presence of a neuroinflammatory process in MDD and for TSPO as a therapeutic target (71). Trials of anti-inflammatory agents in MDD have indicated that

they might be most effective in a subset of individuals with heightened inflammation, suggesting that a more targeted 'personalised' strategy might be a successful approach to treating depression. It will therefore be important for future research to determine whether patients with elevated TSPO would benefit from anti-inflammatory treatment. A potential contribution of suicidality to the elevated TSPO in MDD warrants further research in adequately powered studies.

Holmes SE et al. PET imaging of translocator protein in major depression

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Holmes SE et al. PET imaging of translocator protein in major depression

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Financial Disclosures

Holmes SE et al. PET imaging of translocator protein in major depression

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Conflict of Interest

The authors report no biomedical financial interests or potential conflicts of interest.

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Figure legends:

Figure 1: Regional mean $[^{11}C](R)$-PK11195 BP_{ND} in MDD patients and controls, showing statistically significant elevations in ACC but not PFC or insula. BP_{ND}, binding potential; ACC, anterior cingulate cortex; PFC, prefrontal cortex. * indicates significant at $p<0.05$.

Figure 2: Regional $[^{11}C](R)$-PK11195 BP_{ND} in controls, MDD patients with suicidal thoughts and MDD patients without suicidal thoughts. Horizontal bars indicate means. Open circles represent controls (n=16), closed triangles represent MDD patients with suicidal thoughts (n=9) and closed circles represent MDD patients without suicidal thoughts (n=6). * indicates significant at $p<0.05$.
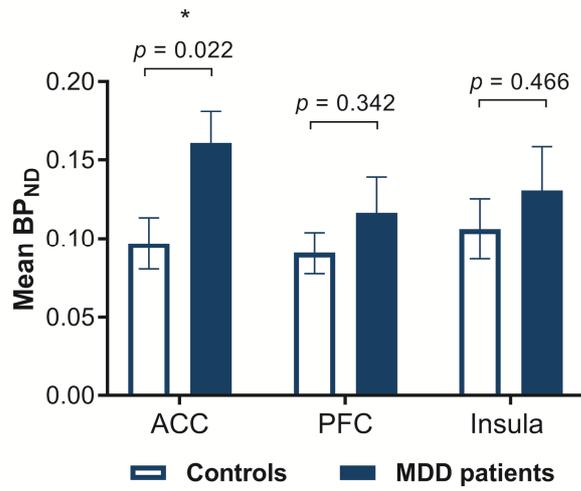
Tables

Table 1: Participant characteristics

Characteristic	All subjects			MDD patients		
	MDD patients (n=14)	Controls (n=13)	*p*-value	With suicidal thoughts (n=9)	Without suicidal thoughts (n=5)	*p*-value
Sex (M:F)	7:7	7:6	-	5:4	2:3	-
Age (yrs)	30 (12)	33 (11)	0.673	33 (14)	27 (5)	0.404
BMI (kg/m^2)	23 (6)	23 (3)	0.615	25 (4)	24 (4)	0.496
Smokers	0	0	-	0	0	-
Age at onset (yrs)	20 (8)	-	-	22 (10)	18 (4)	0.435
Duration of illness (yrs)	11 (10)	-	-	11 (12)	9 (5)	0.705
MADRS	31 (4)	-	-	32 (4)	29 (5)	0.369
HAM-D	20 (3)	-	-	20 (3)	21 (3)	0.596
Injected mass of PK11195 (µg)	1.66 (0.88)	2.03 (2.06)	0.547	1.78 (1.07)	1.45 (0.37)	0.418

Values presented as mean (SD)

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Table 2: Regional TSPO availability ($[^{11}C](R)$-PK11195 BP_{ND}) in MDD patients, healthy controls, and MDD patients stratified by presence or absence of suicidal thinking

Region	MDD patients (n=14)	Healthy controls (n=13)	MDD patients vs healthy controls		MDD without suicidal thoughts (n=5)	MDD with suicidal thoughts (n=9)	MDD with suicidal thoughts vs healthy controls		MDD with vs without suicidal thoughts	
			Difference (%)	Signif (p)			Difference (%)	Signif (p)	Difference (%)	Signif (p)
ACC	0.162 (0.077)	0.097 (0.059)	67%	0.022*	0.092 (0.060)	0.201 (0.055)	107%	0.001*	118%	0.008*
PFC	0.116 (0.085)	0.090 (0.048)	29%	0.342	0.064 (0.074)	0.145 (0.079)	61%	0.179	129%	0.096
Insula	0.131 (0.103)	0.106 (0.068)	24%	0.466	0.051 (0.091)	0.176 (0.083)	66%	0.143	245%	0.023*

