Quantitation of cannabinoid CB₁ receptors in healthy human brain using positron emission tomography and an inverse agonist radioligand

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A B S T R A C T

[¹¹C]MePPEP is a high affinity, CB₁ receptor-selective, inverse agonist that has been studied in rodents and monkeys. We examined the ability of [¹¹C]MePPEP to quantify CB₁ receptors in human brain as distribution volume calculated with the “gold standard” method of compartmental modeling and compared results with the simple measure of brain uptake. A total of 17 healthy subjects participated in 26 positron emission tomography (PET) scans, with 8 having two PET scans to assess retest variability. After injection of [¹¹C]MePPEP, brain uptake of radioactivity was high (e.g., 3.6 SUV in putamen at ~60 min) and washed out very slowly. A two-tissue compartment model yielded values of distribution volume (which is proportional to receptor density) that were both well identified (SE 5%) and stable between 60 and 210 min. The simple measure of brain uptake (average concentration of radioactivity between 40 and 80 min) had good retest variability (~8%) and moderate intersubject variability (16%, coefficient of variation). In contrast, distribution volume had two-fold greater retest variability (~15%) and, thus, less precision. In addition, distribution volume had three-fold greater intersubject variability (~52%). The decreased precision of distribution volume compared to brain uptake was likely due to the slow washout of radioactivity from brain and to noise in measurements of the low concentrations of [¹¹C]MePPEP in plasma. These results suggest that brain uptake can be used for within subject studies (e.g., to measure receptor occupancy by medications) but that distribution volume remains the gold standard for accurate measurements between groups.

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Introduction

Cannabinoid CB₁ receptors, which mediate the psychotropic actions of marijuana, are widely distributed in brain and may well be the most abundant G protein coupled receptor in brain (for review see Howlett et al., 2002). CB₁ receptors are primarily located presynaptically and inhibit the release of several neurotransmitters, including GABA, glutamate, and dopamine. Endogenous transmitters (e.g., "endocannabinoids" such as anandamide and 2-arachidonoylglycerol) are released from postsynaptic sites and act via “retrograde neurotransmission” on presynaptically located CB₁ receptors. The CB₁ receptor is thought to have an important role in normal physiology (e.g., appetite and memory) and may be involved in the pathophysiology of some neuropsychiatric (schizophrenia) (Eggan et al., 2008) and transmission failures (depression, anxiety, and suicidal thoughts) (Jones, 2008).

Clinical research and/or use of two inverse agonists (rimonabant and taranabant) were discontinued in 2008 because of psychiatric side effects (depression, anxiety, and suicidal thoughts) (Jones, 2008).

Development of a positron emission tomographic (PET) radioligand for the CB₁ receptor has been difficult, primarily because of the high lipophilicity of the available ligands. High lipophilicity tends to cause high nonspecific retention in the abundant fat of brain and also causes high binding to plasma proteins, the latter of which limits access of the radioligand to brain. High lipophilicity likely caused the failure of two early radioligands, which had either high nonspecific binding in brain or low delivery to brain (Gatley et al., 1996; Gatley et al., 1998). More recently, three PET radioligands have successfully imaged CB₁ receptors in monkey and/or human brain: [¹⁸F]MK-9470 (Burns et al., 2007), [¹¹C]JHU75528 (Horti et al., 2006; Wong et al., 2008), and [¹¹C]MePPEP (Yasuno et al., 2008). Results quantified with the “gold standard” method of compartmental modeling using concurrent brain and plasma data have been fully reported only for [¹¹C]MePPEP, and that quantitation was done only in animals. Furthermore, prior studies of [¹⁸F]MK-9470 in human subjects quantified CB₁ receptors using only brain uptake, without correction...
for the concentration of radioligand in plasma. Thus, the current study also evaluated the accuracy and precision of brain uptake as a proxy for distribution volume calculated with compartmental modeling.

MePPEP has high affinity ($K_D = 0.14$ nM in human cerebellum; unpublished data) and selectivity for the CB1 receptor (Terry et al., 2008; Yasuno et al., 2008). The majority of brain uptake (65 to 85%) in mice, rats, and monkeys represents specific binding to CB1 receptors. Specific binding was determined by pharmacological blockade or by genetic knockout of the CB1 receptor. Receptor binding in both rat and monkey was quantified with compartmental modeling, using serial measurements of both radioactivity in brain and the concentration of unchanged parent radioligand in plasma.

Similar to our prior experiments in rat and monkey brain, we sought in the current study to evaluate $[^{11}C]$MePPEP in healthy human brain using compartmental modeling. The outcome measure was total distribution volume ($V_T$), which equals the ratio at equilibrium of radioactivity in brain to the concentration of parent radioligand in plasma. We scanned eight subjects for 150 min and found that the intersubject variability (coefficient of variation, COV) of $V_T$ was higher than that of brain uptake, and higher than that of other commonly used PET tracers (10–20%). To evaluate the cause for the high variability in $V_T$, we measured the retest variability within subjects to separately assess the reproducibility of brain and plasma data. We also wondered if a longer acquisition time would provide better identifiability and smaller intersubject variability of $V_T$. Therefore, we scanned for a longer time and surprisingly found that meaningful brain data were still provided at 210 min using a radioligand labeled with $^{11}$C, which has a half-life of only 20.4 min.

