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Failure to detect amphetamine-induced dopamine release in the cortex with [^{11}C]FLB 457 positron emission tomography (PET): methodological considerations

Joshua Gertler¹, Savannah Tollefson¹, Rehima Jordan¹, Michael Himes¹, N. Scott Mason¹, W. Gordon Frankle³, and Rajesh Narendran^{1,2}

⁽¹⁾Department of Radiology, University of Pittsburgh, Pittsburgh, PA

⁽²⁾Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA

⁽³⁾Department of Psychiatry, NYU Langone Medical Center, New York, NY, USA

Abstract

Studies in nonhuman primates and humans have demonstrated that amphetamine-induced dopamine release in the cortex can be measured with [^{11}C]FLB 457 and PET imaging. This technique has been successfully used in recent clinical studies to show decreased dopamine transmission in the prefrontal cortex in schizophrenia and alcohol dependence. Here, we present data from a cohort of twelve healthy controls in whom an oral amphetamine challenge (0.5 mg kg⁻¹) did not lead to a significant reduction in [^{11}C]FLB 457 BP_{ND} (i.e., binding potential relative to non-displaceable uptake). Two factors that likely contributed to the inability to displace [^{11}C]FLB 457 BP_{ND} in this cohort relative to successful cohorts are: (1) the acquisition of the baseline and post-amphetamine scans on different days as opposed to the same day and (2) the initiation of the post-amphetamine [^{11}C]FLB 457 scan at ~ 5 hours as opposed to ~ 3 hours following oral amphetamine. Furthermore, we show [^{11}C]FLB 457 reproducibility data from a legacy dataset to support greater variability in cortical BP_{ND} when the test and re-test scans are acquired on different days as compared to the same day. These results highlight the methodological challenges that continue to plague the field with respect to imaging dopamine release in the cortex.

Keywords

[^{11}C]FLB 457; positron emission tomography; d-amphetamine; cortical dopamine

INTRODUCTION

[^{11}C]FLB 457 is high affinity dopamine D_{2/3} antagonist radiotracer that allows the for measurement of D_{2/3} receptors in extrastriatal regions with relatively low receptor density

Corresponding author: R. Narendran, MD, Department of Radiology and Psychiatry, University of Pittsburgh, University of Pittsburgh PET Facility, UPMC Presbyterian University Hospital B-938, Pittsburgh, PA 15213, Telephone: (412)-647-5176, Fax: (412)-647-0700, narendranr@upmc.edu.

DISCLOSURES

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(Halldin et al., 1995). Studies in healthy humans by three independent groups have demonstrated an 8–15% reduction in [^{11}C]FLB 457 BP_{ND} (i.e., binding potential relative to non-displaceable uptake) in cortical regions following a 0.5 g kg^{-1} oral amphetamine challenge (Narendran et al., 2009; Narendran et al., 2014; Sandiego et al., 2015; Sandiego et al., 2017; Slifstein et al., 2015). This observation in healthy subjects has recently been contrasted with the inability to displace [^{11}C]FLB 457 in patients with schizophrenia and alcohol dependence, and is interpreted as a decrease in cortical dopamine transmission in these disorders (Narendran et al., 2014; Slifstein et al., 2015). Nevertheless, concerns remain with respect to the vulnerability of the *in vivo* binding of [^{11}C]FLB 457 to endogenous competition by dopamine, because not all published studies in humans have replicated this observation (Aalto et al., 2009; Montgomery et al., 2007). Previously, failures to replicate this finding has been attributed to methodological issues (reviewed in detail in Narendran et al., 2009), such as differences in the challenge used to release cortical dopamine (oral vs. intravenous amphetamine, or oral methylphenidate); administration of a higher injected FLB 457 mass (Montgomery et al., 2007); and increased between-subject variability in amphetamine plasma levels during the post-amphetamine scan (Aalto et al., 2009). We present, for the first time, results from a cohort of healthy controls in whom we were unable to displace [^{11}C]FLB 457 binding following an oral amphetamine challenge. Two changes were implemented to the imaging protocol for this cohort compared to previous [^{11}C]FLB 457 oral amphetamine PET studies (Narendran et al., 2009; Narendran et al., 2014; Sandiego et al., 2015; Slifstein et al., 2015). First, the baseline and post-amphetamine scans were acquired on two different days as opposed to the same day. Second, the post-amphetamine [^{11}C]FLB 457 scan was initiated ~ 5 hours as opposed to ~ 3 hours after oral amphetamine. These changes were made so that individuals could be scanned with the striatal $\text{D}_{2/3}$ agonist radiotracer [^{11}C]NPA before the [^{11}C]FLB 457. [^{11}C]NPA can be used to measure $\text{D}_{2/3}$ receptors in the striatum but not in the cortex (Narendran et al., 2010). Such an approach, in which the baseline and post-amphetamine PET scans are acquired on separate days, if successful, would have allowed for the measurement of dopamine release in both the striatum and cortex with a single amphetamine exposure. The need to avoid repeated exposures to amphetamine is desirable for individuals with schizophrenia and addiction, because they are at risk for psychostimulant-induced exacerbation in psychosis and relapse. In this paper, we present the amphetamine-induced displacement of [^{11}C]FLB 457 BP_{ND} and the reproducibility of [^{11}C]FLB 457 binding measures when scans (i.e., the baseline and post-amphetamine scans for dopamine release; and the test and retest scans for reproducibility) are acquired on two different days. This reproducibility data from scans acquired on two different days is then contrasted with published data that was acquired on the same day in prior studies (Narendran et al., 2011b).

