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# Positron Emission Tomography Imaging of Amphetamine-Induced Dopamine Release in the Human Cortex: A Comparative Evaluation of the High Affinity dopamine $D_{2/3}$ Radiotracers [$^{11}$C]FLB 457 and [$^{11}$C]Fallypride

**RAJESH NARENDRAN,[1,2]* W. GORDON FRANKLE,[1,2] N. SCOTT MASON,[1] EUGENII A. RABINER,[3] ROGER N. GUNN,[3] GRAHAM E. SEARLE,[3] SHIVANGI VORA,[1] MARALEE LITSCHGE,[1] STEVE KENDRO,[1] THOMAS B. COOPER,[4] CHESTER A. MATHIS,[1] AND MARC LARUELLE[3,5]**

[1]*Department of Radiology, University of Pittsburgh, Pittsburgh, Pennsylvania*
[2]*Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania*
[3]*Clinical Imaging Center, GlaxoSmithKline, London, United Kingdom*
[4]*Department of Psychiatry, Columbia University, New York, New York*
[5]*Department of Neurosciences, Imperial College London, United Kingdom*

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*ABSTRACT* The use of PET and SPECT endogenous competition binding techniques has contributed to the understanding of the role of dopamine in several neuropsychiatric disorders. An important limitation of these imaging studies is the fact that measurements of acute changes in synaptic dopamine have been restricted to the striatum. The ligands previously used, such as [$^{11}$C]raclopride and [$^{123}$I]IBZM, do not provide sufficient signal to noise ratio to quantify $D_2$ receptors in extrastriatal areas, such as cortex, where the concentration of $D_2$ receptors is much lower than in the striatum. Given the importance of cortical DA function in cognition, a method to measure cortical dopamine function in humans would be highly desirable. The goal of this study was to compare the ability of two high affinity DA $D_2$ radioligands [$^{11}$C]FLB 457 and [$^{11}$C]fallypride to measure amphetamine-induced changes in DA transmission in the human cortex. $D_2$ receptor availability was measured in the cortical regions of interest with PET in 12 healthy volunteers under control and postamphetamine conditions (0.5 mg kg$^{-1}$, oral), using both [$^{11}$C]FLB 457 and [$^{11}$C]fallypride (four scans per subjects). Kinetic modeling with an arterial input function was used to derive the binding potential ($BP_{ND}$) in eight cortical regions. Under controlled conditions, [$^{11}$C]FLB 457 $BP_{ND}$ was 30–70% higher compared with [$^{11}$C]fallypride $BP_{ND}$ in cortical regions. Amphetamine induced DA release led to a significant decrease in [$^{11}$C]FLB 457 $BP_{ND}$ in five out the eight cortical regions evaluated. In contrast, no significant decrease in [$^{11}$C]fallypride $BP_{ND}$ was detected in cortex following amphetamine. The difference between [$^{11}$C]FLB 457 and [$^{11}$C]fallypride ability to detect changes in the cortical $D_2$ receptor availability following amphetamine is related to the higher signal to noise ratio provided by [$^{11}$C]FLB 457. These findings suggest that [$^{11}$C]FLB 457 is superior to [$^{11}$C]fallypride for measurement of changes in cortical synaptic dopamine. **Synapse 63:447–461, 2009.** 

## INTRODUCTION

Over the last few years, several groups have demonstrated that PET and SPECT neuroreceptor imaging techniques can be used, not only to measure

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*Correspondence to: Raj Narendran, MD, University of Pittsburgh, UPMC Presbyterian, PET Facility, B938, 200 Lothrop Street, Pittsburgh, PA 15213, USA. E-mail: narendranr@upmc.edu

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receptor parameters but also to detect acute fluctuations in the concentration of endogenous transmitters in the vicinity of the receptors (for review, see Laruelle, 2000). For example, the displacement of the $D_{2/3}$ (hereafter referred to as $D_2$) receptor antagonists [$^{11}$C]raclopride and [$^{123}$I]IBZM following acute administration of amphetamine has been well validated as a noninvasive measure of the change in extracellular DA concentration induced by the challenge drug (Breier et al., 1997; Carson et al., 1997; Endres et al., 1997; Kegeles et al., 1999; Laruelle et al., 1995, 1997; Villemagne et al., 1999). Using these endogenous competition PET techniques, several groups have reported abnormal dopamine transmission in clinical populations such as schizophrenia (Breier et al., 1997; Laruelle et al., 1996) and addictive disorders (Malison et al., 1999; Martinez et al., 2005, 2007; Volkow et al., 1997). An important limitation of the use of [$^{123}$I]IBZM and [$^{11}$C]raclopride in these studies is the fact that measurements of $D_2$ receptors and amphetamine-induced DA release were restricted mostly to the striatum because of their relatively low signal to noise ratio in the extrastriatal regions.

Since the introduction of the high affinity $D_2$ receptor PET radiotracers [$^{11}$C]FLB 457 (Halldin et al., 1995; in vitro KD = 0.018 nM) and [$^{18}$F]fallypride (Mukherjee et al., 1995, 2004; in vitro KD = 0.030 nM), several groups have confirmed their increased signal to noise ratio relative to [$^{11}$C]raclopride and [$^{123}$I]IBZM and reported on their ability to measure $D_2$ receptors in several extrastriatal regions of interest (Olsson et al., 2004; Slifstein et al., 2004a; Suhara et al., 1999). The question of whether the signal to noise ratio of the radiotracers [$^{11}$C]FLB 457 and [$^{18}$F]fallypride is sufficient to image amphetamine-induced dopamine transmission in relatively low $D_2$ receptor density prefrontal cortical regions of interest, such as the dorsolateral prefrontal cortex (DLPFC), orbital frontal cortex (OFC), medial prefrontal cortex (MPFC), and anterior cingulate cortex (ACC), is still unresolved in the literature.

The advantage of [$^{18}$F]fallypride compared with [$^{11}$C]FLB 457 to image DA transmission is primarily based on the fact that it is labeled with the relatively slowly decaying isotope F-18 and its uptake and washout kinetics are relatively fast compared with [$^{11}$C]FLB 457: the combination of both factors allows for the measurement of $D_2$ receptor BP in both striatal and extrastriatal areas (Cropley et al., 2008; Mukherjee et al., 1997; Price et al., 1997; Riccardi et al., 2005; Slifstein et al., 2007a). Nevertheless, the use of [$^{18}$F]fallypride to measure amphetamine induced DA release in the cortex is impaired by its relatively low signal to noise ratio in the cortical ROIs ($BP_{ND} \leq 0.5$; Cropley et al., 2008; Riccardi et al., 2005; Slifstein et al., 2007a). Furthermore, the use of F-18 makes it impossible to perform a baseline scan and postamphetamine scan the same day, a paradigm typically used with C-11 labeled radiotracers. The acquisition of baseline and postamphetamine scans on two different days increases the within-subject variability for the radiotracer and contributes to a lower sensitivity for the technique to detect amphetamine-induced changes in $BP_{ND}$. In contrast, [$^{11}$C]FLB 457 is labeled with the rapidly decaying isotope C-11 and its kinetic of uptake is slower. Therefore, $^{11}$C]FLB 457 can only be used for quantification of $D_2$ receptor parameters in extrastriatal areas. Several groups have attempted to use [$^{11}$C]FLB 457 to study stimulant-induced DA release in the extrastriatal regions and reported inconsistent results (Aalto et al., 2005; Chou et al., 2000; Montgomery et al., 2006; Okauchi et al., 2001; Tsukada et al., 2005). The major limitations of these studies with respect to the vulnerability of the in vivo binding of [$^{11}$C]FLB 457 following a psychostimulant challenge can be summarized as follows:

1. Administration of pharmacological (or nontracer) doses of [$^{11}$C]FLB 457 in both human and nonhuman primate PET studies. This limitation is based on previous studies, which demonstrate that the injected mass of [$^{11}$C]FLB 457 should be restricted to less than 1.6 nmoles ($\sim$0.6 $\mu$g) per 70 kg human subject to accomplish less than 5% of occupancy at the $D_2$ receptors (Olsson et al., 2004; Sudo et al., 2001). A review of the published literatures suggests that very few investigations (3 out of 16 studies, Table V) have been performed with such a strict restriction on injected mass (Ito et al., 2001; Sudo et al., 2001; Suhara et al., 2002).
2. The failure to quantify amphetamine (or stimulant)-induced changes in the cerebellum distribution volume ($V_{T\ CER}$ or $V_{ND}$ for binding in the cerebellum or reference region is taken as a measure of nonspecfic binding) by using reference region analysis instead of kinetic analysis with arterial input function (3 out of 16 studies, Table V), may have led to an underestimation of change in $BP_{ND}$ in the regions of interest. This issue has been underscored by several recent studies demonstrating a decrease in [$^{11}$C]FLB 457 $V_{TCER}$ following a psychostimulant or $D_2$ antagonist drug challenge (Ahmad et al., 2006; Asselin et al., 2007; Montgomery et al., 2006).
3. Scanning protocols which collect data for <90 min may not be sufficient for robust quantification of the $BP_{ND}$ in the extrastriatal regions (2 out of 16 studies, Table V).

