We quantified the binding potentials ($BP_{ND}$) of $[^{11}C]$yohimbine binding in rat brain to alpha-2 adrenoceptors to evaluate $[^{11}C]$yohimbine as an in vivo marker of noradrenergic neurotransmission and to examine its sensitivity to the level of noradrenaline. Dual $[^{11}C]$yohimbine dynamic positron emission tomography (PET) recordings were applied to five Sprague Dawley rats at baseline, followed by acute amphetamine administration (2 mg/kg) to induce elevation of the endogenous level of noradrenaline. The volume of distribution ($V_v$) of $[^{11}C]$yohimbine was obtained using Logan plot with arterial plasma input. Because alpha-2 adrenoceptors are distributed throughout the brain, the estimation of the $BP_{ND}$ is complicated by the absence of an anatomic region of no displaceable binding. We used the Inhibition plot to acquire the reference volume, $V_{ND}$, from which we calculated the $BP_{ND}$. Acute pharmacological challenge with amphetamine induced a significant decline of $[^{11}C]$yohimbine $BP_{ND}$ of ~38% in all volumes of interest. The $BP_{ND}$ was greatest in the thalamus and striatum, followed in descending order by, frontal cortex, pons, and cerebellum. The experimental data demonstrate that $[^{11}C]$yohimbine binding is sensitive to a challenge known to increase the extracellular level of noradrenaline, which can benefit future PET investigations of pathologic conditions related to disrupted noradrenergic neurotransmission.

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**Keywords:** $[^{11}C]$yohimbine; alpha-2 adrenoceptors; amphetamine challenge; Inhibition plot; reference region

**INTRODUCTION**

Dysfunction of noradrenergic neurotransmission is implicated in a range of brain disorders, such as major depression and neurodegeneration.1,2 Full understanding of the regulation of noradrenergic neurotransmission has been hindered by the lack of selective tracers of noradrenaline’s receptors *in vivo*. Yohimbine is an alkaloid herbal compound, which can be extracted from the West African plants, *Pausinystalia Yohimbe* tree and the roots of *Rauwolfia Serpentina*.3 In *vitro*, yohimbine binds to the alpha-2 adrenoceptors (α2R) with high affinity, whereas it binds to the α1 adrenoceptors and the 5-HT1A, 5-HT1B, and 5-HT1D receptors with lower affinity. Yohimbine is an antagonist of the α2R and a partial agonist of the 5-HT1A receptors.4 However, at the low concentration used in positron emission tomography (PET), the radioligand $[^{11}C]$yohimbine binds with high selectivity to the α2R.5 Recent PET studies of pig brain showed that $[^{11}C]$yohimbine binding is a sensitive marker of noradrenaline release, as shown by the significant decrease of the volume of distribution ($V_v$) in response to acute amphetamine treatment.5,6 These findings suggest that $[^{11}C]$yohimbine can be used to advantage in studies of the normal function and pathophysiologic alterations of noradrenergic neurotransmission.

In the present study, we determined the $[^{11}C]$yohimbine binding potential ($BP_{ND}$) in the absence of a region devoid of specific binding sites, i.e., a ‘reference’ region. The $BP_{ND}$ reflects the ratio at steady state of specifically bound to non-displaceable tracer quantities in the tissue.

To determine the $BP_{ND}$, it is necessary to know the steady-state volume of distribution of the non-displaceable partition volume ($V_{ND}$), as well as the total volume of distribution in a given brain region ($V_v$). There are two ways to obtain the $V_{ND}$ value, either by the identification of a brain region without binding sites,7,8 or by complete blocking of the binding sites in the brain tissue as a whole. For tracers that bind to differentially distributed receptors, such as radioligands of the dopamine receptors, the cerebellum is such a reference tissue with little specific binding. In contrast, the α2R are distributed throughout the brain with no well-defined region(s) of non-displaceable (i.e., non-specifically bound) accumulation. In this case, the alternative approach is to block the displaceable binding with a suitable unlabeled ligand. Such an approach is presented here in the form of the Inhibition plot,6,9 derived to yield the volume of non-displaceable distribution ($V_{ND}$) from the linear relationship of the values of $V_v$ in the challenge condition to those of the baseline condition.

