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Test-retest reproducibility of the metabotropic glutamate receptor 5 ligand [^{18}F]FPEB with bolus plus constant infusion in human

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Abstract

Purpose—[^{18}F]FPEB is a promising PET radioligand for the metabotropic glutamate receptor 5 (mGluR5), a potential target for the treatment of neuropsychiatric diseases. The purpose of this study was to evaluate the test-retest reproducibility of [^{18}F]FPEB in the human brain.

Methods—Seven healthy male subjects were scanned twice, 3–11 weeks apart. Dynamic data were acquired using bolus plus infusion of 162 ± 32 MBq [^{18}F]FPEB. Four methods were used to estimate volume of distribution (V_T): equilibrium analysis (EQ) using arterial (EQ_A) or venous input data (EQ_V), MA1, and two-tissue compartment model (2T). Binding potential (BP_{ND}) was also estimated using cerebellar white matter (CWM) or grey matter (CGM) as a reference region using EQ, 2T and MA1. Absolute test-retest variability (aTRV) of V_T and BP_{ND} were calculated for each method. Venous blood measurements (C_V) were compared with arterial input (C_A) to examine their usability for EQ analysis.

Results—Regional V_T estimated by the four methods displayed a high degree of agreement (r^2 ranging from 0.83 to 0.99 between methods), although EQ_A and EQ_V overestimated V_T by a mean of 9% and 7%, respectively, compared to 2T. Mean aTRV of V_T were 11% by EQ_A, 12% by EQ_V, 14% by MA1 and 14% by 2T. Regional BP_{ND} also agreed well between methods and mean aTRV of BP_{ND} was 8–12% (CWM) and 7–9% (CGM). Venous and arterial blood concentrations of [^{18}F]FPEB were well matched during equilibrium ($C_V = 1.01 \cdot C_A$, $r^2 = 0.95$).

Conclusion—[^{18}F]FPEB binding shows good test-retest variability with minor differences between analysis methods. Venous blood can be used as an alternative for input function

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CONFLICT OF INTEREST

Authors report no conflict of interest relevant to this article.

measurement instead of arterial blood in EQ analysis. Thus, [^{18}F]FPEB is an excellent PET imaging tracer for mGluR5 in humans.

Keywords

[^{18}F]FPEB; human; metabotropic glutamate receptor 5; positron emission tomography; reproducibility

INTRODUCTION

Glutamate is the most abundant excitatory neurotransmitter in the human brain, mediating 70–90% of synaptic transmission [1]. Physiological functions regulated by glutamate include brain development, learning and memory, and long-term potentiation [1–3]. Not surprisingly, dysfunction in the glutamate system is associated with a variety of pathologic conditions such as drug addiction, schizophrenia, anxiety, mood disorders, pain, epilepsy, stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease, and Fragile X syndrome [4, 5].

Glutamate receptors are classified into ionotropic receptors (iGluRs) and metabotropic receptors (mGluRs). iGluRs mediate fast excitatory neurotransmission and NMDA, AMPA, and kainate receptors fall into this category; while the mGluRs mediate slower, modulatory neurotransmission and are comprised of eight subtypes (mGluR1-8) organized into three groups (I, II, and III) based on their pharmacological and signal transduction properties. mGluRs are coupled to their associated iGluRs by a second messenger cascade and modulate function of iGluRs [6, 7]. iGluRs have received great attention as therapeutic targets for drug addiction and various forms of relapse-like behavior based on evidence from animal studies. However, iGluR antagonists have shown serious side effects in humans and interest has shifted to modulating glutamate transmission via mGluRs [5].

Group I mGluRs (mGluR1 and 5) are mainly located postsynaptically at the periphery of postsynaptic densities, while group II and III mGluRs are present primarily in the presynaptic terminals [1, 8]. mGluR5 is structurally linked to the NMDA receptor; it produces positive modulation of NMDA receptor function, and activation of NMDA receptors enhances mGluR5 responses [5, 6, 9]. This synergistic action activates a number of Ca^{2+} -regulated proteins and leads to an increase in AMPA receptor activity [6, 9]. Selective mGluR5 ligands have been shown or are believed to have therapeutic potential for neuropsychiatric diseases through this receptor crosstalk. For example, mGluR5 agonists and positive allosteric modulators are believed to improve memory function in Alzheimer's disease by increasing neuronal excitability and synaptic plasticity, while mGluR5 antagonists and negative allosteric modulators could be potential treatments for epilepsy and multiple sclerosis by way of their neuroprotective effects against glutamate excitotoxicity [4].

The ability to detect changes in mGluR5 availability *in vivo* could be valuable for elucidating functional and dysfunctional glutamate transmission in the brain and facilitating therapeutic drug development targeting mGluR5. [^{18}F]FPEB is a promising PET radioligand that binds to mGluR5 with high specificity and selectivity [10, 11]. The purpose of this

study was to evaluate test-retest reproducibility of [^{18}F]FPEB binding using high resolution PET imaging in healthy humans. Kinetic modeling and equilibrium analysis methods to quantify [^{18}F]FPEB binding were evaluated. Specifically, we tested if a shorter equilibrium measurement, instead of a 2-hour dynamic PET scan, was sufficient to derive reliable binding parameters. Venous input functions were also compared with arterial data for equilibrium analysis to examine their usability in estimating [^{18}F]FPEB volume of distribution (V_T).

