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Evaluation of dopamine D_{2/3} specific binding in the cerebellum for the PET radiotracer [¹¹C]FLB 457: implications for measuring cortical dopamine release

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Abstract

In a recent PET study we demonstrated the ability to measure amphetamine-induced DA release in the human cortex with the dopamine D_{2/3} radioligand [¹¹C]FLB 457. As previous studies in animals have shown that a relatively high fraction of the [¹¹C]FLB 457 signal in the cerebellum represents specific binding to D_{2/3} receptors, there was concern that the use of the cerebellum as a measure of nonspecific binding (i.e., reference region) to derive [¹¹C]FLB 457 binding potential (BP_{ND}) would bias cortical dopamine release measurements. Thus, we evaluated the fractional contribution of specific binding to D_{2/3} receptors in the human cerebellum for [¹¹C]FLB 457.

Six healthy human subjects (5M/1F) were studied twice with [¹¹C]FLB 457, once at baseline and again following a single oral dose of 15 mg of aripiprazole, a D_{2/3} partial agonist. [¹¹C]FLB 457 distribution volume (V_T) was estimated using kinetic analysis in the cortical regions of interest and potential reference regions. The change in [¹¹C]FLB 457 V_T following aripiprazole ranged from −33 to −42% in the cortical regions of interest (ROIs). The aripiprazole-induced change in [¹¹C]FLB 457 V_T in three potential reference regions suggests significant specific binding in the cerebellum (CER, −17 ± 12%), but not pons (PON, −10 ± 10%) and centrum semiovale (CESVL, −3 ± 12%). Nevertheless, a re-analysis of the published [¹¹C]FLB 457 test-retest and amphetamine studies suggests that the use of the PON V_T and CESVL V_T as an estimate of nonspecific binding to derive [¹¹C]FLB 457 BP_{ND} in dopamine release studies is unlikely to be successful because it leads to less reproducible outcome measures, which in turn diminishes the ability to measure dopamine release in the cortex.

D_{2/3} blocking studies with aripiprazole and [¹¹C]FLB 457 suggest specific binding to D_{2/3} receptors in the cerebellum. These data also suggest that the contribution of specific binding to D_{2/3} receptors in the cerebellum is lower than that in the cortical ROIs and that CER V_T is mostly representative of nonspecific binding. Nevertheless, caution is advised when using reference tissue methods that rely solely on the cerebellum signal as an input function to quantify [¹¹C]FLB 457 BP_{ND}.

Keywords

PET; dopamine; D_{2/3} receptors; [¹¹C]FLB 457; human cortex

Introduction

The study of dopamine transmission in the human cortex is of interest in several neurological and psychiatric disorders such as Parkinson's disease, schizophrenia and addiction. In a previous study we demonstrated the vulnerability of the in vivo binding of [¹¹C]FLB 457 to endogenous competition by dopamine following an oral amphetamine challenge (Narendran et al., 2009). The results of this study, demonstrating a significant reduction in the in vivo binding of [¹¹C]FLB 457 following amphetamine (0.5 mg/kg), support the validation of [¹¹C]FLB 457 PET as a technique to measure cortical dopamine release. One validation issue discussed in our previous report that required further investigation was the fact that some (Asselin et al., 2007; Delforge et al., 1999), but not all (Farde et al., 1997), studies suggest 60 to 75% of the binding of [¹¹C]FLB 457 in the cerebellum is specific to D_{2/3} receptors. The presence of a relatively large fraction of [¹¹C]FLB 457 specific binding to D_{2/3} receptors in the cerebellum would pose a problem when cerebellum V_T is used as a measure of nonspecific binding. This assumption would underestimate the [¹¹C]FLB 457 BP_{ND} (i.e., binding potential relative to non displaceable uptake, which is the ratio of the equilibrium specific to non specific binding) in the cortical regions of interest. Furthermore, specific binding to D_{2/3} receptors in the cerebellum that is displaceable by dopamine following an amphetamine challenge could bias amphetamine-induced dopamine release measurements (i.e., Δ [¹¹C]FLB 457 BP_{ND}) in the cortex. Thus, a critical step to validate [¹¹C]FLB 457 as a tool to measure dopamine release in the cortex is to understand the fractional contribution of [¹¹C]FLB 457 binding that is specific to D_{2/3} receptors in the human cerebellum. A relatively low contribution of D_{2/3} specific binding in the cerebellum would allow for the continued use of this region as a reference region for quantitation of [¹¹C]FLB 457 BP_{ND}. To evaluate this issue we studied six healthy human subjects with [¹¹C]FLB 457 before and after pre-treatment with aripiprazole (15 mg, oral), a highly selective D_{2/3} partial agonist drug.