**Methods**

**Radioligand preparation**

$[^{11}C]$MePPEP ($\left(3R,5R\right)$)-5-(3-methoxy-phenyl)-3-((R)-1-phenyl-ethylamino)-1-(4-trifluoromethyl-phenyl)-pyrrolidin-2-one) was prepared by $[^{11}C]$methylation of its desmethyl analogue with $[^{11}C]$-iodomethane (Donohue et al., 2008). The preparation is described in detail in our Investigational New Drug Application #79,948, submitted to the US Food and Drug Administration and a copy of which is available at [http://psdp.med.unc.edu/snid/](http://psdp.med.unc.edu/snid/). The radioligand was obtained in high radiochemical purity (>99%) and had specific radioactivity at time of injection of $81 \pm 40$ GBq/μmol ($n = 26$ batches).

**Human subjects**

Eight healthy subjects (4 females and 4 males; age, $38 \pm 12$ years; body weight, $71 \pm 18$ kg) participated in the initial studies lasting 150 min. Ten healthy subjects (4 females and 6 males; age $27 \pm 5$ years; body weight, $78 \pm 13$ kg) participated in studies lasting 210 min, with 8 subjects completing retest studies. One of these subjects participated in a 150 minute study as well as the 210 minute retest studies; therefore, 17 subjects were studied in total. All subjects were free of current medical and psychiatric illness based on history, physical examination, electrocardiogram, urinalysis including drug screening, and blood tests (complete blood count, serum chemistries, thyroid function test, and antibody screening for syphilis, HIV, and hepatitis B). The subjects’ vital signs were recorded before $[^{11}C]$MePPEP injection and at 15, 30, 90, and 120 min after injection. Subjects returned to repeat urinalysis and blood tests about 24 h after the PET scan.

**PET scans**

After injection of $[^{11}C]$MePPEP (626 ± 120 MBq) in the eight initial subjects, dynamic PET scans were acquired in 3D mode with a GE Advance camera (GE Healthcare; Waukesha, WI) for 150 min in 39 frames of increasing duration from 30 s to 5 min. After injection of $[^{11}C]$MePPEP (657 ± 76 MBq) in the other ten subjects, dynamic PET scans were acquired over 210 min in two 90 minute sessions, consisting of 45 frames of increasing duration from 30 s to 5 min; subjects were allowed a 30 minute rest period outside the camera between the two sessions. Data were reconstructed with 3D filtered back-projection and a Hanning filter, resulting in an image resolution of 7.0 mm full width at half maximum. All PET images were corrected for attenuation and scatter. Head movement was restricted with a thermoplastic mask and was further corrected by intrasubject alignment during reconstruction. Subjects participating in the retest studies had the test scan in the morning and the retest scan in the afternoon.

**Measurement of $[^{11}C]$MePPEP in plasma**

Blood samples (1 mL each) were drawn from the radial artery at 15 second intervals until 2 min, followed by 3 mL samples at 3, 5, 10, 20, 30, 45, and 60 min, and 6 mL samples at 75, 90, and 120 min. The plasma time-activity curve was corrected for the fraction of unchanged radioligand by radio–HPLC separation, as previously described (Imaizumi et al., 2007).

The plasma free fraction of $[^{11}C]$MePPEP was measured by ultrafiltration through Centrifree membrane filters, as previously described (Gandelman et al., 1994). Plasma samples from each subject were stored in a −80 °C freezer, and free fractions were measured on a day other than the PET scan. Measurements were performed either two or three times for each sample with batches of plasma samples receiving a common preparation of $[^{11}C]$MePPEP each time. The formulation of $[^{11}C]$MePPEP that was used to measure plasma free fraction did not contain Tween 80, which may affect plasma protein binding and whose presence would not be representative of in vivo conditions.

**Image analysis**

PET images were spatially normalized to a standard anatomic orientation (Montreal Neurological Institute, or MNI, space) based on transformation parameters from a magnetic resonance image (MRI). Either 1.5 T (1.2 mm per slice) or 3.0 T (1.0 mm per slice) axial MRIs were obtained using spoiled gradient recall acquisition in the steady state protocol. First, the summed PET image of all time frames was coregistered to the MRI of the individual subject using a 6-parameter algorithm. Second, the individual's MRI was coregistered to the template MRI in MNI space using a 12-parameter algorithm. The coregistration information from the PET to subject MRI and from the subject MRI to template MRI were combined and applied to resample the individual's dynamic PET image into MNI space in a single step. All coregistration and spatial normalization of images were performed using the FSL Library (FMRI Software Library; Oxford, UK). Normalizing PET images to the MNI template allowed use of a set of predefined volumes of interest from the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). Six regions were defined from these preset volumes and analyzed: prefrontal cortex (303 cm³), occipital cortex (172 cm³), hippocampus including parahippocampus (32 cm³), putamen (17 cm³), thalamus (17 cm³), and cerebellum (171 cm³). Two additional regions, pons (6.5 cm³) and white matter (8.3 cm³), were not available from the AAL atlas, and thus were manually drawn on the MNI template and added to the AAL library of predefined volumes.

Concentration of radioactivity in brain was normalized for injected dose and body weight, and expressed as standardized uptake value (SUV).
Calculation of distribution volume using metabolite-corrected input function

The input function was analyzed as linear interpolation of the concentrations of [11C]MePPEP in arterial plasma before the peak, and a tri-exponential fit of concentrations after the peak. Rate constants ($K_1$, $k_2$, $k_3$, and $k_4$) in standard one- and two-tissue compartment models (Innis et al., 2007) were calculated with weighted least squares and the Marquardt optimizer. Brain data of each frame were weighted by assuming that the standard deviation of the data is proportional to the inverse square root of noise equivalent counts. To correct the brain data for its vascular component, radioactivity in serial whole blood was measured and subtracted from the PET measurements, assuming that cerebral blood volume is 5% of total brain volume. Image and kinetic analyses were performed using PMOD 2.95 (pixel-wise modeling software; PMOD Technologies Ltd., Zurich, Switzerland) (Burger et al., 1998).

