

Kinetic analysis of the translocator protein positron emission tomography ligand [¹⁸F]GE-180 in the human brain

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Disclosure/Conflict of Interest

GE Healthcare provided [¹⁸F]GE-180 free of charge. Will Trigg is an employee of GE Healthcare. Joel Raffel has received funding for his salary, from GE Healthcare Ltd with support from Fast Forward LLC (National Multiple Sclerosis Society).

ABSTRACT

Purpose: Positron emission tomography can image neuroinflammation by targeting the translocator protein (TSPO), which is upregulated in activated microglia. The high nonspecific binding of the first-generation TSPO radioligand [^{11}C]PK-11195 limits accurate quantification. [^{18}F]GE-180, a novel TSPO ligand, displays superior binding to [^{11}C]PK-11195 in vitro. Our objectives were to: (1) evaluate tracer characteristics of [^{18}F]GE-180 in the brains of healthy human participants; (2) investigate whether the TSPO Ala147Thr polymorphism influences outcome measures.

Methods: Ten participants (5 high affinity binders, HABs, and 5 medium affinity binders, MABs) had dynamic PET scans with arterial sampling after injection of [^{18}F]GE-180. Kinetic modelling of time activity curves with one- and two-tissue-compartmental models and Logan graphical analysis was applied to the data. Our primary outcome measure was the total volume of distribution (V_T) across various regions of interest (ROIs). Our secondary outcome measures were standardised uptake values (SUV), distributed volume ratios (DVR) and SUVR using a pseudoreference region.

Results: The two-tissue-compartment model (2TC) was the best model. The average regional delivery rate constant (K_1) was $0.01 \text{ mL cm}^{-3} \text{ min}^{-1}$ indicating low extraction across the blood-brain barrier (1%). Estimated median V_T was also low across all ROIs, ranging from 0.16 mL cm^{-3} in the striatum and 0.38 mL cm^{-3} in the thalamus. There was no significant difference in V_T between HABs and MABs across all ROIs.

Conclusions: A reversible 2TC model fitted the data well and determined that the tracer has a low first pass extraction ($\sim 1\%$) and low V_T estimates in healthy volunteers. There was no observable dependency on the rs6971 polymorphism as compared to other 2nd generation TSPO PET tracers. Investigation of [^{18}F]GE-180 in disease populations is needed to determine suitability for quantitative assessment of TSPO expression.

Keywords

Positron emission tomography (PET), GE180, translocator protein (TSPO), kinetic analysis, quantification, neuroinflammation

INTRODUCTION

The translocator protein (TSPO) is a mitochondrial transporter involved in varied intracellular processes, but its expression in the central nervous system (CNS) is relatively low under normal physiological conditions(1). However, activation of microglial cells caused by inflammatory stimuli results in significant upregulation of TSPO expression(2). TSPO quantification with positron emission tomography (PET) provides a measure of intrinsic neuroinflammation in a variety of CNS diseases. Early PET studies used the isoquinoline [^{11}C]PK-11195 to measure TSPO binding and detected elevations across a range of conditions including multiple sclerosis(3), Huntington's disease(4), Alzheimer's disease(5, 6), traumatic brain injury(7) and ischaemic stroke(8). However, the ligand can be limited by a high non-specific signal, making non-standard approaches to data analysis necessary(9). In addition, [^{11}C]PK-11195 is a carbon-11 compound, with a half-life of 20.3 minutes, restricting its use to locations with an on-site cyclotron.

A number of second generation TSPO ligands have been developed recently with the promise of improved signal to noise and greater specific binding. [^{18}F]GE-180 is a novel fluorinated radiotracer that binds to the TSPO with high affinity(10). Developed from a series of tricyclic indoles, [^{18}F]GE-180 has demonstrated superior specific binding affinity to [^{11}C]PK-11195 in animal models of acute neuroinflammation(11) and stroke(12). The fluorine-18 radiolabelling, with a half-life of 109.8 minutes, also makes [^{18}F]GE-180 more suitable than carbon-11 based compounds for long-distance distribution enabling widespread clinical use.

Other second generation TSPO radiotracers (e.g. [^{11}C]PBR-28, [^{18}F]PBR-06, [^{11}C]-DAA1106, [^{11}C]-DPA713, [^{18}F] FEPPA) show binding affinities influenced by a TSPO polymorphism expressed by individuals which have been classified as high-affinity binders (HABs), mixed affinity binders (MABs) and low affinity binders (LABs)(13). Expression of the TSPO Ala147Thr polymorphism results in MAB or LAB depending on whether one or two copies are present(14). Here, we report a study in healthy participants using [^{18}F]GE-180 PET imaging. Our primary aim

was to investigate tracer kinetics and quantification in healthy human subjects. Our secondary aim was to investigate whether there were differences in binding between HABs and MABs.

MATERIALS AND METHODS

Human Subjects

This study was approved by the Westminster Research Ethics Committee, London (13/LO/1596), the Riverside Research Ethics Committee (13/LO/1916), and the Administration of Radioactive Substances Advisory Committee (no. 631/336/30788). Research was conducted in accordance with the Helsinki Declaration(15). All participants gave written, informed consent.

Ten healthy volunteers (7 males), mean age 41 ± 9 , 28–56 years, mean weight 81.8 ± 13 kg, were included in the study. A screening assessment was carried out that included full medical and drug history, blood pressure, height, weight, Allen's test for patency of the ulnar anastomosis, and the Structured Clinical Interview for DSM disorders (SCID). Blood samples were taken for analysis of full blood count, renal profile, clotting screen and TSPO genotyping. Exclusion criteria included pregnancy, a history of prior or current psychiatric or neurological disease, abuse of alcohol or drugs or contraindication to arterial line placement.