In this study, the authors compared the in vivo binding characteristics of [$^{11}$C]FLB 457 and [$^{11}$C]fallypride in the same healthy human subjects before and after an amphetamine challenge. The choice of [$^{11}$C]fallypride (a novel first-in-human radio-

ligand with the C-11 label) rather than [$^{18}$F]fallypride was based on the fact that we were interested in measuring only cortical binding and it allowed for two PET scans (a baseline and postamphetamine) to be performed the same day.

The primary objectives of this human PET study were as follows:

1. To assess the signal to noise ratio ($BP_{ND}$) of [$^{11}$C]FLB 457 and [$^{11}$C]fallypride in the cortical regions of interest.
2. To assess the vulnerability of [$^{11}$C]FLB 457 and [$^{11}$C]fallypride binding to endogenous competition by DA following an acute amphetamine challenge (0.5 mg kg$^{-1}$) in the same subjects in the cortical regions of interest.

## METHODS

### General design

The study was approved by the Institutional Review Board of the University of Pittsburgh Medical Center. A total of 48 PET scans were acquired for this study in 12 healthy control subjects over 24 experimental sessions. Each experimental session included two PET scans: a baseline scan and a postamphetamine scan with the same radiotracer. All subjects returned for a second experimental session in a minimum of one week (but no longer than 3 weeks) identical to the first, but with the other radiotracer (a total of four scans per subject). The sequence of the radiotracers was counterbalanced across subjects—half received two scans with [$^{11}$C]FLB 457 during the first experimental session, whereas the other half received two scans with [$^{11}$C]fallypride during the first experimental session—to prevent bias in the between radiotracer comparison. The postamphetamine scan occurred 3 h following the administration of 0.5 mg kg$^{-1}$ oral d-amphetamine (dexedrine). The timing was based on the time for d-amphetamine to reach $C_{max}$ and remain at a relatively constant level in the plasma (Angrist et al., 1987; Cropley et al., 2008; Riccardi et al., 2005).

### Radiolabeling

Radiolabeling of [$^{11}$C]FLB 457 and [$^{11}$C]fallypride were performed using previously published procedures (Halldin et al., 1995; Mukherjee et al., 2004).

### PET protocol

Imaging experiments were conducted on the ECAT EXACT HR+ consistent with previously described image acquisition protocols (Abi-Dargham et al., 2000). Briefly, following completion of a transmission scan (~10 min) for attenuation correction of the emission data, subjects received either an intravenous injection of [$^{11}$C]FLB 457 or [$^{11}$C]fallypride as a bolus over 20 s. Based on previously published studies, the maximum injected mass for [$^{11}$C]FLB 457 and [$^{11}$C]fallypride was restricted to 0.6 and 1.2 μg, respectively (Cropley et al., 2008; Riccardi et al., 2005; Sudo et al., 2001). Emission data were collected for 90 min. The postamphetamine scan was performed 3 h after the administration of 0.5 mg kg$^{-1}$ PO of amphetamine.

### Input function measurement

Following radiotracer injection, arterial samples were collected manually approximately every 6 s for the first 2 min and thereafter at longer intervals. A total of 35 samples were obtained per scan. Following centrifugation, plasma was collected in 200 μl aliquots and activities were counted in a gamma counter.

To determine the plasma activity representing unmetabolized parent compound ([$^{11}$C]FLB 457 or [$^{11}$C]fallypride) six samples (collected at 2, 10, 20, 40, 60, and 75 min) were further processed using HPLC methods described previously (Olsson et al., 1999; Slifstein et al., 2004b). Briefly, the supernatant obtained after centrifugation was deproteinized with acetonitrile. The acetonitrile-plasma mixture was then analyzed by reverse-phase HPLC methods. [$^{11}$C]FLB 457 analyses were performed on a Prodigy ODS-3, (5 μ, 4.6 × 250 mm) eluted at 2.0 ml/min with 23/77 acetonitrile/ammonium formate buffer (pH = 4.2). [$^{11}$C]Fallypride analyses were performed on a Prodigy ODS-3, (5 μ, 4.6 × 250 mm) eluted at 2.0 ml/min with 45/55 acetonitrile/ammonium formate buffer (pH 4.2). The radioactivity corresponding to the parent compound was then expressed as the percentage of the total radioactivity in the sample.

For [$^{11}$C]FLB 457 the six measured parent fractions were fitted using a Hill model (Gunn et al., 1998; Hill, 1910; Wu et al., 2007). For [$^{11}$C]fallypride the parent fractions were fitted to the sum of two exponentials. The smallest exponential of the fraction of the parent curve, $\lambda_{par}$, was constrained to the difference between $\lambda_{cer}$ (the terminal rate of washout of the cerebellar activity) and $\lambda_{tot}$ (the smallest elimination rate constant of the total plasma) (Abi-Dargham et al., 1999).

The input function was then calculated as the product of total counts and interpolated parent fraction at each time point. The measured input function values were fitted to a sum of three exponentials from the time of peak plasma activity and the fitted values were used as the input to the kinetic analysis. The clearance of the parent compound (L/h) was calculated as the ratio of the injected dose to the area under the curve of the input function (Abi-Dargham et al., 1994).

For the determination of the plasma free fraction ($f_P$), triplicate aliquots of plasma collected prior to injection were mixed with the radiotracer, pipetted into ultrafiltration units (Amicon Centrifree; Millipore, Bedford, MA), and centrifuged at room temperature (30 min at 6000 rpm). At the end of centrifugation, the plasma and ultrafiltrate activities were counted in a gamma counter, and $f_P$ was calculated as the ratio of activity in the ultrafiltrate to total activity (Gandelman et al., 1994). Triplicate aliquots of saline solution mixed with the radiotracer were also processed to determine the filter retention of the free tracer.

In the postamphetamine condition, amphetamine plasma levels were measured in three arterial samples obtained at time 0, 45, and 90 min relative to the PET scan as previously described (Reimer et al., 1993). These data ensured that differences in plasma amphetamine concentration did not bias the radiotracer comparison.

### MRI acquisition and segmentation procedures

To provide an anatomical framework for analysis of the PET data, MRI scans were obtained using a 1.5 T GE Medical Systems (Milwaukee, WI) Signa Scanner and a 3D spoiled gradient recalled sequence. MRI segmentation was performed using the FAST automated segmentation tool (Zhang et al., 2001) implemented in the FMRIB Software Library (v4.0, Smith et al., 2004).

### Image analysis

PET data were reconstructed using filtered backprojection (Fourier rebinning/2D backprojection, 3 mm Hann filter) and corrected for photon attenuation ($^{68}$Ge/$^{68}$Ga rods), scatter (Watson, 2000), and radioactive decay. Reconstructed image files were then processed with the image analysis software MEDx (Sensor Systems, Sterling, Virginia) and SPM2 (www.fil.ion.ucl.ac.uk/spm). Frame-to-frame motion correction for head movement and MR-PET image alignment were performed using a mutual information algorithm implemented in SPM2.

Time activity curves were generated for the eight cortical regions of interest using the criteria and methods outlined in (Abi-Dargham et al., 2000, 2002). Sampled cortical regions ($n$ = 8) included the medial temporal lobe MTL (which included the hippocampus, amygdala, parahippocampal gyri, and entorrhinal cortex), anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), orbital frontal cortex (OFC), medial prefrontal cortex (MPFC), temporal cortex (TC), parietal cortex (PC), and occipital cortex (OC). The cerebellum was subsampled in 15 consecutive coronal MRI slices caudal to the cerebellar penduncle and used as a reference region. The subsampling included only the gray matter and excluded the vermis, white matter, and the cerebro-cerebellar fissure.

For bilateral regions, right and left values were averaged. The contribution of plasma total activity to the regional activity was calculated assuming a 5% blood volume in the regions of interest (Mintun et al., 1984) and tissue activities were calculated as the total regional activities minus the plasma contribution.

### Derivation of binding parameters

We denote here the outcome variables using the recently issued consensus nomenclature for in vivo imaging of reversibly binding radioligands (for details refer to Innis et al., 2007).

