

Mapping α_2 adrenoceptors of human brain with ^{11}C -yohimbine

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Abstract

A previous study from this laboratory suggested that ^{11}C -yohimbine, a selective α_2 adrenoceptor antagonist, is an appropriate ligand for positron emission tomography (PET) of α_2 -adrenoceptors that passes readily from blood to brain tissue in pigs but not in rodents. To test the usefulness in humans, we obtained blood-brain clearances, volumes of distribution, and receptor availability by means of PET with ^{11}C -yohimbine in healthy male adults.

Methods: We recorded the distribution of ^{11}C -yohimbine with ninety minutes dynamic PET and sampled arterial blood to measure intact ^{11}C -yohimbine in plasma. For analysis, we co-registered PET images to individual magnetic resonance images (MRI) and automatically identified 27 volumes of interest. We used one tissue compartment graphical analysis, with six linearized solutions of the fundamental binding equation, with the metabolite-corrected arterial plasma curves as input function, to estimate the kinetic parameters of ^{11}C -yohimbine. With the lowest steady-state value of V_T , determined in corpus callosum, we calculated a binding potential (receptor availability) of the radioligand in other regions.

Results: The linear regressions yielded similar estimates of the kinetic parameters. The cortical values of V_T ranged from 0.82 ml cm^{-3} in right frontal cortex to 0.46 ml cm^{-3} in corpus callosum, with intermediate V_T values in subcortical structures. Binding potentials averaged 0.6-0.8 in cortex and 0.2-0.5 in subcortical regions.

Conclusion: The maps of ^{11}C -yohimbine binding to α_2 adrenoceptors in human brain had the highest values in cortical areas and hippocampus, with moderate values in subcortical structures, as found also *in vitro*. The results confirm the usefulness of tracer ^{11}C -yohimbine for mapping α_2 adrenoceptors in human brain *in vivo*.

Keywords

^{11}C -yohimbine, α_2 adrenoceptors, positron emission tomography, brain, noradrenaline.

INTRODUCTION

Among the α_2 adrenoceptors, the autoreceptors are located presynaptically on noradrenergic neurons where they regulate firing of noradrenergic neurons, as well as noradrenaline (NA) synthesis and release (1). Three α_2 -adrenoceptor subtypes have been identified, including the cloned human α_2 -adrenoceptors $\alpha_{2.C10}$, $\alpha_{2.C2}$, and $\alpha_{2.C4}$, labeled according to the chromosomal localization of the genes (2-4). The three α_2 -adrenoceptor subtypes are known as α_{2a} , α_{2b} and α_{2c} in the pharmacological nomenclature (5).

Dense expression of α_2 -adrenoceptors occurs in the cerebral cortex, hippocampal formation, hypothalamus, and locus coeruleus, with intermediate-to-moderate binding in subcortical structures, and low expression in white matter (6,7). Degeneration of the noradrenergic neurotransmitter system and specific loss of α_2 -adrenoceptors is implicated in neurodegenerative disorders (8). Imaging of α_2 -adrenoceptors therefore is essential to the understanding of the role of the noradrenergic system in these disorders. However, no positron emission tomography (PET) radioligands selective for α_2 -adrenoceptors have passed successfully from preclinical evaluation to clinical usefulness (9, 10).

We recently developed ^{11}C -yohimbine for use with PET. Yohimbine binds to all α_2 -adrenoceptor subtypes and has moderate affinity for the 5HT_{1A} receptors as well (11). In porcine brain, ^{11}C -yohimbine enters brain tissue readily and binds specifically in brain areas with known high densities of α_2 -adrenoceptors. In rat brain, ^{11}C -yohimbine is excluded from entry into brain tissue by the action of permeability glycoprotein (Pgp) (12). In porcine brain, unlabeled yohimbine and RX821002, a highly selective α_2 -adrenoceptor antagonist, both displace ^{11}C -yohimbine binding (11). The results prompted us to test ^{11}C -yohimbine as a radioligand for use in humans, particularly with respect to the ability of the tracer to readily cross the blood-brain barrier.

MATERIALS AND METHODS

Subjects

The Committee on Ethics of Central Denmark Region approved this study, and all subjects provided a written informed consent prior to participation in this study. None of the subjects had any neurological or psychiatric disorder. We recruited young male subjects (n=6) with mean age 28.7 (4.1) years and age range 24-35 years to participate in the study. Subject #6 was excluded from analysis following the PET session, as the subject received a dose of radioactivity almost one order of magnitude lower than other subjects, combined with the subsequent observation of the highest rate of metabolism of yohimbine in the circulation, corresponding to the CYP2D6 status given in Table 1, resulting in the almost complete disappearance of radioactivity from the circulation.