MATERIALS AND METHODS

Subjects

Seven healthy male subjects (mean age 27 ± 7 y, range 19 – 38 y, body weight 83 ± 17 kg, body mass index 26 ± 4 kg/m²) were studied under a protocol approved by the Yale Human Investigation Committee, the Yale-New Haven Hospital Radiation Safety Committee, and the Yale University Radiation Safety Committee. Subjects were examined by a physician to exclude major medical conditions or neurologic disorders. Electrocardiography, complete blood counts, serum chemistries, thyroid function test, liver function test, urinalysis and urine toxicology screening were performed during screening. The Structured Clinical Interview for DSM-IV axis I Disorders (SCID-CV) [12] was administered to rule out axis I psychiatric disorders. Written informed consent was obtained from all subjects before the study after complete explanation of the study procedures.

Brain magnetic resonance imaging

Brain magnetic resonance (MR) scans were collected using a 3T scanner (Trio, Siemens Medical Systems, Erlangen, Germany) for registration with PET and region-of-interest (ROI) definition. MR scans were performed within 2–3 weeks of the first PET scan, except in one subject whose MR scan occurred 3 months before the first PET scan. A 3D T1-weighted gradient-echo (MPRAGE) with 1 mm³ isometric resolution (FA=7°, TE=3.34 ms, TI=1100 ms, and TR=2500 ms) was used.

Brain [^{18}F]FPEB PET scanning

[^{18}F]FPEB was synthesized using a previously published method [13]. Average specific activity was 195 ± 47 MBq/nmol ($n = 14$) at the end of synthesis. To evaluate test-retest variability, two scans were performed on separate days, 3 to 11 weeks apart. All procedures for the test and retest scans were identical. PET scans were acquired on a High Resolution Research Tomograph (HRRT) scanner (Siemens/CTI, Knoxville, TN), with spatial resolution (full width at half maximum) of 2–3 mm. A radial artery catheter was inserted at the wrist area to measure the metabolite-corrected input function. A venous catheter was inserted at the antecubital area on the same side as the arterial catheter for intravenous administration of [^{18}F]FPEB. Another venous catheter was placed on the contralateral side for venous sampling to compare the venous blood-derived input function with the arterial blood-derived input function.

Before the scan, an optical motion-tracking tool was fastened to the subject's head via a Lycra swim cap. A 6-min transmission scan using an orbiting ^{137}Cs point-source was

obtained for attenuation correction. Emission data were acquired in list mode for 120 min along with bolus plus infusion (B/I) administration of [^{18}F]FPEB. The injected dose was 162 ± 32 MBq with specific activity of 145 ± 50 MBq/nmol at the time of injection ($n = 14$). The injected mass was 0.32 ± 0.14 μg . For the B/I paradigm, a K_{bol} value of 190 min was used based on our previous study [14]. For [^{18}F]FPEB administration, PTFE tubing was used because [^{18}F]FPEB was found to stick to typical plastic catheter tubing during the slow infusion, which prevented the full dose delivery of [^{18}F]FPEB. Dynamic scan data were reconstructed with corrections for attenuation, normalization, randoms, scatter, deadtime, and motion (Vicra, NDI Systems, Waterloo, Ontario), using the ordered-subset expectation maximization (OSEM)-based MOLAR algorithm [15].

Input function measurement

The arterial input function was obtained from the time-activity curve (TAC) of the metabolite-corrected arterial concentration of [^{18}F]FPEB (C_A), with measurement of plasma free fraction (f_p) and unchanged fraction of [^{18}F]FPEB. Arterial blood samples were taken immediately prior to [^{18}F]FPEB injection and during the scan. To measure f_p , triplicate 300 μL aliquots of plasma separated from blood collected prior to tracer injection and mixed with the radiotracer were pipetted into ultrafiltration units and centrifuged at room temperature (20 min at 1228 g). Plasma and ultrafiltrate activities were counted, and f_p was calculated as the ratio of ultrafiltrate activity to total plasma activity. The same procedure was applied to arterial and venous blood.

For the first 7 min postinjection, continuous arterial blood activity measurements were made with an automated blood counting system (PBS-101, Veenstra Instruments, Joure, The Netherlands). Thirteen individual blood samples were drawn at 3, 8, 12, 15, 20, 25, 30, 45, 60, 75, 90, 105, and 120 min after the administration of [^{18}F]FPEB, and whole blood and plasma were counted. HPLC was performed at selected time points and the parent fraction was determined by the ratio of counts in the parent peak to the total counts collected. The parent fractions were fitted to a 3-parameter function (1 exponential plus a constant) bounded to be ≤ 1 . The final metabolite-corrected arterial concentration of [^{18}F]FPEB was calculated from the product of plasma radioactivity and the parent fractions.

Full arterial input functions were not available for retest scans of three subjects, because two subjects were unable to tolerate arterial lines and the other subject had failure in the automated blood counting system (arterial samples during the equilibrium period were available for this subject). For these 3 cases, the input functions used for equilibrium analysis using arterial data (EQ_A), two-tissue compartment model (2T), and multilinear analysis 1 (MA1) were calculated from each subject's test data, scaled by the ratio of injected doses in the test and retest scans.