The regional tissue distribution volume ($V_T$, ml cm$^{-3}$) was defined as the ratio of the ligand concentration in a region ($C_T$, μCi g$^{-1}$) to the concentration of unmetabolized ligand in arterial plasma ($C_P$, μCi ml$^{-1}$) at equilibrium,

$$V_T = \frac{C_T}{C_P} \tag{1}$$

In the cerebellum, a region with negligible $D_2$ receptor density, $V_T$ includes the free and nonspecific binding, and $V_T$ in the cerebellum ($V_{T\ CER}$) is equal to the nondisplaceable distribution volume ($V_{ND}$). The region of interest $V_T$ ($V_{T\ ROI}$) includes $V_{ND}$ and the specific binding distribution volume or $BP_P$ (binding potential in which specific binding is compared with total plasma concentration). Assuming that $V_{ND}$ is equal in both regions, $BP_P$ was derived as the difference between $V_{T\ ROI}$ and $V_{T\ CER}$.

Under the assumption of a competitive interaction between the radiotracer and DA, $BP_P$ is related to receptor parameters and endogenous DA by

$$V_{T\,ROI} - V_{T\,CER} = BP_P = f_P \times \frac{B_{max}}{K'_D} = f_P \times \frac{B_{max}}{K_D\left(1 + F_{DA}/K_I\right)} \tag{2}$$

where $f_P$ is defined as free fraction of radioligand in the plasma, $B_{max}$ is the concentration of $D_2$ receptors(nM/g of tissue), $K_D$ is the in vivo equilibrium dissociation constant of the radiotracer (nM/ml of brain water), $K'_D$ is $K_D$ in the presence of the competitor DA (nM/ml of brain water), $F_{DA}$ is the free concentration of endogenous DA in the vicinity of the receptors, and $K_I$ is the inhibition constant of DA for the binding of the radiotracer (Laruelle et al., 1994, 1998).

A preferred outcome measure for endogenous competition experiments is the $BP_{ND}$ (binding potential in which specific binding is compared with nondisplaceable uptake). $BP_{ND}$ was calculated as the ratio of $BP_P$ to $V_{T\ CER}$. $BP_{ND}$ is related to receptor parameters and endogenous DA by

*TABLE I. Baseline scan parameters and plasma analysis (n = 12 subjects)*

| | [$^{11}$C]FLB 457 | | [$^{11}$C]fallypride | |
|---|---|---|---|---|
| | Baseline | Postamphetamine | Baseline | Postamphetamine |
| Injected dose (m Ci) | 4.7 ± 0.9 | 4.3 ± 0.9 | 5.0 ± 0.6 | 4.7 ± 0.7 |
| SA (Ci/mmoles) | 4761 ± 2374 | 3460 ± 1291 | 2105 ± 638 | 1640 ± 417[a] |
| Injected mass (μg) | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.9 ± 0.2 | 1.1 ± 0.1[a] |
| Plasma free fraction ($f_P$, %) | 60.0 ± 15.5 | 59.0 ± 15.3 | 13.8 ± 5.0 | 13.3 ± 5.5 |
| Clearance (L/h) | 97 ± 21 | 105 ± 20 | 34 ± 6 | 36 ± 8 |
| Cerebellum VT (ml cm−3) | 5.73 ± 1.20 | 5.68 ± 1.05 | 1.02 ± 0.34 | 1.02 ± 0.31 |
| Non displaceable free fraction ($f_{ND}$, %) | 10.8 ± 3.2 | 10.6 ± 2.7 | 14.0 ± 4.56 | 13.8 ± 6.2 |
| Plasma amphetamine (0 min, ng ml$^{-1}$) | | 71.9 ± 6.9 | | 76.2 ± 10.3 |
| Plasma amphetamine (45 min, ng ml$^{-1}$) | | 66.6 ± 7.2 | | 68.3 ± 7.6 |
| Plasma amphetamine (90 min, ng ml$^{-1}$) | | 65.4 ± 9.7 | | 65.5 ± 8.3 |

[a]$P < 0.05$, paired $t$-test, baseline compared with postamphetamine.

$$\frac{V_{T\,ROI} - V_{T\,CER}}{V_{T\,CER}} = BP_{ND} = f_{ND} \times \frac{B_{max}}{K'_D} = f_{ND} \times \frac{B_{max}}{K_D\left(1 + F_{DA}/K_I\right)} \quad (3)$$

where $f_{ND}$ is the free fraction in the nonspecific distribution volume of the brain ($f_{ND} = f_P/V_{ND}$) (Laruelle et al., 1994). The change in $BP_{ND}$ elicited by amphetamine ($\Delta BP_{ND}$) was calculated as the difference between $BP_{ND}$ measured in the postamphetamine condition ($BP_{ND_{AMPH}}$) and $BP_{ND}$ measured in the baseline condition on that day ($BP_{ND_{BASE}}$) and expressed as a percentage of $BP_{ND_{BASE}}$:

$$\Delta BP_{ND} = 100 \times \frac{BP_{ND_{AMPH}} - BP_{ND_{BASE}}}{BP_{ND_{BASE}}} \quad (4)$$

Equation 3 demonstrates that the increase in $F_{DA}$ resulting from the challenge translates into a decrease in $BP_{ND}$. Equation 3 also indicates that the attribution of changes in $BP_{ND}$ solely to changes in $F_{DA}$ requires the assumption that all other parameters of this equation remain constant, including the nonspecific binding ($f_{ND}$). $\Delta BP_{ND}$ is generally preferred to $\Delta BP_P$ to measure the effect of amphetamine, because the test–retest reproducibility of $BP_{ND}$ is generally better than that of $BP_P$. The effect of amphetamine on plasma clearance ($C_L$), $f_P$, $f_{ND}$, and $V_{ND}$ was also expressed relative to the preamphetamine value measured the same day.

Derivation of [$^{11}$C]FLB 457 and [$^{11}$C]fallypride $V_T$ was performed using kinetic analysis and the arterial input function. A two-tissue compartment model (2TCM) described the cerebellum and regions of interest for both tracers (Montgomery et al., 2006; Mukherjee et al., 2002; Olsson et al., 1999).

### Statistical analysis

Data were analyzed with repeated measure ANOVA (RM ANOVA) and paired $t$-test as specified. For the regions of interest a false discovery rate (FDR) correction with $\alpha = 0.05$ was applied to correct for multiple comparisons (Benjamini and Hochberg, 1995).

## RESULTS

### Demographics

Twelve subjects (5 males/7 females; 1 AA/11 C) participated in the study. The mean age of the subjects was 28 ± 10 (range 19–48). The mean BMI of the subjects was 24 ± 3 (range 19–27). All twelve subjects who participated in the study were nonsmokers.

### Baseline scan parameters

#### Injected dose and mass

The mean injected dose, mass, and specific activity at the time of injection for the baseline and postamphetamine condition for both radiotracers [$^{11}$C]FLB 457 and [$^{11}$C]fallypride are listed in Table I.

No significant differences were observed between the baseline and postamphetamine condition in injected dose and injected mass for [$^{11}$C]FLB 457.

No significant differences were observed between the baseline and postamphetamine condition in injected radiation dose for [$^{11}$C]fallypride. A slightly but significantly higher mass dose of [$^{11}$C]fallypride was injected during the postamphetamine condition (1.1 ± 0.1) relative to the baseline condition (0.9 ± 0.1). Nevertheless, as the total injected mass was <1.2 μg (mass required for less than 5% of occupancy $D_2$ receptor, tracer dose; unpublished data from Columbia University, NY, NY) in both conditions (baseline and postamphetamine) it is unlikely to have had a significant impact on the measurement of [$^{11}$C]fallypride $\Delta BP_{ND}$ following amphetamine.

### Plasma analysis

#### Clearance

Under baseline conditions, [$^{11}$C]FLB 457 plasma $C_L$ was significantly faster than [$^{11}$C]fallypride plasma $C_L$ (RM ANOVA, $P < 0.001$). Amphetamine did not significantly alter the plasma $C_L$ for [$^{11}$C]FLB 457 or [$^{11}$C]fallypride (individual values listed in Table I).

### Free fraction in plasma

Under baseline conditions, [$^{11}$C]FLB 457 $f_P$ was significantly higher than [$^{11}$C]fallypride $f_P$ (RM ANOVA, $P < 0.001$). Amphetamine did not significantly alter $f_P$ for [$^{11}$C]FLB 457 or [$^{11}$C]fallypride (Table I).

### Amphetamine plasma levels

No significant differences in the amphetamine plasma levels were observed between the postamphetamine [$^{11}$C]FLB 457 and [$^{11}$C]fallypride scans (RM ANOVA, $P = 0.72$). In addition, the amphetamine plasma levels were relatively stable throughout the entire duration of the postamphetamine scan for both radioligands (Table I).

## Regions of interest volumes

The volumes of the eight cortical regions of interest and reference region are provided in Table II.