STUDY DESIGN AND METHODS

All data were acquired in imaging protocols approved by the University of Pittsburgh Institutional Review Board.

1. [^{11}C]FLB 457 amphetamine studies:

Healthy control subjects underwent two experimental sessions. Each experimental session included two PET scans: a 60-minute [^{11}C]-(-)-N-propylnorpomorphine (NPA) scan and a 90-minute [^{11}C]FLB 457 scan. The [^{11}C]NPA scan always preceded [^{11}C]FLB 457 in subjects, because there was concern that FLB 457, which has a relatively high affinity for $\text{D}_{2/3}$ receptors, could lead to residual occupancy and lower [^{11}C]NPA BP_{ND} in the striatum. Subjects were scanned under baseline and post-amphetamine conditions in the first and second experimental sessions. The first and second experimental session were separated by 10 ± 7 days (range 4 to 28 days). The post-amphetamine [^{11}C]NPA and [^{11}C]FLB 457 PET scans were initiated approximately 185 ± 37 and 303 ± 23 minutes after the administration of d-amphetamine (0.5 mg kg^{-1} , oral). Amphetamine plasma levels were measured in arterial samples, both at the beginning and completion of each PET scan. We only present the [^{11}C]FLB 457 data in this manuscript, because the [^{11}C]NPA results in the striatum are published in (Frankle et al., 2017).

The [^{11}C]FLB 457 amphetamine imaging protocol, plasma and image analyses were conducted according to methods described previously (Narendran et al., 2009; Narendran et al., 2014). Regions of interest included the medial temporal lobe [MTL], dorsolateral prefrontal cortex [DLPFC], orbital frontal cortex [OFC], medial prefrontal cortex [MPFC], anterior cingulate cortex [ACC], temporal cortex [TEMP], parietal cortex [PAR], and occipital cortex [OCC]. The cerebellum [CER] was used as the reference region. PET outcome variables were defined in accordance to the consensus nomenclature for *in vivo* imaging of reversibly binding radioligands (Innis et al., 2007). [^{11}C]FLB 457 PET data were also modeled using the simplified reference tissue method (SRTM), because a prior study was able to demonstrate an amphetamine-induced reduction in [^{11}C]FLB 457 BP_{ND} using SRTM but not kinetic modeling (Sandiego et al., 2015). The primary outcome measure for this study was amphetamine-induced change in BP_{ND} ($\Delta \text{BP}_{\text{ND}}$). This was calculated as the difference between BP_{ND} measured in the post-amphetamine condition ($\text{BP}_{\text{ND AMPH}}$) and BP_{ND} measured in the baseline condition ($\text{BP}_{\text{ND BASE}}$), and expressed as a percentage of $\text{BP}_{\text{ND BASE}}$.

2. [^{11}C]FLB 457 reproducibility studies:

We hypothesized that the inability to displace [^{11}C]FLB 457 following oral amphetamine in a different day scanning paradigm is due to the greater variability associated with BP_{ND} in cortical regions when measured on different days as opposed to the same day. To evaluate this issue, we examined the test-retest variability for [^{11}C]FLB 457 binding measures in seven subjects in whom we had a second [^{11}C]FLB 457 baseline scan (re-test scan condition) acquired 13 ± 4 days (range 7 to 17 days) following their participation in a [^{11}C]FLB 457 amphetamine same day PET protocol. The first baseline scan done prior to amphetamine was used as the test scan condition. This allowed for the computation of test-retest variability for the baseline [^{11}C]FLB 457 scans acquired on different days.