To determine the minimum scanning time necessary to obtain stable values of distribution volume, we analyzed the PET data from each subject after removing variable durations of the terminal portion of the scan. We analyzed brain data of all subjects from 0–30 min to 0–150 or 0–210 min, depending on the total acquisition length, with 10 minute decrements.

Statistical analysis

Goodness-of-fit by the compartment models was compared with $F$ statistics (Hawkins et al., 1986). A value of $P<0.05$ was considered significant. Goodness-of-fit by nonlinear least squares analysis was evaluated using the Akaike Information Criterion (AIC) (Akaike, 1974) and the model selection criteria (MSC). The most appropriate model is that with the smallest AIC and the largest MSC values.

The identifiability of the kinetic variables was calculated as the standard error, which reflects the diagonal of the covariance matrix (Carson, 1986). Identifiability was expressed as a percentage and equals the ratio of the standard error to the rate constant itself. A lower percentage indicates better identifiability. Identifiability of $V_T$ was calculated from the covariance matrix using the generalized form of error propagation equation (Bevington and Robinson, 2003), where correlations among the rate constants were taken into account.

Group data are expressed as mean±SD. Group analysis of brain data does not include white matter, as it does not contain significant amounts of CB1 receptors. The variability between subjects or intersubject variability (COV) was calculated as SD divided by the mean.

The retest variability of scans within a single subject was defined as the absolute difference between the test and retest studies, divided by the mean of the two. Intraclass correlation coefficient (ICC) was calculated for retest studies and compares the relative variation within subjects to between subjects. Values of ICC were calculated by ($BSMSS-WSMSS)/(BSMSS+WSMSS)$, where $BSMSS =$ mean of summed squares between subjects, and $WSMSS =$ mean of summed squares within subjects. Values between 0 and 1 indicate that variability is higher between subjects than within subjects; values close to 1 suggest good reliability. Values between $-1$ and 0 indicate that variability is higher within subjects than between subjects and suggest poor reliability.

Results

Pharmacological effects

$[11C]$MePPEP caused no pharmacological effects based on subjective reports, electrocardiogram, blood pressure, pulse, and respiration rate. In addition, no effects were noted in any of the blood and urine tests acquired about 24 h after radioligand injection. The injected radioactivity of $[11C]$MePPEP was 651±90 MBq, which corresponds to $9.4±3.3$ nmol of MePPEP ($n=26$ injections in 17 subjects).
subjects). Thus, an uptake of 4 SUV in brain would correspond to a receptor occupancy of 0.3%, assuming the $B_{\text{max}}$ is 1.81 pmol/mg protein in brain (Abood et al., 1997) and that all MePPEP in brain were bound to CB$_1$ receptors.

**Brain imaging**

After injection of $[^{11}C]$MePPEP, all subjects showed high concentrations of radioactivity in brain that decreased slowly over time. Radioactivity in brain peaked by 60 min and was ~3.0 SUV for all areas of neocortex (Figs. 1 and 2). Areas with a high density of CB$_1$ receptors (e.g., putamen) had an even greater concentration of radioactivity, peaking over 4.0 SUV in some subjects. Radioactivity in brain decreased slowly, remaining within ~10% of the peak by 2.5 h and within ~20% of the peak by 3.5 h. We averaged radioactivity concentration from 40 to 80 min after injection to represent brain uptake (brain uptake40–80; Tables 1 and 3).

Two regions of the brain consistently demonstrated less uptake of radioactivity than other regions. The first region, pons, had peak uptake of ~2.2 SUV at 16±10 min. After the peak, washout of radioactivity from the pons was 1.5 to 2 times faster than other regions from 60 to 150 min after injection. The second region, white matter, typically peaked at ~1.5 SUV about 30 min after injection and remained constant until the end of the scan.

**Plasma analysis**

The concentration of $[^{11}C]$MePPEP in arterial plasma peaked at 1–2 min, and then rapidly declined because of distribution in the body, followed by a slow terminal phase of clearance. To quantify the exposure of brain to $[^{11}C]$MePPEP we fit the concentration of $[^{11}C]$MePPEP after its peak to a tri-exponential curve (Fig. 3A). Of the three associated half-lives, the first two (~0.6 and 6.7 min) largely reflected distribution, and the last (~3700 min) reflected clearance (i.e., metabolism and elimination). The long value of this last half-life accounted for ~55% of the total area under the curve integrated to infinity.

Several radiometabolites of $[^{11}C]$MePPEP appeared in plasma (Figs. 3B, C). The main radiometabolite eluted earlier than $[^{11}C]$MePPEP on reverse phase HPLC and was, therefore, less lipophilic than the parent compound. The concentration of this radiometabolite peaked within 20 min and declined for the remainder of the scan. Other radiometabolites were detected throughout the scan in varying concentrations and with varying elution times on HPLC. One of these radiometabolites (Fig. 3C, metabolite F) eluted later than $[^{11}C]$MePPEP and was, therefore, more lipophilic than the parent compound. This radiometabolite was only apparent during part of the scan and never accounted for more than 3% of radioactivity in plasma. After 60 min, $[^{11}C]$MePPEP remained at about 12% of total radioactivity in plasma.

The free fraction of $[^{11}C]$MePPEP in plasma ($f_p$) was very low. The average $f_p$ was 0.05±0.01 in 12 subjects. In the retest study, $f_p$ from morning and afternoon plasma samples were identical (expressed as a second decimal of a percent) for each subject.

