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Imaging dopamine transmission in the frontal cortex: a simultaneous microdiaysis and [11C]FLB 457 PET study

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Abstract

In a recent human PET study we demonstrated the ability to detect amphetamine-induced dopamine release in the prefrontal cortex as a reduction in the binding of the dopamine $D_{2/3}$ radioligand [^{11}C]FLB 457. A key requirement for validating this paradigm for use in clinical studies is demonstrating that the changes in [^{11}C]FLB 457 binding observed with PET following amphetamine are related to changes in dialysate dopamine concentration as measured with microdialysis. Microdialysis and PET experiments were performed to compare, in five rhesus monkeys, amphetamine-induced dopamine release and [^{11}C]FLB 457 displacement in the frontal cortex after three doses of amphetamine (0.3 mg/kg, 0.5 mg/kg, and 1 mg/kg). Amphetamine led to a significant dose-dependent increase in dialysate dopamine (DA, 0.3 mg/kg: 999 ± 287%; 0.5 mg/kg: 1320 ± 432%; 1.0 mg/kg: 2355 ± 1026%) as measured with microdiaysis and decrease in [^{11}C]FLB 457 binding potential ($BP_{ND,}$ 0.3 mg/kg: −6 ± 6%; 0.5 mg/kg: −16 ± 4%; 1.0 mg/kg: −24 ± 2%) as measured with PET. The relationship between amphetamine-induced peak ΔDA and Δ[^{11}C]FLB 457 BP_{ND} in the frontal cortex was linear. The results of this study clearly demonstrate that the magnitude of dialysate dopamine release is correlated with the magnitude of the reduction in [^{11}C]FLB 457 BP_{ND} in the frontal cortex. The use of the [^{11}C]FLB 457-amphetamine imaging paradigm in humans should allow for characterization of prefrontal cortical dopamine release in neuropsychiatric disorders such as schizophrenia and addiction.

Keywords

[^{11}C]FLB 457; Positron emission tomography; microdialysis

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INTRODUCTION

The displacement of the *in vivo* binding of $D_{2/3}$ receptor-specific SPECT and PET radiotracers by an acute amphetamine challenge is used as a non-invasive measure to quantify dopamine release in the striatum. Combined microdialysis and imaging studies in non-human primates have validated this approach by demonstrating a linear dose response relationship in which 1% reduction in [^{123}I]IBZM or [^{11}C]raclopride binding potential (BP_{ND}) corresponds to a 40% increase in extracellular dopamine[1, 2]. In the past two decades numerous clinical investigations have used this approach to report abnormal amphetamine-induced dopamine release in the striatum in neuropsychiatric disorders such as schizophrenia[2, 3], ADHD[4] and addiction[5, 6]. The advancement of the PET radioligands [^{11}C]FLB 457 (K_D 0.06 nM) and [^{18}F]fallypride (K_D = 0.14 nM), which bind to $D_{2/3}$ receptors with a relatively high affinity led to the characterization of amphetamine-induced dopamine release in extrastriatal regions such as the midbrain, thalamus, hippocampus, and cortex[7-10]. We recently demonstrated the ability to detect amphetamine-induced dopamine release in the human cortex with [^{11}C]FLB 457 and PET[9]. In this study, amphetamine led to a significant reduction (range 4 to 13%) in [^{11}C]FLB 457 BP_{ND} in several cortical regions of interest including the dorsolateral prefrontal cortex, medial prefrontal cortex, and anterior cingulate cortex. Since this initial report we have conducted a series of studies to validate the use of [^{11}C]FLB 457 PET as a non invasive tool to measure dopamine release in the cortex. The results of these [^{11}C]FLB 457 studies suggest good reproducibility (< 15%) for BP_{ND} in the cortical regions of interest[11], no carryover mass induced decrease in BP_{ND} in the imaging paradigm that is used to measure amphetamine-induced dopamine release in the cortex[11], and a small but significant fraction of $D_{2/3}$ receptor specific binding in the cerebellum (i.e., the reference region which is used as an estimate of non-specific binding), which may impact the measurement (ΔBP_{ND}) of dopamine release in the cortex[12].

The [^{11}C]FLB 457 PET-amphetamine paradigm was developed to demonstrate differences in amphetamine-induced dopamine release between patients and healthy controls in clinical imaging studies. Hence, a critical step in its validation is to document that the relationship between increases in dopamine and decreases in [^{11}C]FLB 457 BP_{ND} in the cortex are amphetamine dose-dependent and linear as demonstrated in the striatum. In addition, there are unique challenges to demonstrating this relationship in the cortex as opposed the striatum because there is less dopamine in the cortex[13, 14]. Finally, another observation that required investigation is the magnitude of amphetamine (0.5 mg/kg) -induced displacement of [^{11}C]FLB 457 in the frontal cortex (~12 to 15%)[9] in humans for it is comparable to that reported in the striatum (~10%) with [^{11}C]raclopride and [^{123}I]IBZMfor review, see [15]. Although this seems discrepant based on the fact that the striatum receives greater dopaminergic projections from the ventral tegmental area/ substantia nigra relative to the frontal cortex, a possible explanation for the same percent reduction in binding potential after amphetamine is related to the relatively large differences in baseline dopamine between these regions. In other words, the greater vulnerability of the *in vivo* binding of a $D_{2/3}$ radioligand in the cortex compared to the striatum is explained by less occupancy of $D_{2/3}$ receptors by dopamine at baseline in the cortex. Therefore, in order to gain a better

understanding of these issues we conducted simultaneous microdialysis and PET imaging in non-human primates.

In summary, the primary objective of this study was to "calibrate" [^{11}C]FLB 457 displacement in the frontal cortex, i.e. to establish the quantitative relationship between relative change in dialysate DA and change in [^{11}C]FLB 457 BP_{ND}. In addition, the secondary objective was to contrast the sensitivity of dopamine to displace $D_{2/3}$ radiotracer binding in the cortex with that in the striatum.