Venous input functions were obtained and compared with arterial input function, in order to determine if less invasive venous blood samples could be used instead of arterial ones when estimating V_T using equilibrium analysis (EQ). The parent fractions were averaged from 90 to 120 min. The venous input function was obtained from the time-activity curve (TAC) of the metabolite-corrected venous concentration of [^{18}F]FPEB (C_V). This curve was only

obtained for the latter portion of the curve, specifically from five individual blood samples manually drawn at 60, 75, 90, 105, and 120 min.

The relative difference in f_p between arterial and venous blood was calculated as $[(\text{arterial } f_p - \text{venous } f_p) / \text{arterial } f_p] \cdot 100 (\%)$. The relative difference between C_A and C_V was calculated as $[(C_A - C_V) / C_A] \cdot 100 (\%)$.

ROI delineation

ROIs from the AAL (Anatomical Automatic Labeling for SPM2) template were used. In order to compute TACs for these ROIs, summed PET images from 0 to 10 min post-injection (p.i.) were registered to each subject's T1-weighted MR images using a six-parameter mutual information algorithm (FLIRT, FSL 3.2, Analysis Group, FMRIB, Oxford, UK), which was then registered to the MR template by nonlinear transformation using the Bioimagesuite software (version 2.5; <http://www.bioimagesuite.com>). The ROIs were: caudate (16 cm³), cerebellar grey matter (CGM, 84 cm³), cerebellar white matter (CWM, 6 cm³), anterior cingulate cortex (22 cm³), posterior cingulate cortex (6 cm³), frontal cortex (256 cm³), hippocampus (15 cm³), occipital cortex (81 cm³), pallidum (5 cm³), parietal cortex (65 cm³), putamen (17 cm³), temporal cortex (172 cm³), and thalamus (17 cm³).

Kinetic analysis

The distribution volume (V_T) of [¹⁸F]FPEB was estimated by four different analysis methods: 2 equilibrium analysis [16, 17] methods using arterial and venous blood data (EQ_A and EQ_V, respectively), 2T, and MA1 ($t^* = 30$ min) [18]. With EQ, once equilibrium is achieved in the tissue and the blood, V_T can be measured directly from the concentration ratio of a tracer in the tissue (C_T) to that in plasma. V_T was measured as the ratio of the average C_T and C_A values from 90 to 120 min p.i. (EQ_{A,90–120}). Different time windows were also evaluated, in order to test if earlier and/or shorter scans are feasible. Specifically, 30-min time windows were tested by shifting the starting time by 10 min down to 60 min (80–110 min, 70–100 min, and 60–90 min). Fifteen-minute time windows of 105–120 min, 90–105 min, 75–90 min, and 60–75 min were also tested. V_T was also measured using venous data (EQ_{V,90–120}). The quality of equilibrium was assessed by calculating the rate of change (% change/h) for C_T , C_A , and C_V .

In addition, V_T corrected for f_p (V_T/f_p) was calculated from the four analysis methods, in order to test if f_p correction affected test-retest reliability.

Even though a validated reference region is not available for [¹⁸F]FPEB [19, 20], it is sometimes the case that calculation of an apparent binding potential using a region with a small quantity of specific binding can be useful. Therefore, apparent binding potential

(BP_{ND}) was calculated as $\frac{V_T}{V_{T,CWM}} - 1$ using CWM as a reference region. This region showed the lowest uptake of [¹⁸F]FPEB in our previous study [14]. BP_{ND} was also calculated using CGM as a reference region for comparison.

Test-retest reliability

To evaluate the test-retest reliability of [^{18}F]FPEB binding parameters, percent test-retest variability (TRV) of V_T , V_T/f_p , and BP_{ND} were calculated for each subject:

$$TRV(\%) = \frac{(T - RT)}{\left(\frac{T + RT}{2}\right)} * 100$$

where T and RT refer to values from the test and retest scans, respectively.

Statistics to evaluate test-retest reliability of V_T , V_T/f_p , and BP_{ND} of [^{18}F]FPEB were mean and standard deviation (SD) of TRV, and mean of absolute TRV (aTRV). Both relative and absolute TRV were evaluated since they have been used in the literature previously. Mean TRV estimates any systematic trend of [^{18}F]FPEB binding values between the test and the retest scans; if binding values across the ROIs tend to be higher in the test scan than the retest, mean TRV will be positive. If there is no such trend, it will be 0. SD of TRV estimates uncertainty in the percent change between the two scans. Mean of aTRV combines both trend and variability of change in [^{18}F]FPEB binding, and thus can be complex if there is a systematic trend. In the absence of such a trend, the mean of aTRV is approximately equal to the percent uncertainty in one single scan value, and is thus smaller than the SD of TRV, which is the percent uncertainty in the difference between the two scans. The intersubject variability was measured by the coefficient of variation (CoV) for all ROIs.

Statistical analysis

Paired t-tests were used to compare [^{18}F]FPEB injection parameters, including injected dose, specific activity, injected mass, f_p , V_T , V_T/f_p and BP_{ND} between the test and the retest studies. A p value less than 0.05 was considered statistically significant.