The reproducibility of volume of distribution expressed relative to total plasma ligand concentration (V_T), binding potential expressed relative to total plasma ligand concentration (BP_P), and BP_{ND} were evaluated for their *variability* and *reliability* (see Narendran et al.,

2011b for detailed methods). The test-retest variability (VAR) was calculated as the absolute value of the difference between the test and retest, divided by the mean of the test and retest values. The reliability of the measurements was assessed by the intraclass correlation coefficient (ICC).

STATISTICAL ANALYSIS

All statistical analyses were performed with IBM SPSS Statistics v.23. Scan parameters (such as injected dose, mass, specific activity, plasma clearance and V_{ND}) between scan conditions were contrasted with paired t-tests. Amphetamine effect on the dependent variable, BP_{ND} , was analyzed using a linear mixed model (LMM) with regions of interest as a repeated measure and condition as the fixed factor. Regions and condition by regions interaction were included in the model as explanatory variables. Linear mixed models with the same structure were also used to examine the effect of smoking on BP_{ND} and modeling method on test-retest variability.

RESULTS

1. [^{11}C]FLB 457 amphetamine studies

Demographics: 12 subjects (8 males/4 females; 2 African American/10 Caucasian) participated in the study. The mean age of the subjects was 26 ± 4 years. Six subjects who participated in the study were tobacco users (smokers).

Scan parameters: No significant differences were observed between the baseline and post-amphetamine [^{11}C]FLB 457 injected dose (baseline: 7.8 ± 1.3 mCi, post-amphetamine: 7.4 ± 1.2 mCi), specific activity (baseline: 11018 ± 6855 Ci mmol $^{-1}$, post-amphetamine: 8415 ± 4656 Ci mmol $^{-1}$) and injected mass (baseline: 0.3 ± 0.2 μ g, post-amphetamine: 0.4 ± 0.2 μ g).

Plasma Analysis: Amphetamine did not significantly alter the [^{11}C]FLB 457 plasma clearance (C_L , baseline: 84 ± 21 L h $^{-1}$, post-amphetamine 73 ± 27 L h $^{-1}$) or free fraction (f_p , baseline: $40 \pm 9\%$, post-amphetamine: $39 \pm 8\%$). The mean amphetamine levels at the time of initiation ($t = 0$ -min) and completion ($t = 90$ -min) of the post-amphetamine [^{11}C]FLB 457 PET scan were 67.6 ± 8.8 and 58.0 ± 9.7 ng mL $^{-1}$ (data available from $n=10$ out of 12 subjects).

Cerebellum distribution volume (V_{ND}): Amphetamine did not significantly alter the V_{ND} for [^{11}C]FLB 457 (baseline: 4.51 ± 0.88 mL cm $^{-3}$, post-amphetamine: 4.37 ± 1.00 mL cm $^{-3}$, paired t-test, $p = 0.47$)

Region of interest analysis: No significant differences were observed between the [^{11}C]FLB 457 BP_{ND} measured in the baseline and post-amphetamine conditions in the cortical regions (LMM, effect of condition, $F(1, 22) = 0.09$, $p = 0.76$; effect of region, $F(7, 154) = 186.89$, $p < 0.001$; region x condition interaction, $F(7, 154) = 0.36$, $p = 0.93$; also see individual paired t-tests p values > 0.1 in all cortical regions in **Table 1**). Derivation of BP_{ND} with the SRTM using the cerebellum as an input function provided similar results (see **Table**

2). Pearson product moment correlations between amphetamine plasma levels ($t=0$ min and 90 min) and BP_{ND} were not significant (data not shown, all p -values > 0.3)

Effect of smoking on BP_{ND} : No significant differences in BP_{ND} were observed between smokers and non-smokers [LMM, effect of smoking, $F(1, 10) = 1.06$, $p = 0.33$, effect of region, $F(7, 70) = 1.31$, $p = 0.26$, region $\times$ smoking interaction, $F(7, 70) = 0.62$, $p = 0.74$]. Unpaired t -tests in individual cortical regions were all $p > 0.1$ when contrasting BP_{ND} in smokers vs. non-smokers (data not shown).

2. [^{11}C]FLB 457 reproducibility studies

Baseline scan parameters: The mean injected dose for [^{11}C]FLB 457 in the test and retest conditions were 7.1 ± 1.9 mCi and 7.9 ± 1.2 mCi. The mean injected specific activity in the test and retest conditions were 7971 ± 4631 Ci mmol $^{-1}$ and 12474 ± 7847 Ci mmol $^{-1}$. The mean injected mass for [^{11}C]FLB 457 in the test and retest conditions were 0.4 ± 0.2 μg and 0.3 ± 0.1 μg . There were no significant differences in injected dose or mass between the test and the retest conditions (paired t -test, $p > 0.05$).