MATERIALS AND METHODS

General Design

All experiments were performed under a protocol that was reviewed and approved by the University of Pittsburgh Institutional Animal Care Use Committee. A total of 54 PET scans in 5 adult male rhesus monkeys (denoted A, B, C, D and E; average age of the animals was 10.7 ± 0.7 years; average weight of the animals was 9.0 ± 0.7 kg) are reported here. These 54 PET scans were acquired in 27 experimental sessions over the course of 18 months. A minimum of one week was allowed between experimental sessions in the same animal. Each experimental session included 2 PET scans: a test-scan and a re-test scan in the reproducibility studies (n=10 scans); and a baseline scan and a post-amphetamine scan in the amphetamine studies (n = 44 scans). Animals were euthanized following successful completion of the research procedures.

1. [^{11}C]FLB 457 test-retest studies—3 animals were scanned with [^{11}C]FLB 457 PET under test and re-test conditions. The aim of these studies was to determine the length of the inter-scan interval (i.e., time in minutes between the start of the test and re-test scans) that is associated with no decrease in [^{11}C]FLB 457 $BP_{ND.}$ The inter-scan interval was a concern as the FLB 457 mass carried over from the baseline to post-amphetamine PET scan in itself could lead to a reduction in [^{11}C]FLB 457 BP_{ND} as it has a relatively high affinity for $D_{2/3}$ receptors[9]. Thus, the animals were studied under a test and re-test condition both 135 min (A and B) and 195 min apart (A, B, and C) to determine the appropriate inter-scan interval for the amphetamine studies.

2. [^{11}C]FLB 457 amphetamine studies—Five animals were scanned with [^{11}C]FLB 457 and PET in a baseline and post-amphetamine condition after 0.3, 0.5 and 1.0 mg/kg of intravenous d-amphetamine. Amphetamine was administered approximately thirty minutes prior to the post-amphetamine scan. Informed by the test-retest studies, the inter-scan interval between the baseline and post-amphetamine scans was maintained at 195 min. All animals underwent simultaneous dopamine microdialysis from the frontal cortex (medial wall of dorsal prefrontal cortex) during the baseline and post-amphetamine conditions. The experiments with the amphetamine 0.5 mg/kg dose were repeated in 4/5 animals because this dose is also used in our human imaging paradigm[16, 17]. The aims of these amphetamine experiments were to demonstrate (a) a dose-dependent increase in dialysate dopamine (ΔDA) (b) a dose-dependent decrease in [^{11}C]FLB 457 BP_{ND} (ΔBP_{ND}) and (c) a linear relationship between ΔDA and Δ BP_{ND}.

Simultaneous microdialysis and PET data were successfully acquired in 14/20 experiments. Failure of microdialysis experiments was generally due to the inability to obtain baseline dopamine measurements. The most common reason for failure of PET experiments was the inability to synthesize an adequate dose (~1 mCi) of [^{11}C]FLB 457 at the mass limit (0.008 μg/kg) that was required for tracer dose[18]. Microdialysis/PET experiments that failed to provide usable data for a given dose of amphetamine during the simultaneous session were repeated at least once in a separate (i.e., non-simultaneous) session to make up for the lost data point in each animal. An exception was a microdialysis experiment with amphetamine 0.5 mg/kg in animal B, which could not be repeated due to removal of that animal from the study because of a fatal non-study related cardiac event. The breakdown of n=20 PET and n=18 microdialysis experiments, fourteen of which were acquired in simultaneous sessions in five animals is provided in Table 1.

3. [^{11}C]raclopride amphetamine studies—Two animals (D and E) were scanned with [^{11}C]raclopride in a baseline and post-amphetamine condition after 0.5 mg/kg of intravenous amphetamine (n=2 experiments). Both of these animals underwent dopamine microdialysis from the striatum in separate experimental sessions (non-simultaneous) following the same dose of amphetamine (n=5 experiments). The aim of these experiments was to contrast the concentration of dopamine that is required to displace [^{11}C]raclopride BP_{ND} in the striatum with that of [^{11}C]FLB 457 BP_{ND} in the cortex in the same experimental subjects and context.

Microdialysis methods

The microdialysis methods used in the construction of guide cannulae and probes have been previously described[19-23]. All animals underwent a stereotactic surgical procedure for placement of a polysulfone recording chamber (Crist Instruments, Damascus, MD) that directs the insertion of the microdialysis probes[19]. Microdialysis probe trajectories to access the regions of interest (frontal cortex or striatum) were guided by a post-surgical computerized tomography (CT) scan co-registered to the subject's MRI. Under ketamine anesthesia, microdialysis probes were placed in sites, which had not been previously sampled, the day before the experiment as previously described[19]. Two probes were placed – one in the right and another in the left region of interest. The data from the right and left regions of interest were averaged when the microdialysis was successful from both of the probes. After lowering the probe, a protective cap was used to cover the chamber overnight. The following day, the cap was removed, and inlet and outlet lines were attached to the probe for microdialysis. Probes were held in place by a snug fit and were prevented from rotating by a tab. The solution used to perfuse the probe at 1.0 μl/min was in mM: KCl 2.4, NaCl 137, $CaCl_2$ 1.2, $MgCl_2$ 1.2, and NaH_2PO_4 0.9, pH 7.4; ascorbate 0.2. Dopamine in the perfusates was then determined using High-Performance liquid chromatography (HPLC) as previously described[19]. Using these procedures dialysate dopamine was sampled every 10 minutes from the regions of interest during the baseline and post-amphetamine conditions. Dialysate dopamine concentration was measured as nanomolar (nM) in dialysate without correction for *in vitro* recovery. There are two reasons for not using in-vitro recovery corrected values to estimate extracellular dopamine (ECF DA) concentrations. First, an aqueous solution is a poor model of the actual extracellular environment in which diffusion

occurs within a tortuous environment dominated by cell surface glycosaminoglycans with fixed anionic sites [24]. Secondly, it has been previously demonstrated that the "no-net-flux" in-vivo estimates of true extacellular concentrations correlate better with uncorrected dialysate levels than with in-vitro calibration corrected dialysate measures[25]. The time constraints of the combined microdialysis PET procedures did not permit the use of "no-net-flux" extracellular values. Because we did not perform any correction for in vitro recovery and did not adopt the "no-net flux" methodology, we refer to the dopamine concentration that was measured during the microdialysis experiments as change in 'dialysate dopamine' rather than 'extracellular dopamine' in this manuscript.