RESULTS

Injection parameters were similar between the test and retest scans (Table 1). Mean tissue time-activity curves of all ROIs from the 14 test and retest scans are shown in Figure 1. High uptake areas were the anterior cingulate, frontal, temporal, parietal, and occipital cortices, hippocampus, caudate nucleus, and putamen. Medium uptake was observed in the posterior cingulate cortex, pallidum, and thalamus. Uptake was low in the CGM and the lowest in CWM.

For most regions, equilibrium was reached by 90 min. In all regions except CWM, the rate of change of C_T between 90 and 120 min was $-5 \pm 9\%/h$ (mean $\pm$ SD), which was smaller in high-uptake regions (mean of $-3\%/h$) than medium-uptake regions (mean of $-8\%/h$). For CWM, C_T change rate was $-15 \pm 26\%/h$.

Comparison of 2T, MA1, and EQ methods for V_T calculation

Regional V_T estimates derived from four different analysis methods are shown in Table 2. By any method, anterior cingulate cortex had the highest V_T and cerebellar white matter had the lowest among the ROIs examined. Values of V_T estimated by different methods agreed

well with each other (Figure 2). For example, V_T values estimated by MA1 had excellent correlation with those estimated by 2T ($V_{T(MA1)} = 0.99 \cdot V_{T(2T)}$, $r^2 = 0.99$). Values of V_T estimated by $EQ_{A,90-120}$ also had good correlation with those from 2T ($V_{T(EQ_{A,90-120})} = 1.09 \cdot V_{T(2T)}$, $r^2 = 0.93$), although $EQ_{A,90-120}$ overestimated V_T in all ROIs by a mean of 9%. Values of V_T estimated by $EQ_{V,90-120}$ had somewhat worse correlation with those by 2T ($V_{T(EQ_{V,90-120})} = 1.07 \cdot V_{T(2T)}$, $r^2 = 0.83$), and overestimated V_T in all ROIs by a mean of 7%. The intersubject variability measured by mean CoV across the ROIs was smallest for 2T (13.7%) and MA1 (13.5%), and largest for $EQ_{V,90-120}$ (22.2%). Mean CoV for $EQ_{A,90-120}$ was 18.2%. Regional BP_{ND} values derived from three methods using CWM as reference region are shown in Table S1 and Figure S1 of the Online Resource.

Test-retest variability (TRV) of V_T

Mean TRV of V_T ranged from -13% to -9% for 2T, from -12% to -9% for MA1, from -6% to -2% for $EQ_{A,90-120}$, and from 0% to 4% for $EQ_{V,90-120}$ across the ROIs (Table 3). $EQ_{A,90-120}$ and $EQ_{V,90-120}$ had the smallest mean TRV. Test V_T values were lower than those of retest in most ROIs of 6/7 subjects analyzed by 2T and MA1, but this difference was not significant.

SD of TRV ranged from 13% to 18% for 2T, 11% to 16% for MA1, 11% to 18% for $EQ_{A,90-120}$, and 13% to 22% for $EQ_{V,90-120}$ (Table 4). Between methods there was little overall difference in SD of TRV. Mean aTRV ranged from 12% to 16% for 2T and MA1, and 9% to 12% for $EQ_{A,90-120}$, and 10% to 16% for $EQ_{V,90-120}$ (Table 5). The EQ methods thus provided slightly better results than the kinetic methods. TRV of BP_{ND} values are shown in the Table S2 of the Online Resource.

Plasma free fraction correction

Plasma free fraction (f_p) values were available from 12 scans in 7 subjects for the arterial blood, while all 14 values were available for the venous blood. In the test scans, f_p values were $5.3\% \pm 0.9\%$ and $4.7\% \pm 0.9\%$, respectively, in the arterial and venous blood ($p = 0.182$), while arterial and venous f_p values were $5.1\% \pm 0.3\%$ and $4.8\% \pm 1.0\%$, respectively, in the retest scans ($p = 0.723$). The overall relative difference in f_p between the arterial and venous blood was $5.0\% \pm 15.9\%$ (data from 12 studies of 7 subjects). Mean TRV, SD of TRV, and mean of aTRV of f_p were -6%, 14%, and 13% for arterial blood and -2%, 33%, and 25% for the venous blood, respectively.

When V_T was corrected for f_p (V_T/f_p), the intersubject variability was similar to that of V_T . Mean CoV across the ROIs were 14.5% for 2T, 14.5% for MA1, 18.0% for $EQ_{A,90-120}$, and 21.1% for $EQ_{V,90-120}$. With V_T/f_p , mean TRV was improved for 2T, MA1, and $EQ_{A,90-120}$, across almost all ROIs, but SD of TRV and mean aTRV were increased (Tables 3 through 5).

Comparison of arterial and venous concentration of [^{18}F]FPEB

The arterial and the venous plasma concentrations of [^{18}F]FPEB reached a common level during equilibrium. The correlation was excellent and the venous concentration (C_V) was 1% higher than the arterial concentration (C_A) at 90–120 min p.i. ($C_V = 1.01 \cdot C_A$, $r^2 = 0.95$, Figure 3(a)). In fact, the agreement of the arterial and the venous concentrations occurred by

~ 60 min p.i. The relative differences were $2.8\% \pm 9\%$ (mean $\pm$ SD) at 60–65 min p.i. and $-0.3\% \pm 9\%$ at 115–120 min p.i. (Figure 3(b)).