Plasma analysis: The mean [^{11}C]FLB 457 f_p in the test and retest conditions were $37 \pm 6\%$ and $36 \pm 7\%$. The mean [^{11}C]FLB 457 C_L in the test and retest conditions were 55 ± 19 L h $^{-1}$ and 59 ± 16 L h $^{-1}$. There were no significant differences in f_p or C_L between the test and the retest conditions.

Brain analysis: The mean regional V_T , BP_P , BP_{ND} and their corresponding VAR and ICC derived using kinetic analysis are provided in **Table 3**. No differences in BP_{ND} were noted between test and retest conditions (paired t -test, $p > 0.1$ in all cortical regions). The VAR and ICC for SRTM derived BP_{ND} is provided in **Table 4**. The test-retest variability for regional BP_{ND} derived using SRTM (10 to 18%) was numerically, but not statistically better (LMM, effect of analysis method, $F(1, 12) = 1.1$, $p = 0.31$; effect of region, $F(7, 84) = 1.6$, $p = 0.14$; region $\times$ condition interaction, $F(7, 84) = 0.58$, $p = 0.77$) compared to that derived using kinetic analyses (18 to 26%).

DISCUSSION

We failed to detect oral amphetamine-induced dopamine release in the cortex with [^{11}C]FLB 457 PET in a cohort of healthy individuals who underwent the baseline and post-amphetamine scan on different days. The use of the SRTM to derive BP_{ND} did not alter this result. This analysis was conducted because, in a recent report, amphetamine-induced displacement of [^{11}C]FLB 457 BP_{ND} was detected with SRTM, but not kinetic analyses (Sandiego et al., 2015). The inability to displace [^{11}C]FLB 457 BP_{ND} in the cortex following an oral amphetamine challenge was unexpected, because three independent groups have replicated this observation in five different cohorts of healthy controls (**Table 5**) (Narendran et al., 2009; Narendran et al., 2014; Sandiego et al., 2015; Sandiego et al., 2017; Slifstein et al., 2015). The scanning of subjects with [^{11}C]NPA before [^{11}C]FLB 457 in the amphetamine PET studies allowed for us to contrast BP_{ND} in the striatum with that in the cortex. The amphetamine challenge that failed to displace [^{11}C]FLB 457 BP_{ND} in the cortical regions (mean $BP_{ND} = -5$ to $+5\%$; see Table 1) led to a significant decrease in

[¹¹C]NPA BP_{ND} in the striatal sub-regions in the same subjects (mean BP_{ND} = -19 to -26%, $p < 0.001$) (Frankle et al., 2017). This highlights the continued difficulties in using PET to measure dopamine release in the cortex compared to the striatum. Measuring dopamine release in the striatum is not only robust and easy to replicate, but also minimally influenced by technical factors, such as the D_{2/3} radioligand and/or psychostimulant challenge (methylphenidate vs. amphetamine) used in the paradigm, route of administration of the challenge (oral vs. intravenous), and modeling method used to derive BP_{ND} (arterial input vs. reference tissue-based analyses).

The major difference between the current cohort that failed to displace [¹¹C]FLB 457 and successful cohorts is the acquisition of PET scans (baseline and post-amphetamine) on different days rather than the same day. This led us to postulate that the reproducibility for [¹¹C]FLB 457 BP_{ND} is worse when the scans are performed on different days as opposed to the same day. Consistent with this notion, the test-retest variability for [¹¹C]FLB 457 BP_{ND} was ~2-fold higher when scans were performed on different days compared to the same day, when nearly identical image acquisition and analyses methods were used (see Table 6). Such differences between the same day and different day test-retest variability have not been evidenced with [¹¹C]raclopride, the reference radiotracer used to measure D_{2/3} receptors in the striatum. [¹¹C]raclopride PET studies conducted by the imaging group at Turku in Finland have reported the same day (2.5 hours apart) and different day (five weeks apart) test-retest variability to be within 5 to 6% and 4 to 9% for BP_{ND} in the striatal subdivisions (Alakurtti et al., 2011; Alakurtti et al., 2015). Next, we wanted to understand whether technical challenges involved with characterizing the metabolite-corrected arterial input function for [¹¹C]FLB 457 across two different days contributes to poor reproducibility. This led us to use the cerebellum time activity curve as an input to derive [¹¹C]FLB 457 BP_{ND} with SRTM. The test-retest variability for BP_{ND} in the eight cortical regions derived using SRTM (10 to 18%) was numerically, but not statistically improved compared to the kinetic analyses (18 to 26%). The minimal improvement in reproducibility noted with SRTM suggests a possible role for other factors contributing to poor reproducibility in the different day scan protocol. Unfortunately, these other factors, which may include, but are not limited to the anticipation/stress/pain levels associated with arterial line insertions, sleep quality/quantity, last use of caffeine/nicotine/alcohol use and stage of the menstrual cycle, etc., tend to be difficult to control across different days in human studies. The poor reproducibility in the different day scan paradigm may also be a physiological consequence of the relatively low baseline dopamine concentrations in the cortex, which is less stable when measured across days. Consistent with this notion, D_{2/3} PET studies that have attempted to measure baseline dopamine concentrations in the cortex with the dopamine depleting agent alpha-methyl-para-tyrosine (AMPT) have been unsuccessful due to the need to measure a relatively small effect (BP_{ND}) with increased variability (Cropley et al., 2008; Frankle et al., 2010).