Amphetamine-induced increases in dopamine concentration (Δ DA) in dialysate were expressed as a percent increase in dopamine release relative to the mean baseline dopamine concentration (measured from −30 to 0 min before amphetamine). The peak Δ DA (%) in the frontal cortex and striatum was used as the primary outcome measure for microdialysis in correlations with amphetamine-induced displacement of [^{11}C]FLB 457 and [^{11}C]raclopride. Our rationale for choosing the peak DA release rather than an average DA release (which is comparable to the area under curve because dialysate samples were collected at regular 10 minute intervals) was based on the fact that there is a significant temporal discrepancy between the change in dialysate dopamine (as measured with microdialysis) and reduction in $D_{2/3}$ radiotracer binding potential (as measured with PET) due to the agonist-induced internalization of $D_{2/3}$ receptors following an acute amphetamine challenge[1, 26-29]. Given this temporal disconnect between the microdialysis and PET outcome measures, it is likely that the reduction in BP_{ND} following amphetamine is represented better by the peak rather than an average Δ DA (%) release. Furthermore, the use of peak DA release as the primary outcome measure in correlations with ΔBP_{ND} is consistent with our previous report that evaluated amphetamine-induced dopamine release in the striatum[1]. The average Δ DA in the frontal cortex and striatum was calculated and used as a secondary outcome measure for microdialysis in the correlations with amphetamine-induced displacement of [^{11}C]FLB 457 and [^{11}C]raclopride.

Imaging methods

Structural MRI—Each animal underwent a structural MRI scan using a Siemens 3T Allegra scanner for proper localization of regions of interest prior to the PET and microdialysis procedures[30].

Radiochemistry—The synthesis of [^{11}C]FLB 457 and [^{11}C]raclopride were carried out as previously described[31, 32].

PET imaging protocol—Fasted animals were immobilized with ketamine (10 mg kg^{-1} intramuscularly, approximately 90 minutes prior to the first [^{11}C]FLB 457 injection) and anesthetized with 1 to 2% isoflurane via endotracheal tube. The animals' vital signs were monitored every 10 min and temperature was kept constant at 37°C with heated water blankets. An intravenous perfusion line was placed in each animal for hydration, injection of radiotracers and amphetamine. Head was positioned at the center of the field of view as defined by imbedded laser lines. PET imaging was performed with ECAT EXACT HR+

scanner (Siemens/CTI, Knoxville, TN). A 10 min transmission scan was obtained prior to radiotracer injection for attenuation correction. Activity was injected intravenously over 30 s as a bolus. The injected FLB 457 and raclopride mass had upper limits of 0.008 μg/kg and 0.1 μg/kg respectively. Emission data was collected in 3D mode for 75 min as successive frames (20 frames) of increasing duration for both [^{11}C]FLB 457 and [^{11}C]raclopride.

Image analysis—PET data were reconstructed using filtered back-projection and corrected for photon attenuation, scatter[33], and radioactive decay. Reconstructed image files were then processed with the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) and SPM2 (www.fil.ion.ucl.ac.uk/spm). MR-PET image alignment was performed using a mutual information algorithm implemented in SPM2. Regions of interest including the frontal cortex (the frontal cortex was drawn as a single region to include the dorsolateral prefrontal cortex, medial prefrontal cortex, orbital frontal cortex and anterior cingulate cortex), striatum and cerebellum were subsampled on the structural MRI and then transferred to the co-registered PET scan. Time activity curves were generated for the frontal cortex (for [^{11}C]FLB 457) or striatum (for [^{11}C]raclopride) and cerebellum using the criteria and methods outlined previously[34]. Activity from the right and left regions of interest was averaged.

The outcome measure BP_{ND} (= f_{ND} *B_{avail}/K_D, where B_{avail} is the density of $D_{2/3}$ receptors available to bind the radioligand *in vivo*, K_D is the disassociation constant and f_{ND} is the free fraction in the non displaceable compartment) was derived for [^{11}C]FLB 457 and [^{11}C]raclopride using the simplified reference tissue model with the cerebellum as an input function[35]. The relative change in BP_{ND} (ΔBP_{ND}) for test-retest and amphetamine studies were calculated as the difference between BP_{ND} measured in the second scan ($BP_{ND\ SECOND\text{-}SCAN}$) and BP_{ND} measured in the first scan ($BP_{ND\ FIRST\text{-}SCAN}$), and expressed as a percentage of $BP_{ND\ FIRST\text{-}SCAN}$.

$$\Delta BP_{ND} = 100\% * \frac{BP_{ND_{\mathrm{SECOND-SCAN}}} - BP_{ND_{\mathrm{FIRST-SCAN}}}}{BP_{ND_{\mathrm{FIRST-SCAN}}}}$$

Amphetamine plasma levels—Amphetamine plasma levels were measured in the PET experiments in three venous samples – 5 min, 35 min, and 70 min after the amphetamine injection to contrast the concentrations at the 0.3, 0.5, and 1 mg/kg doses. The analysis of these samples were conducted using previously described methods[36]. For technical reasons, amphetamine levels were not obtained in one of the 0.5 mg/kg dose experiments.

Statistical analysis

All statistical analysis was performed with PASW Statistics 18.0. The differences in scan variables (such as injected mass and dose) between the baseline and amphetamine conditions were contrasted using paired t tests. The effect of amphetamine on Δ DA (expressed as % of baseline levels) was evaluated using a non-parametric one-way (Kruskal-Wallis) analysis of variance ANOVA with amphetamine dose as factor. The effect of amphetamine on BP_{ND} was evaluated using a repeated measures ANOVA (RM ANOVA),

with the BP_{ND} as dependent variable, the baseline and post-amphetamine conditions as within subject factors, and the amphetamine dose and animal as between subject factors. The significance levels for condition, condition*dose, condition*animal, condition*dose*animal interactions are reported. Correlations between Δ DA and Δ BP_{ND} were performed using Pearson product moment correlation coefficient. A two-tailed $p = 0.05$ was selected as the significance level for all tests. All values are expressed as mean ± standard deviation unless specified. The limited number of experiments that were performed with [^{11}C]raclopride and amphetamine at 0.5 mg/kg precluded meaningful statistical analysis.