Radiochemistry, Metabolites and CYP2D6 Status

The synthesis of ¹¹C-yohimbine is described in detail elsewhere (11). Briefly, cyclotron-produced ¹¹C-carbon dioxide was converted to ¹¹C-methyl iodide and trapped in DMSO (300 µl) containing NaOH (1 µl, 3 mol/L) and yohimbic acid (1 mg) in a 1-ml vial. This mixture was heated at 80°C for 3 min. Purification of ¹¹C-yohimbine was performed by semipreparative high performance liquid chromatography (HPLC). The mobile phase, consisting of 75% aqueous 70 mmol/L Na₂HPO₄ and 25% ethanol, was delivered at a rate of 5 ml/min to a LUNA C18(2) (Phenomenex, 250 x 10 mm) semipreparative column with online radio and ultraviolet (UV)-visible (280 nm) detection. The fraction containing ¹¹C-yohimbine (retention time, 7–8 min) was collected and diluted with 5 ml sterile saline, filtered through a sterile 0.22 µm filter to obtain a total of 10 ml product solution. This procedure gave 1-2 GBq ¹¹C-yohimbine with radiochemical purity > 99% in a sterile formulation ready for injection. With regard to metabolites of ¹¹C-yohimbine in circulation, the fraction of untransformed ¹¹C-yohimbine was measured by radio-HPLC in extracts of plasma from samples taken at 2, 5, 10, 20, 40, 60, and 90 minutes after injection. We also genotyped the cytochrome P450 (CYP) system with regards to the CYP2D6 isoform at the laboratory Fildelfia, Denmark.

Yohimbic acid monohydrate, yohimbine hydrochloride, dimethylsulfoxide (DMSO) and acetonitrile (MeCN) were purchased from Sigma Aldrich.

Image Acquisition, Registration and Segmentation

Subjects reclined the scanner, ECAT High Resolution Research Tomograph (HRRT, CTI/Siemens, Knoxville, TN, USA), and the head was positioned and immobilized in a custom-built head-holder. A 6 minutes long transmission scan was obtained and a 90 minutes dynamic PET scan consisting of 28 frames increasing in duration from 15 seconds in the beginning to 10 minutes at the end of the scan (8 x 15 seconds, 4 x 30 seconds, 6 x 60 seconds, 4 x 300 seconds, 6 x 600 seconds), was recorded in 3D mode upon administration of ^{11}C -yohimbine. An OSEM3D-OP algorithm including point spread function modeling, with 10 iterations and 16 subsets, was used for reconstruction resulting in $256 \times 256 \times 207$ images at a resolution of 1.8mm at full width half maximum. Blood samples were collected at 5-second intervals in the beginning increasing to intervals of 10 minutes at end of scan. Subjects underwent T1 weighted magnetic resonance imaging (MRI) scans. We used PMOD v.3.5 software and the module PNEURO (PMOD Technologies Ltd., Zurich, Switzerland) for model based image co-registration and segmentation of volumes of interests (VOIs). First, we averaged the dynamic images from frames 5 to 25 to provide a sufficient anatomical PET image. We then co-registered the anatomical PET images to the individual anatomical MRIs by a rigid co-registration method using a mutual information algorithm. Individual MRI scans were spatially normalized to the MNI T1 template and the transformation matrices were used to bring the dynamic PET into MNI space. Volumes of interest (VOIs) were automatically outlined on the normalized MRI according to the Hammers maximum probability atlas, which is implemented in PMOD (13). The VOIs were intersected with a 50% gray matter probability mask and then applied to the spatially normalized dynamic PET scans to obtain time activity curves (TACs) for 27 selected VOIs (see Table 2).

Kinetic Analysis of ¹¹C-Yohimbine Uptake and Distribution

The kinetic parameters of ¹¹C-yohimbine in the human brain were estimated with nonlinear one-tissue and two-tissue compartment models. There were no significant differences between estimates of ¹¹C-yohimbine volumes of distribution or Akaike's information criterion (AIC) scores between these models. However, the non-linear two-tissue compartment model produced non-physiological estimates of the kinetic parameters in some instances. For simplicity of analysis and illustration, we chose to compare the results of six linearizations of the one-tissue compartment model, as presented in the paragraphs below.

The distribution of a tracer as a function of time is governed by linear differential equations with constant transfer coefficients. In the case of a tracer that enters a brain tissue compartment in which bound and unbound tracer are in steady-state, the distribution of the tracer is governed by the exchange between the circulation and the tissue compartment across the blood-brain and other potential barriers:

$$\frac{dm_1}{dt} = V_o \frac{dc_a}{dt} \quad \text{Eq.1}$$

where m_1 (kBq/ml) is the tracer quantity in the vascular compartment with the volume V_o (ml/cm³) and the concentration $c_a(t)$ (kBq/ml) as function of time, and

$$\frac{dm_2}{dt} = K_1 c_a - k'_2 m_2 \quad \text{Eq.2}$$

where m_2 (kBq/ml) is the quantity of tracer in the tissue compartment, K_1 (ml/cm³/min) is the unidirectional clearance of tracer from the circulation, and k'_2 (min⁻¹) is the rate constant of tracer efflux from the tissue compartment, equal to the ratio of the unidirectional clearance K_1 and the total steady-state volume of distribution V_T (ml/cm³), here defined as

$$k'_2 = \frac{k_2}{1 + BP_{ND}} = \frac{K_1}{V_{ND} (1 + BP_{ND})} = \frac{K_1}{V_T} \quad \text{Eq.3}$$

where k_2 (min⁻¹) is the rate constant of efflux of dissolved (unbound) tracer from the tissue compartment where the physical volume of distribution of tracer in solution is the volume of non-displaceable tracer V_{ND} (ml/cm³) (from which it cannot be displaced by blockade of the binding) and the binding potential, or steady-state ratio between bound and unbound tracer, is BP_{ND} .