EQ analysis using various time windows

Earlier time windows to estimate V_T by EQ_A and EQ_V were evaluated to see if scanning could be moved earlier. Time windows of 80–110 min, 70–100 min, and 60–90 min were examined. With both EQ_A and EQ_V , V_T estimated using 60–90 min p.i. data demonstrated excellent correlation with $V_{T,90-120}$ ($V_{T,60-90} = 1.00 \cdot V_{T,90-120}$, $r^2 = 0.96$ with EQ_A ; $V_{T,60-90} = 1.02 \cdot V_{T,90-120}$, $r^2 = 0.98$ with EQ_V). The percent difference between the 60–90 and 90–120 min values was $-1\% \pm 7\%$ with EQ_A and $-3\% \pm 5\%$ with EQ_V . Shorter time windows were also evaluated to test if scanning time could be shortened. Fifteen-minute time windows of 105–120 min, 90–105 min, 75–90 min, and 60–75 min were examined. The correlation with $V_{T,90-120}$ was excellent even for $V_{T,60-75}$ ($V_{T,60-75} = 0.99 \cdot V_{T,90-120}$, $r^2 = 0.97$ with EQ_A , Figure 4; $V_{T,60-75} = 1.01 \cdot V_{T,90-120}$, $r^2 = 0.97$ with EQ_V). The difference between $V_{T,60-75}$ and $V_{T,90-120}$ was $0\% \pm 6\%$ with EQ_A and $-3\% \pm 7\%$ with EQ_V . Both earlier and shorter time windows did not affect the reproducibility of $[^{18}F]FPEB$ V_T . For example, with EQ_A , the mean TRV, SD TRV and mean of aTRV were -2% , 16% , and 12% for $V_{T,60-90}$ and -1% , 15% , and 12% for $V_{T,60-75}$, compared to -4% , 14% , and 11% for $V_{T,90-120}$. Also, the quality of V_T images created using earlier and shorter time windows was comparable to those using 90–120 min data (Figure 5). Characteristics of BP_{ND} with shorter and earlier time windows are shown in Figure S2 of the Online Resource.

DISCUSSION

The purpose of this study was to evaluate the test-retest reproducibility of $[^{18}F]FPEB$ binding parameters in the human brain using high resolution PET with bolus plus constant infusion of the radiotracer. Equilibrium analysis using arterial plasma concentration (EQ_A) provided $[^{18}F]FPEB$ binding parameters with good reproducibility: mean aTRV of 11% for V_T and 12% for BP_{ND} across the brain areas examined. The 2T and MA1 kinetic models produced slightly worse reproducibility than EQ_A . Regional V_T values and their reproducibility obtained in the present study agreed well with the results of a previous study in human using bolus injections of $[^{18}F]FPEB$ [21]. Reproducibility of $[^{18}F]FPEB$ binding parameters appear to be better than that of $[^{11}C]ABP688$, another mGluR5 radioligand widely used in human. High variability was reported with $[^{11}C]ABP688$ in human, with mean aTRV of up to 20% for BP_{ND} [22], or from 23 to 73% [23] in each region. Results from test-retest studies in human have not been published to date with other mGluR5 radioligands such as $[^{18}F]SP203$ or $[^{18}F]PSS232$.

Regional V_T values estimated by four different methods agreed well with each other. Compared to 2T, $EQ_{A,90-120}$ and $EQ_{V,90-120}$ overestimated V_T in all ROIs by a mean of 9% and 7% , respectively. Values of BP_{ND} estimated by three different methods also agreed well with each other. Compared to 2T, EQ_{90-120} underestimated BP_{ND} by a mean of 11% . This underestimation of regional BP_{ND} results from overestimation of CBW V_T by $EQ_{A,90-120}$ and $EQ_{V,90-120}$ (19% and 17% , respectively), which was larger than the mean

overestimation of the other ROIs (9% and 7%, respectively). These results are consistent with our previous study [14].

In principle, V_T corrected for plasma free fraction (f_p) of a radioligand (V_T/f_p) is a more accurate estimate for receptor binding than V_T itself, because V_T values should be linearly proportional to f_p . Correction for f_p is particularly useful if there are between-group differences in f_p and if the f_p measurement is reliable. However, V_T/f_p is not always a more useful measure than V_T , especially if additional noise is introduced by including f_p , such as when this measurement is not reliable. The f_p of [^{18}F]FPEB is relatively low and [^{18}F]FPEB appears to be a highly lipophilic (“sticky”) compound that adsorbs to the ultrafiltration devices used for f_p analysis, as well as to certain infusion tubing. This may explain the larger intersubject variability of V_T/f_p compared to that of V_T in this study.

As is often the case in multi-day studies, full arterial blood samples were not available for the retest scans of 3 subjects. For these scans, input functions were created by scaling the arterial curve from the test study by the ratio of the injection doses. The mean and SD of TRV of V_T by 2T and MA1 were similar between these 3 subjects who used scaled input function for their retest studies (‘scaled input group’) and the 4 subjects who had original arterial input function for both test and retest studies (‘original input group’). However, mean TRV of V_T by $\text{EQ}_{A,90-120}$ was larger in absolute value for the scaled input group compared to the original input group (−9% vs. −1%). This does not appear to be due to scaling, because the mean TRV of V_T by $\text{EQ}_{V,90-120}$ was also larger for the former compared to the latter (7% vs. −2%; venous input functions were original for all subjects.).