**Kinetic analysis based on 150 min of scan data**

For this kinetic analysis, we combined results from 8 subjects who were scanned once for 150 min with the baseline scan of 10 subjects who were scanned for 210 min. The subject who was scanned for 210 min in the retest study. For the latter, we used only 150 min of the baseline scan.

The identifiability of $V_r$ is inversely related to the standard error (SE). For each brain region, the SE is expressed as percent and is listed below the variable itself. Intersubject variability is the SD divided by the mean, and is expressed as percent.

### Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>Brain uptake40–80</th>
<th>Distribution volume: 150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. (SUV)</td>
<td>Intersubject variability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$V_r$ (mL·cm$^{-3}$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intersubject variability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>2.89</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>22.4±12.8</td>
<td>57%</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>2.96</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>14.6±0.1</td>
<td>42%</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.26</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>26.8±23.2</td>
<td>57%</td>
</tr>
<tr>
<td>Putamen</td>
<td>3.59</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>29.1±17.4</td>
<td>60%</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.42</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>11.2±5.0</td>
<td>44%</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.55</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>14.4±6.8</td>
<td>47%</td>
</tr>
<tr>
<td>Pons</td>
<td>1.94</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>7.2±4.0</td>
<td>56%</td>
</tr>
<tr>
<td>White matter</td>
<td>1.19</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>11.4±8.4</td>
<td>73%</td>
</tr>
</tbody>
</table>

Brain uptake and $V_r$ (mean±SD) came from 7 subjects scanned for only 150 min and from 10 subjects scanned for 210 min in the retest study. For the latter, we used only 150 min of the baseline scan.

The unconstrained two-tissue compartment model provided a significantly better fit to the data in all subjects than did the one-tissue compartment model, consistent with the presence of both specific and nonspecific binding in brain. Although the one-tissue model estimated $K_1$, $K_2$, and $V_1$ with reasonable identifiability (SE 1%–6%), the curves significantly deviated from the measured brain data, especially in regions with low CB$_1$ receptor density. Compared to the one-tissue model, the two-tissue model had a statistically better fit to measured data by F-test ($P<0.05$), lower AIC scores (197 vs. 248, on average), and higher MSC scores (4.0 vs. 2.6, on average) for all brain regions.

For the two-tissue compartment model, we assessed the utility of constraining nondisplicable uptake ($V_{ND}=K_1/K_2$) to a single value...
Table 2

<table>
<thead>
<tr>
<th>Region</th>
<th>Rate constant</th>
<th>SE</th>
<th>SE</th>
<th>SE</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal</td>
<td>$K_1$ (mL·cm⁻³·min⁻¹)</td>
<td>0.08 ± 0.02</td>
<td>0.07 ± 0.09</td>
<td>0.385 ± 0.521</td>
<td>0.046 ± 0.076</td>
</tr>
<tr>
<td>cortex</td>
<td>$k_2$ (min⁻¹)</td>
<td>4%</td>
<td>79%</td>
<td>40%</td>
<td>59%</td>
</tr>
<tr>
<td>Occipital</td>
<td></td>
<td>0.10 ± 0.03</td>
<td>0.13 ± 0.17</td>
<td>0.341 ± 0.368</td>
<td>0.025 ± 0.012</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>$k_3$ (min⁻¹)</td>
<td>4%</td>
<td>29%</td>
<td>21%</td>
<td>13%</td>
</tr>
<tr>
<td>Putamen</td>
<td>$k_4$ (min⁻¹)</td>
<td>0.10 ± 0.02</td>
<td>0.07 ± 0.08</td>
<td>0.293 ± 0.268</td>
<td>0.033 ± 0.042</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td>5%</td>
<td>90%</td>
<td>74%</td>
<td>42%</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.08</td>
<td>0.212 ± 0.176</td>
<td>0.026 ± 0.017</td>
</tr>
<tr>
<td>Pons</td>
<td></td>
<td>4%</td>
<td>25%</td>
<td>24%</td>
<td>12%</td>
</tr>
<tr>
<td>White matter</td>
<td></td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.04</td>
<td>0.267 ± 0.501</td>
<td>0.059 ± 0.129</td>
</tr>
</tbody>
</table>

The subject sample is the same as for Table 1: 7 subjects scanned for 150 min and 10 subjects scanned for 210 min in the retest study. For the latter, we used only the initial 150 min of the baseline scan.

The identifiability of rate constants is inversely related to the standard error (SE). For each brain region, the SE is expressed as percent and is listed below the variable itself. Values are mean ± SD.

which is the dissociation rate constant from the specific compartment, was low and ranged from 0.011 to 0.046 min⁻¹, with an average of ~ 0.027 min⁻¹. Finally, the estimated ratio of specific to nondisplaceable uptake ($BP_{ND} = k_1 / k_2$) was ~ 19 in healthy human brain.

determined from all regions except white matter. When compared by F-test, the unconstrained model fit the data significantly better than the constrained model in the majority of regions, and the AIC and MSC scores favored the unconstrained model. For these reasons, we used the unconstrained two-tissue compartment model for additional analyses.