RESULTS

1. [^{11}C]FLB 457 test-retest studies

Injected dose and mass—The mean injected dose for [^{11}C]FLB 457 was 1.08 ± 0.04 mCi (40.0 ± 1.5 MBq) and 1.03 ± 0.12 mCi (38.1 ± 4.4 MBq) for the test and re-test conditions (n=5/condition; t= 0.68, df= 4, p= 0.53). The mean specific activity at the time of injection for [^{11}C]FLB 457 was 10244 ± 2117 Ci/mmol (379 ± 78 GBq/μmol) and 10912 ± 4096 Ci/mmol (404 ± 152 GBq/μmol) for the test and re-test conditions (t= 0.67, df= 4, p= 0.54). The mean injected mass for [^{11}C]FLB 457 was 0.04 ± 0.01 μg and 0.04 ± 0.02 μg for the test and re-test conditions (t= 0.28, df= 4, p =0.79)

Binding potential—[^{11}C]FLB 457 BP_{ND} in the frontal cortex under test and re-test conditions, and Δ BP_{ND} after an inter-scan interval of 135 min and 195 min are shown in Table 2. As the change in [^{11}C]FLB 457 BP_{ND} was relatively small when the inter-scan interval was 195 min, we used this protocol for the amphetamine studies.

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

Injected dose and mass—The mean injected dose for [^{11}C]FLB 457 was 1.28 ± 0.37 mCi (47.4 ± 13.7 MBq) and 1.27 ± 0.36 mCi (47.0 ± 13.3 MBq) for the baseline and post-amphetamine conditions (n=20/condition; t= 0.78, df= 19, p= 0.94). The mean specific activity at the time of injection for [^{11}C]FLB 457 was 8708 ± 3867 Ci/mmol (322 ± 143 GBq/μmol) and 8379 ± 4326 Ci/mmol (310 ± 160 GBq/μmol) for the baseline and post-amphetamine conditions (t = 0.22, df = 19, p = 0.83). The mean injected mass for [^{11}C]FLB 457 was 0.06 ± 0.01 μg and 0.06 ± 0.01 μg for the baseline and post-amphetamine conditions (t= 0.49, df= 19, p= 0.63).

Binding potential and dialysate dopamine—[^{11}C]FLB 457 BP_{ND} under baseline and post-amphetamine conditions, and Δ BP_{ND} after amphetamine 0.3, 0.5, and 1.0 mg/kg in the animals are shown in Table 3. Also, included in Table 3 are the frontal cortical dopamine concentrations (nM) at baseline, the peak Δ DA and average Δ DA after amphetamine as measured using microdialysis.

Amphetamine significantly increased dopamine concentrations in the dialysate in a dose-dependent manner (one-way ANOVA, peak Δ DA: H=7.895, df=2, p=0.019; average Δ DA: H=7.604, df=2, p =0.022) as shown in Figure 1 and Table 3. In addition, it significantly reduced [^{11}C]FLB 457 BP_{ND} in a dose-dependent manner as well, data shown in Table 3

(n=20 experiments, RM ANOVA; [^{11}C]FLB 457 BP_{ND} as dependent variable; baseline versus amphetamine condition, effect of amphetamine on condition: F= 179.59, df= 1, error df= 8, p< 0.0001; condition* dose interaction: F= 13.05, df= 2, error df= 8, p =0.003; condition*animal interaction: F= 1.25, df= 4, error df= 8, p=0.36; condition* dose* animal interaction: F=0.54, df= 5, error df= 8, p= 0.75).

Correlational analyses of the microdialysis and PET outcome measures from simultaneous sessions (n= 14 experiments; 3 experiments at 0.3 mg/kg; 7 experiments at 0.5 mg/kg and 4 experiments at 1.0 mg/kg) demonstrated a significant relationship between amphetamine-induced Δ in peak DA and Δ BP_{ND} (y = 575.87 + 57.00x, r^2= 0.32, p =0.035, see Figure 2A). A linear relationship between these outcome measures was also evident when data from all the microdialysis (n=18) and PET (n=20) experiments were included (y = 434.44 + 74.34x, r^2 =0.89 see Figure 2B). There was also a significant relationship between the Δ in average DA and Δ BP_{ND} (data from n=14 simultaneous experiments, y = 453.63 + 45.60x, r^2= 0.29, p =0.049; all data from n=18 microdialysis and n=20 PET experiments, y = 286.59 + 61.85x, r^2 =0.88).

Amphetamine plasma levels—Amphetamine plasma levels obtained at 5 min, 35 min, and 70 min were all linearly correlated with dose (r^2= 0.56, 0.80 and 0.86 respectively, all p values < 0.01). The mean amphetamine plasma levels obtained at these time points after doses 0.3, 0.5, and 1.0 mg/kg in the [^{11}C]FLB 457 PET experiments are shown in Figure 3.

3. [^{11}C]raclopride amphetamine studies

Injected dose and mass—The mean injected dose for [^{11}C]raclopride was 5.15 ± 0.27 mCi (190.5 ± 10.0 MBq) and 5.21 ± 0.06 mCi (192.8 ± 2.2 MBq) for the baseline and post-amphetamine conditions (n=2/condition). The mean specific activity at the time of injection for [^{11}C]raclopride was 2275 ± 205 Ci/mmol (84 ± 8 GBq/μmol) and 3100 ± 1782 Ci/mmol (114 ± 66 GBq/μmol) for the baseline and post-amphetamine conditions. The mean injected mass for [^{11}C]raclopride was 0.79 ± 0.03 μg and 0.70 ± 0.39 μg for the baseline and post-amphetamine conditions.

Binding potential and dialysate dopamine—[^{11}C]raclopride BP_{ND} under baseline and post-amphetamine conditions, and Δ BP_{ND} after amphetamine 0.5 mg/kg in both animals are shown in Table 4. Also, included in Table 4 are the striatal dopamine concentrations (nM) at baseline, the peak Δ DA and average Δ DA after amphetamine in the same animals.