When solved, the two differential equations yield,

$$m_1 = V_o c_a \quad \text{Eq.4}$$

and

$$m_2 = K_1 \int_0^T c_a dt - k'_2 \int_0^T m_2 dt \quad \text{Eq.5}$$

from which the unknown quantity m_2 can be eliminated by substitution of $m_2 = m - m_1$,

$$m \left(1 - \frac{m_1}{m}\right) = K_1 \int_0^T c_a dt - k'_2 \int_0^T \left[m \left(1 - \frac{m_1}{m}\right)\right] dt \quad \text{Eq.6}$$

which can be further reduced when the m_1/m ratio approaches zero,

$$m = K_1 \int_0^T c_a dt - k'_2 \int_0^T m dt \quad \text{Eq.7}$$

at a time that can be judged from the ratio as a function of time,

$$\frac{m_1}{m} = \frac{V_o c_a}{m} = \frac{V_o}{V} \quad \text{Eq.8}$$

where V is an apparent volume of distribution (ml/cm³) that equals the ratio of the total quantity of tracer in the tissue and the arterial concentration of the tracer as a function of time. With a vascular volume close to 3% in brain tissue, the volume V , equal to the ratio m/c_a , has to exceed no more than 3 ml cm⁻³ for the vascular tracer in brain to be less than 1% of the total radioactivity in the brain. After that time, the transfer of ¹¹C-yohimbine to the tissue from the vascular compartment can be described with a single-compartment equation that replaces the two-compartment equation for times great enough to render the radioactivity in the vascular volume negligible in relation to the total radioactivity in the tissue, which then obeys the equation,

$$m(T) = K_1 \int_0^T C_a(t) dt - k'_2 \int_0^T m(t) dt \quad \text{Eq.9}$$

Rearrangement of this basic equation yields linear plots with positive or negative slopes. Here, we compare six different linear plots that estimate the kinetic parameters that govern uptake and distribution of ¹¹C-yohimbine in brain (14-16).

The kinetic variables calculated from the radioactivity records include the apparent volume of distribution as a function of the duration of circulation of the tracer (in units of ml cm⁻³),

$$V_{\text{app}}(T) \equiv \frac{\int_0^T m(t) dt}{\int_0^T C_a(t) dt} \quad \text{Eq.10}$$

as well as the apparent net clearance of the tracer from plasma to brain as a function of circulation time (in units of ml/cm³/min),

$$K_{\text{app}}(T) \equiv \frac{m(T)}{\int_0^T C_a(t) dt} \quad \text{Eq.11}$$

and the apparent residence time in the tissue (in units of minutes),

$$\Theta(T) \equiv \frac{\int_0^T m(t) dt}{m(T)} \quad \text{Eq.12}$$

such that $V_{\text{app}}(T) = K_{\text{app}}(T) \Theta(T)$. When the primary variables are combined, they define two plots of negative slope (N1-2), or four plots of positive slope (P1-4). In the “clearance plot” (N1), the ordinate intercept is the apparent clearance, $K_{\text{app}}(T)$, of tracer from plasma to brain, and the slope is $-k'_2$, such that the abscissa intercept is the estimate of the steady-state volume of distribution V_T (17, 18)

$$\text{(N1)} \quad K_{\text{app}}(T) = K_1 - k'_2 V_{\text{app}}(T) = k'_2 (V_T - V_{\text{app}}(T)) \quad \text{Eq.13}$$

while in the reverse “reciprocal ratio plot” (N2) (19), $V_{\text{app}}(T)$ is plotted against $K_{\text{app}}(T)$, such that the ordinate intercept is the estimate of the steady-state volume of distribution,

$$\text{(N2)} \quad V_{\text{app}}(T) = V_T - \left(\frac{1}{k'_2}\right) K_{\text{app}}(T) \quad \text{Eq.14}$$

The first of the positive slope plots is the “ratio plot” (P1) (17) where the inverse of the unidirectional clearance K_1 is the ordinate intercept and the slope is the inverse of the steady-state volume of distribution V_T ,

$$\text{(P1)} \quad \frac{1}{K_{\text{app}}(T)} = \frac{\Theta(T)}{V_T} + \frac{1}{K_1} \quad \text{Eq.15}$$

such that the second of the positive slope plots, the Logan plot (P2), is obtained by reversing the equation, as

originally published by Logan et al. (1990) (20),

$$(P2) \quad \Theta(T) = V_T \left[\frac{1}{K_{app}(T)} \right] - \frac{1}{k'_2} \quad \text{Eq.16}$$

where $\Theta(T)$ is plotted against the inverse of clearance, such that the positive slope represents the steady-state volume of distribution V_T . The plot becomes linear when the system has reached steady-state.