TSPO Genotyping

DNA was extracted using the Qiagen QIAmp DNA blood mini kit. TSPO genotyping of the c.439A>G (p.Thr147A1a) (SNP rs6971) was performed using a TaqMan Allelic Discrimination assay. Low affinity binders (n=1) were excluded from the imaging component of the study. During the study design it was felt that on ethical and economical grounds we should not expose LABs to this novel tracer and instead focus on MABs and HABs. Of the 10 participants eligible for imaging, 5 were high-affinity binders and 5 were mixed affinity binders.

Synthesis of [^{18}F]GE-180

[^{18}F] fluorine was produced by the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction on a GE PETtrace 8 cyclotron (The Grove Centre, Amersham, UK). All radiochemistry was performed on the GE FASTlab synthesizer with single use cassettes. Average synthesis time was 43 mins, radiochemical yield 43% and purity was greater than 95%(16). The radiotracer was manufactured by GE Healthcare

(The Grove Centre, Amersham, UK), transported to Hammersmith Hospital, London, and used within 8 hours of manufacture.

Positron Emission Tomography (PET) Scanning and Image Reconstruction

All participants were scanned at the Clinical Imaging Facility, Imperial College London, Hammersmith Hospital. Prior to PET scanning, an arterial cannula was inserted under local anaesthetic (2% lidocaine) into the radial artery to allow arterial blood sampling. An antecubital venous cannula was inserted for radiotracer administration.

PET studies were performed on a Biograph 6 (6 slice CT) scanner after administration of 182 ± 3.1 MBq via intravenous bolus injection given over ten seconds followed by a 10 mL saline flush. A low dose CT scan preceded the PET acquisition to allow correction for tissue attenuation. Emission data were then acquired over 90 minutes in list mode and reconstructed as 24 temporal frames (6x15, 3x60, 5x120, 5x300, 5x600 seconds) using filtered back projection (matrix size 168x168, zoom 2.6, 5mm Gaussian filter, pixel size 1.56x1.56, slice thickness 3mm) with and without attenuation correction. Standard corrections for scatter, decay and random counts were applied.

Whole Blood, Plasma Activity and Parent Fraction of [^{18}F]GE-180

Arterial blood activity was measured every second for the first 15 minutes of the 90 minute scan using an automated blood sampling system (ABSS Allog, Mariefred, Sweden) connected to the participant via a 1.5 m x 1.0mm diameter polytetrafluoroethylene (PTFE) line (blood withdrawal rate 2.5 ml/min). In addition, manual blood samples (10mL) were collected from the radial artery at 0, 5, 10, 15, 30, 50, 70, 90 mins to assay whole blood and plasma activity. Plasma was obtained by centrifugation over 3 minutes at 1800 g. Activity of whole blood and plasma was measured in a Well Counter (CAPRAC -®T/Well Counter) over 10-60 seconds. The first 15 minutes of continuous whole blood activity was combined with the discrete samples to generate the whole blood activity curve for use in data modelling. The continuous plasma-over-blood ratio

was estimated using a constant model and a total plasma activity curve was obtained by correcting the whole blood curve for this partition between plasma and blood.

The parent fraction of [^{18}F]GE-180 was measured by high-performance liquid chromatography (HPLC) (Agilent 1100 and 1260 series) of discrete plasma samples (3.5 mL). The parent fraction was fitted to a single exponential plus a constant model. The total plasma activity was multiplied by this parent fraction and then smoothed post peak by fitting to a tri-exponential function to generate an arterial parent plasma input function. A delay correction of up to +30 seconds was applied to the input function prior to fitting the kinetic modelling. This was performed to account for delay in the 1.5 m tube (28.3 seconds) and delay between the radial artery and the brain.

Magnetic Resonance Imaging (MRI)

To provide additional anatomical information to aid analysis of the PET data, each subject had a structural T1-weighted MRI scan on a Siemens Verio 3T scanner (matrix size 256x256, 1x1x1 mm voxel size, TR 9.63 ms, TE 4.74 ms, flip angle 9 degrees).

Data Analysis

A high level overview of the data analysis is provided in Figure 1. We used the PET data analysis and kinetic modelling toolkit, MIAKATTM (www.miakat.org), which incorporates software from SPM5 (Wellcome Trust Centre for Neuroimaging) and FSL (FMRIB, University of Oxford)(17). The brain was initially extracted from the T1-weighted MR image. The CIC neuroanatomical atlas(18) was non-linearly registered to the individual's extracted brain in order to generate a personalised set of anatomically parcellated regions(18). Frame-by-frame motion correction of the dynamic (non attenuated-corrected) PET data was performed using mutual information co-registration of the individual frames to a reference frame. An average motion-corrected PET image was generated and used for co-registration with the T1-weighted MR image. Finally, regional tissue time activity curves (TACs) were generated for each region of

interest (ROI) defined from the CIC atlas that had been transformed into each individual's image space.

Datasets were analysed with one- and two-tissue-compartment models and the Logan graphical method, using the metabolite-corrected plasma input function as previously described(19) with a fixed 5% blood volume correction.

The primary outcome measures were the radioligand delivery rate constant (K_1 ; $\text{mL cm}^{-3} \text{ min}^{-1}$), total distribution volume (V_T ; mL cm^{-3}), and standardised uptake values (SUV). The SUV ratio and distribution volume ratios (DVR) were estimated using cortical grey matter as a pseudo reference region. This region was chosen as there is no true reference region for TSPO, but previous work has used a cortical grey matter reference as in healthy brain there is usually low TSPO expression (20).