Amphetamine plasma levels—The mean amphetamine plasma levels at 5 min, 35 min, and 70 min for the [^{11}C]raclopride experiments (n=2) were 165 ± 26 ng/mL, 154 ± 45 ng/mL and 124 ± 42 ng/mL. These values were not significantly different than the mean amphetamine plasma levels in the [^{11}C]FLB 457-amphetamine experiments (n=8 experiments at 0.5 mg/kg dose, 182 ± 49 ng/mL, 144 ± 47 ng/mL and 129 ± 39 ng/mL).

DISCUSSION

In a previous report, we demonstrated the ability of amphetamine to displace the *in vivo* binding of [^{11}C]FLB 457 in the prefrontal cortical regions such as dorsolateral prefrontal cortex, medial prefrontal cortex and anterior cingulate cortex in healthy humans. Since, this report these findings have been replicated in independent cohorts by both us[37] and others[38]. An important validation step in using [^{11}C]FLB 457 as a tool to measure cortical DA release requires the demonstration of a linear relationship between increases in dialysate DA and decreases in [^{11}C]FLB 457 binding. The demonstration of such a relationship would support the use of this technique to detect differences in cortical dopamine release between patients and controls. The confirmation of a dose-effect relationship between amphetamine and changes in radiotracer binding led to the success of [^{123}I]IBZM and [^{11}C]raclopride as imaging tools to measure striatal DA release in clinical studies[1, 2]. At the same time, the inability to demonstrate such a doseresponse relationship led to the failure of other dopamine $D_{2/3}$ imaging agents such as [^{11}C]NMSP[39, 40] and [^{123}I]IBF[1]. The results of this simultaneous microdialysis and PET imaging study in non-human primates showed a linear relationship between increases in dialysate dopamine and decreases in [^{11}C]FLB 457 BP_{ND} in the frontal cortex consistent with that reported in the striatum[1, 2]. This supports the use of the [^{11}C]FLB 457 – amphetamine imaging paradigm as a non-invasive tool to measure cortical dopamine release.

In this study, the correlation between microdialysis and PET outcome measures in the frontal cortex suggests that a 1% reduction in [^{11}C]FLB 457 binding corresponds to 57% increase in dialysate peak dopamine concentration (Figure 2). These data are comparable with the 1:40 to 1:64 (Δ BP_{ND}: ΔDA) ratio that has been reported for [^{123}I]IBZM and [^{11}C]raclopride in the striatum[1, 2]. Although, the correlations between Δ DA and Δ BP_{ND} within a particular region is useful to interpret imaging data, a simple contrast of this ratio from the striatum and cortex is less informative. This is because the normalization of amphetamine-induced increases in dopamine to the baseline dopamine in the dialysis outcome measure (Δ DA) used in these correlations fails to reflect the relatively large difference in the concentration of dopamine that is released in the striatum versus frontal cortex. In contrast, the relationship between the absolute increase in dialysate dopamine concentration and decrease in $D_{2/3}$ radiotracer binding potential in the striatum and frontal cortex represents the sensitivity of dopamine to inhibit radiotracer binding in these regions. To establish this relationship, two of the five animals underwent striatal dopamine microdialysis and [^{11}C]raclopride PET scans, both before and after amphetamine 0.5 mg/kg. It was necessary to use [^{11}C]raclopride because the relatively slow kinetics of [^{11}C]FLB 457 does not allow for the estimation of BP_{ND} in the striatum within the duration of a typical 60 to 120 min [C-11] PET scan. The absolute increase in peak dialysate dopamine concentration (i.e., difference between post-amphetamine and baseline dopamine concentration) following amphetamine 0.5 mg/kg was calculated for the frontal cortex and striatum using the data in Tables 3 and 4. These data suggest that 14.2 nM increase in dialysate dopamine is associated with 1% reduction in [^{11}C]raclopride BP_{ND} in the striatum (calculated as absolute increase in peak dopamine concentration/ [^{11}C]raclopride displacement = 523 nM/36.8%); and 0.21 nM increase in dialysate dopamine is associated

with 1% reduction in [^{11}C]FLB 457 BP_{ND} in the frontal cortex (3.3 nM/15.5%). Thus, dopamine is more efficient in inhibiting $D_{2/3}$ receptor binding in the cortex compared to the striatum. The likely reason for the increased sensitivity of dopamine to inhibit cortical $D_{2/3}$ binding is that baseline dopamine levels are lower in this region compared to the striatum. Consistent with this notion are the results of imaging studies in healthy humans that have failed to detect significant occupancy of $D_{2/3}$ receptors by dopamine in the cortex with the alpha-methyl-para-tyrosine (AMPT)-induced dopamine depletion paradigm[8, 41, 42]. The same AMPT-induced dopamine depletion paradigm suggests that 10-15% of $D_{2/3}$ receptors are occupied by dopamine in the striatum[43, 44]. This explains the similar reduction in BP_{ND} (~10%) observed in both these regions in humans after amphetamine (oral, 0.5 mg/kg) despite the fact that dopamine release is much lower in the cortex compared to the striatum (150-fold difference in absolute concentration change, based on the above data). In summary, despite the differences in sensitivity between striatum and cortex, [^{11}C]FLB 457-amphetamine studies in neuropsychiatric disorders such as schizophrenia and addiction should be able to use the 1: 57 ratio established in this study to quantitate the differences in cortical dopamine release that exist between patients and controls.