The third positive slope or “reciprocal time” plot originally was derived by Reith et al., 1990 (21), who plotted the reciprocal of $\Theta(T)$ against the reciprocal of $V_{app}(T)$,

$$(P3) \quad \frac{1}{\Theta(T)} = K_1 \left[\frac{1}{V_{app}(T)} \right] - k'_2 \quad \text{Eq.17}$$

where the steady-state volume is obtained from the slope and the ordinate intercept. In the last of the four positive slope plots, the axes of P3 have been reversed,

$$(P4) \quad \frac{1}{V_{app}(T)} = \left(\frac{1}{K_1} \right) \left[\frac{1}{\Theta(T)} \right] + \frac{1}{V_T} \quad \text{Eq.18}$$

where the steady-state volume of distribution V_T is the reciprocal of the ordinate intercept. We calculated binding potentials (BP_{ND}) as the ratio of the volume V_T in ROIs with specifically displaceable binding and the volume V_{ND} of distribution in a chosen reference region with specifically non-displaceable binding, here selected as the volume determined for corpus callosum ($BP_{ND} = 0$) (22).

$$BP_{ND} = \frac{V_T}{V_{ND}} - 1 \quad \text{Eq.19}$$

Data Analysis

Analyses were performed in GraphPad Prism 6.0 and results are reported as mean (\pm standard error of mean). The correlation of estimates of V_T between the different linear models was tested by linear regression of frontal cortex V_T determined with P4 versus the other models.

RESULTS

Brain Time-Activity Curves and Metabolites

The tracer readily entered brain tissue, as revealed by the standardized uptake value (SUV) curves and the estimates of the unidirectional blood-brain clearances (K_1) of the tracer. Standardized uptake value (SUV) curves for all 27 volumes-of-interest (VOIs) of one subject are shown in Figure 1A. We used the fraction of radioactivity in plasma, relative to the radioactivity in a given VOI, as shown in Figure 1B, to determine the time after which the fraction would be negligible and the plots henceforth linear, exemplified by frontal cortex and hippocampus, where, on average, the vascular tracer amounted to less than 6% after 12.5 minutes. The fraction of intact tracer declined in plasma as function of time. When we genotyped the cytochrome P450 mixed-function oxidase CYP2D6 in all subjects, we classified two subjects as fast metabolizers and three subjects as moderate metabolizers. We classified one subject with no functional CYP2D6 genetic variants as non-metabolizer as shown in Table 1 and Figure 2.

Blood-Brain Clearances, Efflux Rates, Volumes of Distribution, and Binding Potentials of ^{14}C -Yohimbine

After the time of negligible tracer in the vascular space (12.5 minutes), the N1-P4 plots based on unmetabolized tracer in plasma assumed linearity and yielded comparable estimates of the three parameters K_1 , V_T , and k'_2 as shown in Figure 3. In line with this, we noted a highly significant correlation between estimates of V_T in the frontal cortex determined with plot P4 method and the remaining plots with $P < 0.0001$ and mean $R^2 = 0.9974$ (± 0.0007). As the estimates were comparable, only V_T and K_1 values from plot P4 and the efflux rate constant k'_2 from plot N2 are presented here. We calculated BP_{ND} values listed in Table 2 for selected VOIs from the ratios of V_T estimates to the estimate in corpus callosum. Mean values of V_T ranged from 0.82 (± 0.16) ml cm^{-3} in right frontal cortex to 0.46 (± 0.09) ml cm^{-3} in corpus callosum, as also listed in Table 2. Estimates of K_1 and k'_2 are listed in Table 2 for selected VOIs.

The estimates of K_1 and k'_2 ranged from 0.020 (± 0.003) ml $\text{cm}^{-3} \text{min}^{-1}$ and 0.023 (± 0.001) min^{-1} , respectively, in right frontal cortex, to 0.009 (± 0.001) ml $\text{cm}^{-3} \text{min}^{-1}$ and 0.019 (± 0.003) min^{-1} , respectively in corpus callosum. The estimates of K_1 are consistent with blood-brain extraction fractions of 5%. A parametric image of the average

estimates of V_T in five subjects obtained with the plot P2 (Logan plot) is shown in Figure 4. In the sixth subject (subject #6), the parametric image was of unacceptably poor quality because of a combination of minimum dose of radioactivity and maximum rate of metabolism in plasma. Mean values of BP_{ND} , calculated and averaged from estimates of V_T obtained from regions including the corpus callosum as reference by means of plot P4, are listed in Table 2 where they range from 0.6-0.8 in cortex to 0.2-0.5 in subcortical regions.