Time Stability Analysis

To investigate the stability of V_T over different scan durations, a time stability analysis was performed by analyzing data for total time windows that ranged between 40-90 minutes in 10 minute increments.

Statistical Analysis

To determine the most appropriate compartmental model the Akaike information criterion (AIC) was used for model selection(21), where lower AIC was indicative of a more parsimonious model. To compare characteristics between genetic groups (HAB/MAB), Fisher's exact test (gender) and the Mann Whitney U test (age weight, injected dose) were used. To evaluate differences between genetic groups in time activity curves for blood data, a repeated measures analysis of variance (ANOVA) was used, with time as the within-subjects factor and genotype as the between-subjects factor. A repeated measures ANOVA was also used to compare outcome measures across multiple ROIs, where ROI was used as the within-subjects factor.

RESULTS

Injection of [^{18}F]GE-180 caused no pharmacological effects based on patient reports, blood pressure, pulse, respiration rate and oxygen saturation after radioligand administration. There were no significant differences in gender, age or weight between HABs and MABs. We looked for correlations between age and our principal outcome measure. We found no significant correlations between age and V_t in both HABs and MABs in any of the ROIs studied (HABs: Spearman's $\rho = -0.3-0.7$ $p=0.188-0.873$, MABs: Spearman's $\rho = 0.1-0.8$, $p=0.104-0.94$). There was also no significant relationship between age and outcome measures when added as a covariate in the repeated measures ANCOVAs performed. Demographic data are provided in Supplementary Table 2.

Plasma Data

In plasma, the concentration of [^{18}F]GE-180 peaked at about 45 seconds and was followed by a rapid decrease (Figure 2A). The fraction of unchanged [^{18}F]GE-180 over time is shown in Figure 2B. The parent compound accounted for 65.0-81.7% (min-max across participants) of the total concentration in plasma at 30 minutes, and 57.3-78.3% at 90 minutes. Three polar radioactive metabolites were identified over the course of the 90 minute scanning window (<10% of parent compound). The plasma to blood ratio remained constant at ≈ 1.69 (Figure 2C) across individuals. There were no significant differences in profiles of plasma over blood ($F(1,48)=0.407$, $p=0.541$), parent fraction ($F(1,48)=0.871$, $p=0.378$) or parent in plasma between genetic groups ($F(1,48)=0.130$, $p=0.728$). There was no interaction of genotype with time for any of these profiles ($p>0.204$). The parent in plasma profile for HABs and MABs is shown in Supplementary Figure 1.

Tissue Data

The concentration of the ligand in the brain peaked at around one minute followed by rapid washout for all participants. Group-averaged tissue TACs for frontal grey matter and thalamus are shown in Figures 3A and B. A 60-90 minute SUV image for a representative MAB subject are shown in Figure 3C. Overall, there was low uptake of the tracer in brain with images being

dominated by signal from blood vessels. There were no significant differences in SUV curves between genetic groups ($F(1,48)=1.396$, $p=0.271$). There was no interaction of genotype with time ($P=0.684$).

Kinetic Analysis

The results of the kinetic modeling are shown in Table 2. The two-tissue-compartment (2TC) model was superior to the one-tissue-compartment model (1TC), as judged by a lower AIC, in all ROIs except the striatum. Example model fits for 1TC and 2TC are shown in Figure 4A for a representative MAB subject. The first 10 minutes of Figure 4A and 4C are shown in more details in Supplementary Figure 2. The 2TC generally showed a good fit to the data excepting an initial small mismatch in the blood volume peak, which should not impact on V_T estimates. When we included the 2TC-fit model, it did not outperform the 2TC-fix based on the AIC. Blood volume estimates ranged from 6.3-10.5% (mean 8.4%) across all ROIs. Therefore the 2TC-fix was selected as the model to use for further analysis and gave average rate constants (across all regions and participants) of $K_1=0.013 \text{ mL cm}^{-3} \text{ min}^{-1}$, $k_2=0.229 \text{ min}^{-1}$, $k_3=0.035 \text{ min}^{-1}$, and $k_4=0.010 \text{ min}^{-1}$, resulting in $V_T=0.311 \text{ mL cm}^{-3}$ (Table 2).

The delivery rate constant, K_1 , was low across all ROIs in all participants, indicating low extraction across the blood-brain barrier. This is consistent with the low tissue uptake observed in the images and the predominance of the vasculature structures. There was no significant effect of genetic group on any of the four rate constants. The Logan graphical method was also used to estimate the V_T in each ROI (Table 2). A representative plot is shown in Figure 4B. Pooled V_T estimates from the 2TC were positively correlated with V_T Logan (Pearson's $r=0.630$ $p<0.0001$, regression equation $V_T \text{ Logan} = 0.3 * V_{T-2TC} + 0.19$). Tissue TAC minus whole blood radioactivity demonstrates that approximately 20% of the activity in a typical ROI comes from blood (Fig 4C).

Time Stability Analysis and Outcome Measures

The time stability analysis of the 2TC model demonstrated an increasing V_T for each successive time window analysed (shown for two ROIs in Figure 4D). A comparison of six outcome measures for a number of ROIs is shown in Figure 5. For all six outcome measures, no significant effect of genetic group was found ($p>0.186$), nor any interaction between genetic group and ROI ($p=0.468$).