In contrast to the 6 to 24% decrease in [^{11}C]FLB 457 BP_{ND} observed after 0.3 to 1.0 mg/kg of amphetamine, the mean change in [^{11}C]FLB 457 BP_{ND} in the absence of amphetamine (test-retest scans, 195 min apart) was only 0.5 ± 1.7%. Due to a concern that the FLB457 mass carried over from the baseline to the post-amphetamine scan in itself would lead to a reduction in BP_{ND}, the demonstration of no effect on [^{11}C]FLB 457 BP_{ND} in the imaging paradigm that is used to measure amphetamine induced dopamine release was necessary. Consistent with this concern, the test and retest scans performed 135 min apart showed a reduction in [^{11}C]FLB 457 BP_{ND} (− 7.2 ± 4.2%). Thus, it is necessary for future [^{11}C]FLB 457-amphetamine studies in non human primates to adhere to an inter-scan interval of 195 min to minimize the impact of carry over mass on BP_{ND}. Two previous studies have evaluated amphetamine and methamphetamine-induced displacement of [^{11}C]FLB 457 in non-human primates and reported contradictory results. Chou and colleagues reported a 10% reduction in the cortex to cerebellum [^{11}C]FLB 457 binding ratio (measured at 15 minutes and 3 hours) after 1 mg/kg of intravenous amphetamine. But, limitations such as the relatively small number of experimental observations (n=3), administration of higher injected mass for [^{11}C]FLB 457 (ten-fold higher than current study), and measurement of specific to non-specific binding ratios under non-equilibrium conditions (no modeling approach was used to derive BP_{ND}) make it difficult to compare these results with the current study[45]. Another non-human primate study that used intravenous methamphetamine (2 mg/kg) to measure dopamine release reported a non significant reduction (− 3.0 ± 5.1%) in [^{11}C]FLB 457 BP_{ND} in the frontal cortex[46]. Despite the several strengths of this study that demonstrated low variability for [^{11}C]FLB 457 BP_{ND} in the frontal cortex in test-retest studies, administration of less injected mass (only two-fold higher than current study), and use of robust modeling methodology to derive BP_{ND}, the investigation failed to report results consistent with this study. One reason for the inability to detect a significant effect for methamphetamine is inadequate power due to the limited number of experimental observations (n=3 animals in which Δ BP_{ND} was +6, −4, and −12% after methamphetamine). Another possible reason is that methamphetamine-induced changes in

non-specific binding (V_{ND}) may have led to an underestimation of ΔBP_{ND}. This is a limitation of the current study as well, in which amphetamine-induced changes in V_{ND} and its potential impact on the outcome measure, ΔBP_{ND} were not evaluated. However, recent data from $D_{2/3}$ blocking (antipsychotic occupancy) studies inform us that the contribution of the $D_{2/3}$ specific component to [^{11}C]FLB 457 binding in the human cerebellum is relatively small (7 to 14%) compared to the cortex (> 80%)[12]. Consistent with this observation previous [^{11}C]FLB 457-amphetamine studies in humans have not reported a significant reduction in cerebellum V_{ND}[9, 47, 48]. Thus, the methods of this imaging study, which were attentive to inadequate power and the effect of carry over mass were successful in demonstrating a dose-dependent effect for amphetamine on [^{11}C]FLB 457 BP_{ND}.

Another methodological consideration that deserves discussion is the use of ketamine as a pre-anesthetic in animals for the imaging/microdialysis experiments. Previous PET studies have shown that ketamine when co-administered with amphetamine can lead to a two-fold increase in stimulated dopamine release in the striatum [49]. It is not known whether ketamine has the same modulatory effect on dopamine release in the prefrontal cortex. Nevertheless, if we were to assume that ketamine pre-treatment led to greater amphetamine-induced dopamine release in the cortex in these studies, it is likely that that a smaller displacement of [^{11}C]FLB 457 binding (i.e., less dopamine release) will be measured in non-anesthetized humans. Somewhat consistent with this notion the same 0.5 mg/kg dose of amphetamine leads to ~ 16% and 10% reduction in [^{11}C]FLB 457 BP_{ND} in anesthetized primates and non-anesthetized humans. Although its possible that differences in the route of administration of amphetamine (intravenous versus oral) may have contributed to the differential displacement in primates and humans. Nevertheless, it is likely that the relationship between amphetamine-induced increases in dialysate dopamine and decrease in [^{11}C]FLB 457 BP_{ND} is preserved irrespective of anesthesia or route of administration of amphetamine. Thus, [^{11}C]FLB 457-amphetamine studies in humans should be able to use the quantitative relationship established between microdialysis and PET in this study to interpret their data.

The strengths of the microdialysis experiments were the ability to repeat experiments in the same animal after different doses of amphetamine, the use of a post-surgical CT scan to guide probe trajectories to access the frontal cortex, implantation of the probes a day before microdialysis to reduce changes in dopamine related to acute implantation, and simultaneous performance of dialysis during PET scans. The microdialysis experiments demonstrated a dose-dependent increase in dialysate dopamine for amphetamine in the frontal cortex. The mean increase in dialysate peak dopamine in this study was 999%, 1320%, and 2355% after 0.3, 0.5, and 1.0 mg/kg of amphetamine (Table 3). These results that suggest a linear relationship between amphetamine dose and dialysate peak dopamine in the frontal cortex are consistent with that observed in the striatum in previous studies (Figure 2)[1, 2]. In this study, dopamine release (peak Δ DA) in the striatum (3244%) was 2.5-fold higher than that in the frontal cortex (1320%). Two previous microdialysis studies in non-human primates suggest a 4 and 10-fold higher dopamine release in the striatum relative to the frontal cortex[13, 50]. One reason for a lack of consistency in the ratio of striatal/frontal cortical dopamine release between studies is the greater between-subject variability in dopamine release observed in the frontal cortex as opposed to the striatum[12]. This issue is likely exacerbated in non-human primate studies, which evaluate a small number of animals. In

rodent studies where sample sizes are relatively large, amphetamine studies support a striatal/frontal cortical dopamine release ratio of 2 to 4[51, 52], which is in line with the values reported in this study.

One of the limitations of the current study was the lack of measurements of dopamine release in prefrontal cortical subdivisions such as dorsolateral prefrontal cortex, orbital frontal cortex, medial prefrontal cortex, and anterior cingulate cortex. We decided against this approach as the [^{11}C]FLB 457 reproducibility studies in non human primates showed a relatively high test-retest variability for BP_{ND} in the prefrontal cortical subdivisions when they were analyzed using the method used in the human studies. This is likely due to the poor separation of gray and white matter voxels in the primate cortex. Nevertheless, considering that the magnitude of amphetamine-induced displacement of [^{11}C]FLB 457 in the prefrontal cortical subdivisions in humans is not that different (~10% in an extended human [^{11}C]FLB 457-amphetamine dataset in n =24 healthy individuals, unpublished data) and that dopamine in different prefrontal cortical regions likely comes from axons from the same population of dopamine neurons it seems reasonable to use the data that was acquired from the frontal cortex region as a whole in this study to inform the magnitude of dopamine release in the human subdivisions.