## Reference region analysis

### Cerebellum distribution volume

Under baseline conditions, [$^{11}$C]FLB 457 $V_{ND}$ was significantly higher than [$^{11}$C]fallypride $V_{ND}$ (RM ANOVA, $P < 0.001$). Amphetamine did not significantly alter the $V_{ND}$ for [$^{11}$C]FLB 457 or [$^{11}$C]fallypride (Table I)

### Free fraction in nondisplaceable compartment ($f_{ND}$)

Under baseline conditions, [$^{11}$C]FLB 457 $f_{ND}$ was not significantly different than [$^{11}$C]fallypride $f_{ND}$ (RM ANOVA, $P = 0.12$). Amphetamine did not significantly alter the $f_{ND}$ for [$^{11}$C]FLB 457 or [$^{11}$C]fallypride (Table I). The lack of any change in $f_{ND}$ following amphetamine for both radioligands validated our assumptions for using $\Delta BP_{ND}$ as an outcome measure to detect changes in free dopamine concentrations following amphetamine ($F_{DA}$, see Eq. 3).

*TABLE II. Regional volumes*

| Region | Volume (mm$^3$) |
|---|---|
| Medial temporal lobe | 15,383 ± 3386 |
| Anterior cingulate cortex | 3945 ± 1231 |
| Dorsolateral prefrontal cortex | 21,451 ± 4886 |
| Orbital frontal cortex | 9640 ± 2661 |
| Medial prefrontal cortex | 4779 ± 1227 |
| Temporal cortex | 37,099 ± 4854 |
| Parietal cortex | 74,053 ± 15,018 |
| Occipital cortex | 53,459 ± 14,564 |
| Cerebellum | 21,640 ± 7586 |

## Region of interest analysis: binding potential $BP_{ND}$

The values of $BP_{ND}$ and $\Delta BP_{ND}$ for each region are given in Table III.

### Comparison of baseline [$^{11}$C]FLB 457 and [$^{11}$C]fallypride $BP_{ND}$

Under baseline conditions, [$^{11}$C]FLB 457 $BP_{ND}$ was significantly higher than [$^{11}$C]fallypride $BP_{ND}$ (RM ANOVA, $BP_{ND}$ as dependent variable; tracer factor, $P = 0.012$; tracer × region interaction, $P = 0.03$; see Fig. 1)

### Amphetamine-induced changes in [$^{11}$C]FLB 457 $BP_{ND}$

Amphetamine produced a statistically significant decrease in $BP_{ND}$ in the MTL, ACC, DLPFC, MPFC, and PC (paired $t$-tests, $P < 0.05$; see Table III, Figs. 2 and 3). These results remained significant after correction for multiple comparisons using the false discovery rate. Nonsignificant decreases in $BP_{ND}$ were observed in the remaining regions (OFC, TC, and OCC).

The regional differences in $\Delta BP_{ND}$ were evaluated with repeated measures ANOVA, with regional $\Delta BP_{ND}$ as repeated factor. No significant between-region differences in $\Delta BP_{ND}$ were observed (RM ANOVA, $P = 0.5$)

### Amphetamine-induced changes in [$^{11}$C]fallypride $BP_{ND}$

The values of $BP_{ND}$ and $\Delta BP_{ND}$ for each region are given in Table III and Fig. 3). Amphetamine produced a statistically significant decrease in $BP_{ND}$ only in the

*TABLE III. Effect of amphetamine on [$^{11}$C]FLB 457 and [$^{11}$C]fallypride $BP_{ND}$*

| | [$^{11}$C]FLB 457 $BP_{ND}$ | | | | [$^{11}$C]fallypride $BP_{ND}$ | | | |
|---|---|---|---|---|---|---|---|---|
| Region | Baseline | Postamphetamine | Difference (%) | $P$ values | Baseline | Postamphetamine | Difference (%) | $P$ values |
| Medial temporal lobe | 1.51 ± 0.28 | 1.40 ± 0.24 | −7.0 ± 6.1 | 0.005[a] | 1.16 ± 0.17 | 1.11 ± 0.24 | −4.4 ± 11.8 | 0.256 |
| Anterior cingulate cortex | 0.94 ± 0.37 | 0.85 ± 0.28 | −8.1 ± 8.2 | 0.024[a] | 0.61 ± 0.24 | 0.55 ± 0.27 | −8.7 ± 21.2 | 0.216 |
| Dorsolateral prefrontal cortex | 0.58 ± 0.32 | 0.50 ± 0.26 | −12.6 ± 15.2 | 0.011[a] | 0.36 ± 0.19 | 0.31 ± 0.19 | −9.7 ± 25.3 | 0.053 |
| Orbital frontal cortex | 0.67 ± 0.34 | 0.61 ± 0.29 | −5.1 ± 21.6 | 0.224 | 0.44 ± 0.19 | 0.40 ± 0.22 | −8.0 ± 27.4 | 0.246 |
| Medial prefrontal cortex | 0.78 ± 0.35 | 0.69 ± 0.30 | −11.3 ± 14.3 | 0.021[a] | 0.47 ± 0.20 | 0.43 ± 0.22 | −6.9 ± 17.6 | 0.166 |
| Temporal cortex | 1.72 ± 0.59 | 1.63 ± 0.49 | −4.2 ± 9.1 | 0.146 | 1.12 ± 0.29 | 1.03 ± 0.31 | −7.5 ± 10.9 | 0.048 |
| Parietal cortex | 0.57 ± 0.32 | 0.48 ± 0.26 | −12.4 ± 13.4 | 0.005[a] | 0.33 ± 0.19 | 0.32 ± 0.23 | −10.0 ± 33.4 | 0.536 |
| Occipital cortex | 0.51 ± 0.26 | 0.46 ± 0.23 | −4.7 ± 19.6 | 0.057 | 0.32 ± 0.18 | 0.31 ± 0.23 | −8.7 ± 30.0 | 0.576 |

Values are mean ± SD; $n = 12$ subjects. $P$ values indicate paired $t$-tests.
[a]Significant following multiple corrections using FDR.

TC (paired $t$-tests, $P < 0.048$). This result was not significant after correction for multiple comparisons using the false discovery rate (Table III).

No significant differences were observed between [$^{11}$C]FLB 457 $\Delta BP_{ND}$ and [$^{11}$C]fallypride $\Delta BP_{ND}$ (RM ANOVA; $\Delta BP_{ND}$ as dependent variable; tracer factor, $P = 0.97$).

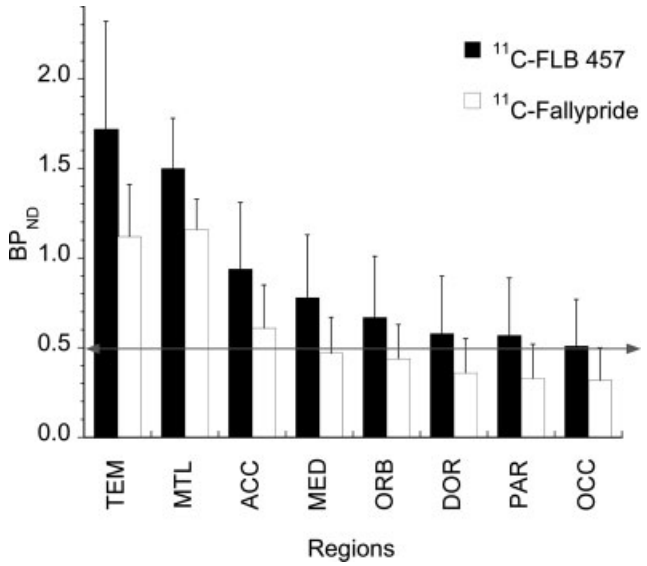


Fig. 1. Comparison of the baseline $BP_{ND}$ for [$^{11}$C]FLB 457 and [$^{11}$C]fallypride ($n = 12$ subjects). The [$^{11}$C]FLB 457 $BP_{ND}$ is 1.6-fold (average across eight ROIs) greater than [$^{11}$C]falypride $BP_{ND}$. Note: gray arrow illustrates [$^{11}$C]FLB 457 $BP_{ND} > 0.5$ in all ROIs evaluated in this study. Regions include TEM (Temporal cortex); MTL (medial temporal lobe); ACC (anterior cingulate cortex); MPFC (medial prefrontal cortex); ORB (orbital frontal cortex); DOR (dorsolateral prefrontal cortex); PAR (Parietal cortex); and OCC (Occipital cortex).

## Region of interest analysis: binding potential $BP_P$

### Comparison of baseline [$^{11}$C]FLB 457 and [$^{11}$C]fallypride $BP_{ND}$

Table IV lists the values of [$^{11}$C]FLB 457 and [$^{11}$C]fallypride $BP_P$ under baseline and postamphetamine conditions. Under baseline conditions, [$^{11}$C]FLB 457 $BP_P$ was significantly higher than [$^{11}$C]fallypride $BP_{ND}$ (RM ANOVA, $BP_{ND}$ as dependent variable; tracer factor, $P < 0.0001$; tracer $\times$ region interaction, $P < 0.0001$).