DISCUSSION

Here, we describe the characterization and quantification of the novel TSPO tracer [^{18}F]GE-180 for the first time in the normal healthy human brain. We generated arterial parent plasma and whole blood input functions and fitted brain TACs to one- and two-tissue compartmental kinetic models and Logan graphical analysis to generate outcome measures across regions and individuals. The following key outcome measures were generated from the analysis: delivery (K_1), volume of distribution (V_T), standardised uptake values (SUV), distribution volume ratios (DVR) and SUV with cortical grey matter as a pseudo reference region (SUVR). In addition, we investigated whether the TSPO Ala147Thr polymorphism in the TSPO binding site influenced these outcome measures.(14, 22)

There was consistent and stable metabolism of [^{18}F]GE-180 parent compound across all individuals. There was no difference in blood or plasma activity between the two genotypes and there were only moderate levels of detectable metabolites in all individuals with 70% of the intact parent tracer remaining at 90 min. SUV images across all individuals demonstrated low uptake of the tracer in brain tissue with significant tracer activity apparent in the blood compartments of the brain. The low brain uptake could reduce the signal to noise ratio for this tracer and might mean that variation in the activity within the blood across the groups could bias results. In addition, the low uptake might make the tracer more susceptible to showing increased uptake when there is BBB breakdown.

Analysis of the tracer compartmental modelling showed that the reversible two tissue compartment model provided the best overall fit in the majority of cases. There was a small discrepancy in the model fit at the initial sharp peak of the curve i.e. <5 mins of data acquisition. It is possible that this may be due to increased dispersion of [^{18}F]GE-180 in the vascular bed, although this is difficult to quantify precisely. In addition, the 1.5m line that was used from the radial artery to the arterial blood detection machine may have impacted the model fit. However, this discrepancy should not impact in a significant way on the estimation of V_T as this is based on the integral of the impulse response function (i.e integral/area under curve of plasma input

function) (23). K_1 could be affected by dispersion but would still remain small after any correction and therefore the interpretation of low brain delivery of this tracer is still valid.

Using the 2TC model, the initial rate constant K_1 was consistently low suggesting a low extraction fraction and delivery into brain tissue. Theoretically this could be due to a number of reasons: (1) low lipophilicity - however pre-clinical work has suggested that this tracer is relatively lipophilic (logD at pH 7.4 is 2.95), making this unlikely; (2) [^{18}F]GE-180 could be a substrate for xenobiotic pumps at the blood brain barrier such as the three major ABC transporters, p-glycoprotein-ABCB1 and ABCC1, ABCG2 as can be seen with other tracers with low BBB penetration (24); (3) the low K_1 could be due to increased plasma protein binding although the relationship here is complex and high plasma protein binding does not always lead to low brain penetration. Most molecules to a greater or lesser extent bind to human serum albumin and some tracers also bind to α_1 -acid glycoprotein(25). However in the case of [^{18}F]GE-180, the binding affinity to these or other plasma proteins may be considerable. A limitation of this study is that the protein binding of [^{18}F]GE-180 was not measured. However in vitro work suggests that in humans the plasma free fraction is approximately 3%.

The median volume of distribution of [^{18}F]GE-180 using the 2TC model across all subjects and brain regions ranged from 0.16-0.38 mL cm⁻³. There was little variability across brain regions. V_T estimates were lower than those observed for some other second generation TSPO tracers (e.g. [^{11}C]-PBR-28: 4.1 ± 1.6 mL cm⁻³ in grey matter(26) and [^{11}C]PK-11195: 0.72-1.06 mL cm⁻³ (27)).

Our time stability analysis demonstrated that V_T did not reach a stable estimate during our 90 minute scan, continually increasing over the 90 minute scanning window. This might lead to an underestimation of V_T . A scan time of 90 minutes was originally selected based on pre-clinical studies and consideration of what would be acceptable for individuals. However, our results suggest that a longer scanning duration might give a more 'stable' V_T estimate. This ongoing increase in V_T could have been caused by the accumulation of radiometabolites in brain.

However, only modest levels of metabolites were detected in the blood and earlier pre-clinical work demonstrated very low penetration of any metabolite into the brain with 94% parent at 60 minutes(28). Metabolites have not formally been identified but all those observed in this study have been more polar than the parent. It is believed that the 2 main routes of metabolism are O-demethylation and hydroxylation of the aliphatic ring(28). Other metabolites could be a combination of the 2 processes or hydroxylation at different sites. The question of accumulation of metabolites in the brain is most relevant when brain uptake is high, which is not the case here. It is also worth noting that these time stability results are consistent with other TSPO tracers which also do not achieve a stable estimate within a 2-hour scanning window e.g. [¹¹C]PBR28(19).

We did not find evidence of an effect of TSPO genotype on any of our outcome measures. We observed no difference in K_1 , V_T , DVR, SUV or SUVR between MABs and HABs. This was an unexpected finding as in vitro work with cold GE-180 displacing [3H]PK11195 has shown a binding affinity of 15:1 between HABs and LABs (D Owen, personal communication). Although we did not acquire PET scans on LABs we would still have expected to see an effect of HABs vs. MABs on our outcome measures. Our expectation was that MABs would show an intermediate binding between HABs i.e. around 50% of the HAB binding (13). The fact that we did not observe this difference in vivo may be a consequence of the low uptake seen with this tracer. Genotype effects may emerge in other groups, for example older individuals or diseased individuals where we may expect microglial TSPO expression to be higher. We did consider the affect that increasing age of our participants could have on TSPO binding as has been shown previously (29), however we did not find any correlations between age and V_t in all ROIs.

In summary, we report the first PET studies of [¹⁸F]GE-180 in humans. Administration of the tracer was used safely and the scan was tolerated well by all participants. A reversible 2TC model fitted the data well and determined that the tracer has a low first pass extraction (~1%) and low V_T estimates in healthy volunteers. There was no observable dependency on the rs6971 polymorphism as compared to other 2nd generation TSPO PET tracers. A low first pass

extraction combined with a tissue signal with a relatively large blood component suggests similarities to [^{11}C]PK-11195 in vivo. However more human work with [^{18}F]GE-180 would be informative including studies in patients with neuroinflammatory conditions, subjects of varying ages and a competition study to more clearly delineate specific binding and in disease states to assess signal in the presence of upregulated TSPO.