Values presented as mean (SD). ACC, anterior cingulate cortex; PFC, prefrontal cortex; BP_{ND}, binding potential; MDD, major depressive disorder. *indicates significant at p<0.0
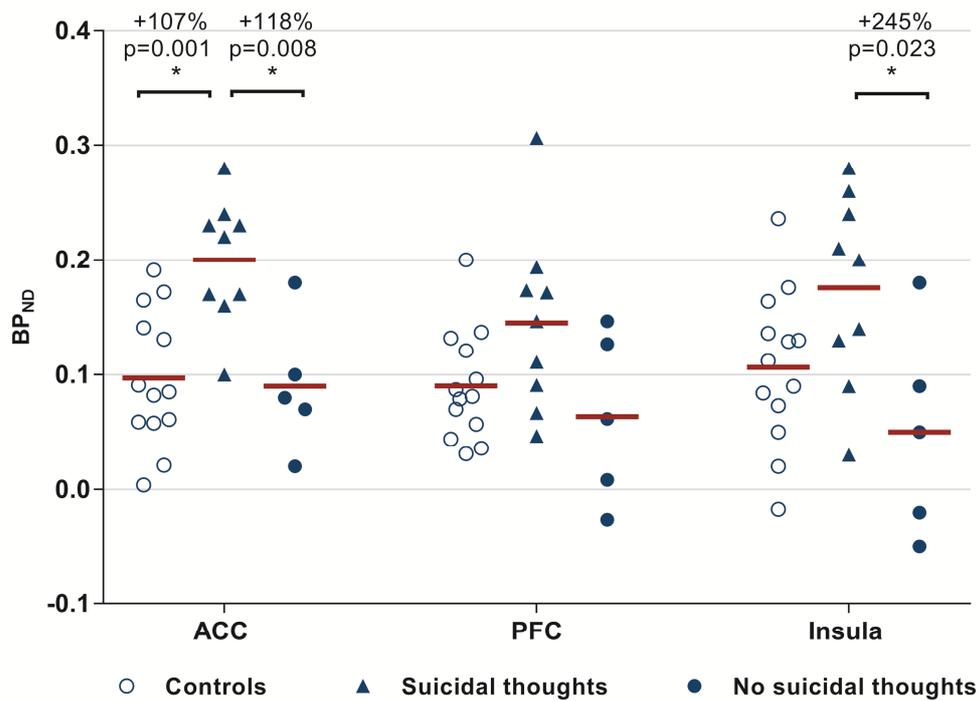
Elevated Translocator Protein in Anterior Cingulate in Major Depression and a Role for Inflammation in Suicidal Thinking: A Positron Emission Tomography Study

Supplemental Information

Past antidepressant use in MDD patients

Table S1. Details of previous antidepressants for each patient and number of months since last use, where applicable.

Patient	Previous antidepressants	Months without antidepressant
1	Citalopram	48
2	Citalopram	12
3	-	-
4	-	-
5	Sertraline, citalopram, fluoxetine, venlafaxine	12
6	-	-
7	Fluoxetine	60
8	-	-
9	Fluoxetine	8
10	Amitriptyline, mirtazapine	25
11	Reboxetine, sertraline, paroxetine	24
12	-	-
13	-	-
14	-	-

Methodological considerations

$[^{11}C](R)$-PK11195 was chosen in this study because, unlike $[^{11}C]$PBR-28, $[^{18}F]$DPA-714 and other second generation TSPO radioligands, its differences in binding affinity in humans due to the polymorphism rs6971 are negligible (1). This allowed us to include all eligible participants in this study regardless of binding affinity, including the approximately 10% of the population who are low affinity binders and therefore excluded from studies using second generation tracers (2).

We decided not to use an arterial input function due to the likelihood that the requirement for arterial cannulation would further limit recruitment of an already difficult to recruit clinical population (drug-free patients with major depression of at least moderate severity).

1

Therefore the quantification of regional $[^{11}C](R)$-PK11195 binding in the brain had to use a reference tissue input function. This brought in further requirements such as that the reference input is not affected by the disease, that its displaceable binding is insignificant relative to that in the target area and that of homogeneity of the non-displaceable binding across the brain. However, at the same time, results obtained from reference tissue analyses have proven to be more robust than those from plasma input function kinetic models in cases where it had been difficult to get reliable measurements of the fractions of unmetabolised tracer in plasma or of the plasma free fraction (3).