The arterial and the venous plasma concentrations of [^{18}F]FPEB reached a common level during equilibrium. The correlation between them was excellent and the venous concentrations were only 1% higher than the arterial concentration on average at 90–120 min. This result is different from that of our previous study, which observed a mean 7% lower concentration in the venous plasma [14]. The difference may have originated from two sources. Study subjects were different and those who participated in the previous study had tissue TACs that were mostly increasing during the 90–120 min period, while subjects in the present study had stable or somewhat decreasing TACs. In addition, the fitting method for metabolite analysis in the venous blood was simplified in the current analysis (see Methods).

Equilibrium was achieved in most regions. The mean negative rate of concentration change ($-5 \pm 9\%/h$) between 90 to 120 min suggests that, in this population, the bolus portion of the tracer delivery (K_{bol}) might be slightly too large. Interestingly, V_T from EQ methods showed better stability, with mean % difference of 60–90 vs. 90–120 min values of −1% and −3% for EQ_A and EQ_V , respectively. This is consistent with theoretical expectations, whereby the magnitude of bias of the tissue-to-plasma ratio compared to true V_T in non-equilibrium conditions is nonlinearly related to plasma and tissue clearance rates [16]. Note that CWM showed poorer equilibrium, which is not surprising since it has the lowest V_T value and also has smaller rate constants, as it is a white matter region. If CWM is used as a reference region, BP_{ND} values do not show the same stability as V_T values, with mean % difference of 60–90 vs. 90–120 min values of +8% (Online Resources), consistent with larger percent reductions in the denominator (CWM) than the numerators. Moving the equilibrium period

later than 120 min would likely allow the CWM to obtain adequate stability, if it is to be used as reference region in estimating BP_{ND} by EQ.

CWM was selected as a reference region in our study, because it showed the lowest V_T (5.7 ± 0.6 with kinetic modeling and 6.7 ± 0.9 with EQ_A). In another [¹⁸F]FPEB study in human [21], pons was used to calculate BP_{ND} , since it was the region with the lowest V_T in that study (~ 5 mL/cm³). CGM V_T was higher than the CWM V_T values, indicating that there is either specific binding in the CGM, or that the non-displaceable volume of distribution V_{ND} is different between the two regions. If the V_{ND} is the same between the two regions, then BP_{ND} in CGM is 0.8 ± 0.1 by 2T, 0.7 ± 0.1 by MA1 and 0.8 ± 0.2 with EQ_A. An *in vitro* binding study revealed small but positive specific binding of [¹⁸F]FPEB in the cerebellar tissue of human as well as rat and rhesus monkey [19]. In vivo blocking study is not yet available for [¹⁸F]FPEB. Similarly, for [¹¹C]ABP688 studies [24–26], CGM has been used as a reference region, but studies indicate the presence of specific binding of [¹¹C]ABP688 in CGM [20, 27, 28]. Thus, based on our findings and other published data, it appears that there is specific mGluR5 binding in the cerebellum. However, it still may be useful to estimate BP_{ND} using the cerebellum as a reference region, so long as investigators clearly spell out their assumptions in its use. In this case, a careful balance must be considered as to whether to use CGM or CWM as a reference region. For example, with [¹⁸F]FPEB, selecting CWM as a reference region may include the least possible specific binding but have poorer test-retest variability than selecting CGM for EQ analysis, due to slower kinetics in the white matter than the grey matter as well as noise effects due to region size differences (see Online Resource Table S2 and S3). To avoid this issue, using V_T as an outcome measure may be advantageous, as it is not influenced by the specific binding in the cerebellum. However, V_T is more sensitive to errors in the input function. BP_{ND} may be a better measure if there is no difference in specific binding between groups or patient states in the reference region. From a test-retest reproducibility point of view, [¹⁸F]FPEB BP_{ND} was better than V_T with 2T and MA1 analyses, while BP_{ND} was equivalent to V_T with EQ. Further, since a portion of the V_T value reflects non-displaceable binding (V_{ND}), comparable TRV of BP_{ND} and V_T means greater signal-to-noise contrast for BP_{ND} , since 100% of the BP_{ND} value is the receptor “signal” (assuming the reference region is correct).

Earlier as well as shorter scanning showed promising results with [¹⁸F]FPEB, in that the 60–75 min results were comparable to those of 90–120 min. The correlation of [¹⁸F]FPEB binding (V_T and BP_{ND} by EQ analysis) was excellent and the test-retest reproducibility was similar between those two time windows. Earlier and shorter scanning could bring more convenience for patients, reduce motion issues during scanning, and enhance efficiency of the scanning schedule. However, it may be important to validate the optimal equilibrium in each patient population.

In this study, using a bolus plus constant infusion, we demonstrated test-retest reproducibility of [¹⁸F]FPEB to be very good and in agreement between venous and arterial blood measurements during equilibrium. Considering this test-retest reproducibility and the simplicity of scanning and analysis at equilibrium that does not require arterial blood sampling, EQ is considered to be the method of choice to estimate mGluR5 availability in the human brain using [¹⁸F]FPEB.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Mean tissue time-activity curves expressed as standard uptake value (n = 14 scans of 7 subjects)

Fig. 2.