Methods

1. Aripiprazole study

The study was approved by the Institutional Review Board of the University of Pittsburgh. A total of 12 PET scans were acquired for this study in six healthy control subjects (1 female/5 males; 1 African American/5 Caucasian; 24 ± 2 years of age; 6 non-smokers) over six experimental sessions. Each experimental session included two [¹¹C]FLB 457 PET scans: a baseline scan and a post aripiprazole scan which was performed 3 hours after oral administration of aripiprazole. The post-aripiprazole scan duration was optimized to correspond with time to peak concentration for aripiprazole, which has been reported to be at 3 to 5 hours (Abilify®, U.S. Full prescribing information)

PET Protocol—Radiolabeling of [¹¹C]FLB 457 was performed as outlined in previously published procedures (Halldin et al., 1995). Imaging experiments were conducted on the ECAT EXACT HR+ consistent with previously described image acquisition protocols (Narendran et al., 2010). Briefly, following completion of a transmission scan (~10 min) for attenuation correction of the emission data, subjects received an intravenous injection of [¹¹C]FLB 457 as a bolus over 20 sec. Consistent with previous studies, the maximum

injected mass for [^{11}C]FLB 457 was restricted to 0.6 μg (Narendran et al., 2009; Narendran et al., 2010; Sudo et al., 2001). Emission data were collected for 90 min.

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 [^{11}C]FLB 457 parent compound, six samples (collected at 2, 10, 20, 40, 60 and 75 min) were further processed using HPLC methods (Narendran et al., 2009; Olsson et al., 1999). For [^{11}C]FLB 457 the six measured parent fractions were fitted to the sum of two exponentials. The smallest exponential of the fraction of the parent curve, λ_{par} , was constrained to the difference between λ_{cer} (the terminal rate of washout of the cerebellar activity) and λ_{tot} (the smallest elimination rate constant of the total plasma) as described in (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). In addition, measurement of plasma free fraction (f_p) for [^{11}C]FLB 457 was performed as previously described (Narendran et al., 2009).

MRI Protocol—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 (4.0, Smith et al., 2004).

Analysis of PET data—PET data were reconstructed and processed with the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) and SPM2 (www.fil.ion.ucl.ac.uk/spm) as described in (Narendran et al., 2009). 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 and three potential reference regions using the criteria and methods outlined in (Narendran et al., 2010). Sampled cortical regions included the 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). The potential reference regions included the cerebellum (CER), pons (PON), and centrum semiovale (CESVL, an expansive region of white matter region sub sampled in the dorsal core of the frontal and parietal lobes).

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.

The primary outcome measure provided is regional tissue distribution volume (V_T , mL cm^{-3}). The definition of this outcome measure is described in the consensus nomenclature for PET studies manuscript (Innis et al., 2007). Derivation of [^{11}C]FLB 457 V_T in the regions of interest and reference (CER, PON, CESVL) were performed using a two tissue

compartment kinetic analysis and the arterial input function (Narendran et al., 2009; Olsson et al., 1999).

Outcome measures and statistical analysis—The aripiprazole-induced change in [¹¹C]FLB 457 V_T was calculated as the difference between V_T measured in the post aripiprazole scan ($V_{T \text{ ARIPIPRAZOLE}}$) and V_T measured in the baseline scan ($V_{T \text{ BASELINE}}$), and expressed in percentage of $V_{T \text{ BASELINE}}$.

$$\text{Aripiprazole-induced } \Delta V_T = 100 * \frac{V_{T \text{ ARIPIPRAZOLE}} - V_{T \text{ BASELINE}}}{V_{T \text{ BASELINE}}} \quad \text{Eq. 1}$$

Furthermore, to arrive at receptor occupancy and V_{ND} independent of choice of reference region, the baseline and post-aripiprazole V_T values from the eight cortical regions of interest for each subject were analyzed using Lassen plots as described in (Cunningham et al., 2010). Briefly, the equation for the line [$y=mx+b$], where $y=[V_{T \text{ BASELINE}} - V_{T \text{ ARIPIPRAZOLE}}]$, and $x=V_{T \text{ BASELINE}}$, produces a linear relationship with x intercept equal to V_{ND} and slope of line equal to receptor occupancy (m). This approach assumes that there is uniform receptor occupancy and non-displaceable binding across the cortical regions of interest.

