Supplementary Online Content

Setiawan E, Wilson AA, Mizrahi R, et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry*. Published online January 28, 2015. doi:10.1001/jamapsychiatry.2014.2427.

eAppendix. Methods, Results, and Discussion

eTable. Relationship Between Regional TSPO V_T and MDE Group Characteristics

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Methods, Results, and Discussion

I. Methods.
Participant Recruitment

All participants were recruited through advertisements in the community and our tertiary care mood disorders clinic. No participants were withdrawn from medication in order to participate in the study. The most common reason that subjects were not taking antidepressant medication at the time of enrollment was that they felt their previous assessment had been too brief and wanted a second opinion with the study psychiatrist, JHM (14/20, 70%) and the second most common reason was past history of non-response to treatment (4/20, 20%).

Additional screening criteria included a standardized medical history questionnaire regarding past illnesses clustered by organ system, including history of autoimmune disorders. Each subject also completed a TSH, CBC and ESR. Subjects were also asked about recent symptoms of illness in the previous 2 weeks prior to scan and those with such histories were excluded from study.

In addition to the clinical measures previously described, measures of anxiety in MDE subjects also included the Hospital Anxiety and Depression Scale[1] and the State Trait Anxiety Inventory.[2]

Radiosynthesis of [^{18}F]FEPPA

Details of [^{18}F]FEPPA radiosynthesis have been described previously.[3] Briefly, [^{18}F]-fluoride is dried, then reacted with the tosylate precursor in acetonitrile for 10 minutes at 90°C. The product is purified by high-performance liquid chromatography and formulated in buffered saline containing 5% to 10% ethanol, then cold sterilized by passing through a 0.22-μ filter. The final formulation is sterile, pyrogen free, with a pH of 5 to 8.[4]

Image Acquisition and Analysis

[^{18}F]FEPPA was of high radiochemical purity (>96%) and high specific activity (119 ± 127 TBq/mmol). The scan duration was 125 minutes following the injection of [^{18}F]FEPPA. The images were reconstructed into 34 time frames. Frames were acquired as followed: 1 frame of variable length (dependent on the time between the start of acquisition and the arrival of [^{18}F]FEPPA in the tomograph field of view), 5 x 30, 1 x 45, 2 x 60, 1 x 90, 1 x 120, 1 x 210, and 22 x 300 seconds. The PET images were obtained using 3D HRRT brain tomography (CPS/Siemens, Knoxville, TN, USA), which measures radioactivity in 207 slices with an interslice distance of 1.22mm. The emission list mode data were rebinned into a series of 3D sinograms. The 3D sinograms were gap filled, scatter corrected, and Fourier rebinned into 2D sinograms. The images were reconstructed from the 2D sinograms using a 2D filtered-back projection algorithm, with a HANN filter at Nyquist cutoff frequency. The reconstructed image has 256 x 256 x 207 cubic voxels measuring 1.22 x 1.22 x 1.22 mm^3 and the resulting reconstructed resolution

is close to isotropic 4.4mm, full width at half maximum in plane and 4.5mm full width at half maximum axially, averaged over measurements from the center of the transaxial FOV to 10cm off-center in 1.0cm increments.

An automatic blood sampling system (ABSS, Model #PBS-101, Veenstra Instruments, Joure, The Netherlands) was used to measure arterial blood radioactivity continuously at a rate of 2.5mL/min for the first 22.5 minutes. In addition, manual blood samples were obtained at 2.5, 7, 12, 15, 30, 45, 60, 90, and 120 minutes. These samples were used to determine the temporal evolution of 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.[4] An aliquot of each blood sample was taken to measure radioactivity concentration in total blood. The remaining blood was centrifuged (1,500 g, 5 minutes) and a plasma aliquot counted together with the total blood sample using a Packard Cobra II g counter cross-calibrated with the PET system. The blood-to-plasma ratios were determined from the manual samples to correct the blood radioactivity time–activity curve (TAC) measured by automatic sampling and to generate the plasma radioactivity curve. A biexponential function was used to fit the blood-to-plasma ratios. The remaining volume of each manual plasma sample was used to determine parent radioligand and its metabolites in plasma. A Hill function was used to fit the percentage of unmetabolized tracer. A metabolite corrected plasma curve was generated by the product of the dispersion corrected blood curve with the two curves (plasma-to-blood ratio and percentage of parent radiotracer), which was then used as the input function for the kinetic analysis.

Magnetic Resonance Image and Regions of Interest Delineation

For the anatomical delineation of regions of interest (ROIs), a brain magnetic resonance image was acquired for each subject. 2D axial proton density magnetic resonance images were 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 x 256, and field of view = 22cm). Regions of interest were generated using a semi-automated method (ROMI, Toronto, Canada) in which regions of a template MRI are located on to the individual MRI based on transformations and deformations to match the template image to the individual coregistered MRI followed by a step of grey matter voxel selection which incorporates the probability of grey matter based on the segmentation of the individual MRI as previously described.[5, 6] Regions selected included the anterior cingulate cortex, prefrontal cortex, insula, temporal cortex, parietal cortex, occipital cortex, hippocampus, thalamus, dorsal putamen, dorsal caudate and ventral striatum.

The subregions of the prefrontal cortex were defined based upon their cytoarchitectural differentiation from surrounding tissue, which was then mapped onto the external morphology of the cortex.[7-9] The dorsolateral prefrontal cortex includes cytoarchitectonic areas 9, 46, 8, and transitional areas 9-10, 46-10, 9-8. The orbitofrontal cortex includes cytoarchitectonic areas 47, 11 and transitional area 47-10. The ventrolateral prefrontal cortex includes cytoarchitectonic areas 45, and transitional areas 9-10 and 9-45. The

medial prefrontal cortex includes cytoarchitectonic areas 32, 12 and transitional areas 32-9 and 12-10. It is bounded anteriorly by the frontal pole which includes area 10.