DISCUSSION

Animal studies from this laboratory suggest that ^{11}C -yohimbine is a selective and displaceable α_2 -adrenoceptor antagonist when used in tracer concentration (11). To measure the accumulation of the tracer in human brain, we determined the uptake and binding of ^{11}C -yohimbine in brain of five healthy male volunteers (subject #6 was excluded). The results revealed about 5% extraction of the tracer, sufficient for determination of regionally averaged uptake in five volunteers, and similarly only acceptable for voxel-based mapping in five of the six volunteers.

Linearized solutions of the underlying differential equations such as the Logan plot (P2) were used here to graphically estimate the kinetic parameters of ^{11}C -yohimbine (17-21). We analyzed the present data with six different linearized solutions, including the Logan plot, to identify the plot most suitable to future applications of ^{11}C -yohimbine. All solutions, here abbreviated N1-P4, of which P2 is the Logan plot, adequately fitted the data after the plots assumed linearity approximately 12.5 minutes after administration of the bolus of radioligand as shown in Figure 3. The estimates of ^{11}C -yohimbine V_T in frontal cortex determined with the plot P4 significantly correlated with the five other linearizations ($P < 0.0001$). The estimates ranged from 0.82 ml cm^{-3} in right frontal cortex to 0.72 ml cm^{-3} in left insula and 0.77 ml cm^{-3} in left hippocampus. Subcortical structures, including thalamus, caudate nucleus, putamen, and amygdala had lower estimates of V_T . Surprisingly, white matter, excluding corpus callosum, also had a moderate volume of distribution of ^{11}C -yohimbine (0.59 ml cm^{-3}), which may reflect the influence of partial volume.

Obtained from the linearized plots, the estimates of unidirectional blood-brain clearance of ^{11}C -yohimbine, K_1 , had a two-fold range whereas the rate of washout of the tracer had a narrow range from right frontal cortex to corpus callosum (see table 2). In the study by Hume et al. (23), K_1 values in frontal cortex of another PET ligand for α_2 -adrenoceptors, ^{11}C -RS-15385-197, were $0.009 \text{ ml/cm}^3/\text{min}$, which are close to K_1 in corpus callosum in the current study. In contrast, tracer ^{11}C -mirtazapine had K_1 values approximately 10 times higher than ^{11}C -yohimbine. However, ^{11}C -mirtazapine lacks selectivity for α_2 -adrenoceptors (9).

Although the distribution volumes of ^{11}C -yohimbine are modest compared to other PET radioligands and

to ^{11}C -mirtazapine, its binding potential is comparable to other noradrenergic tracers in successful use. For example the binding potential of (S,S)- ^{11}C -methylreboxetine, an antagonist of noradrenaline transporters, is 0.52 in thalamus, which receives noradrenergic innervation (24).

Here, we calculated binding potentials as estimates of receptor availability as the ratio of V_T estimated in VOIs with assumed displaceable binding, compared to a chosen reference region, corpus callosum. Hume et al. (23), used thalamus as a reference region based on the assumption that it contains few α_2 -adrenoceptor binding sites. However, some thalamic nuclei show dense expression of α_2 -adrenoceptors, which may complicate the choice of thalamus as a reference region (6). In-vitro studies have shown that white matter and corpus callosum has negligible α_2 -adrenoceptor binding sites, and thus may be considered for use as a reference region. The tentative choice of corpus callosum as reference region here was based on the finding of the lowest V_T , as estimated with the plot P4. The high selectivity of the PET ligand, ^{11}C -yohimbine, for α_2 -adrenoceptors (11), and highest V_T values in regions with known high density of α_2 -adrenoceptors, indicates that it is useful as a PET-ligand for in vivo imaging of α_2 -adrenoceptors in human.

The CYP-450 system and specifically the CYP2D6 enzyme in the liver are involved in the metabolism of yohimbine in the circulation, yielding two metabolites, 10-OH-yohimbine and 11-OH-yohimbine (25). We genotyped the CYP2D6 enzyme and classified subjects as fast, moderate, or low metabolizers, depending on the number of functional CYP2D6 variants, with one subject classified as non-metabolizer. As expected, the fraction of unchanged ^{11}C -yohimbine remained close to unity in this subject, while moderate and fast metabolizers had substantial breakdown of ^{11}C -yohimbine. The results are consistent with an earlier study of yohimbine metabolism (25), which also identified one subject as a non-metabolizer. Yohimbine metabolites may have some action at α_2 -adrenoceptors, albeit with lower affinity when compared to native yohimbine (26). Labeled 11-OH-yohimbine could hypothetically contribute to radioactivity in brain, as samples of CSF in previous studies have revealed transport across the blood-brain barrier (27). We plan to study radioactively labeled metabolites of yohimbine to estimate the degree to which they cross the blood-brain barrier and bind to targets in the brain.

The α_2 -adrenoceptors play important roles in the regulation of noradrenergic neurotransmission and are implicated in the pathology of neurodegenerative and neuropsychiatric disorders (27). It is likely, therefore, that in

vivo imaging of α_2 -adrenoceptors with ^{11}C -yohimbine will provide insights into the underlying mechanisms of these disorders.