All statistical analyses were performed with two-tailed paired t test as specified.

2. Reanalysis of test-retest and amphetamine data with alternative reference regions (PON, CESVL)

In our previous [¹¹C]FLB 457 test-retest (Narendran et al., 2010) and amphetamine studies (Narendran et al., 2009), we utilized the cerebellum V_T as an estimate of nonspecific binding to derive [¹¹C]FLB 457 BP_{ND} . Here, we wanted to understand whether the use of alternative reference regions such as the PON or CESVL would lead to improved test-retest variability for [¹¹C]FLB 457 BP_{ND} and allow for reliable detection of amphetamine-induced displacement of [¹¹C]FLB 457 BP_{ND} in the cortical ROIs. Thus, [¹¹C]FLB 457 BP_{ND} in the test-retest (n = 6 subjects) and amphetamine datasets (n = 11 subjects, age < 40 years old) were derived both using the PON V_T and CESVL V_T as an estimate of nonspecific binding. In the test-retest data, variability was then calculated as the absolute value of the difference between the test and retest, divided by the mean of the test and retest values. In the amphetamine data, amphetamine-induced change in BP_{ND} (ΔBP_{ND}) was then calculated as the difference between baseline and post-amphetamine BP_{ND} values, divided by the baseline BP_{ND} . Both these outcome measures, i.e., test-retest variability and amphetamine-induced ΔBP_{ND} , derived using the PON V_T and CESVL V_T in the corresponding datasets were then contrasted with the previously published data that used the cerebellum V_T as an estimate of nonspecific binding.

Results

1. Aripiprazole study

Scan parameters and plasma analysis—The mean injected dose, mass and specific activity at the time of injection for the baseline and post-aripiprazole condition for [¹¹C]FLB 457 are listed in Table 1. No significant differences were observed between the baseline and post-aripiprazole condition in any of these variables. No significant difference in the plasma free fraction or clearance was observed between the [¹¹C]FLB 457 baseline and post-aripiprazole conditions (Table 1)

Brain analysis—The regional V_T values for the baseline and post-aripiprazole scans and corresponding changes are provided in Table 2. Aripiprazole produced a statistically significant decrease in V_T in all the cortical regions of interest and the cerebellum (paired t-tests, $p < 0.05$; see Table 2), but not in pons and centrum semiovale.

Lassen plot derived receptor occupancy and V_{ND} derived using V_T values from the eight cortical regions of interest are shown in Table 3.

2. Reanalysis of test-retest and amphetamine data with alternative reference regions (PON, CESVL)

Test-retest variability for [^{11}C]FLB 457 BP_{ND} in the cortical ROIs derived using CER V_T , PON V_T and CESVL V_T are shown in Table 4

Amphetamine-induced displacement of [^{11}C]FLB 457 BP_{ND} in the cortical ROIs derived using CER V_T , PON V_T and CESVL V_T are shown in Table 5.