The poor reproducibility for baseline [¹¹C]FLB 457 BP_{ND} in the different day scan paradigm reported here is also inconsistent with the low test-retest variability reported for the post-amphetamine BP_{ND} when measured on different days (Narendran et al., 2013). The exact reasons that lead to poor reproducibility in the baseline (18 to 26% in cortical ROIs in Table 3), but not post-amphetamine (4 to 11% in cortical ROIs) condition when the test and

retest scans are performed on different days is not clear. Nevertheless, the divergence in reproducibility between the baseline and post-amphetamine conditions when scans are acquired on different days poses a problem for [^{11}C]FLB 457 amphetamine studies, because

BP_{ND} is likely to be highly variable. The effect of this variability would result in diminished power to detect between-group differences in clinical studies. This result also has implications for longitudinal studies that have used [^{11}C]FLB 457 and amphetamine to measure dopamine release on two different days. For example, a recent study that documented amphetamine-induced displacement of [^{11}C]FLB 457 before and after three weeks of guanfacine treatment reported that guanfacine led to a decrease in dopamine release (BP_{ND}) (Sandiego et al., 2017). However, the smaller BP_{ND} measured in the guanfacine-treated relative to the untreated condition was attributable to a non-significant 4–12% decrease in baseline (not post-amphetamine) BP_{ND} after chronic guanfacine treatment. The reproducibility for BP_{ND} , which is poor in the baseline and good in post-amphetamine conditions may have introduced a bias on BP_{ND} measured after guanfacine treatment. To exclude such bias, future longitudinal studies that use [^{11}C]FLB 457 and amphetamine should consider the inclusion of a placebo-control group to examine the impact of reproducibility on baseline, post-amphetamine, and percent change in BP_{ND} .

The mean amphetamine plasma level at the time of initiation and completion of the post-amphetamine [^{11}C]FLB 457 PET scan in this study was 68 ± 9 and 58 ± 10 ng mL $^{-1}$ ($t = 0$ - and 90-min relative to post-amphetamine scan). These values are ~ 20% lower than the mean amphetamine plasma values (80 ± 10 and 70 ± 10) reported in our largest healthy control cohort that was successful in displacing [^{11}C]FLB 457 BP_{ND} (Narendran et al., 2014). This difference between means was anticipated because the post-amphetamine scan was initiated at five hours, as opposed to three hours, following the oral amphetamine challenge in this study. This adjustment from three to five hours between baseline and post-amphetamine scan was done to accommodate the [^{11}C]NPA scan, which was necessary to measure striatal dopamine release. Studies with $\text{D}_{2/3}$ radiotracers in the striatum (and globus pallidus) have shown that it takes 24–48 hours for BP_{ND} to return back to baseline following an amphetamine challenge (Cardenas et al., 2004; Carson R, 2001; Laruelle et al., 1997; Narendran et al., 2007). These investigations have also shown that the BP_{ND} measured following an acute amphetamine challenge in the striatum is essentially unchanged for the first six hours. It is not known whether the same holds true for [^{11}C]FLB 457 in a region such as the prefrontal cortex, where the clearance of dopamine from the synapse does not occur via dopamine transporters. In the absence of these data, one cannot exclude the possibility that lower amphetamine plasma levels during the [^{11}C]FLB 457 scan led to the inability to detect dopamine release in this study. We found no relationship between amphetamine plasma levels and BP_{ND} to support this notion. This is likely due to the low variability in amphetamine plasma levels seen in this study. Lastly, we cannot exclude the contribution from unique dopamine clearance mechanisms in the cortex (compared to primarily dopamine transporter-mediated uptake in striatum) that include diffusion into the extracellular space, monoamine oxidase -A (MAO-A), catechol-O-methyl-transferase (COMT), norepinephrine transporter (NET), etc., leading to a greater degradation of synaptic dopamine at ~ 5 vs. 3 hours, and influencing the ability to detect a change in BP_{ND} (Kaenmaki et al., 2010; Tammimaki et al., 2016).