CONCLUSION

Here, we present ^{11}C -yohimbine as a PET radioligand for vivo imaging of α_2 -adrenoceptors in human. The relative distribution of ^{11}C -yohimbine is similar to that predicted from in-vitro studies, with highest binding in cortex and hippocampus, and the binding suggests that ^{11}C -yohimbine may be a suitable ligand for future studies of role of α_2 -adrenoceptors in neurodegenerative and neuropsychiatric disorders of human brain.

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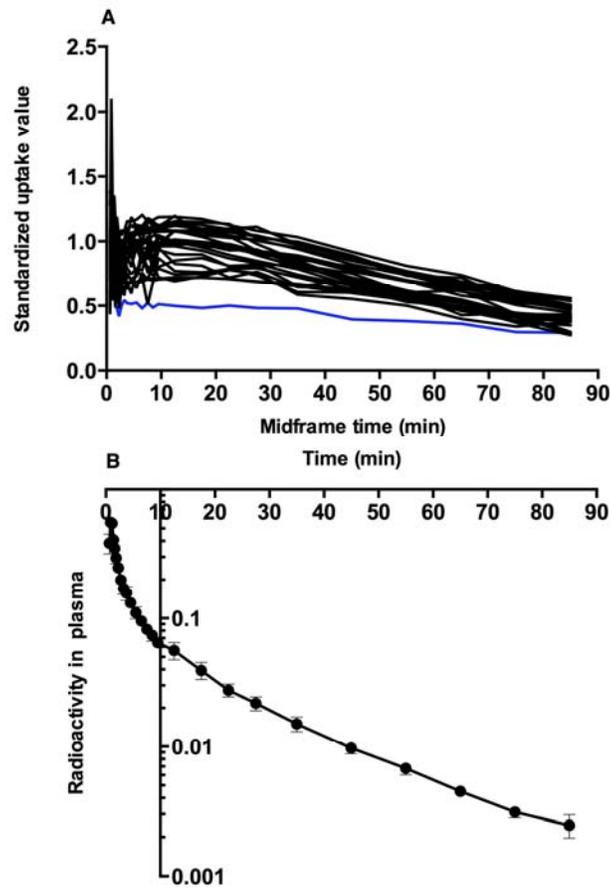


FIGURE 1: Panel A: Examples of standardized uptake value curves for all 27 VOIs of ^{11}C -yohimbine presented for subject 5 (abscissa is mid-frame time (minutes) and ordinate is standardized uptake value of radioactivity in VOIs). The blue line shows corpus callosum, which was used as a reference region for calculation of binding potentials (BP_{ND}). Panel B: Radioactivity in plasma, expressed as a fraction of radioactivity in VOIs is shown on the ordinate, and the abscissa represents time (minutes).

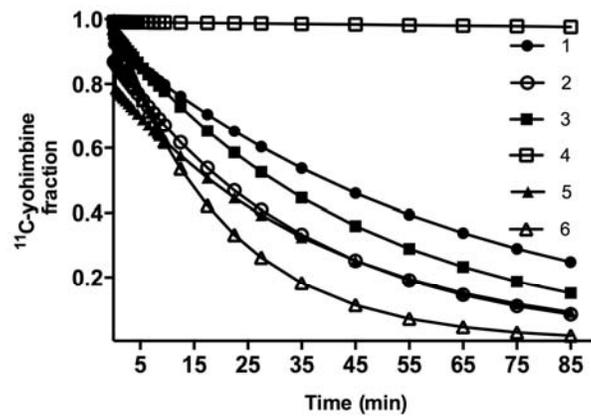


FIGURE 2: Fractions of unchanged ^{11}C -yohimbine were interpolated to match time points of tissue TACs, presented here for all six subjects. The ordinate represents intact ^{11}C -yohimbine and abscissa shows time (minutes).