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Author Contributions Statement

CF contributed to acquisition of data and analysis of data, and drafted the article. GSc contributed to analysis of data, and drafted the article. JR contributed to acquisition of data and analysis of data and drafted the article. SR contributed to acquisition of data and revised the article. CC contributed to analysis and interpretation of the data, and revised the article. AJ contributed to analysis of data and revised the article. GSe contributed to analysis of data and revised the article. DB revised the article. RN revised the article. WT revised the article. RG contributed to analysis and interpretation of data, and revised the article. DJS designed the study, contributed to analysis of the data and revised the article.

Compliance with Ethical Standards:

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Conflict of Interest: GE Healthcare provided [^{18}F]GE-180 free of charge. Will Trigg is an employee of GE Healthcare. Joel Raffel has received funding for his salary, from GE Healthcare Ltd with support from Fast Forward LLC (National Multiple Sclerosis Society). All other authors declare no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

All authors approved the manuscript.

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FIGURES

Figure 1. Overview of data analysis. We used the MIAKAT™ analysis toolkit for image processing (A), blood data processing (B) and kinetic modelling (C) of PET data.

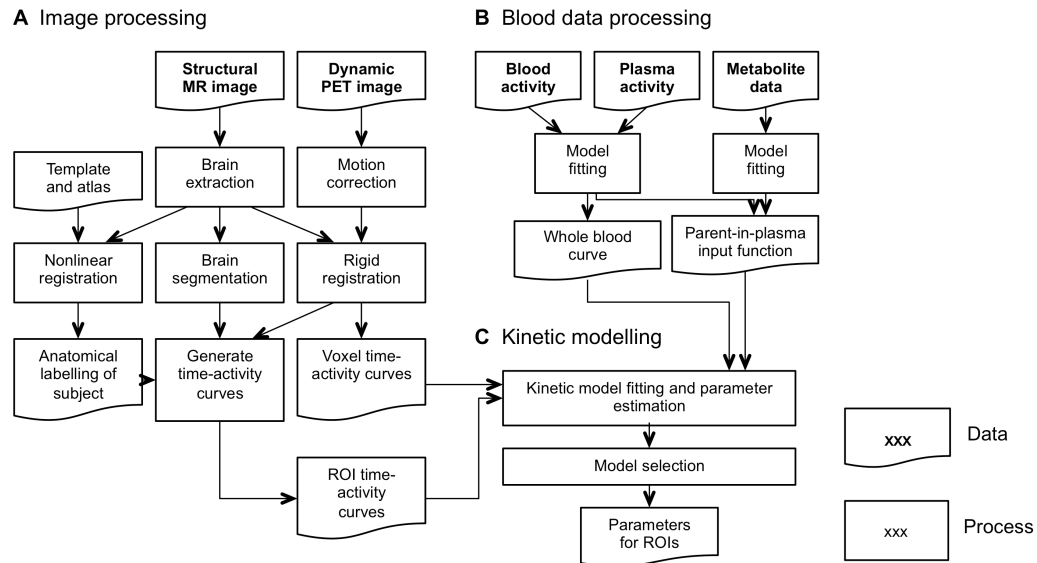


Figure 2. Blood data. (A) Whole blood (red) and parent in plasma (black) curves for one subject. (B) The fraction of unchanged parent compound over time, for high affinity binders (HAB, red) and medium affinity binders (MAB, blue). (C) Plasma to blood ratio over time, for HAB and MAB groups. Values plotted for B and C are mean $\pm$ standard error of the mean.

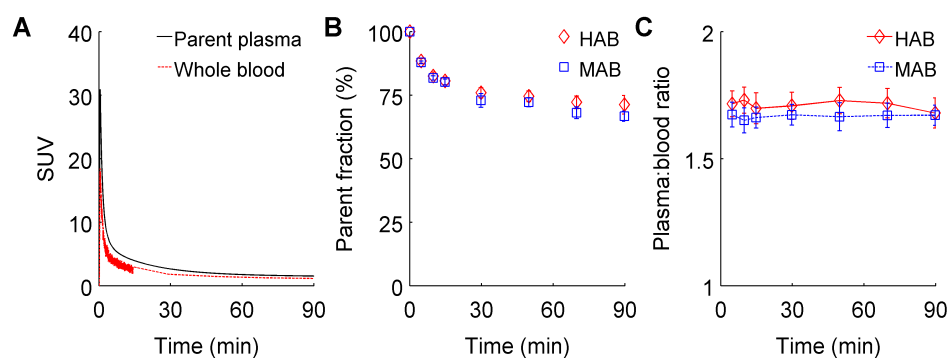


Figure 3. Imaging data. Time activity curves (TACs) are shown for high affinity binders (HAB) and medium affinity binders (MAB), in frontal grey matter (A) and thalamus (B). Standardised uptake values (SUV) are plotted as mean $\pm$ standard error of the mean. (C) SUV image calculated from the 60-90 minute PET frames, superimposed on the co-registered T1-weighted MR, for a representative MAB subject.