Time activity curves from each ROI were used to estimate the V_T using a two-tissue compartment model, which has been shown previously as the optimum outcome for [^{18}F]FEPPA quantification.[4]

Sample Size Estimation

Sample size for the primary hypothesis was determined based on the ability to detect at least a 20 per cent difference in mean TSPO V_T in the brain between healthy and MDD groups (an effect size of approximately 0.8 when applying a standard deviation of 2.5 TSPO V_T). Assuming an alpha coefficient of 0.05 and 20 individuals per group, the power was 85%.

Serum Samples and Analyses

Whole blood samples were collected on the PET scan day. Blood was allowed to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Serum was removed and divided into aliquots and stored at -80°C. IL-1β, IL-6 and TNFα we analyzed using the Human Adipokine Magnetic Bead Panel 2 (Milliplex) and high sensitivity C-reactive protein was run on an automated platform using Tina-quant Cardiac C-reactive Protein (Latex) High Sensitive (Roche Diagnostics).

Additional Exploratory Analyses

Since clinical information regarding the MDE subjects was recorded and this was the first study to assess TSPO V_T in a substantial clinical sample, the relationship between TSPO V_T in the primary regions of interest and other clinical variables was also explored. These included age of onset, duration of current episode, history of previous antidepressant trial, suicidal ideation, history of suicide attempts, and state and trait anxiety. In the MDE group only, partial correlations, controlling for rs6971 genotype, were used to quantitate the relationship between TSPO V_T and age of onset, duration of current MDE, measures of state and trait anxiety and suicidal ideation. MANOVAs were conducted to determine the effects of the presence of a previous AD trial, presence of current suicidal ideation or presence of suicide attempts on TSPO V_T in the primary regions, with genotype included in the model.

II. Results

While the 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, $F_{15,23} = 4.46$, $P = 0.001$). There was no significant interaction between diagnosis and genotype ($F_{15,22}$, P=0.33), thus this interaction was removed from the model. When age was included as a covariate the effect of diagnosis and genotype remained significant (effect of diagnosis,

$F_{15,22}$=4.69, *P*=0.001; effect of genotype, $F_{15,22}$=2.72, *P*=0.016) but age was not a significant predictor of TSPO V_T ($F_{15,22}$=1.65, *P*=0.14).

While, in MDE subjects, BMI was significantly, negatively correlated with TSPO V_T in the insula, after correcting for rs6971 genotype (r=-0.605, *P*=0.006, see Figure 2b) this relationship was not present in the healthy subjects (partial correlation coefficient, after correcting for rs6971 genotype, PFC, r=0.16, *P*=0.52; ACC, r=-0.058, *P*=0.81; insula, r=-0.021, *P*=0.93). Furthermore, the correlations in the insula were significantly different between groups (z=-1.98, *P*=0.048, 2-tailed).

None of the additional clinical variables has a significant relationship to TSPO V_T in the primary regions of interest (see Supplementary Table 1). In the MDE group, the presence (N=9) or absence (N=11) of a previous anti-depressant trial did not affect TSPO V_T in the primary regions of interest (effect of previous AD trial, $F_{3,14} = 0.48$, P = 0.70). Additionally, neither the presence of current suicidal ideation (N=14, $F_{3,15} = 0.50$, P = 0.69) nor previous suicide attempts (N=4, $F_{3,15} = 0.25$, P = 0.86) were significantly related to TSPO V_T in the primary regions of interest. Additional exploratory analyses in the MDE group are summarized in Supplementary Table 1.

III. Discussion

Another limitation common to PET imaging is that our primary outcome measure was total distribution volume (V_T) which has the advantage of being computationally efficient but includes both a specific (V_S) and non-specific ($V_{(F+NS)}$) component. However, for [^{18}F]FEPPA, approximately 85% of V_T is composed of V_S,[4] hence it is very unlikely that changes in $V_{(F+NS)}$ can account for a 30% rise in V_T. For example, $V_{(F+NS)}$ would need to triple (i.e. increase by 200%) to raise V_T by 30%.

References

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eTable. Relationship Between Regional TSPO V_T and MDE Group Characteristics

Characteristic[a]	Prefrontal Cortex	Anterior Cingulate Cortex	Insula
Age of onset MDE (yrs)	-0.11 (0.65)	-0.14 (0.57)	-0.14 (0.57)
No. of MDEs	-0.09 (0.72)	-0.11 (0.66)	-0.08 (0.73)
Duration of current MDE (wks)	-0.19 (0.44)	-0.05 (0.84)	-0.17 (0.49)
HDRS-suicide	-0.01 (0.96)	-0.10 (0.69)	-0.08 (0.74)
HADS-Anxiety	0.03 (0.90)	0.08 (0.79)	0.10 (0.73)
STAI-Trait	0.34 (0.24)	0.27 (0.35)	0.32 (0.27)
STAI-State	0.14 (0.62)	0.19 (0.50)	0.20 (0.47)

[a]Values represent partial correlation coefficient (P-value), controlling for rs6971 polymorphism.
Abbreviations: HADS, Hospital Anxiety and Depression Scale; HDRS, 17-item Hamilton Depression Rating Scale; MDE, major depressive episode; STAI, State-Trait Anxiety Index; TSPO VT, translocator protein density.