As TSPO expression is ubiquitous throughout the brain, there is no ideal reference region for PET studies assessing microglial activation with TSPO radioligands. An alternative is to use a pseudo-reference region, and our methodology used cerebellar grey matter (GM). Labelling of TSPO in post mortem human brain with $[^{3}H]$PK11195 found for the cerebellar cortex binding densities of 660 ± 85 fmol/mg protein in the granular cell layer, 191 ± 55 fmol/mg protein in the molecular cell layer and 41 ± 32 fmol/mg protein in white matter (4). For comparison, the highest binding densities were found in the dorsomedial thalamic nucleus (1912 ± 412 fmol/mg protein) and in inferior olivary nucleus of the medulla (1655 ± 355 fmol/mg protein). This amount of specific binding in the cerebellum causes an underestimation of the specific binding in the target regions of the brain, if a cerebellar input function is chosen for a reference tissue model.

Therefore, data driven approaches have been developed to extract the reference tissue kinetics from dynamic brain scans with $[^{11}C](R)$-PK11195 on the voxel level (5-7). These methods do not rely on an anatomically delineated region of interest for the definition of the reference region. Instead, they group voxels together based on their similarity between the voxel time-activity curves.

Superv**ised cluster analysis (SVC6)**

We therefore also analysed our data using the alternative approach of supervised cluster reference input function (SVC6), a data-driven modelling method which segments voxels in the raw dynamic data into six pre-defined tissue classes (normal grey and white matter, blood pool, muscle, skull and pathological tissue with high TSPO density) based on their time activity curves, then extracts as a

reference region a cluster of GM voxels which exhibit kinetic behaviour closest to that of GM in a population of healthy controls.

Overall, BP_{ND} in our regions of interest derived from SVC6 were modestly underestimated and had higher variance (data not shown), with an associated reduced power to discriminate between-group differences, compared to BP_{ND} derived using the cerebellar GM input function. We therefore chose to present the latter data in our manuscript, in concurrence with papers which have concluded that the cerebellum is the preferred reference region over a supervised cluster region for $[^{11}C](R)$-PK11195 (8-10).

Validity of cerebellum as pseudo-reference region in MDD

The use of a pseudo-reference region such as the cerebellum is acceptable as long as there is no significant systematic difference in cerebellar uptake between healthy subjects and patients with MDD such as might occur if the cerebellum is involved in the disease process associated with MDD. To the best of our knowledge there are no published post mortem data on microglia in the cerebellum in MDD to support or refute the validity of cerebellar grey matter as a pseudo-reference region for TSPO imaging. However, using SVC6, cerebellum BP_{ND} values in our study were approximately mean-zero (in fact slightly negative) and not different (p=0.77) between healthy controls (-0.051±0.057; n=13) and patients with MDD (-0.042±0.095; n=14) (see Figure S1, below). We therefore found no evidence within our data to suggest that the study findings are confounded by a systematic difference in cerebellar TSPO binding between the control and patient groups.
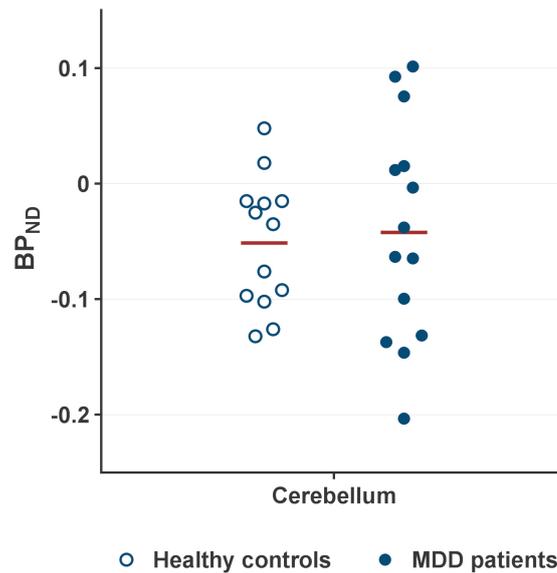
Figure S1. Cerebellum BP$_{ND}$ values in healthy controls and MDD patients.