Comparison of V_T by different analysis methods. Each point represents regional V_T of each subject (13 ROIs, 14 test and retest studies of 7 subjects). 2T: two-tissue compartment model, MA1: multilinear analysis 1, t^* : starting time, EQ_{A,90-120}: equilibrium analysis using arterial input function from 90 to 120 min p.i., EQ_{V,90-120}: equilibrium analysis using venous input function from 90 to 120 min p.i. Solid lines in the figures are linear fits of data.

Fig. 3. Comparison of arterial versus venous concentration of [^{18}F]FPEB during equilibrium. (a) Arterial-venous differences were calculated every 5 min from 60 to 120 min p.i. Data are from 11 scans in 7 subjects. (b) Each point represents metabolite-corrected plasma concentration of [^{18}F]FPEB averaged from 90–120 min p.i.

Fig. 4.

V_T estimation by the equilibrium method (EQ_A) using an earlier and shorter time window. Each point represents V_T of each ROI of each subject (13 ROIs, 14 studies of 7 subjects).

Fig. 5.
 V_T parametric images of a representative subject created with EQ analysis using data from 90 to 120 min (upper panel) and from 60 to 75 min (lower panel) p.i.

Table 1

Study parameters

Injection parameters	Test	Retest	<i>p</i> value
Specific activity at the end of synthesis (MBq/nmol)	198 ± 40	192 ± 56	0.86
Specific activity at the time of injection (MBq/nmol)	155 ± 42	135 ± 59	0.55
Injection dose (MBq)	164 ± 27	161 ± 38	0.87
Injection mass (µg)	0.28 ± 0.08	0.36 ± 0.18	0.32

Numbers are mean ± standard deviation, n=7.

Table 2

V_T and $V_T/\bar{f}_p$ results by different analysis methods.

ROI	V_T					$V_T/\bar{f}_p$				
	2T	MA1 ($r^*=30$)	EQ _A (90–120 min)	EQ _V (90–120 min)	2T	MA1 ($r^*=30$)	EQ _A (90–120 min)	EQ _V (90–120 min)	EQ _V (90–120 min)	EQ _V (90–120 min)
anterior cingulate	30.4 ± 4.1	30.0 ± 3.7	31.8 ± 5.8	31.3 ± 6.9	569 ± 64	564 ± 66	599 ± 97	678 ± 144		
posterior cingulate	16.2 ± 3.8	16.5 ± 3.8	19.1 ± 5.3	18.8 ± 5.8	309 ± 75	311 ± 77	362 ± 105	405 ± 106		
frontal	23.2 ± 3.0	22.9 ± 2.7	25.8 ± 4.2	25.4 ± 5.5	434 ± 45	431 ± 43	486 ± 67	553 ± 125		
temporal	27.3 ± 4.2	26.7 ± 3.7	28.6 ± 5.3	28.1 ± 6.5	514 ± 70	501 ± 59	537 ± 85	611 ± 143		
parietal	23.2 ± 3.8	23.0 ± 3.5	25.9 ± 5.2	25.6 ± 6.4	438 ± 62	431 ± 53	486 ± 83	557 ± 147		
occipital	22.4 ± 3.6	22.4 ± 3.5	25.3 ± 5.1	24.9 ± 6.3	423 ± 54	420 ± 54	475 ± 83	542 ± 142		
hippocampus	23.4 ± 3.1	23.4 ± 3.0	23.3 ± 4.3	22.9 ± 4.9	448 ± 69	443 ± 70	440 ± 83	496 ± 100		
caudate nucleus	25.1 ± 2.9	24.9 ± 3.0	27.2 ± 4.7	26.7 ± 5.3	478 ± 85	474 ± 86	516 ± 112	577 ± 89		
putamen	26.1 ± 3.3	25.7 ± 3.0	28.9 ± 4.6	28.3 ± 5.3	492 ± 70	487 ± 70	546 ± 92	614 ± 100		
pallidum	14.9 ± 2.0	14.7 ± 1.9	16.9 ± 2.9	16.7 ± 3.6	278 ± 37	277 ± 38	320 ± 51	362 ± 76		
Thalamus	17.0 ± 1.9	17.1 ± 1.8	20.0 ± 3.0	19.7 ± 3.7	321 ± 36	322 ± 38	378 ± 56	427 ± 82		
cerebellar grey matter	10.0 ± 1.4	9.9 ± 1.3	11.8 ± 2.0	11.6 ± 2.5	187 ± 28	188 ± 29	222 ± 41	251 ± 50		
cerebellar white matter	5.7 ± 0.6	5.7 ± 0.6	6.7 ± 0.9	6.6 ± 1.2	107 ± 17	109 ± 17	127 ± 23	143 ± 23		

n = 7

Numbers are mean ± standard deviation.

V_T and $V_T/\bar{f}_p$ values were averaged across test and retest scans, and the mean and SD was calculated across the 7 subjects. Retest V_T and $V_T/\bar{f}_p$ by 2T, MA1, and EQA of 3 subjects were estimated using the input function from the test study of each subject scaled by the ratio of the injection doses.