After 150 min of scanning, the value of $K_1$ in all regions except white matter for all subjects ranged from 0.07 to 0.10 mL·cm⁻³·min⁻¹, with an average of 0.09 mL·cm⁻³·min⁻¹ (Table 2). Assuming that cerebral blood flow is ~ 0.5 mL·cm⁻³·min⁻¹, the extraction fraction ($\text{extraction} = K_1 / \text{flow}$) of $[^{11}C]$MePPEP from plasma to brain was ~ 18%. The value of $k_2$ in all regions except white matter ranged from 0.05 to 0.17 min⁻¹, with an average of ~ 0.11 min⁻¹. Thus, the value of nondisplaceable distribution volume ($V_{ND} = K_1 / k_2$) was ~ 2.1 mL·cm⁻³. The value of $k_3$, which is defined as $k_3 = \frac{B_{max} - B_{ND}}{B_{ND}}$, ranged from 0.177 to 0.385 min⁻¹, with an average value of ~ 0.276 min⁻¹. The value of $k_4$,

Fig. 3. Concentration of $[^{11}C]$MePPEP and its percentage composition in arterial plasma. (A) Average concentration of $[^{11}C]$MePPEP in arterial plasma from 17 subjects is plotted over time after injection. Data after the peak (about 1 min) were fitted to a tri-exponential curve (−). Symbols (●) and error bars represent mean and SD. (B) Percent composition of parent radioligand (●) and radiometabolites (○) in arterial plasma from 17 subjects are plotted over time after injection. Data after the peak (about 1 min) were fitted to a tri-exponential curve (−). (C) This radiochromatogram illustrates plasma composition from one subject, 30 min after injection of $[^{11}C]$MePPEP. Radioactivity was measured in counts per second (cps). Peaks are labeled with increasing lipophilicity from A to F. Peak E represents $[^{11}C]$MePPEP. cps = counts per second.

Fig. 4. Value of distribution volume ($V_T$) and its identifiability as function of duration of image acquisition for putamen, a high binding region. $V_T$ (●) was calculated using an unconstrained two-tissue compartment model with increasingly truncated acquisition times. Values are normalized to the value attained with 150 min of imaging and are plotted with the y-axis on left. The corresponding SE (○), which is inversely proportional to identifiability, is plotted with the y-axis on right. (A) The points represent an average of 15 subjects scanned for a total of 150 min. Two subjects scanned for only 150 min had a slow washout, and did not have stable measurements of $V_T$ until 120 min. (B) The points represent an average of 10 subjects scanned for a total of 210 min. Other brain regions demonstrated similar or better stability over time.
To determine the minimal scanning time necessary to obtain stable values of \( V_T \), we calculated \( V_T \) and its identifiability using increasingly truncated durations of brain data. The value of \( V_T \) was stably identified after \(~60\) min of scanning and had good identifiability (i.e., low percentage values) after \(~70\) min (Fig. 4).

Although the identifiability of \( V_T \) was good after \( 150 \) min of scanning (SE \(~5\%\) or less), we observed a high intersubject variability of \( V_T \) among the initial eight subjects (\(~61\%\)). In contrast, the intersubject variability of brain uptake at \( 40-80 \) was much lower (\(17\%\)). \( V_T \) is calculated from both brain and plasma data. Although high intersubject variability of \( V_T \) might reflect actual variations in CB1 receptor density in this population, we wondered if it was caused by noise in the measurements of either brain radioactivity or the concentration of \([^{11}C]MePPEP\) in plasma. To assess the relative contributions of noise in brain and plasma data, we performed a retest study and assumed that the concentration of CB1 receptors in brain and the clearance of radioligand from plasma were unchanged in each subject within a single day. Furthermore, although \( V_T \) appeared to be stably determined after \(~60\) min, we increased the imaging time of both the test and retest scans to \(210\) min to see if values of \( V_T \) both remained stable during this extra \(60\) min of imaging and reduced its variability.

The high intersubject variability of \( V_T \) might have also been caused by variations in plasma free fraction of \([^{11}C]MePPEP\). However, the intersubject variability of \( V_T \) for \(~8\%\); Table 3) in all eight brain regions of the 12 subjects in whom \( f_p \) was measured, using \(150\) min of image data. Thus, correction of \( V_T \) for individual values of plasma protein binding neither increased nor decreased intersubject variability.

### Retest variability using \(210\) min of scan data

Similar to the results using \(150\) min of scan data, those from \(210\) min showed higher intersubject variability of \( V_T \) (\(~39\%)\) than of brain uptake \(40-80\) (\(~15\%)\) in a similar manner. The retest variability (i.e., within subject) of \( V_T \) (\(~15\%)\) was nearly twice that of brain uptake \(40-80\) (\(~8\%\); Table 3). However, the retest variability of the plasma measurements was extraordinarily high. The exposure to the brain is the area under the curve (AUC) of \([^{11}C]MePPEP\) in arterial plasma extrapolated to infinity. The retest variability of plasma AUC was \(~5\%\) and the ICC was \(~0.02\). Since \( V_T \) equals the ratio of brain AUC to plasma AUC, we suspect that the primary cause of the high intersubject variability of \( V_T \) was uncertainty or noise in the measurement of plasma AUC. Furthermore, the terminal clearance of \([^{11}C]MePPEP\) was very slow (half-life of \(3700\) min (see above)), several times the physical half-life of \([^{11}C]\) itself (\(20.4\) min).

Similar to the data from \(150\) min of scanning, we increasingly truncated the data from \(210\) min to determine minimal scan duration to obtain stable values of \( V_T \). We again found that \( V_T \) was relatively stable after \(~60\) min and had good identifiability after \(~80\) min (Fig. 4). We compared the \( V_T \), retest variability, intersubject variability, and ICC acquired after \(210\) min to that of the data truncated to \(150\) min within the same subjects and found that they were essentially identical (change \(~3\%)\).