Effect of age on group comparison

Because there was no significant main effect of age on BP$_{ND}$, the main analysis did not include age as a covariate. However, for the sake of comparison we performed a secondary analysis with BP$_{ND}$ in ACC, PFC and insula as the dependent variables, group (MDD or healthy controls) as the fixed independent variable, and introducing age as a covariate. This did not materially alter the statistical significances of the main effect of group ($F_{3, 22}=5.40$, $p=0.006$) or the between group differences: the elevation in the ACC remained of large effect size and statistically significant ($F_{1, 24}=6.77$, $p=0.016$; partial $h^2=0.220$; Cohen's $d=0.95$), and of small effect size and not statistically significant in PFC ($F_{1, 24}=1.28$, $p=0.268$; partial $h^2=0.051$; Cohen's $d=0.38$) and insula ($F_{1, 24}=0.727$, $p=0.402$; partial $h^2=0.029$; Cohen's $d=0.29$).

Voxel-based morphometry

A post-hoc voxel-based morphometry (VBM) analysis was carried out to examine possible differences in grey matter volume between MDD patients and controls. Image pre-processing was conducted using SPM12 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, UK) running in MATLAB R2015a (Mathworks,

Natick, Massachusetts). After realignment, the structural T1-weighted images were segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). A template was created and the deformations that best aligned the images were estimated using DARTEL (diffeomorphic registration). Then, spatially normalised and smoothed Jacobian scaled GM images were generated using the deformation images calculated in the previous step. For each volunteer GM tissue volumes were calculated. Finally, a GM analysis mask (thresholded at a belonging probability >0.2) was created in order to avoid instabilities that might occur in the analysis if the background is included. After pre-processing, a voxel-wise two-sample t-test was run in SPM12 comparing the smoothed, modulated, normalised, grey-matter images of our two groups. In this analysis we used the previously created GM analysis mask for explicit masking and the previously calculated tissue volumes for global calculation. Clusters of voxels were considered significant at a cluster-size threshold of pFWEc<0.05 and a height-threshold of p<0.001 (uncorrected).

Additional non-hypothesized regions

Table S2. TSPO availability ([^{11}C](R)-PK11195 BP$_{ND}$) for additional regions

Region	MDD patients (n=14)	Healthy controls (n=13)	% difference	Significance (p)
Parietal cortex	0.081 (0.077)	0.074 (0.024)	9%	0.76
Occipital cortex	0.058 (0.046)	0.048 (0.029)	20%	0.51
PCC	0.109 (0.064)	0.068 (0.024)	59%	0.04*
Hippocampus	-0.001 (0.082)	-0.027 (0.063)	-	0.37
Amygdala	0.017 (0.078)	-0.030 (0.053)	-	0.08
Thalamus	0.198 (0.080)	0.172 (0.071)	15%	0.38
Caudate	-0.084 (0.093)	-0.128 (0.104)	-	0.25
Putamen	0.103 (0.069)	0.088 (0.053)	17%	9.54
Brainstem	0.154 (0.051)	0.115 (0.066)	34%	0.10

Values presented as mean (SD). PCC: posterior cingulate cortex. P-values obtained from independent-samples t-tests. *significant at p<0.05, without correction for multiple comparisons.
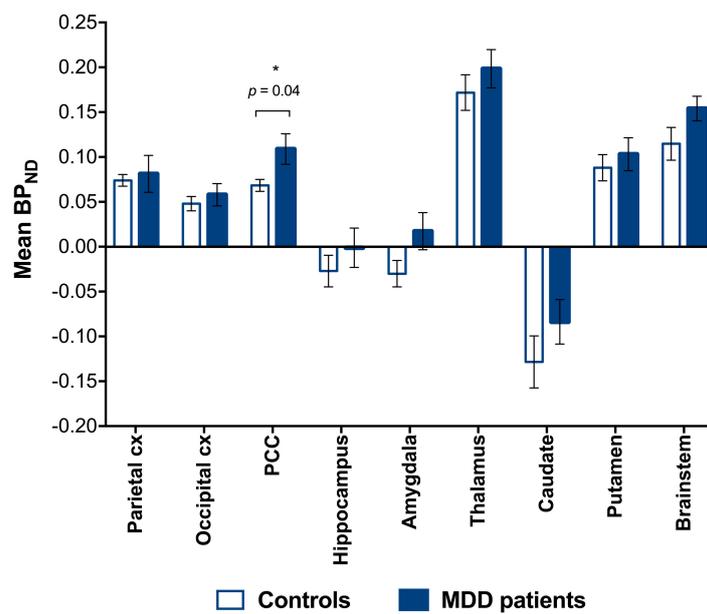
Figure S2. TSPO availability ([^{11}C](R)-PK11195 BP$_{ND}$) for additional regions

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