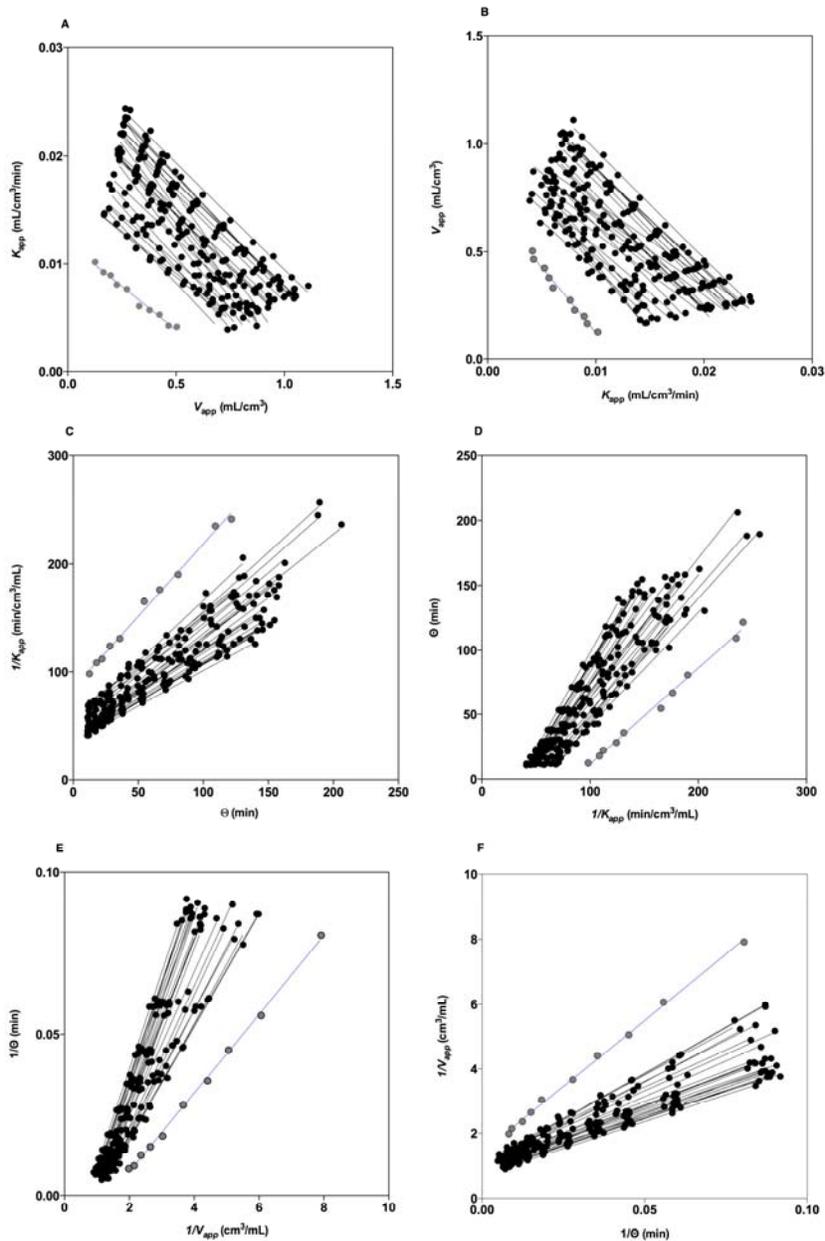


FIGURE 3: Six linearized solutions were used to derive linear regression estimates of the kinetic parameters, using metabolite corrected plasma curves as input function. (Panel A shows plot N1, panel B is plot N2, panel C is plot P1, panel D is plot P2, panel E is plot P3, panel F is plot P4)(please see text for equations and description)). The blue lines represent corpus callosum.

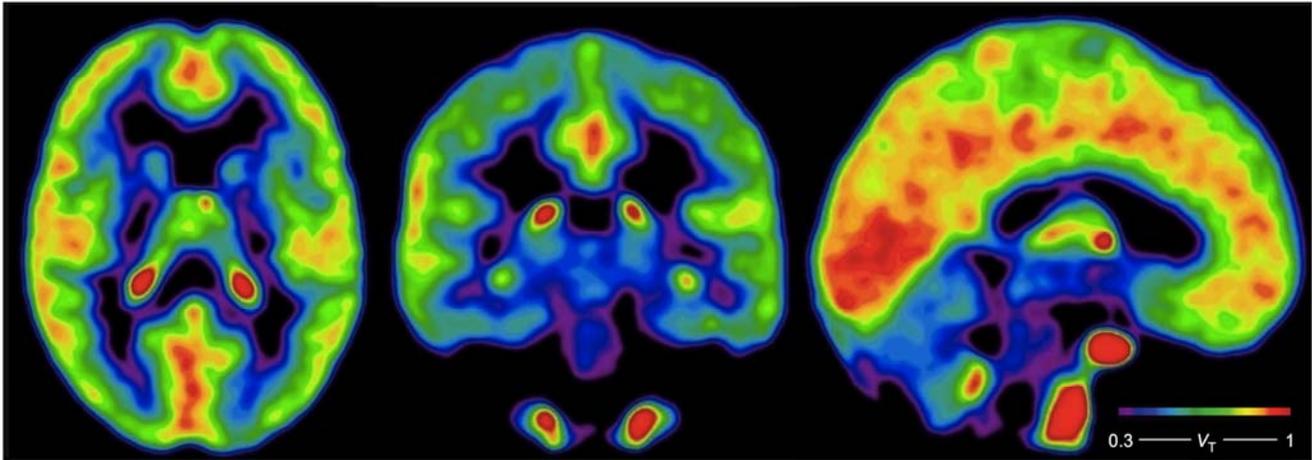


FIGURE 4: Average parametric images of voxel-wise V_T estimates in five of the six subjects, estimated with the P2 plot (Logan plot). Color bar gives estimates of V_T in units of ml cm^{-3} .

TABLE 1
 Injected radioactivity dose (MBq), yohimbine mass (nmol) and CYP2D6 status.