### Amphetamine-induced changes in [$^{11}$C]FLB 457 $BP_P$

The values of $BP_P$ and $\Delta BP_P$ for each region are given in Table IV. Amphetamine produced a statistically significant decrease in $BP_P$ only in the PC (paired $t$-tests, $P < 0.05$; Table IV). This result was not significant after correction for multiple comparisons using the false discovery rate.

### Amphetamine-induced changes in [$^{11}$C]fallypride $BP_P$

The values of $BP_P$ and $\Delta BP_P$ for each region are given in Table IV. Amphetamine did not produce a statistically significant decrease in $BP_P$ in any of the regions (paired $t$-tests, $P < 0.05$; Table IV).

Amphetamine induced decrease in [$^{11}$C]FLB 457 or [$^{11}$C]fallypride $BP_P$ or $BP_{ND}$ in the cortical regions of interest were not significantly correlated with the subjects' age or plasma amphetamine levels achieved during the scan ($P > 0.05$, Pearson Correlation Coefficient).

In addition, the order of the sequence (first or second) in which subjects received radiotracer had no

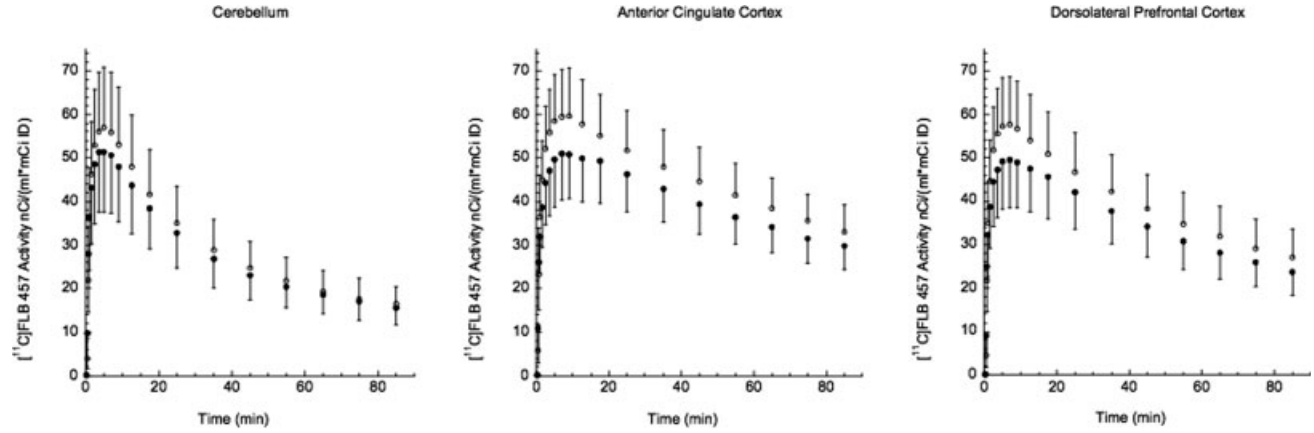


Fig. 2. Amphetamine-induced displacement of [$^{11}$C]FLB 457; mean $\pm$ SD, baseline (open circles, $n = 12$ subjects) and postamphetamine (closed circles, $n = 12$ subjects) [$^{11}$C]FLB 457 time activity curves (TAC) normalized to injected doses are shown for Cerebellum (reference region), Anterior Cingulate Cortex (ACC) and Dorsolateral prefrontal cortex (DLPFC). Note the significant decreases in TAC following amphetamine in ACC and DLPFC but not in cerebellum.

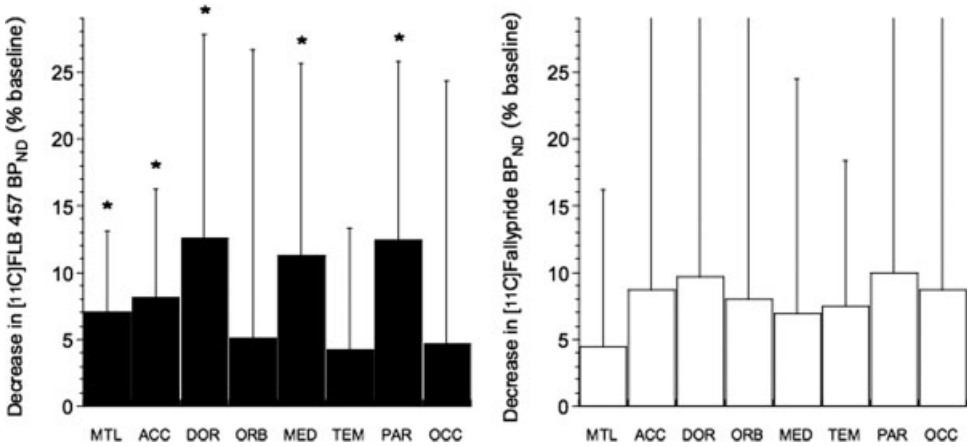


Fig. 3. Mean ± SD decrease in [$^{11}$C]FLB 457 (black bars) and [$^{11}$C]fallypride (white bars) $D_2$ receptor $BP_{ND}$ in eight cortical regions of interest in 12 healthy subjects after the administration of 0.5 mg kg$^{-1}$ amphetamine. * represents significant decrease ($P < 0.05$) following the FDR correction for multiple comparisons.

*TABLE IV. Effect of amphetamine on [$^{11}$C]FLB 457 and [$^{11}$C]fallypride BPP*

| Region | [$^{11}$C]FLB 457 $BP_P$ | | | | [$^{11}$C]fallypride $BP_P$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | Post amphetamine | Difference (%) | *P* Values | Baseline | Post amphetamine | Difference (%) | *P* Values |
| Medial temporal lobe | 8.60 ± 2.26 | 7.88 ± 1.72 | −6.6 ± 12.4 | 0.112 | 1.17 ± 0.38 | 1.12 ± 0.39 | −4.5 ± 11.6 | 0.266 |
| Anterior cingulate cortex | 5.32 ± 2.26 | 4.71 ± 1.41 | −7.4 ± 15.2 | 0.145 | 0.60 ± 0.25 | 0.53 ± 0.22 | −9.5 ± 16.6 | 0.073 |
| Dorsolateral prefrontal cortex | 3.27 ± 2.04 | 2.73 ± 1.37 | −12.0 ± 19.8 | 0.066 | 0.36 ± 0.21 | 0.31 ± 0.19 | −9.9 ± 25.4 | 0.129 |
| Orbital frontal cortex | 3.78 ± 2.08 | 3.43 ± 1.62 | −5.6 ± 20.5 | 0.312 | 0.43 ± 0.21 | 0.39 ± 0.22 | −8.8 ± 24.4 | 0.236 |
| Medial prefrontal cortex | 4.40 ± 2.17 | 3.76 ± 1.47 | −10.3 ± 21.3 | 0.111 | 0.46 ± 0.20 | 0.42 ± 0.19 | −7.6 ± 13.8 | 0.091 |
| Temporal cortex | 9.73 ± 3.63 | 9.11 ± 2.67 | −3.1 ± 19.4 | 0.391 | 1.12 ± 0.40 | 1.04 ± 0.39 | −7.4 ± 12.3 | 0.135 |
| Parietal cortex | 3.23 ± 1.99 | 2.68 ± 1.34 | −11.9 ± 17.5 | 0.037 | 0.33 ± 0.20 | 0.30 ± 0.18 | −11.1 ± 30.1 | 0.196 |
| Occipital cortex | 2.86 ± 1.56 | 2.57 ± 1.21 | −4.5 ± 21.6 | 0.107 | 0.32 ± 0.17 | 0.29 ± 0.18 | −9.7 ± 28.0 | 0.256 |

Values are mean ± SD; $n = 12$ subjects. *P* values indicate paired *t*-tests.
[c]Significant following multiple corrections using FDR.

significant effect on amphetamine-induced reduction in $BP_{ND}$ or $BP_P$ for [$^{11}$C]FLB 457 or [$^{11}$C]fallypride (RM ANOVA, $P > 0.05$).

## DISCUSSION

The fundamental aim of this investigation was to compare the in vivo specific binding of [$^{11}$C]FLB 457 and [$^{11}$C]fallypride in terms of vulnerability to endogenous competition by DA. Endogenous competition experiments were performed in the same human subjects, under carefully controlled conditions, and in counterbalanced sequence. The results demonstrate that in vivo [$^{11}$C]FLB 457 has a higher signal to noise ratio than [$^{11}$C]fallypride, a finding consistent with the previously published human PET studies performed with both radioligands (Table V). These results also demonstrate for the first time, unequivocally, that this enhanced signal to noise ratio allows for the measurement of amphetamine induced DA release in the human cortex with [$^{11}$C]FLB 457 and PET. This observation is also consistent with the previous studies in the literature that have reported more success in measuring changes in cortical DA transmission with [$^{11}$C]FLB 457 (Aalto et al., 2005; Montgomery et al., 2006) than with [$^{18}$F]fallypride (Cropley et al., 2008; Riccardi et al., 2005; Slifstein et al., 2007a).