In summary, the results of this study support a linear relationship between increases in dialysate dopamine and reduction in [^{11}C]FLB 457 binding in the frontal cortex. These results are highly consistent with previous studies with [^{123}I]IBZM and [^{11}C]raclopride that have demonstrated this relationship in the striatum. They also suggest that dopamine is more potent in inhibiting cortical relative to striatal $D_{2/3}$ receptor binding, likely due to the difference in baseline dopamine between these regions. The use of the [^{11}C]FLB 457-amphetamine imaging paradigm in humans should allow for the evaluation of cortical dopamine transmission in neuropsychiatric disorders such as ADHD, schizophrenia, and drug addiction.

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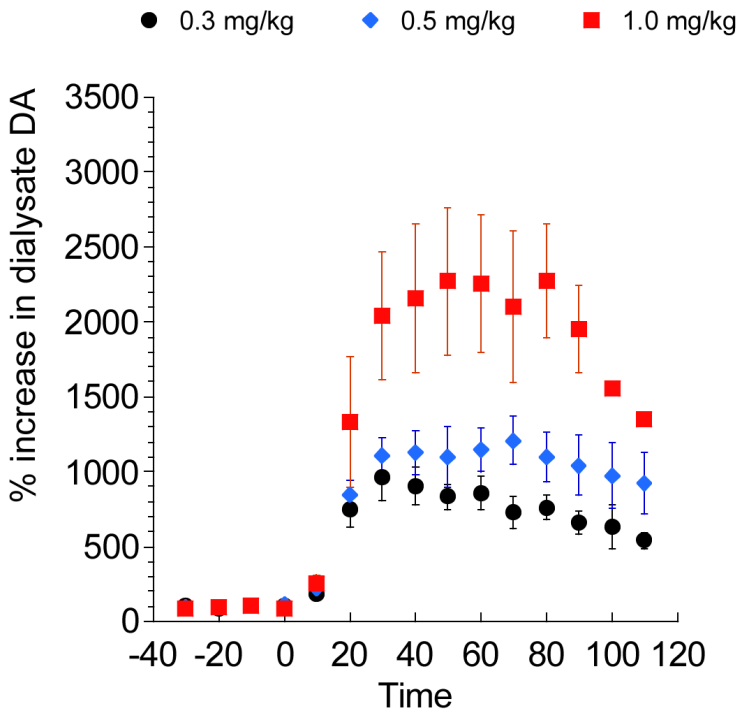

Figure 1.

shows a dose-dependent increase in dialysate DA (% mean ± standard error) in the frontal cortex after amphetamine doses 0.3, 0.5 and 1.0 mg/kg, which was injected at time 0 intravenously.

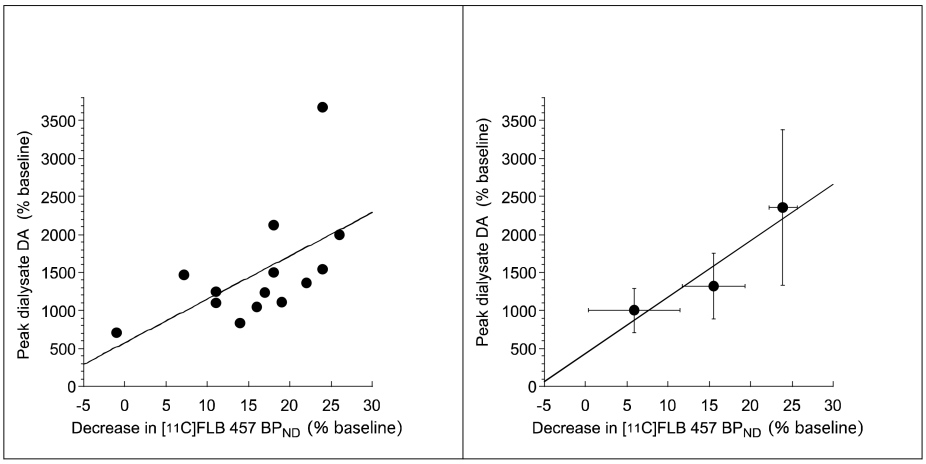

Figure 2.

shows the linear relationship between amphetamine induced increases in dialysate DA (%) and decreases in [^{11}C]FLB 457 BP_{ND} (%) using data acquired in simultaneous microdialysis and PET sessions. Figure 2B also supports a linear relationship between ΔDA and Δ [^{11}C]FLB 457 BP_{ND} when data from all the microdialysis and PET experiments are included irrespective of whether they were conducted in simultaneous sessions. The vertical error bars (Y-axis) in the figure represent the standard deviation for peak ΔDA and the horizontal error bars (X-axis) represent the standard deviation for Δ [^{11}C]FLB 457 BP_{ND}

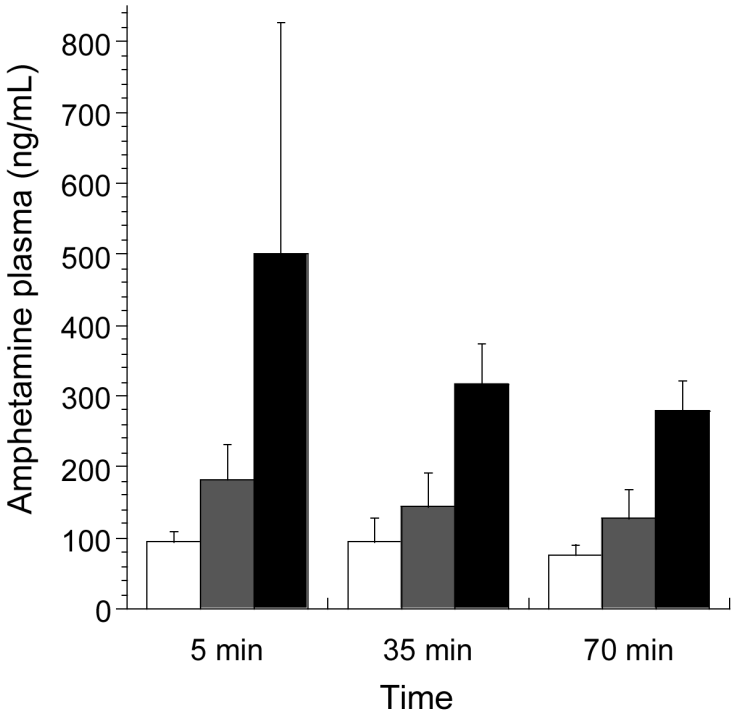

Figure 3.