Subject	MBq	Yohimbine (nmol)	CYP2D6
1	641	30.3	*1 *2 (F)
2	441	4.6	*4 *1 (M)
3	635	7	*1 *4 (M)
4	715	21.4	*4 *4 (N)
5	507	11.2	*2 *5 (M)
6	169	11	*2 *2 (F)

Fast (F), moderate (M) or non (N) metabolizers of ¹¹C-yohimbine.

TABLE 2
Kinetic parameters of ^{11}C -yohimbine (mean (\pm sem))

VOIs	V_T	K_1	k'_2	BP
Amygdala_L	0.55 (\pm 0.11)	0.014 (\pm 0.002)	0.026 (\pm 0.002)	0.22 (\pm 0.12)
Amygdala_R	0.55 (\pm 0.10)	0.014 (\pm 0.002)	0.025 (\pm 0.001)	0.23 (\pm 0.07)
Caudate_Nucl_L	0.55 (\pm 0.10)	0.016 (\pm 0.003)	0.029 (\pm 0.002)	0.24 (\pm 0.13)
Caudate_Nucl_R	0.56 (\pm 0.12)	0.016 (\pm 0.003)	0.028 (\pm 0.002)	0.23 (\pm 0.11)
Cerebellum_L	0.57 (\pm 0.10)	0.019 (\pm 0.003)	0.032 (\pm 0.002)	0.28 (\pm 0.11)
Cerebellum_R	0.59 (\pm 0.11)	0.020 (\pm 0.003)	0.032 (\pm 0.002)	0.32 (\pm 0.11)
Corp_Callosum	0.46 (\pm 0.09)	0.009 (\pm 0.001)	0.019 (\pm 0.003)	---
Frontal_lobe_L	0.80 (\pm 0.16)	0.020 (\pm 0.003)	0.023 (\pm 0.001)	0.77 (\pm 0.14)
Frontal_Lobe_R	0.82 (\pm 0.16)	0.020 (\pm 0.003)	0.023 (\pm 0.001)	0.81 (\pm 0.13)
Gyrus_cinguli_L	0.80 (\pm 0.16)	0.020 (\pm 0.003)	0.025 (\pm 0.001)	0.77 (\pm 0.14)
Gyrus_cinguli_R	0.80 (\pm 0.15)	0.020 (\pm 0.003)	0.024 (\pm 0.001)	0.77 (\pm 0.14)
Hippocampus_L	0.77 (\pm 0.13)	0.016 (\pm 0.003)	0.021 (\pm 0.003)	0.73 (\pm 0.14)
Hippocampus_R	0.82 (\pm 0.16)	0.014 (\pm 0.002)	0.018 (\pm 0.003)	0.82 (\pm 0.21)
Insula_L	0.72 (\pm 0.14)	0.017 (\pm 0.003)	0.023 (\pm 0.001)	0.61 (\pm 0.15)
Insula_R	0.73 (\pm 0.13)	0.017 (\pm 0.003)	0.023 (\pm 0.001)	0.62 (\pm 0.12)
Occipital_lobe_L	0.78 (\pm 0.15)	0.020 (\pm 0.003)	0.024 (\pm 0.001)	0.73 (\pm 0.14)
Occipital_lobe_R	0.82 (\pm 0.17)	0.020 (\pm 0.004)	0.024 (\pm 0.001)	0.80 (\pm 0.12)
Parietal_lobe_L	0.78 (\pm 0.15)	0.019 (\pm 0.003)	0.023 (\pm 0.001)	0.72 (\pm 0.13)
Parietal_lobe_R	0.79 (\pm 0.16)	0.019 (\pm 0.003)	0.023 (\pm 0.001)	0.75 (\pm 0.11)
Putamen_L	0.62 (\pm 0.12)	0.021 (\pm 0.004)	0.032 (\pm 0.002)	0.40 (\pm 0.15)
Putamen_R	0.60 (\pm 0.12)	0.020 (\pm 0.004)	0.033 (\pm 0.002)	0.33 (\pm 0.12)
Temporal_lobe_L	0.75 (\pm 0.13)	0.017 (\pm 0.003)	0.022 (\pm 0.001)	0.67 (\pm 0.13)
Temporal_lobe_R	0.75 (\pm 0.14)	0.017 (\pm 0.003)	0.022 (\pm 0.001)	0.66 (\pm 0.12)
Thalamus_L	0.62 (\pm 0.12)	0.018 (\pm 0.003)	0.029 (\pm 0.002)	0.37 (\pm 0.09)
Thalamus_R	0.65 (\pm 0.10)	0.019 (\pm 0.004)	0.027 (\pm 0.002)	0.46 (\pm 0.11)
White_matter_L	0.59 (\pm 0.11)	0.012 (\pm 0.002)	0.020 (\pm 0.001)	0.30 (\pm 0.10)
White_matter_R	0.59 (\pm 0.12)	0.012 (\pm 0.002)	0.019 (\pm 0.001)	0.30 (\pm 0.09)

Steady-state volume of distribution (V_T , ml/cm 3).

Uptake of tracer in brain (K_1 , ml/cm 3 /min),

Washout of tracer (k'_2 , min $^{-1}$),

Binding potentials (BP).