### Can brain uptake substitute for distribution volume?

Brain uptake not corrected for plasma measurements has been used with another radioligand to measure CB1 receptor availability in human brain (Burns et al., 2007; Van Laere et al., 2008). Furthermore, we found that the intersubject variability and retest variability of brain uptake was much better than that of \( V_T \). To evaluate whether brain uptake is an accurate measurement of receptor density, we simulated increased and decreased receptor densities by corresponding changes in \( k_d \). We used the average input function and rate constants for prefrontal cortex from the 17 subjects scanned for \(150\) min. Brain uptake was calculated for three time intervals: \(40-80\), \(0-210\), and \(150-180\) min.

Brain uptake for all three time intervals followed the pattern of increasing or decreasing receptor density but underestimated the change (Fig. 5). For example, a \(50\%\) increase in receptor density yielded only \(13-30\%\) decrease of brain uptake during these three time intervals, while \( V_T \) increased by \(45\%\). In addition, a \(50\%\) decrease in receptor density yielded a \(25-44\%\) decrease of brain uptake during these three time intervals, while \( V_T \) decreased by \(45\%\).

In turn, we wanted to know the impact that good precision and poor accuracy has on using brain uptake to measure changes in CB1 receptor density, so we calculated the expected number of subjects needed to detect these simulated outcome measurements. Estimation of sample sizes for a two-tailed independent samples \(t\)-test was made assuming \(\alpha = 0.05\) (probability of type I error) and \(\beta = 0.20\) (probability of type II error, i.e., power of \(80\%)\). Intersubject variability from our measurements from \(17\) subjects was used to estimate the pooled standard deviation of outcome (\( V_T \) and brain uptake), and results from

### Table 3

<table>
<thead>
<tr>
<th>Region</th>
<th>Brain uptake (40-80)</th>
<th>Intersubject variability</th>
<th>Retest variability</th>
<th>ICC</th>
<th>Distribution volume</th>
<th>( V_T ) ((\text{mL} \cdot \text{cm}^{-3}))</th>
<th>Intersubject variability</th>
<th>Retest variability</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal</td>
<td>2.94</td>
<td>17%</td>
<td>8%</td>
<td>0.78</td>
<td>17.8 ± 6.4</td>
<td>2%</td>
<td>36%</td>
<td>15%</td>
<td>0.91</td>
</tr>
<tr>
<td>Occipital</td>
<td>2.95</td>
<td>16%</td>
<td>8%</td>
<td>0.75</td>
<td>12.3 ± 4.2</td>
<td>2%</td>
<td>34%</td>
<td>12%</td>
<td>0.93</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.21</td>
<td>13%</td>
<td>7%</td>
<td>0.73</td>
<td>18.5 ± 9.4</td>
<td>5%</td>
<td>51%</td>
<td>21%</td>
<td>0.81</td>
</tr>
<tr>
<td>Putamen</td>
<td>3.60</td>
<td>17%</td>
<td>8%</td>
<td>0.75</td>
<td>22.4 ± 7.8</td>
<td>3%</td>
<td>35%</td>
<td>17%</td>
<td>0.87</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.46</td>
<td>15%</td>
<td>8%</td>
<td>0.75</td>
<td>9.6 ± 3.3</td>
<td>3%</td>
<td>34%</td>
<td>11%</td>
<td>0.94</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.47</td>
<td>18%</td>
<td>8%</td>
<td>0.79</td>
<td>11.7 ± 3.4</td>
<td>3%</td>
<td>29%</td>
<td>16%</td>
<td>0.82</td>
</tr>
<tr>
<td>Pons</td>
<td>1.98</td>
<td>19%</td>
<td>8%</td>
<td>0.85</td>
<td>5.9 ± 1.3</td>
<td>6%</td>
<td>22%</td>
<td>13%</td>
<td>0.82</td>
</tr>
<tr>
<td>White matter</td>
<td>1.13</td>
<td>13%</td>
<td>8%</td>
<td>0.71</td>
<td>8.7 ± 7.0</td>
<td>8%</td>
<td>80%</td>
<td>108%</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Brain uptake and \( V_T \) reflect only the baseline scan from \(8\) healthy subjects (mean ± SD).

The identifiability of \( V_T \) is inversely related to the standard error (SE). For each brain region, the SE is expressed as percent and is listed below the variable itself. Intersubject variability is the SD divided by the mean, and is expressed as percent.

Retest variability is the absolute value of the difference between test and retest, divided by their mean, and is expressed as percent.

* Brain uptake is the average radioactivity from 40 to 80 min after injection.
our simulations of varying receptor density determined the measured
difference to be detected. We found that 25 subjects would be needed
when measuring brain uptake:50 vs. 26 subjects when measuring \( V_f \)
if a 50% increase of receptor density is expected. However, 8 subjects
would be needed when measuring brain uptake:80 vs. 26 subjects
when measuring \( V_f \) if a 50% decrease of receptor density is expected.