V_T : total volume of distribution, $\bar{f}_p$: plasma free fraction, ROI: region of interest, 2T: two-tissue compartment model, MA1: multilinear analysis 1, t^* : starting time, EQA: equilibrium analysis using arterial input function, EQV: equilibrium analysis using venous input function.

Table 3

Mean Test-Retest Variability (TRV) of V_T and V_T/f_p by different analysis methods.

ROI	Mean TRV of V_T (%)				Mean TRV of V_T/f_p (%)			
	2T	MA1 ($t^*=30$)	EQ _A (90–120 min)	EQ _V (90–120 min)	2T	MA1 ($t^*=30$)	EQ _A (90–120 min)	EQ _V (90–120 min)
anterior cingulate	-10	-9	-3	3	-6	-5	2	5
posterior cingulate	-11	-10	-4	1	-6	-6	0	4
frontal	-11	-11	-4	2	-6	-6	0	4
temporal	-9	-12	-4	2	-5	-7	1	4
parietal	-10	-12	-5	1	-5	-7	0	4
occipital	-12	-12	-5	1	-7	-8	-1	3
hippocampus	-10	-10	-2	4	-6	-5	3	6
caudate nucleus	-13	-12	-4	2	-8	-7	1	4
putamen	-10	-11	-4	2	-5	-6	1	4
pallidum	-12	-12	-6	0	-7	-7	-1	2
thalamus	-10	-10	-4	2	-6	-6	0	4
cerebellar grey matter	-10	-10	-5	1	-5	-5	-1	3
cerebellar white matter	-10	-9	-4	1	-5	-4	0	3
Mean of all ROIs	-11	-11	-4	2	-6	-6	0	4

n = 7

For 2T, MA1 and EQ_A, values of 3 subjects were calculated using the retest V_T estimated using scaled input function from the test study of each subject by the injection dose.

TRV: test-retest variability, ROI: region of interest, 2T: two-tissue compartment model, MA1: multilinear analysis 1, t^* : starting time, EQ_A: equilibrium analysis using arterial input function, EQV: equilibrium analysis using venous input function.

Table 4

SD of Test-Retest Variability (TRV) of V_T and V_T/f_p by different analysis methods.

ROI	SD of TRV of V_T (%)				SD of TRV of V_T/f_p (%)			
	2T	MA1 ($t^*=30$)	EQA (90–120 min)	EQV (90–120 min)	2T	MA1 ($t^*=30$)	EQA (90–120 min)	EQV (90–120 min)
anterior cingulate	17	16	14	13	20	20	20	33
posterior cingulate	17	16	14	16	18	18	19	34
frontal	16	16	14	13	20	19	20	32
temporal	17	14	13	13	21	18	20	34
parietal	13	11	11	14	17	19	17	35
occipital	14	13	12	15	17	16	18	35
hippocampus	16	16	13	13	21	21	20	35
caudate nucleus	13	12	12	15	16	15	17	36
putamen	15	14	13	14	19	19	20	34
pallidum	17	16	14	14	21	21	21	33
thalamus	15	15	14	14	17	18	19	32
cerebellar grey matter	13	12	13	15	16	16	19	35
cerebellar white matter	18	16	18	22	20	18	22	39
Mean of all ROIs	16	14	14	15	19	18	19	34

n = 7

For 2T, MA1 and EQA, values of 3 subjects were calculated using the retest V_T estimated using scaled input function from the test study of each subject by the injection dose.

TRV: test-retest variability, SD: standard deviation, ROI: region of interest, 2T: two-tissue compartment model, MA1: multilinear analysis 1, t^* : starting time, EQA: equilibrium analysis using arterial input function, EQV: equilibrium analysis using venous input function.

Table 5

Mean absolute test-retest variability (aTRV) of V_T and V_T/f_p by different analysis methods.

ROI	Mean aTRV of V_T (%)				Mean aTRV of V_T/f_p (%)			
	2T	MA1 ($r^*=30$)	EQ _A (90–120 min)	EQ _V (90–120 min)	2T	MA1 ($r^*=30$)	EQ _A (90–120 min)	EQ _V (90–120 min)
anterior cingulate	15	14	11	11	17	16	17	25
posterior cingulate	15	14	12	12	16	16	16	26
frontal	16	16	11	10	17	16	17	24
temporal	15	15	11	10	19	16	16	25
parietal	12	13	9	10	16	14	14	26
occipital	14	14	10	11	16	15	16	26
hippocampus	14	14	11	12	19	19	17	27
caudate nucleus	14	14	9	11	14	14	14	25
putamen	14	15	11	12	17	16	16	25
pallidum	16	16	12	12	17	17	17	25
thalamus	14	14	11	11	15	15	16	23
cerebellar grey matter	12	12	11	12	14	14	16	25
cerebellar white matter	14	13	11	16	14	13	16	27
Mean of all ROIs	14	14	11	12	16	15	16	25

n = 7

For 2T, MA1 and EQ_A, values of 3 subjects were calculated using the retest V_T/f_p estimated using scaled input function from the test study of each subject by the injection dose.)

aTRV: absolute test-retest variability, ROI: region of interest, 2T: two-tissue compartment model, MA1: multilinear analysis 1, t^* : starting time, EQ_A: equilibrium analysis using arterial input function, EQ_V: equilibrium analysis using venous input function.