Discussion

The primary objective of this blocking study was to evaluate the fractional contribution of $D_{2/3}$ specific binding for [^{11}C]FLB 457 in the cerebellum, which is used as a reference region for this radiotracer. It was important to evaluate the contribution of [^{11}C]FLB 457 binding that is specific to $D_{2/3}$ receptors in humans because animal studies suggest that 60 to 75% of the binding of [^{11}C]FLB 457 in the cerebellum is specific to $D_{2/3}$ receptors (Asselin et al., 2007; Delforge et al., 1999). Furthermore, these reports were in contrast with the only study in humans that evaluated this issue and reported a lack of displaceable $D_{2/3}$ binding in the cerebellum for [^{11}C]FLB 457 following pretreatment with the antipsychotic drug haloperidol (4 mg, single oral dose). Unfortunately a limitation of this previous study was the failure to use arterial input based modeling methods to quantify [^{11}C]FLB 457 binding in the cerebellum (V_T). The main result of this study, which used fully quantitative kinetic modeling methods to measure changes in V_T after the administration of a dopamine $D_{2/3}$ selective partial agonist drug support a small (7 to 14%; derived as CER V_T /Lassen V_{ND} to CER V_T /CESVL V_T) but statistically significant fraction of specific binding to $D_{2/3}$ receptors for [^{11}C]FLB 457 in the human cerebellum. In contrast to these estimates, the Lassen plot derived occupancy in the cortical ROIs was $79 \pm 12\%$, a result that is highly consistent with previous studies that have evaluated the $D_{2/3}$ receptor occupancy of aripiprazole in the striatal and extrastriatal regions (Grunder et al., 2008; Kegeles et al., 2008). In addition, results of this study also suggest that [^{11}C]FLB 457 binding in the pons and centrum semiovale are not displaceable by the $D_{2/3}$ selective drug aripiprazole. This observation led us to explore the use of the pons and centrum semiovale as reference regions for [^{11}C]FLB 457. Specifically, we were interested to know whether the use of the pons or centrum semiovale as a reference region for [^{11}C]FLB 457 would lead to (1) improved reproducibility for [^{11}C]FLB 457 BP_{ND} in the cortical ROIs and (2) allow for reliable detection of amphetamine-induced displacement of [^{11}C]FLB 457 in the cortical ROIs.

In order to evaluate the reproducibility for [^{11}C]FLB 457 V_T in the pons and centrum semiovale a re-analysis was thus performed of the test-retest data that was previously published in (Narendran et al., 2010). In this re-analysis, the test-retest variability for [^{11}C]FLB 457 V_T in the pons and centrum semiovale were $9 \pm 4\%$ and $7 \pm 4\%$ respectively. Both values were higher than the previously published variability for V_T in the cerebellum ($5 \pm 4\%$). One reason for the relatively higher test-retest variability for V_T in the pons ($6946 \pm 1851 \text{ mm}^3$ and centrum semiovale ($6656 \pm 1400 \text{ mm}^3$) may be due to the relatively smaller size of these regions compared to the cerebellum ($17537 \pm 1680 \text{ mm}^3$, data from $n=6$ subjects in Narendran 2010) that renders them more vulnerable to head motion and

partial voluming effects. Furthermore, the centrum semiovale, which is comprised exclusively of white matter, had relatively slow wash out kinetics compared to other gray matter regions (see regional brain time activity curve in Figure 1) that led to less robust quantitation of V_T in this region. Thus, the use of the the pons V_T and centrum semiovale V_T as V_{ND} to compute BP_{ND} in the cortical regions of interest translated to a higher test-retest variability for [^{11}C]FLB 457 BP_{ND} in the cortical regions of interest as shown in Table 4. These results were of concern as a higher test-retest variability for [^{11}C]FLB 457 BP_{ND} in the cortical regions of interest would diminish the ability to detect [^{11}C]FLB 457 displacement in these regions following an amphetamine challenge. Consistent with this prediction, a re-analysis of the [^{11}C]FLB 457-amphetamine data revealed that the number of cortical regions in which amphetamine led to a significant reduction in [^{11}C]FLB 457 binding was lower when the pons V_T (0/8 ROIs) and centrum semiovale V_T (3/8 ROIs) were used as opposed to the cerebellum V_T (5/8 ROIs) to estimate nonspecific binding (see Table 5). These data suggest that the higher within subject variability for [^{11}C]FLB 457 BP_{ND} with the use of the pons and centrum semiovale as reference regions leads to lower power to detect amphetamine-induced dopamine release in the human cortex. Thus, the use the cerebellum as a reference region in future [^{11}C]FLB 457 studies that evaluate cortical dopamine transmission may be necessary despite the presence of a small $D_{2/3}$ specific binding in this region. Two factors support the continued use of the cerebellum as a reference region. First, in the [^{11}C]FLB 457-aripiprazole dataset the baseline [^{11}C]FLB 457 scan V_T values in the cerebellum were reasonably well correlated with the V_T measured in the pons and centrum semiovale and with the V_{ND} derived using Lassen plots—i.e., all independent measures of nondisplaceable binding (see Figure 2). Additionally, data from the previous human studies that have evaluated dopamine release using either amphetamine or methylphenidate do not suggest displaceable binding for [^{11}C]FLB 457 in the cerebellum (Aalto et al., 2008; Montgomery et al., 2007; Narendran et al., 2009). In these studies, the mean change in cerebellum V_T after oral amphetamine (0.5 mg kg^{-1}), intravenous amphetamine (0.3 mg kg^{-1}) and oral methylphenidate (0.4 to 0.6 mg kg^{-1}) was $0 \pm 12\%$, -1% $-5 \pm 9\%$ respectively. Results from these earlier studies suggest that despite the presence of $D_{2/3}$ receptors in the cerebellum confirmed by this current investigation, the amount of dopamine released following a stimulant challenge is not sufficient to displace [^{11}C]FLB 457 specific binding to $D_{2/3}$ receptors in the human cerebellum. Nevertheless, this assumption may not be valid in different patient populations, and future [^{11}C]FLB 457 studies should document amphetamine-induced changes in cerebellum V_T using metabolite corrected arterial input function in patients and controls.