To summarize, our observation of amphetamine-induced DA release reduction of in vivo binding of [$^{11}$C]FLB 457 in the human prefrontal cortex is consistent with previous human studies, in which the binding of this radioligand have been reported to be vulnerable to endogenous competition by DA in the frontal as well as other cortical regions of interest following an acute cognitive (Aalto et al., 2005) or methylphenidate challenge (Montgomery et al., 2006). On the other hand, consistent with the inability to reliably measure amphetamine-induced displacement of [$^{11}$C]fallypride in the human cortex in this study are two out of three investigations with [$^{18}$F]fallypride reporting that [$^{18}$F]fallypride cannot be used to measure amphetamine-induced DA release (greater than 5% decrease in radioligand binding that is statistically significant) in the cortical regions of interest because of its relatively low signal to noise ratio in

*TABLE V. Comparison of in vivo binding parameters of [$^{11}$C]FLB 457 and [18F]/[$^{11}$C]fallypride in published human studies*

| Study | HC ($n$) | Mass (nmoles) | Duration (min) | Analysis | Modeling | Prefrontal cortex $BP_{ND}$ | Anterior cingulate $BP_{ND}$ |
|---|---|---|---|---|---|---|---|
| [$^{11}$C]FLB 457 | | | | | | | |
| Ito et al., 2001 | 7 | 0.7–1.52 | 70 | SUB | KIN | 1.0 ± 0.2 | 0.9 ± 0.2 |
| Sudo et al., 2001 | 8 | 0.5–1.7 | 90 | SUB | SRTM | 1.0 ± 0.2 | – |
| Suhara et al., 2002 | 18 | 0.5–1.67 | 80 | SUB | SRTM | 1.0 ± 0.2 | 1.2 ± 0.2 |
| Olsson et al., 1999 | 8 | 2.4–4.6 | 60 | ROI | KIN | 0.7 ± 0.2 | 0.8 ± 0.2 |
| Vilkman et al., 2000 | 7 | 3.7 ± 1.4 | 70 | ROI | GRA | 0.5 ± 0.1 | |
| Olsson et al., 2004 | 10 | 2.4–4.6 | 60 | ROI | SRTM | 0.7 ± 0.1 | 0.8 ± 0.2 |
| Talvik et al., 2003 | 8 | 1.9 ± 0.4 | 60 | ROI | SRTM | 0.7 ± 0.2 | 0.9 ± 0.4 |
| Aalto et al., 2005 | 12 | 2.2–2.6 | 70 | ROI | SRTM | 0.6 ± 0.2 | 0.9 ± 0.2 |
| Kemppainen et al., 2003 | 11 | 2.2–4.8 | 70 | ROI | SRTM | 0.4 ± 0.1 | 0.4 ± 0.1 |
| Kaasinen et al., 2001 | 12[a] | 3.5 ± 1.2 | 70 | ROI | SRTM | 0.3 ± 0.1 | 0.4 ± 0.1 |
| Kaasinen et al., 2001 | 12[b] | 3.6 ± 1.1 | 70 | ROI | SRTM | 0.5 ± 0.1 | 0.6 ± 0.1 |
| Kaasinen et al., 2000a | 20 | 3.3 ± 1.0 | 70 | ROI | SRTM | 0.4 ± 0.1 | 0.5 ± 0.1 |
| Hagelberg et al., 2004 | 8 | 2.6 ± 0.4 | 70 | ROI | SRTM | 0.8 ± 0.2 | 1.0 ± 0.2 |
| Hagelberg et al., 2004 | 8 | 3.8 ± 1.5 | 70 | ROI | SRTM | 0.7 ± 0.6 | 0.9 ± 0.2 |
| Okubo et al., 1999 | 13 | 1.6–5.0 | 65 | ROI | SRTM | 0.5 ± 0.2 | 0.9 ± 0.4 |
| Montgomery et al., 2006 | 12 | 2.7–10.8 | 90 | ROI | KIN | 0.7 ± 0.1 | 0.9 ± 0.3 |
| This study | 12 | 0.4 ± 0.1 | 90 | SEG | KIN | 0.6 ± 0.3[c] | 0.9 ± 0.3 |
| Average $BP_{ND}$ | | | | | | 0.7 ± 0.2 | 0.8 ± 0.2 |
| [18F]Fallypride | | | | | | | |
| Slifstein et al., 2007a | 15 | <3.3 | 240 | SEG | KIN | 0.4 ± 0.2 | 0.5 ± 0.1 |
| Mukherjee et al., 2002 | 6 | 1.8–2.5 | 180 | ROI | GRA | 0.3 ± 0.1 | – |
| Riccardi et al., 2005 | 14 | <2.0 | 210 | ROI | SRTM | <0.5 | – |
| Cropley et al., 2008 | 14 | <3.3 | 300 | SEG | SRTM | 0.6 ± 0.1[d] | 0.5 ± 0.1 |
| This study | 12 | 0.9 ± 0.2 | 90 | SEG | KIN | 0.4 ± 0.2[c] | 0.6 ± 0.2 |
| Average $BP_{ND}$ | | | | | | 0.4 ± 0.2 | 0.5 ± 0.1 |

$V_{ND}$, nonspecific binding; $BP_{ND}$, binding potential; ROI, full region of interest delineated; SEG, segmentation of gray matter pixels in region of interest; SUB, subsampling of region interest with small circles; KIN, kinetic analysis; SRTM, simplified reference tissue method; GRA, logan graphical method.
[a]Males.
[b]Females.
[c]Weighted average of DLPFC, MPFC, and OFC.
[d]Medial orbital frontal cortex.

these regions (Riccardi et al., 2005; Slifstein et al., 2007a). The third study by Cropley et al. reported a statistically significant decrease of [$^{18}$F]fallypride binding (−13% ± 4%) in the medial OFC but not in the temporal cortex. Other cortical regions such as the DLPFC, MPFC, and ACC were not evaluated in this study either due to relatively low binding potential ($BP_{ND} < 0.5$) or poor reproducibility for $BP_{ND}$ (Cropley et al., 2008). Thus, the success of [$^{11}$C]FLB 457 and failure of [$^{11}$C]fallypride to measure amphetamine-induced DA release in the human cortex was somewhat predicted by the published literature. The choice of [$^{18}$F]fallypride over [$^{11}$C]FLB 457 as a preferred tool to measure amphetamine-induced DA transmission was primarily driven by its promise to evaluate both the high binding striatal and low binding extrastriatal regions of interest such as the cortex at the same time. Unfortunately, the results of this study as well as other recent investigations with [$^{18}$F]fallypride only confirm the fact that measurement of amphetamine induced DA release in both striatum and cortex with one tracer is not currently feasible.

## Methodological considerations

The study was designed to establish the amphetamine effect in the cortical regions of interest for both [$^{11}$C]FLB 457 and [$^{11}$C]fallypride. Because of the between-subject variability in the magnitude of this effect, it was important to study the same subject following the same dose of amphetamine with both radioligands. Although previous results are mixed with respect to sensitization of dopamine transmission following repeated amphetamine exposure (Boileau et al., 2006; Kegeles et al., 1999), this requirement induced the concern that tolerance or sensitization to the amphetamine effect might develop during the course of the study. To alleviate this concern, the radiotracers were administered in counterbalanced fashion. Our results demonstrated a statistically significant decrease in 5/8 ROIs for [$^{11}$C]FLB 457 but not for [$^{11}$C]falypride (other than TC, which failed to survive correction for multiple comparisons). This is likely due to the increased variance in amphetamine-induced $\Delta BP_{ND}$ for [$^{11}$C]fallypride, which was caused by its relatively low signal to noise ratio in the cortical regions of interest ($BP_{ND} < 0.5$ in DLPFC, OFC, MPFC, PC, and OC).

Other issues that are significant and will need to be addressed in the validation of the use of [$^{11}$C]FLB 457 to measure cortical DA transmission are discussed below.