shows concentration of amphetamine in plasma that was reached during the [^{11}C]FLB 457 PET-amphetamine experiments (mean ± standard deviation). The times shown in the X-axis are relative to the post-amphetamine [^{11}C]FLB 457 PET scan

Table 1

Breakdown of PET and microdialysis experiments

Animal	PET AMPH 0.3	AMPH 0.5	AMPH 1.0	Microdialysis AMPH 0.3	AMPH 0.5	AMPH 1.0	Simultaneous* AMPH 0.3	AMPH 0.5	AMPH 1.0
A	1	2	2	1	2	3	1	2	2
B	-	1	1	-	-	1	-	-	1
C	2	2	-	1	2	-	1	2	-
D	2	2	1	1	2	1	1	2	1
E	2	2	-	1	3	-	-	1	-
Total (n)	7	9	4	4	9	5	3	7	4

AMPH 0.3, AMPH 0.5, AMPH 1.0 indicate amphetamine doses 0.3, 0.5 and 1.0 mg/kg

*Experiments in which both microdialysis and PET were successful

Table 2

Carry over mass-induced change in [^{11}C]FLB 457 BP_{ND} in the frontal cortex under test and re-test conditions

Animal	Inter-scan Interval	Test BP_{ND}	Re-Test BP_{ND}	Δ BP_{ND}
A	135 min	1.13	1.02	−10%
B	135 min	0.74	0.71	−4%
Mean +/− SD		**0.94 ± 0.28**	**0.86 ± 0.22**	**−7.2% ± 4.2%**
A	195 min	0.93	0.94	1%
B	195 min	0.86	0.88	2%
C	195 min	0.79	0.78	−1%
Mean +/− SD		**0.86 ± 0.07**	**0.87 ± 0.08**	**0.5% ± 1.7%**

SD-standard deviation

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Table 3

Amphetamine-induced change in [^{11}C]FLB 457 BP_{ND} and dialysate DA in frontal cortex

Amphetamine Dose	Animal	PET Baseline BP_{ND}	AMPH BP_{ND}	Δ BP_{ND}	Microdialysis Baseline DA* (nM)	Δ Peak DA (%)	Δ Average DA (%)
0.3 mg/kg	A	0.85	0.73	−14%	0.53	837	671
PET: n=7experiments/4 animals	C	0.96	0.91	−5%	-	-	-
MD: n=4 experiments/4 animals	C	0.97	0.86	−11%	0.27	1100	924
	D	0.89	0.89	+1%	0.47	707	522
	D	0.65	0.60	−7%	-	-	-
	E	0.81	0.82	+2%	-	-	-
	E	0.97	0.91	−6%	-	-	-
	E	-	-	-	0.39	1352	941
Mean +/− SD		**0.87 ± 0.12**	**0.82 ± 0.11**	**−5.9% ± 5.5%**	**0.42 ± 0.11**	**999 ± 286**	**765 ± 203**
0.5 mg/kg	A	0.97	0.81	−17%	0.51	1239	871
PET: n=9 experiments/5 animals	A	0.78	0.64	−18%	0.23	1500	1178
MD: n=9 experiments /4 animals	B	0.83	0.69	−17%	-	-	-
	C	1.03	0.86	−16%	0.28	1048	745
	C	0.96	0.79	−19%	0.07	1110	856
	D	0.82	0.73	−11%	0.21	1249	996
	D	0.77	0.63	−18%	0.17	2123	1777
	E	1.01	0.94	−7%	0.26	1470	1254
	E	1.17	0.99	−16%	-	-	-
	E	-	-	-	0.20	1589	917
	E	-	-	-	0.45	550	441
Mean +/− SD		**0.93 ± 0.13**	**0.79 ± 0.13**	**−15.5% ± 3.8%**	**0.27 ± 0.14**	**1320 ± 432**	**1004 ± 375**
1 mg/kg	A	0.87	0.68	−22%	0.23	1365	1027
PET: n=4 experiments/3 animals	A	0.87	0.67	−24%	0.11	3670	2996
MD: n=5 experiments /3 animals	A	-	-	-	0.20	3200	2602
	B	0.88	0.66	−24%	0.23	1543	1241
	D	0.81	0.60	−26%	0.38	1997	1608

Amphetamine Dose	Animal	PET Baseline BP_{ND}	AMPH BP_{ND}	Δ BP_{ND}	Microdialysis Baseline DA* (nM)	Δ Peak DA (%)	Δ Average DA (%)
Mean +/− SD		**0.86 ± 0.03**	**0.65 ± 0.04**	**− 23.9% ± 1.7%**	**0.23 ± 0.10**	**2355 ± 1026**	**1895 ± 863**

MD- Microdialysis; AMPH- Amphetamine; SD-Standard Deviation

*Mean values of n=3 to 4 baseline dopamine samples from −30 to 0 minutes before amphetamine

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Table 4

Amphetamine-induced change in [^{11}C]raclopride BP_{ND} and dialysate DA in striatum

Amphetamine Dose	Animal	PET Baseline BPND	AMPH BPND	Δ BP	Microdialysis Baseline DA* (nM)	Δ Peak DA (%)	Δ Average DA (%)
0.5 mg/kg	D	3.48	1.87	−46%	-	-	-
PET: n=2experiments/2 animals	D	-	-	-	8.81	5167	2355
MD: n=5 experiments/2 animals	D	-	-	-	8.36	4688	2819
	E	3.12	2.26	−27%	-	-	-
	E	-	-	-	13.14	2981	2169
	E	-	-	-	28.83	2473	1673
	E	-	-	-	24.05	913	401
Mean +/− SD		**3.30 ± 0.25**	**2.07 ± 0.28**	**−36.8% ± 13.3%**	**16.64 ± 9.29**	**3244 ± 1723**	**1884 ± 925**

MD- Microdialysis; AMPH- Amphetamine

*Mean values of n= 3 to 4 baseline dopamine samples from −30 to 0 minutes before amphetamine