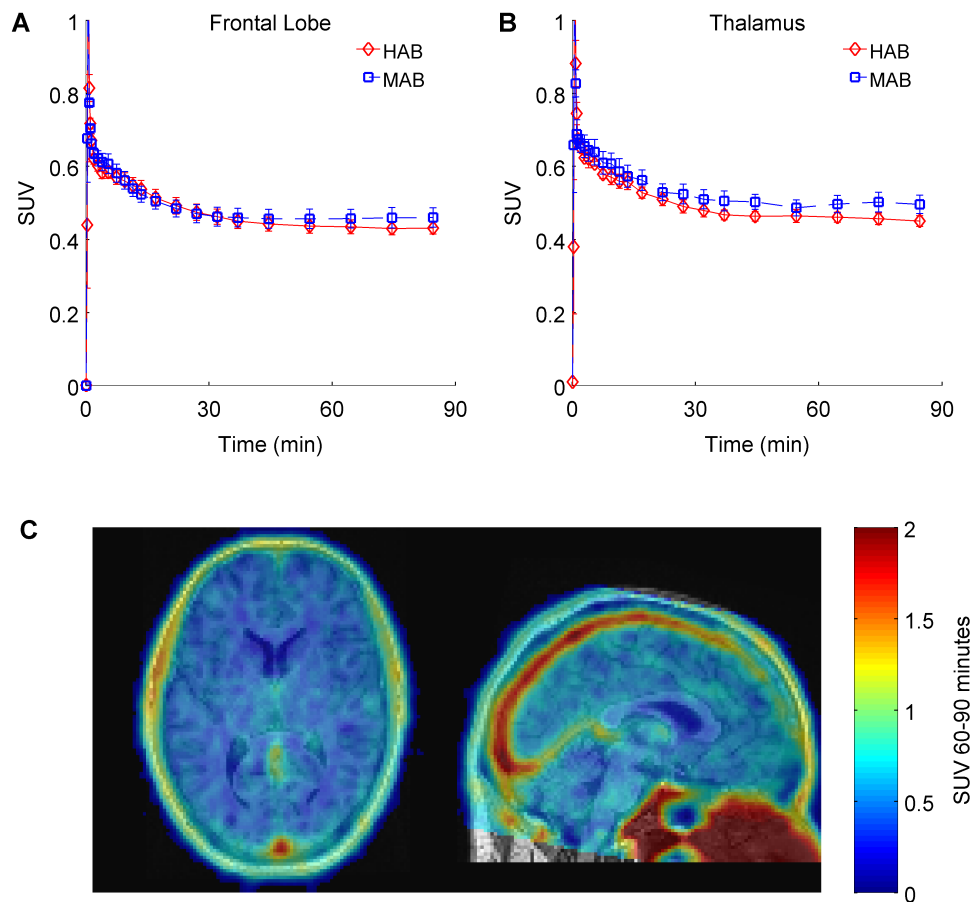


Figure 4. Kinetic modelling and time stability analysis. (A) Model fits are shown for one-tissue compartmental-model (reversible 1TC) black dashed line and two-tissue compartmental-model (reversible 2TC), red solid line) against a time activity curve (TAC) (black dots) for the Parietal Lobe. (B) Logan plot (black dashed line for most linear portion of curve) for the same time activity curve as (A) (black dots). (C) Parietal Lobe TAC (solid black line), 5% whole blood activity curve (red solid) and difference of the two curves (black dashed) highlights the significant contribution of blood signal to the TAC. (D) Estimates of two-tissue compartmental-model V_T calculated for 40-90 minute scan windows in 10 minute increments are plotted as the absolute percentage difference compared to the final 90 minute V_T , for parietal lobe (A) and cerebellum (B) grey matter regions of interest. Values plotted are mean percentage $\pm$ standard error of the mean.