## FLB 457 occupancy

The paradigm we used to study amphetamine induced DA release included two administrations of [$^{11}$C]FLB 457 in the same day 3-h apart. An issue that needs further evaluation is the contribution of the carryover mass of FLB 457 from the first scan to

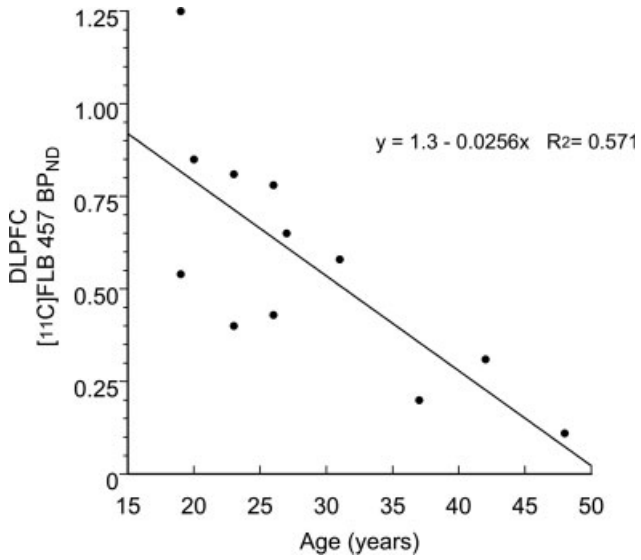


Fig. 4. Effect of age on cortical [$^{11}$C]FLB 457 $BP_{ND}$. Note significant decrease in baseline DLPFC $BP_{ND}$ values in relatively older subjects compared with those less than 35 years of age ($\rho = -0.76$; $P < 0.005$).

the second scan and its impact on $\Delta BP_{ND}$. In general, this particular issue is less of a problem when radioligands such as [$^{11}$C]raclopride, which have relatively low affinity and fast washout kinetics are used to measure amphetamine-induced DA-release. Nevertheless, there are significant advantages to performing two PET scans (such as baseline and postamphetamine) on the same day as this technique yields better within-subject reproducibility. In addition, completing all study procedures in a single day is less invasive (requiring only one A-line, IV, etc.) and less of time commitment for the subjects. This issue was debated extensively in the designing of this study. Our decision to pursue a single day study design (3-h apart) was based on a previous test–retest study that did not detect any carry over mass-induced decreases in [$^{11}$C]FLB 457 $BP_{ND}$ in the frontal and temporal cortex when subjects underwent the test and retest scans 3-h apart (Vilkman et al., 2000). In addition, in a counterbalanced study evaluating DA release following a cognitive task in which subjects received three [$^{11}$C]FLB 457 scans the same day, injected mass was not correlated with decrease in [$^{11}$C]FLB 457 $BP_{ND}$ (Aalto et al., 2005). Consistent with these previous reports we observed no correlation between either the injected mass of Scan 1 or the total injected mass (Scan 1 + 2) and decrease in [$^{11}$C]FLB 457 $BP_{ND}$ in the postamphetamine scan. This was not unexpected as the carrier mass for [$^{11}$C]FLB 457 in the above studies were restricted to 0.6 μg (1.6 nmoles), a tracer dose at which it occupies less than 5% of $D_2$ receptors in vivo (Sudo et al., 2001). Also, consistent with this report, an estimate of the in vivo $D_2$ receptor occupancy of [$^{11}$C]FLB 457 during the peak uptake of the radioligand in the DLPFC suggested 6% ± 2% in the studies in this report (DLPFC $D_2$ receptor occupancy was derived as $100 \times F/(K_D+F)$, where the free concentration of radioligand in the brain, $F$ was obtained by multiplying the measured concentration of [$^{11}$C]FLB 457 in the cerebellum at the time of peak specific binding in DLPFC by $f_{ND}$; and in vivo $K_D$ value of 0.09 nM for [$^{11}$C]FLB 457 in the DLPFC was used as reported by (Suhara et al., 1999). Given that the relatively low occupancy observed at peak uptake of the radioligand (6%) is followed by washout of the radiotracer, it is highly unlikely that the mass carried over contributed significantly to the amphetamine-induced decrease in $BP_{ND}$ measured in the second scan, which was administered 3-h postamphetamine.

### Increased between-subject variance in prefrontal cortical [$^{11}$C]FLB 457 $BP_{ND}$

To reliably and robustly measure amphetamine-induced DA release it is desirable that the specific signal be at least 50% of the nonspecific signal, i.e., $BP_{ND} > 0.5$ (for review, see Laruelle, 2000; Slifstein et al., 2004b). As evidenced in our data, a relatively low baseline [$^{11}$C]FLB 457 $BP_{ND}$ with high variability as was observed in the DLPFC (0.58 ± 0.32), OFC (0.67 ± 0.34), and MPFC (0.78 ± 0.35) could be a problem in measuring amphetamine effect in some subjects in these regions. In our data set this variance in baseline $BP_{ND}$ in the prefrontal cortical regions was driven by age (DLPFC $\rho = -0.76$, $P = 0.005$; OFC $\rho = -0.69$, $P = 0.013$; and MPFC $\rho = -0.67$, $P = 0.018$; where $\rho$ is Pearson Correlation Coefficient; see Fig. 4 in which the DLPFC is shown as an example). This observation is consistent with previous [$^{11}$C]FLB 457 studies demonstrating a significant age related decrease in cortical $D_2$ receptor $BP_{ND}$ that is most pronounced in the frontal cortex (~12% per decade) (Inoue et al., 2001; Kaasinen et al., 2000b, 2002). When we restricted the data to subjects <35 years of age ($n = 9$ subjects), the [$^{11}$C]FLB 457 $BP_{ND}$ values in the DLPFC, OFC, and MPFC were 0.70 ± 0.26, 0.80 ± 0.27, and 0.91 ± 0.30, respectively. These values are comparable to the striatal $BP_{ND}$ (0.7 ± 0.1) values of the SPECT $D_2$ radiotracer [$^{123}$I]IBZM that has been widely used to measure amphetamine-induced DA release in clinical populations such as schizophrenia (Laruelle et al., 1996) and cocaine addiction (Malison et al., 1999). Thus, our relatively small dataset ($n = 12$ subjects) suggests that the use of [$^{11}$C]FLB 457 to measure amphetamine-induced DA release in the prefrontal cortex is likely to be successful only in young adult subjects (<35 years of age). Further studies are necessary to confirm this preliminary observation in a larger representative sample.

### Displacement of [$^{11}$C]FLB 457 in OFC

In our studies amphetamine failed to induce displacement by DA of [$^{11}$C]FLB 457 in the OFC (−5% ± 21%). This result was in contrast to the robust displacements (DLPFC [$^{11}$C]FLB 457 $\Delta BP_{ND}$ −13% ± 15%; MPFC [$^{11}$C]FLB 457 $\Delta BP_{ND}$ −11% ± 14%) observed in the other two prefrontal cortical regions of interest. Nevertheless, this is likely to be a significant issue because the study of DA transmission in the OFC is of interest in addiction (Winstanley, 2007). One possible explanation for not detecting an amphetamine effect in the OFC is that [$^{11}$C]FLB 457 binding is associated with poor within-subject reproducibility in this particular region. A second possible explanation is that amphetamine leads to less displacement of [$^{11}$C]FLB 457 binding in the OFC relative to the DLPFC in healthy controls. Future test–retest studies as well as expansion of our current study cohort are likely to provide us with the information necessary to further understand this discrepant observation in the OFC.

### Magnitude of [$^{11}$C]FLB 457 in the prefrontal cortical regions (DLPFC and MPFC)

Another interesting observation was that the magnitude of the amphetamine-induced displacement of [$^{11}$C]FLB 457 in the DLPFC and MPFC (∼12%) was comparable to that previously reported in the striatum (∼10%) with other $D_2$ antagonist radiotracers (for review, see Laruelle, 2000). Although these data appear discrepant because the striatum receives a greater DA projection from the VTA/substantia nigra, these data are consistent with previous [$^{11}$C]FLB 457 and [$^{18}$F]fallypride studies, which have reported displacement values in the range of 10–15% in regions such as the hippocampus, ACC, DLPFC, and MPFC following amphetamine administration or cognitive stimulation (Aalto et al., 2005; Cropley et al., 2008; Slifstein et al., 2007a). A possible reason for comparable decreases in the striatum and DLPFC/MPFC binding following amphetamine is due to the difference in baseline DA levels between these regions. This hypothesis is consistent with combined microdialysis-PET studies, which have demonstrated that increases in endogenous DA following acute amphetamine challenge leads to a relatively larger change in [$^{18}$F]fallypride $BP_{ND}$ in the cingulate cortex than in the striatum (Slifstein et al., 2007b). In other words, the in vivo binding of the radioligand is more vulnerable or sensitive to endogenous competition by DA in the cortex than in the striatum because of less DA at baseline in the cortex. Combined PET-microdialysis studies with [$^{11}$C]FLB 457 in nonhuman primates are likely to further our understanding of these mechanistic differences between amphetamine-induced DA transmission in the striatum and cortex.