In summary, we demonstrated $D_{2/3}$ specific binding in the human cerebellum, but not the pons and centrum semiovale for the radiotracer [^{11}C]FLB 457 with aripiprazole. These data also suggest that the contribution of [^{11}C]FLB 457 specific binding to $D_{2/3}$ receptors in the cerebellum is lower than that in the cortical ROIs. Thus, the cerebellum V_T mostly represents nonspecific binding, thereby justifying its continued use as a reference region in human studies. Nevertheless, caution is advised when using reference tissue method that rely solely on the cerebellum signal as an input function to quantify [^{11}C]FLB 457 BP_{ND} and cortical dopamine release. The use of alternative reference regions such as the pons and centrum semiovale to quantify [^{11}C]FLB 457 BP_{ND} in dopamine release studies will require a larger number of subjects to be successful because it leads to less reproducible outcome measures, which in turn diminishes the ability to measure dopamine release in the cortex.

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Figure 1. shows the regional brain time activity curve for $[^{11}\text{C}]\text{FLB 457}$ in human brain. Note the relatively slow wash out kinetics in the centrum semiovale (CESVL), which is comprised exclusively of white matter as opposed to the predominantly gray matter regions such as cerebellum (CER), pons, (PON), and dorsolateral prefrontal cortex (DLPFC).

Figure 2. shows that the baseline $[^{11}\text{C}]$ FLB 457 cerebellum V_T is correlated with all three independent measures of nondisplaceable binding (i.e., baseline $[^{11}\text{C}]$ FLB 457 pons V_T , centrum semiovale V_T and $[^{11}\text{C}]$ FLB 457 V_{ND} derived using Lassen plots)

Table 1

[¹¹C]FLB 457 scan parameters and plasma analysis (n=6 subjects)

Scan Parameter	Baseline	Post-ariprazole
Injected dose (mCi)	8.1 ± 1.1	7.7 ± 1.8
SA (Ci/mmoles)	11772 ± 7292	15243 ± 6800
Injected Mass (ug)	0.4 ± 0.2	0.3 ± 0.2
Plasma Free Fraction (fP, %)	38.0% ± 8.8%	36.1% ± 6.9%
Clearance (L/h)	98 ± 25	104 ± 30

Comparisons between baseline and post-ariprazole parameters all $p > 0.05$, paired t-test