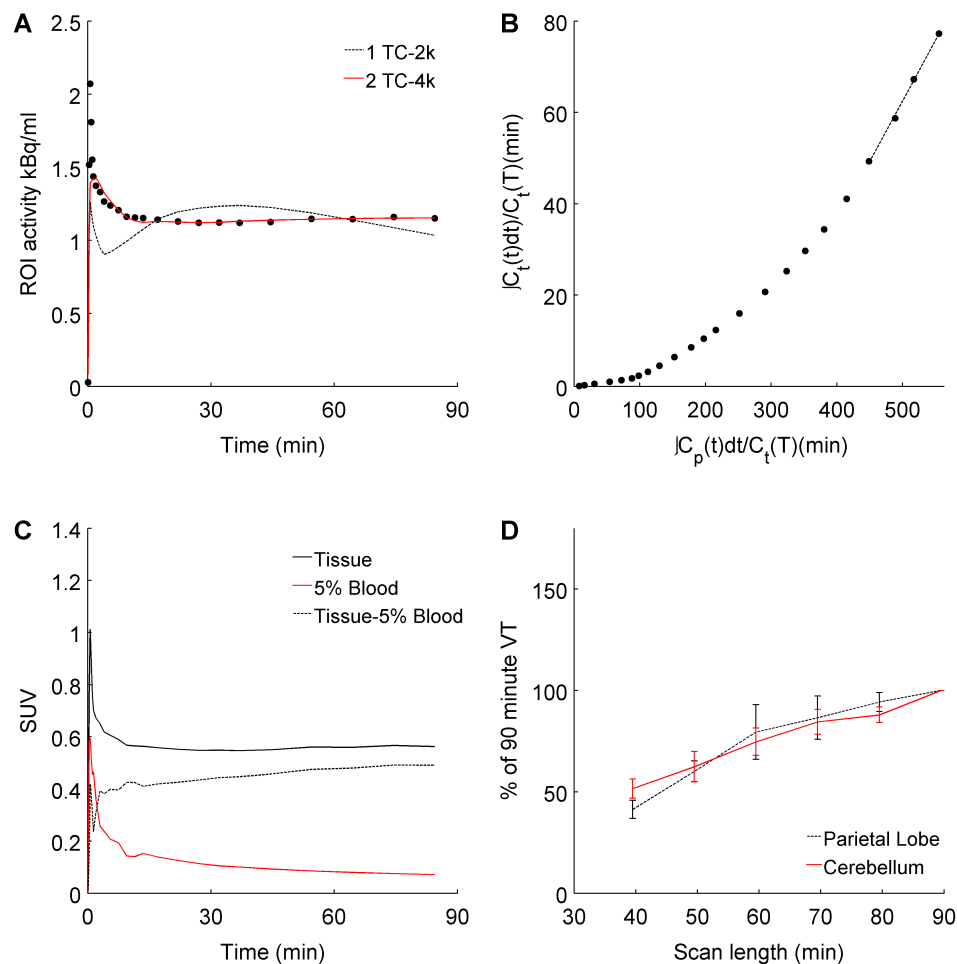
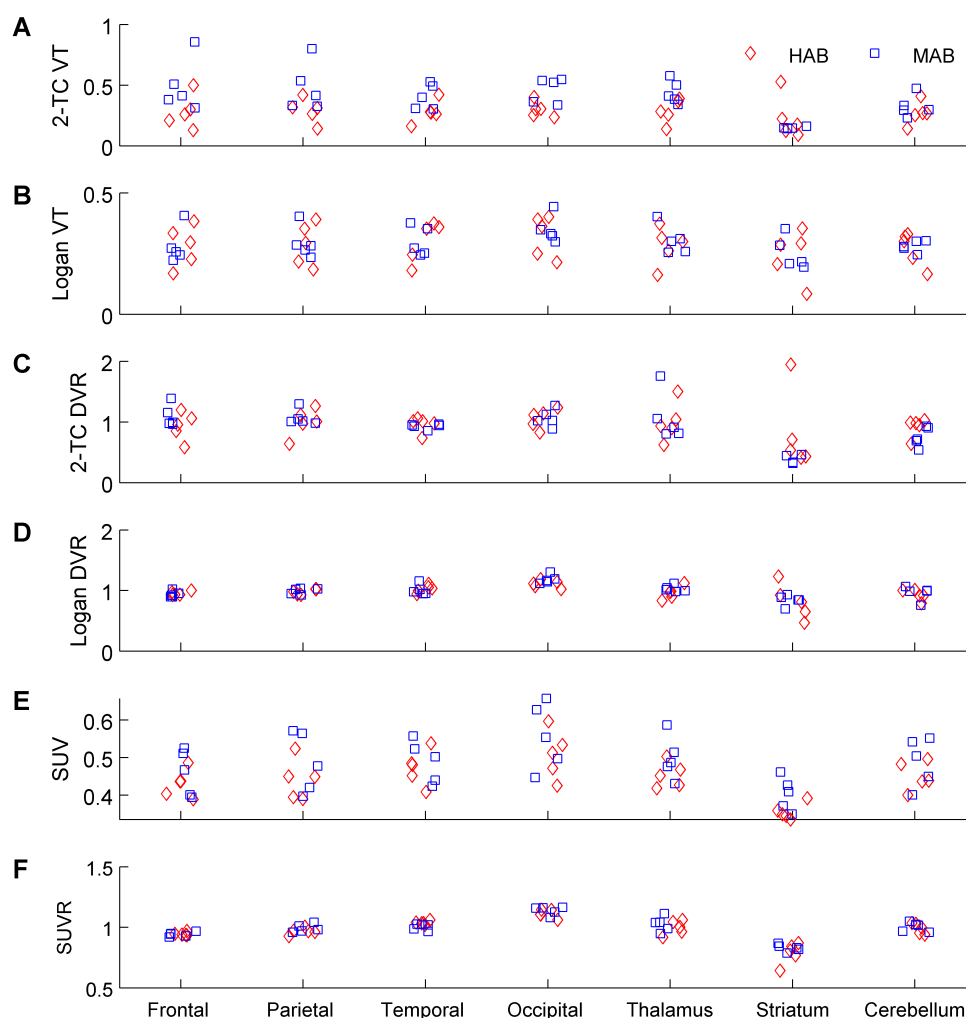


Figure 5. Outcome measures. Six outcome measures are plotted for seven regions of interest, coloured separately for high affinity binders (HAB, red) and medium affinity binders (MAB, blue). (A) Two-tissue-compartmental model (2 TC) volume of distribution (V_T). (B) V_T estimated using Logan plot. (C) Distribution volume ratio (DVR) from 2TC using cortical grey matter as a reference region. (D) DVR estimated using Logan plot and cortical grey matter as a reference region. (E) 60-90 minute standardised uptake values (SUV) (F) 60-90 minute SUV ratios (SUVR) using cortical grey matter as a reference region.



TABLES

Supplementary Table 1. Subject demographics. HAB=high affinity TSPO binders and MAB=mixed affinity TSPO binders