In summary, we failed to displace [^{11}C]FLB 457 following an oral amphetamine challenge in a cohort of healthy individuals who underwent the baseline and post-amphetamine scans on different days. These results, which are inconsistent with our prior reports, highlight the methodological challenges that continue to plague the field with respect to imaging dopamine release in the cortex with PET. In closing, in Table 7 we list a few suggestions to maximize success for future investigations planning to use [^{11}C]FLB 457 PET and oral amphetamine to measure cortical dopamine transmission.

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Table 1.

Effect of amphetamine on [^{11}C]FLB 457 BP_{ND} derived using two-tissue compartment kinetic analyses

	BASE	AMPH	Difference (%)	p-value
Medial Temporal Lobe	1.25 ± 0.27	1.21 ± 0.24	-2.2 ± 11.3	0.42
Anterior Cingulate Cortex	0.74 ± 0.15	0.77 ± 0.17	4.3 ± 15.0	0.44
Dorsolateral Prefrontal Cortex	0.53 ± 0.20	0.49 ± 0.16	-4.2 ± 16.4	0.27
Orbital Frontal Cortex	0.76 ± 0.21	0.77 ± 0.19	2.7 ± 10.8	0.70
Medial Prefrontal Cortex	0.66 ± 0.19	0.62 ± 0.15	-3.8 ± 12.9	0.18
Temporal Cortex	1.50 ± 0.35	1.42 ± 0.33	-4.7 ± 9.9	0.14
Parietal Cortex	0.54 ± 0.21	0.52 ± 0.20	-3.3 ± 11.4	0.28
Occipital Cortex	0.43 ± 0.20	0.42 ± 0.18	0.7 ± 11.8	0.63

Values are the means ± SD; n = 12 subjects. P values indicate paired t-tests.

Table 2.
Effect of amphetamine on [^{11}C]FLB 457 BP_{ND} derived using the simplified reference tissue method

	BASE	AMPH	Difference (%)	p-value
Medial Temporal Lobe	1.12 ± 0.20	1.08 ± 0.15	-3.9 ± 9.1	0.24
Anterior Cingulate Cortex	0.71 ± 0.15	0.73 ± 0.15	6.0 ± 16.7	0.50
Dorsolateral Prefrontal Cortex	0.50 ± 0.17	0.49 ± 0.15	-3.6 ± 15.9	0.49
Orbital Frontal Cortex	0.74 ± 0.20	0.74 ± 0.16	2.1 ± 11.9	0.90
Medial Prefrontal Cortex	0.63 ± 0.17	0.60 ± 0.14	-2.7 ± 17.1	0.31
Temporal Cortex	1.33 ± 0.29	1.28 ± 0.23	-3.3 ± 9.1	0.26
Parietal Cortex	0.52 ± 0.19	0.50 ± 0.18	-3.1 ± 12.1	0.41
Occipital Cortex	0.42 ± 0.19	0.42 ± 0.16	1.3 ± 12.8	0.94

Values are the means ± SD; n = 12 subjects. P values indicate paired t-tests.

Table 3.

Reproducibility of [^{11}C]FLB 457 total distribution volume (V_T , mL cm^{-3}), binding potential relative to plasma concentrations (BP_P , mL cm^{-3}) and binding potential relative to non-specific uptake (BP_{ND} , unitless) derived via kinetic analysis.