### Use of oral rather than intravenous d-amphetamine (amphetamine) as a challenge

Several groups have recently demonstrated the safety and effectiveness of oral amphetamine (dexedrine, 0.5 mg kg$^{-1}$) to measure amphetamine-induced DA release with PET (Cardenas et al., 2004; Cropley et al., 2008; Riccardi et al., 2005; Willeit et al., 2008). In contrast to intravenous amphetamine (0.3 mg kg$^{-1}$), which can lead to high fluctuations in vital signs and EKG parameters, oral amphetamine has been shown to have less effect on these parameters. For example, the mean peak systolic blood pressure following 0.5 mg kg$^{-1}$ oral amphetamine ($n$ = 24 administrations) in this study was 160 ± 13 mm of Hg, as opposed to 180 ± 8 following 0.3 mg kg$^{-1}$ intravenous amphetamine ($n$ = 24 administrations, healthy subjects) in Martinez et al. (2007).

In a recent study of healthy controls (Ziolko et al., 2007; $n$ = 8, healthy women subjects, 27 ± 5 years of age), we were able to successfully displace the binding of [$^{11}$C]raclopride in striatal subdivisions following oral amphetamine 0.5 mg kg$^{-1}$ ([$^{11}$C]raclopride $\Delta BP_{ND}$; RM ANOVA, $F$ = 31.3, df = 4, 13; $P <$ 0.0001). Of significant interest is the fact that the displacements in the three functional subdivisions of the striatum (limbic striatum, LST—15% ± 8%; associative striatum, AST—10% ± 5%, and sensorimotor striatum, SMST—17% ± 5%) were comparable to that reported in a previous [$^{11}$C]raclopride PET study, which used intravenous amphetamine 0.3 mg kg$^{-1}$ (LST −15% ± 10%; AST −8% ± 7%; and SMST −16 ± 9 Martinez et al., 2003). These data clearly demonstrate that oral amphetamine is as effective as intravenous amphetamine in measuring amphetamine-induced DA release with PET.

Higher plasma variance in amphetamine levels between subjects has been mentioned as a criticism for using oral rather than intravenous amphetamine to measure amphetamine-induced DA release with PET. PET studies in which higher plasma variance have been observed following oral amphetamine have typically scanned subjects after 1–2 h (Leyton et al., 2002; Willeit et al., 2008), as opposed to 3 h following amphetamine (Cropley et al., 2008; Riccardi et al., 2005). This is not surprising as pharmacokinetic studies have shown that the time taken for d-amphetamine to reach plasma $C_{max}$ and remain relatively stable is 3–4 h (Angrist et al., 1987). Thus, in the current study subjects were scanned 3 h following amphetamine administration. Another factor that may have driven the variance in plasma amphetamine levels in these studies is the effect of food on gastrointestinal (GI) absorption kinetics of d-amphetamine. In the above studies, subjects were not allowed to eat solid foods 5 h before and 3 h after the dose of amphetamine to reduce the variance in GI

*TABLE VI. Comparison of in vivo affinity of [$^{11}$C]FLB 457 and [18F]fallypride*

| Study | Species | In vivo $K'_D$ (nM) | In vivo $K_D$ (nM) | Method |
|---|---|---|---|---|
| [$^{11}$C]FLB | | | | |
| Olsson et al., 2004 | Human ($n$ = 10) | 0.4 (0.27–0.43) | | TEA |
| Suhara et al., 1999 | Human ($n$ = 8) | 0.9 (0.7–1.1) | | TEA |
| Delforge et al., 2001 | Baboon ($n$ = 4) | 0.3 (0.16–0.40) | | MIA |
| Average | | 0.53 | 0.06[a] | |
| [18F]Fallypride | | | | |
| Christian et al., 2004 | Rhesus ($n$ = 3) | 0.7 (0.38–1) | | MIA |
| Slifstein et al., 2004a | Baboon ($n$ = 3) | 1.3 | 0.2 (0.17 to 0.22) | NLKM, TEA |
| Average | | 1.0 | 0.14[a] | |

$K'_D$ is uncorrected for free fraction of radiotracer in the brain ($f_{ND}$). The corrected value provides in vivo $K_D$ values comparable to in vitro estimates.
TEA, transient equilibrium approach; MIA, multi-injection approach; NLKM, nonlinear kinetic modeling.
[a]The average published in vivo $K'_D$ for both radioligands were corrected for $f_{ND}$ using values from Table I ([$^{11}$C]FLB 457 = 10.8% and [$^{11}$C]fallypride = 14.0%).

absorption kinetics. The between-subject variance in amphetamine plasma levels in our data (for example, at the time of start of the postamphetamine [$^{11}$C]FLB 457 the variance was 72 ± 7 ng ml$^{-1}$; for more detailed data refer to Table I) is no different than that reported following intravenous amphetamine 0.3 mg kg$^{-1}$ (40 ± 13 ng ml$^{-1}$) in Martinez et al. 2003. Thus future studies could propose to use the relatively safer and equally effective oral amphetamine paradigm to study subcortical and cortical DA transmission in clinical populations.

### The use of cerebellum as a reference region for [$^{11}$C]FLB 457

Some, but not all (Farde et al., 1997; Olsson et al., 1999), studies suggest 11–75% of the binding of [$^{11}$C]FLB 457 in the cerebellum is specific to $D_2$ receptors (Ahmad et al., 2006; Asselin et al., 2007; Delforge et al., 1999; Montgomery et al., 2006). We did not observe any change in [$^{11}$C]FLB 457 $V_{T\ CER}$ before and after amphetamine (baseline $V_{T\ CER}$ 5.7 ± 1.2; postamphetamine $V_{T\ CER}$ 5.7 ± 1.1; $n$ = 12 subjects). The nonspecific binding in the cerebellum was comparable to that observed in the pons (baseline $V_{T\ PONS}$ 6.3 ± 1.2; postamphetamine $V_{T\ PONS}$ 6.1 ± 1.2; $n$ = 12 subjects), another brain region, which is relatively devoid of $D_2$ receptors (Hall et al., 1996). One possible reason for the discordant reports in the literature may be related to the differences in delineating the cerebellum such as including the vermis, which has been shown to receive DA input (Melchitzky and Lewis, 2000), or including the cerebro-cerebellar fissue, which may be more prone to spillage (or partial voluming) of signal from the cortex. We performed a subsampling of the cerebellum that carefully avoided both these subregions (vermis and cerebro-cerebellar fissure) and white matter (which may have different blood flow characteristics than the gray matter) Nevertheless, consistent with previous autoradiographic studies demonstrating near negligible $D_2$ receptor binding in the cerebellum (0.1% of that observed in the putamen) for [$^{123}$I]epidepride, the iodinated SPECT derivative of FLB 457 (Hall et al., 1996), our data supports the continued use of the cerebellum as a reference region for [$^{11}$C]FLB 457.

### In vivo $K_D$ for [$^{11}$C]FLB 457 and [$^{11}$C]fallypride

The data from our results also suggests that the in vivo $K_D$ of [$^{11}$C]FLB 457 is 2-fold lower than that of [$^{11}$C]fallypride. This value is derived based on the following equation:

$$\frac{[^{11}\text{C}]\text{FLB457BP}_F}{[^{11}\text{C}]\text{fallprideBP}_F} = \frac{[^{11}\text{C}]\text{fallpride}K_D}{[^{11}\text{C}]\text{FLB457K}_D} = \frac{8.6}{4.3} = 2 \quad (5)$$

where $BP_F$ is specific binding compared with free plasma concentration calculated as $BP_P/f_P$. [$^{11}$C]FLB 457 and [$^{11}$C]fallypride $BP_F$ values in Eq. 5 are the average values across the eight regions of interest evaluated.

Approximately 2-fold lower in vivo $K_D$ for [$^{11}$C]FLB 457 (0.06 nM) relative to [$^{11}$C]fallypride (0.14 nM) is consistent with the in vivo $K_D$ values published in the literature for both these radioligands (Table VI). This enhanced affinity is what leads to the increased signal to noise ratio associated with [$^{11}$C]FLB 457 compared with [$^{11}$C]fallypride in the cortical regions of interest (Table V).

Another point worthy of consideration is the fact that there were differences in the variability but not the magnitude of displacement as measured with [$^{11}$C]FLB 457 and [$^{11}$C]fallypride following an identical dose of amphetamine (as shown in Fig. 3). This observation of lack of differences in the magnitude of displacement of these two radiotracers of different affinity is yet another illustration of the fact that the affinity of the radiotracer does not impact on its vulnerability to endogenous competition by DA, given DA levels are constant and the radioligand is administered at tracer dose (for detailed discussion refer to Laruelle, 2000).

## CONCLUSIONS

Despite the methodological considerations discussed in the previous section, our results clearly demonstrate the superiority of [$^{11}$C]FLB 457 over [$^{11}$C]fallypride to measure amphetamine-induced DA release in the human cortex with PET. Further validation of [$^{11}$C]FLB 457 as a tool to image amphetamine-induced DA release is likely to allow for the measurement of in vivo DA transmission in the prefrontal cortex, which is of extreme interest to the study of several neuropsychiatric disorders such as schizophrenia, addiction, Parkinson's disease, and ADHD.

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