Discussion

The purpose of this study was to evaluate the ability of \([11C]\)
MePPEP to quantify CB1 receptors in human brain in regard to several
characteristics, including minimal time necessary to scan subjects,
retest variability, and intersubject variability. \([11C]\)MePPEP readily
entered human brain with high uptake (\(>3.0 \) SUV) and had a
distribution consistent with that of CB1 receptors (Glass et al., 1997).
The values of distribution volume had good identifiability (SE < 10%)
and were relatively stable after about 60 min of imaging. We found
that scanning up to 210 min provided quantifiable data but did not
significantly improve the identifiability or retest variability of
distribution volume measured using the initial 150 min of data. We
compared brain uptake and the more complicated but theoretically
more accurate distribution volume, which corrects for exposure of the
parent radioligand to brain. The retest variability of brain uptake:40–80
(\(\sim 8\%\)) was better than that of distribution volume (\(\sim 15\%\)), which
might be expected since \( V_d \) includes multiple variables and calcula-
tions. Surprisingly, the intersubject variability of \( V_f \) (\(\sim 52\% \) COV)
was more than three times that of brain uptake:40–80 (\(\sim 16\% \) COV). With
the current data, we cannot determine whether brain uptake or distribu-
tion volume is the more accurate measure of CB1 receptor density,
since we do not know the actual intersubject variability of CB1 receptors
in this group of subjects. Nevertheless, we suspect that noise
in measurement of the input function of distribution volume
decreased our precision in measuring \( V_f \).

Advantages and limitations of \([11C]\)MePPEP

\([11C]\)MePPEP had such high uptake and retention in brain that it
could be meaningfully measured for 210 min, which is greater than
ten times the radioactive half-life of \(^{11}C\). Distribution volume was well
identified (SE \(\sim 5\%\)), showed good time stability after \(\sim 60\) min, and
had good reliability on test–retest study (ICC \(\sim 0.90\)). In addition, the
short half-life of the \(^{11}C\) label allowed us to perform two scans in a
subject per day.

Our assessment also uncovered several limitations of \([11C]\)
MePPEP. The washout of radioactivity from brain was slow and
difficult to quantify. Although distribution volume is typically defined
using rate constants, it may be more easily conceptualized as the
ratio of two areas under the curve (AUC) extrapolated to infinite
time. Distribution volume equals AUC in brain divided by AUC of
parent radioligand in plasma. The slow washout from brain
decreased the precision to extrapolate AUC in brain to infinite time.
Even after 210 min we had observed only about one third of the total
AUC of brain. The slow washout was caused by the high affinity of the
radioligand (\(K_B = 0.14 \pm 0.03 \) nM in human cerebellum, unpublished
data) and by the high density of CB1 receptors in brain. In fact, we
estimate that the density of CB1 receptors, if evenly distributed in the
entire brain, would be more than six times that of dopamine D2
receptors in striatum. The high density of CB1 receptors allowed rapid
rebinding of the radioligand, creating a “synaptic barrier” that
impedes exit of the radioligand from brain to plasma (Frost and
Wagner, 1984).

While high concentrations of radioactivity in brain allowed us to
image for 210 min, low concentrations in plasma restricted our
measuring the input function to only the initial 120 min. The low
concentration of the radioligand was due to distribution and clearance
as well as physical decay of \(^{11}C\). Measurements of such low
concentrations introduced noise in the input function, which then
decreased the precision of \( V_f \). A fluorine–18 labeled version of \([11C]\)
MePPEP may permit a longer acquisition time from both brain and
plasma, and the resulting \( V_f \) might be more precise.

\([11C]\)MePPEP is also flawed by having very low free fraction in
plasma (\(f_p\)). Since only free radioligand in plasma can cross the blood–
brain barrier, distribution volume should be corrected for (i.e., divided
by) \(f_p\) to more accurately measure CB1 receptor density. We wondered
whether correcting for \( f_p \) would decrease retest and intersubject
variability of distribution volume. Correcting both brain uptake and \( V_f \)
for \( f_p \) neither increased nor decreased retest or intersubject variability.
The mean value of \( f_p \) for \([11C]\)MePPEP was 0.05%, which corresponds to
a bound fraction of 99.95%. The impact would seem insignificant if the
bound fraction increased from 99.95% to 99.97%. However, such a
change would nearly halve the value of \( f_p \) to 0.03%, and would then
nearly double the corrected value of \( V_f \). We suspect that the low
values of \( f_p \) will be problematic in clinical studies, because apparently
minute differences may exist between groups. Additionally, if \([11C]\)
MePPEP is used to measure receptor occupancy by medications,
investigators should confirm that the medications do not change the
plasma protein binding of the radioligand.

Precision and accuracy affect sample sizes for clinical studies

Our current results demonstrate that brain uptake has greater
precision than \( V_f \), but brain uptake is theoretically less accurate. We
use “precision” most narrowly in reference to retest reproducibility,
with the assumption that the density and affinity of receptors do not
change between scans. In this study, we also indirectly gauge precision
by intersubject variability, since a less precise measurement will have
greater standard deviation in the population. In comparison to
distribution volume, we found brain uptake had greater precision
because it had better retest reproducibility and as suggested by its
lower intersubject variability. However, high intersubject variability
merely suggests poor precision, since it inseparably incorporates both
precision and accuracy.

We use “accuracy” to refer to the theoretically correct measure-
ment. For example, distribution volume provides a more accurate
measure of receptor density than brain uptake, because distribution
volume corrects for exposure of the brain to the concentration of
radioligand in plasma. The concentration in plasma differs between

![Fig. 5. Simulated changes in brain uptake with variations of receptor density. The average individual kinetic parameters from the prefrontal cortex were used to simulate expected changes in brain uptake from 40 to 80 (○), 0 to 210 (●), and 150 to 180 (□) min. Changes in receptor density were simulated by varying the value of \( k_3 \) from its mean value (set at 100% on the x-axis). As expected, the value of \( V_f \) (shown by a line which has a y-intercept equal to \( V_f/K_d \)) is directly proportional to changes in \( k_3 \).](image)
subjects based on variations in distribution, metabolism, and excretion of the radioligand. Accuracy is more difficult to measure than precision, since we do not know the actual density of CB₁ receptors in these subjects. In this study, we indirectly assessed accuracy in two ways. First, for the retest study, an ICC value of 1 is associated with greatest accuracy. Second, for measurement of minimal time to calculate distribution volume, a time-stable value is consistent with an accurate measurement that is not contaminated by radiometabolites. Nevertheless, both ICC and time stability are merely consistent, with but do not prove, accuracy. For example, our prior ex vivo study of [¹¹C]MePPEP in rat brain showed that distribution volume can also be stable over time even in the presence of a radiometabolite, if its percentage of total radioactivity in brain is constant (Terry et al., 2008).