Region	V_T				BP_P				BP_{ND}				VAR $\pm$ SD (%)	ICC	
	Mean	BSSD CV	WSSD CV	VAR $\pm$ SD (%)	ICC	Mean	BSSD CV	WSSD CV	VAR $\pm$ SD (%)	ICC	Mean	BSSD CV			WSSD CV
CER	3.94	0.25	0.10	15.2 $\pm$ 18.6	0.71	-	-	-	-	-	-	-	-	-	-
MTL	9.16	0.31	0.08	12.1 $\pm$ 8.4	0.88	5.22	0.40	0.10	13.7 $\pm$ 13.8	0.88	1.34	0.29	0.14	21.9 $\pm$ 22.5	0.61
ACC	7.06	0.28	0.10	14.1 $\pm$ 14.5	0.76	3.12	0.36	0.14	21.2 $\pm$ 10.7	0.73	0.80	0.25	0.13	25.9 $\pm$ 11.4	0.57
DLPFC	6.13	0.33	0.09	13.4 $\pm$ 15.3	0.87	2.19	0.55	0.09	15.3 $\pm$ 7.4	0.94	0.54	0.41	0.09	18.2 $\pm$ 14.2	0.91
OFC	7.06	0.30	0.09	11.9 $\pm$ 14.7	0.84	3.12	0.45	0.11	16.7 $\pm$ 9.2	0.88	0.80	0.33	0.11	19.7 $\pm$ 15.7	0.80
MPFC	6.65	0.28	0.09	14.4 $\pm$ 14.7	0.80	2.71	0.40	0.12	22.3 $\pm$ 9.0	0.84	0.70	0.31	0.11	23.0 $\pm$ 15.2	0.77
TC	10.34	0.35	0.07	9.8 $\pm$ 8.4	0.91	6.40	0.44	0.09	13.6 $\pm$ 10.1	0.91	1.62	0.29	0.15	20.8 $\pm$ 23.1	0.59
PC	6.25	0.36	0.09	13.6 $\pm$ 16.4	0.88	2.31	0.59	0.11	18.9 $\pm$ 9.1	0.93	0.56	0.42	0.09	19.2 $\pm$ 14.6	0.91
OC	5.86	0.37	0.09	14.0 $\pm$ 16.4	0.89	1.92	0.69	0.12	21.4 $\pm$ 9.5	0.94	0.46	0.56	0.10	22.4 $\pm$ 17.2	0.93

Values are the mean of 7 subjects with each value measured twice.

Between-subject and within-subject standard deviations were calculated and expressed as fraction of mean value (coefficient of variation). BSSD CV = between subject standard deviation coefficient of variation, WSSD CV = within subject standard deviation coefficient of variation; VAR $\pm$ SD = test/retest variability $\pm$ standard deviation, ICC = intraclass correlation coefficient.

Regions of interest consisted of the cerebellum (CER), medial temporal lobe (MTL), 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).

Reproducibility of [^{11}C]FLB 457 binding potential relative to non-specific uptake (BP_{ND}) derived via SRTM.

Table 4.

Region	Mean	BSSD CV	WSSD CV	VAR $\pm$ SD (%)	ICC
MTL	1.17	0.24	0.05	9.5 $\pm$ 6.9	0.91
ACC	0.76	0.25	0.10	18.3 $\pm$ 7.8	0.74
DLPFC	0.53	0.36	0.07	13.1 $\pm$ 8.9	0.93
OFC	0.76	0.28	0.09	14.1 $\pm$ 12.3	0.81
MPFC	0.67	0.28	0.10	18.4 $\pm$ 12.6	0.79
TC	1.40	0.19	0.05	9.9 $\pm$ 7.1	0.85
PC	0.54	0.37	0.08	14.8 $\pm$ 12.1	0.92
OC	0.45	0.50	0.09	17.2 $\pm$ 14.2	0.94

BSSD CV = between subject standard deviation coefficient of variation, WSSD CV = within subject standard deviation coefficient of variation; VAR $\pm$ SD = test/retest variability $\pm$ standard deviation (%), ICC = intraclass correlation coefficient.

Regions of interest consisted of the cerebellum (CER), medial temporal lobe (MTL), 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).

Table 5.

Amphetamine-induced displacement of [^{11}C]FLB 457 BP_{ND} in the prefrontal cortical regions in healthy controls

Region	This study (Table 2)	Narendran 2009	Narendran 2014	Slifstein 2015	Sandiego 2015 [†]	Sandiego 2017 [†]
DLPFC	-4 ± 16	-13 ± 15	-14 ± 15	-8 ± 11	-12 ± 9	-16 ± 5
OFC	3 ± 11	-8 ± 15*	-9 ± 12	-6 ± 14	-9 ± 23	NA
MPFC	-4 ± 13	-11 ± 14	-12 ± 15	-5 ± 13	-9 ± 13	NA
ACC	4 ± 15	-8 ± 8	-9 ± 18	-5 ± 16	-9 ± 9	-11 ± 3

The post-amphetamine [^{11}C]FLB 457 scan was acquired ~ 5 hours after oral amphetamine (0.5 mg/kg) in the current study Vs. ~ 2.5 to 3 hours in all previously published studies.

[†] BPND is from data modeled using a simplified reference tissue model as opposed to a two-tissue compartment model

* Data shown for OFC is from a re-analysis of the original study data that was published in (Narendran et al., 2011b)

NA-data is not available for these regions

Table 6.