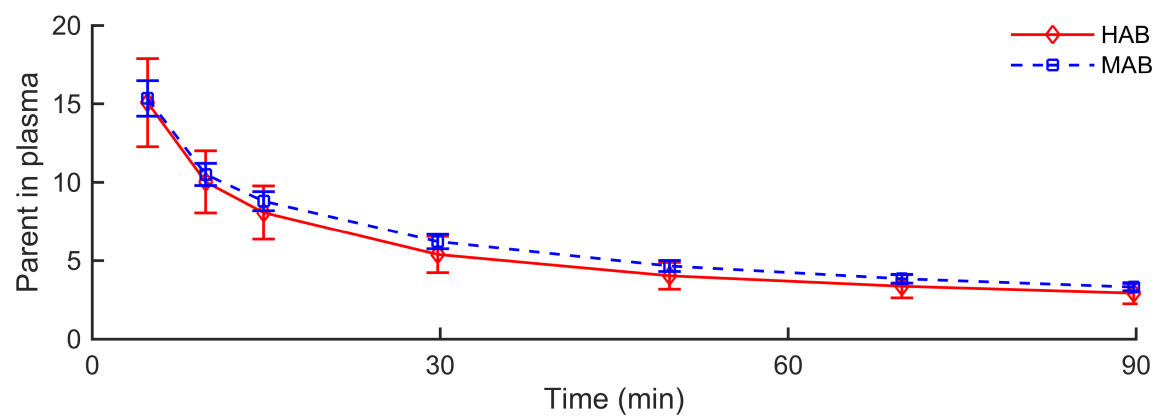
Subject	Genotype	Age (yrs)	Gender	Weight (kg)
1	HAB	47.9	M	97.9
2	HAB	35.5	M	72.1
3	HAB	34.4	M	94.6
4	HAB	48.1	F	69.7
5	HAB	28.4	M	96.6
6	MAB	49.2	M	87.5
7	MAB	39.8	F	70.3
8	MAB	31.0	F	74.2
9	MAB	56.2	M	65.4
10	MAB	39.7	M	90.4

Table 2. Parameter estimates and model fits Parameter estimates and model fits are shown for the one tissue compartment (1-TC), two tissue compartment (2-TC) model and Logan graphical method. Data are presented as median (interquartile range, IQR). For 1-TC versus 2-TC, the right-most column shows the proportion of the ten participants for which the model was the more parsimonious (defined as having the lower akaike information criterion, AIC).

Model	Region	K ₁ (mL/min)	k ₂ (/min)	k ₃ (/min)	k ₄ (/min)	V _T (mL/cm ³)	DVR	AIC wins
1-TC	Frontal Lobe	0.00472(0.0041-0.006)	0.0271(0.023-0.028)			0.171(0.15-0.22)	0.939(0.94-0.95)	2/10
	Parietal Lobe	0.00513(0.004-0.0061)	0.027(0.024-0.028)			0.19(0.16-0.22)	0.969(0.96-1)	1/10
	Temporal Lobe	0.00514(0.0048-0.006)	0.0266(0.025-0.028)			0.182(0.17-0.24)	1.04(1-1)	0/10
	Occipital Lobe	0.00621(0.0057-0.0074)	0.03(0.025-0.032)			0.214(0.19-0.25)	1.12(1.1-1.2)	0/10
	Thalamus	0.00555(0.0046-0.0058)	0.0261(0.024-0.028)			0.182(0.17-0.24)	1.03(0.96-1.1)	0/10
	Striatum	0.00358(0.003-0.0046)	0.0241(0.021-0.025)			0.155(0.14-0.2)	0.826(0.78-0.89)	8/10
	Cerebellum	0.00656(0.0054-0.0076)	0.0339(0.034-0.034)			0.178(0.16-0.22)	0.968(0.91-1)	0/10
2-TC	Frontal Lobe	0.0102(0.0089-0.013)	0.195(0.15-1.6)	0.0301(0.024-0.15)	0.00653(0.004-0.017)	0.346(0.26-0.5)	0.984(0.96-1.2)	8/10
	Parietal Lobe	0.0116(0.011-0.014)	0.192(0.15-0.25)	0.0334(0.025-0.039)	0.00873(0.0045-0.014)	0.33(0.31-0.42)	1.01(0.98-1.1)	9/10
	Temporal Lobe	0.0143(0.012-0.032)	0.217(0.16-1.3)	0.0348(0.03-0.15)	0.00927(0.0063-0.016)	0.306(0.27-0.42)	0.958(0.93-1)	10/10
	Occipital Lobe	0.0238(0.019-0.031)	0.385(0.2-0.62)	0.0395(0.036-0.054)	0.0101(0.0062-0.014)	0.35(0.3-0.52)	1.07(0.97-1.1)	10/10
	Thalamus	0.0118(0.011-0.014)	0.16(0.14-0.2)	0.0315(0.026-0.042)	0.00881(0.0062-0.012)	0.376(0.28-0.41)	0.922(0.81-1.1)	10/10
	Striatum	0.00413(0.0033-0.0088)	1.14(0.66-1.7)	4.89(0.62-9.1)	0.14(0.027-0.21)	0.155(0.14-0.22)	0.451(0.41-0.71)	2/10
	Cerebellum	0.0224(0.016-1.3)	0.319(0.23-78)	0.0331(0.029-0.091)	0.0113(0.0084-0.018)	0.281(0.25-0.33)	0.916(0.69-0.98)	10/10
Logan	Frontal Lobe					0.265(0.23-0.33)	0.931(0.92-0.97)	
	Parietal Lobe					0.284(0.23-0.35)	0.996(0.93-1)	
	Temporal Lobe					0.312(0.24-0.36)	1.01(0.95-1.1)	
	Occipital Lobe					0.34(0.3-0.39)	1.14(1.1-1.2)	
	Thalamus					0.3(0.26-0.31)	0.991(0.98-1)	
	Striatum					0.25(0.21-0.29)	0.844(0.7-0.92)	
	Cerebellum					0.29(0.24-0.3)	0.99(0.91-1)	

Supplementary Figure 1

Parent in plasma for groups HABs and MABs over time



Supplementary Figure 2

First 10 minutes of Figure 4A and 4C shown in more detail:

(A) Model fits are shown for one-tissue compartmental-model (reversible 1TC) black dashed line and two-tissue compartmental-model (reversible 2TC), red solid line) against a time activity curve (TAC) (black dots) for the Parietal Lobe. (C) Parietal Lobe TAC (solid black line), 5% whole blood activity curve (red solid) and difference of the two curves (black dashed) highlights the significant contribution of blood signal to the TAC.