What is the relative sensitivity of brain uptake and distribution volume to discern changes in CB₁ receptor density between groups, and what sample sizes would be required? To estimate sample sizes, we assumed that distribution volume (\(V_T\)) provides the accurate measure of receptor density, though we found it to be less precisely measured than brain uptake\(_{40-80}\), and then simulated the effect of changing receptor density on brain uptake. We found that brain uptake underestimated both increases and decreases in receptor density, and that this underestimation was more severe for increased than decreased receptor density (Fig 5).

For example, a 50% decrease in receptor density would be erroneously measured as only 25% decrease of brain uptake\(_{40-80}\). However, because of its greater precision, brain uptake\(_{40-80}\) would require only 8 subjects per group, while \(V_T\) would require 26 per group to achieve the same statistical endpoint (significance level = 0.05 and power = 80%). In contrast, a 50% increase of receptor density would be erroneously measured as only 13% increase of brain uptake\(_{40-80}\). Despite its greater precision, brain uptake\(_{40-80}\) would require 25 subjects per group, while \(V_T\) would require 26 per group to achieve the same statistical endpoint. In summary, the effects of precision and accuracy have differential effects on the relative sample sizes required for brain uptake and distribution volume.

Brain uptake\(_{20-120}\) was more sensitive than brain uptake\(_{40-80}\) in these simulations, however brain uptake\(_{50-180}\) had superior sensitivity to either time period, reflecting that radioactivity in brain is more dependent on \(k_b\) at later times during the scan. While brain uptake\(_{150-180}\) or perhaps even later time frames, may be more sensitive than brain uptake\(_{40-80}\) in measuring changes in \(k_b\), the added noise at those later time frames may cause their measurements to be less precise.

Based on these results, we recommend using brain uptake\(_{40-80}\) as an outcome measure only for within subject studies (e.g., receptor occupancy), since brain uptake is more reproducible than \(V_T\). However, we recommend that \(V_T\) be used for between subject studies (patients vs. healthy subjects), because it is more accurate than brain uptake. Furthermore, our simulations on the effects of accuracy and precision are merely predictions, which need validation from larger sample sizes of both patients and healthy subjects.

**Comparison with two other CB₁ radioligands**

The first two successful radioligands to image CB₁ receptors in human brain were [¹⁸F]MK-9470 (Burns et al., 2007) and [¹¹C]HU75528 (Horti et al., 2006). Assessed by peak brain uptake, [¹⁸F]MK-9470 has been used to measure receptor occupancy by a nonradioactive inverse agonist (tarabandat, Burns et al., 2007), to show an age-related increase of CB₁ receptors in women but not in men (Van Laere et al., 2008), and to find an association of CB₁ receptor density with novelty seeking personality (Van Laere et al., 2009). Compared to [¹⁸F]MK-9470, [¹¹C]MePPEP has about 2.5 times greater uptake in human brain (~1.4 vs. 3.6 SUV) but comparable retest variability and intersubject variability when brain uptake is used as the outcome measure. However, the washout of radioactivity from brain for [¹⁸F]MK-9470 appears to be even slower than that for [¹¹C] MePPEP. If so, measurement of distribution volume for [¹⁸F]MK-9470 may be quite problematic and have low identifiability and poor time stability.

Results using [¹¹C]HU75528 (also called [¹¹C]OMAR) have been published from baboons as a paper (Horti et al., 2006) and from humans as an abstract (Wong et al., 2008). [¹¹C]HU75528 has low to moderate uptake in baboon brain (peak uptake of ~1.0 SUV) and can be displaced by nonradioactive ligands. The peak uptake in brain of [¹¹C]MePPEP is higher than that of [¹¹C]HU75528: ~4–6 times higher in monkey and ~2 times higher in human. Quantification of [¹¹C]HU75528 using arterial input function has not been fully described.

In summary, although the brain uptake of [¹¹C]MePPEP is greater than that of [¹⁸F]MK-9470 and [¹¹C]HU75528, the more important comparisons may well be resolved only after detailed kinetic analysis are reported for all three radioligands.

**Conclusion**

[¹¹C]MePPEP has high brain uptake and regional distribution in human brain consistent with that of CB₁ receptors. In addition, by standard measures, [¹¹C]MePPEP provides good quantitation of receptor density using both brain and plasma data—i.e., distribution volume is well identified and has relatively stable values after ~60 min of imaging. Nevertheless, the high variability of distribution volume among subjects suggests that it is not as precisely measured as suggested by identifiability. For within subject studies (e.g., receptor occupancy by medications), peak uptake of [¹¹C]MePPEP will be useful, since this outcome measure showed good retest variability. For between subject studies (e.g., patients vs. healthy subjects), distribution volume is theoretically superior to peak uptake. However, the plasma measurements required to calculate distribution volume add moderate noise to this outcome measure. Thus, between subject studies must have adequate sample sizes to detect differences because of the moderately high retest and intersubject variability of distribution volume measured with [¹¹C]MePPEP.

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**References**