Test-Retest Variability for baseline [^{11}C]FLB 457 BP_{ND} derived using kinetic analysis when performed on the same vs. different day

	Same Day VAR $\pm$ SD (%)	Different Day VAR $\pm$ SD (%)
Medial Temporal Lobe	11 $\pm$ 5	22 $\pm$ 23
Anterior Cingulate Cortex	15 $\pm$ 8	26 $\pm$ 11
Dorsolateral prefrontal Cortex	8 $\pm$ 6	18 $\pm$ 14
Orbital Frontal Cortex	7 $\pm$ 6	20 $\pm$ 16
Medial Prefrontal Cortex	6 $\pm$ 4	23 $\pm$ 15
Temporal Cortex	10 $\pm$ 6	21 $\pm$ 23
Parietal Cortex	8.0 $\pm$ 4	19 $\pm$ 15
Occipital Cortex	10 $\pm$ 4	22 $\pm$ 17

Same day test-retest variability data are [^{11}C]FLB 457 data in n=6 subjects from (Narendran et al., 2011b).

Different day test-retest variability data are from n=7 subjects in this study (see Table 3).

Table 7.

Recommendations for the use of [¹¹C]FLB 457 and oral amphetamine to measure cortical dopamine release

	Recommendation	Rationale
1	Perform the baseline and post-amphetamine scans on same day.	This is based on the fact that the reproducibility for [¹¹ C]FLB 457 BP _{ND} is significantly better when scans are performed on same compared to different days (Narendran et al., 2011b and data from this study).
2	Restrict injected mass for [¹¹ C]FLB 457 0.6 micrograms to ensure studies are conducted at tracer dose (i.e., 5% occupancy).	This is necessary to avoid carrier-mass induced changes in BP _{ND} when the baseline and post-amphetamine scans are performed on same day (Sudo et al., 2001).
3	Perform arterial input function based kinetic analysis for modeling of [¹¹ C]FLB 457 data.	[¹¹ C]FLB 457 has been shown to have D _{2/3} specific binding in the cerebellum (Narendran et al., 2011a). Thus, it is necessary to exclude amphetamine-induced changes in cerebellum V _{ND} , which in turn will impact the primary outcome measures, i.e., BP _{ND} . For example, in a clinical PET study, an amphetamine-induced decrease in V _{ND} in patients, but not in controls could bias the between-group comparison of BP _{ND} . However, once no group differences in V _{ND} is established with kinetic analyses, it might be reasonable to use SRTM to derive BP _{ND} and BP _{ND} . The rationale for this is based on a prior study that showed oral amphetamine-induced displacement of BP _{ND} , when BP _{ND} was derived with SRTM, but not kinetic analyses (Sandiego et al., 2015). This is likely due to the lower within-subject variability when BP _{ND} is arrived with the SRTM compared to kinetic analyses. Finally, more advanced modeling methods (such as a modified two-tissue compartment kinetic analyses in which V _{ND} and the specific binding dissociation constant, K _d are fixed to a single value across cortical regions for both baseline and post-amphetamine scans) that account for the D _{2/3} specific binding have been recently used to measure an amphetamine-induced BP _{ND} (Shifstein et al., 2015). Such advanced modeling methods may be robust in detecting relatively small amphetamine-induced changes in BP _{ND} when the baseline BP _{ND} values are relatively small as with [¹¹ C]FLB 457 in the cortex.
4	Use oral d-amphetamine at 0.5 mg kg ⁻¹ as a challenge to stimulate cortical dopamine release (as opposed to the intravenous formulation, or oral methylphenidate).	Oral compared to intravenous amphetamine is associated with less plasma variability during the post-amphetamine scan (Aalto et al., 2009; Narendran et al., 2009). This may be critical to measuring dopamine release in the cortex compared to the striatum (see discussion section). Also, methylphenidate which releases dopamine primarily by inhibiting the dopamine and norepinephrine transporters (as opposed to amphetamine which releases dopamine by additional mechanisms such as vesicular release, reverses dopamine transporter, etc.), and lower doses (less than 0.5 mg kg ⁻¹) of amphetamine may not displace [¹¹ C]FLB 457 BP _{ND} (Montgomery et al., 2007).
5	The post- amphetamine scan should be initiated at ~ 3 hours following the oral amphetamine challenge.	To ensure that the post-amphetamine [¹¹ C]FLB 457 scan corresponds to the peak plasma amphetamine levels (and presumably peak dopamine release in cortex) (Angrist et al., 1987). This is important because it is not known whether amphetamine -induced BP _{ND} is prolonged beyond peak dopamine release in the cortex as demonstrated in the striatum.
6	The [¹¹ C]FLB 457 amphetamine imaging paradigm is more robust when used in younger individuals.	BP _{ND} values in prefrontal cortical regions fall below 0.5 in individuals with > 35 years old (Kaasinen et al., 2002; Kaasinen et al., 2000; Narendran et al., 2009). Displacing a relatively low BP _{ND} with amphetamine leads to increased variability BP _{ND} .