Table 2Aripiprazole-induced change in [^{11}C]FLB 457 V_T

Regions of interest	Baseline V_T	Post-Aripiprazole V_T	ΔV_T (%)	p value
Medial Temporal Lobe	10.99 $\pm$ 3.09	6.52 $\pm$ 2.56	-42.0 $\pm$ 8.4	0.001
Anterior Cingulate Cortex	8.48 $\pm$ 2.35	5.20 $\pm$ 1.87	-38.7 $\pm$ 10.7	0.002
Dorsolateral Prefrontal Cortex	7.29 $\pm$ 2.22	4.83 $\pm$ 1.31	-32.6 $\pm$ 9.9	0.007
Orbital Frontal Cortex	8.77 $\pm$ 2.84	5.23 $\pm$ 1.54	-39.1 $\pm$ 10.7	0.007
Medial Prefrontal Cortex	7.91 $\pm$ 2.41	5.05 $\pm$ 1.34	-34.8 $\pm$ 11.8	0.008
Temporal Cortex	11.86 $\pm$ 3.74	5.80 $\pm$ 1.57	-50.4 $\pm$ 5.4	0.001
Parietal Cortex	7.42 $\pm$ 2.25	4.72 $\pm$ 1.10	-34.4 $\pm$ 11.2	0.007
Occipital Cortex	7.17 $\pm$ 2.22	4.63 $\pm$ 1.20	-34.1 $\pm$ 9.4	0.004
Cerebellum	4.44 $\pm$ 0.92	3.67 $\pm$ 0.82	-17.1 $\pm$ 12.4	0.032
Pons	4.52 $\pm$ 1.37	4.07 $\pm$ 1.43	-10.1 $\pm$ 9.7	0.099
Centrum Semiovale	3.89 $\pm$ 0.95	3.74 $\pm$ 0.80	-2.7 $\pm$ 11.8	0.536

Values are mean $\pm$ standard deviation; n=6 subjects. *p* values indicate paired t-tests

Table 3

Lassen plot analysis of [11C]FLB 457-aripiprazole data

Subjects	Occupancy of D _{2/3} receptors by aripiprazole Slope (m)	[11C]FLB 457 V _{ND} X intercept
1	0.92	4.97
2	0.60	2.91
3	0.71	2.91
4	0.83	5.95
5	0.84	5.45
6	0.85	2.68
Mean ± SD	0.79 ± 0.12	4.14 ± 1.47

Table 4

Re-analysis of test-retest data in n=6 healthy controls from (Narendran et al., 2010)

Regions of interest (ROI)	Test-retest variability of ROI [¹¹ C]FLB 457 BP _{ND} derived using		
	Cerebellum V _T	Pons V _T	Centrum semiovale V _T
Medial Temporal Lobe	11% ± 5%	15% ± 14%	10% ± 8%
Anterior Cingulate Cortex	15% ± 8%	17% ± 13%	12% ± 10%
Dorsolateral Prefrontal Cortex	8% ± 6%	14% ± 11%	13% ± 11%
Orbital Frontal Cortex	7% ± 6%	17% ± 11%	11% ± 8%
Medial Prefrontal Cortex	6% ± 4%	14% ± 10%	9% ± 11%
Temporal Cortex	10% ± 6%	15% ± 13%	10% ± 9%
Parietal Cortex	8% ± 4%	17% ± 14%	12% ± 10%
Occipital Cortex	10% ± 4%	21% ± 14%	15% ± 12%

Test-retest variability is provided as mean ± standard deviation; The test-retest variability was calculated as the absolute value of the difference between the test and retest, divided by the mean of the test and retest values.

Table 5

Re-analysis of amphetamine data in n=11 healthy controls from (Narendran et al., 2009)

Regions of interest (ROI)	Amphetamine-induced change in [11C]FLB 457 BP _{ND} derived using		
	Cerebellum V _T	Pons V _T	Centrum semiovale V _T
Medial Temporal Lobe	- 6.9 ± 6.4 *	0.4 ± 16.8	- 8.2 ± 12.1 *
Anterior Cingulate Cortex	- 8.0 ± 8.5 *	5.6 ± 38.9	- 10.0 ± 13.4 *
Dorsolateral Prefrontal Cortex	-11.0 ± 14.8 *	- 8.4 ± 17.9 †	- 11.1 ± 22.9 †
Orbital Frontal Cortex	- 8.3 ± 15.6 †	- 0.9 ± 19.6	- 8.8 ± 19.5
Medial Prefrontal Cortex	- 9.6 ± 13.8 *	4.2 ± 32.9	- 7.7 ± 29.6 †
Temporal Cortex	- 3.5 ± 9.2	3.5 ± 15.2	- 3.7 ± 19.2
Parietal Cortex	- 12.1 ± 14.0 *	- 31.6 ± 68.5	- 14.3 ± 17.2 *
Occipital Cortex	- 4.2 ± 20.5 †	- 18.3 ± 59.1	- 10.4 ± 16.9 †

Values are mean ± SD; n =11 subjects

*
p < 0.05†
p < 0.1 on paired t-tests