To examine the sensitivity of $[^{11}C]$yohimbine binding to monoaminergic competition, we challenged the binding with acute administration of amphetamine, a drug known to induce the...
release of endogenous monoamines, particularly noradrenaline and dopamine. Amphetamine is a psychostimulant that acts as a substrate for the monoamine re-uptake and vesicular transporters (VMAT-2), leading to reversal of the membrane transport and depletion of synaptic vesicles, and ultimate elevation of the extracellular concentration of endogenous monoamines.10

Here, we apply the Inhibition plot to obtain a steady-state estimate of the \( V_{ND} \) in the absence of a reference region, to examine the specificity of \([11C]\)yohimbine as a marker of \( \alpha_2R \) availability and noradrenaline release in rat brain in vivo.

**MATERIALS AND METHODS**

**Theory**

Kinetic analysis of \([11C]\)yohimbine uptake and distribution. Conventional kinetic analysis of PET radioligand distribution is based on compartmental models described by linear differential equations with transfer coefficients. When the bound and free quantities of tracers are in steady state, the tracer distribution in the brain compartment is dominated by the exchange between the circulation and the tissue compartment across the blood–brain barrier (BBB). The tracer exchange across BBB is governed by the concentration of tracer in plasma (kbq/ml) and the quantity in brain tissue (kbq/cm³).

We applied the one-tissue compartment model, where the distribution of tracer is described by two first order differential equations as follows,

\[
\frac{dm_1}{dt} = V_0 \frac{dC_2}{dt}
\]

and

\[
\frac{dm_2}{dt} = k_1 C_2 - k_2 m_2
\]

where \( m_1 \) is the tracer quantity in the vascular compartment with the volume \( V_0\) and the concentration \( C_2 \) as function of time. The term \( m_2 \) denotes the tracer quantity in the brain tissue, which depends on the clearance \( k_1 \) from plasma to brain and \( k^-1 \) the rate constant of the reverse direction from the tissue compartment. Here, at steady state, \( k_1 \) is defined as,

\[
K_1 = \frac{F}{1 - e^{-PS/F}}
\]

where \( F \) is blood flow, \( PS \) is the permeability-surface area product of the tracer. In addition, \( k_2^-1 \) equals the ratio of \( k_1 \) to total volume of distribution, \( V_T \), at steady state,

\[
k_2^-1 = \frac{k_2}{1 + BP_{ND}} - \frac{k_1}{V_{ND}} = \frac{F}{1 - e^{-PS/F}}
\]

where \( BP_{ND} \) denotes the binding potential, and \( V_{ND} \) is the partition volume of non-displaceable tracer in the tissue compartment. Rearrangement and combination of equations (3) and (4) yield the transfer coefficient \( k_2^-1 \),

\[
k_2^-1 = k_2^-1 (1 + BP_{ND}) = \frac{k_1}{V_{ND}}
\]

Combination of equations (1) and (2) shows that the change of mass of \([11C]\)yohimbine in the brain as a whole obeys the equation,

\[
\frac{dm}{dt} = \frac{dm_1}{dt} + \frac{dm_2}{dt} = V_0 \frac{dC_2}{dt} + k_1 C_2 - k_2 m_2
\]

The total mass of radiotracer, \( m \), is obtained by the integration of equation (6),

\[
m = m_1 + m_2 = V_0 \int_0^T C_2 dt - k_2 \int_0^T m_2 dt,
\]

where the unknown quantity, \( m_2 \), is solved by substitution of \( m_2 = m - m_1 \)

\[
m = V_0 C_2 + k_1 \int_0^T C_2 dt - k_2 \int_0^T m \left( \frac{1 - m}{m} \right) dt
\]

According to equation (8), the kinetics obey a linear relationship when steady state is approximated, which strictly occurs at times when the \( m/m \) ratios are of negligible magnitude. When the ratio \( m/m \) becomes negligible, equation (8) can be simplified to the basic equation for the one-compartment analysis,

\[
m = K_1 \int_0^T C_2 dt - k_2 \int_0^T m dt,
\]

According to equation (9), the mass exchange can be described by a linear equation at the time when \( m/m \) becomes negligible. Here, \( m/m \) ratio of 0.01 was chosen as the threshold of negligible vascular radioactivity, implying that \( m_1 \) is negligible when 1% or less of radiotracer remains in plasma relative to brain tissue.

The dynamic parameters in equation (9) can be estimated after re-arrangement, allowing linear regression combinations of any two of the three kinetic variables, including the apparent volume of distribution, \( V_{app}(T) \).

\[
V_{app}(T) = \frac{\int_0^T m dt}{\int_0^T C_2 dt}
\]

the apparent clearance, \( K_{app}(T) \), of tracer from the vascular compartment to brain tissue,

\[
K_{app}(T) = \frac{m(T)}{V_{app}(T)}
\]

and the apparent residence time of tracer in tissue, \( \Theta(T) \),

\[
\Theta(T) = \frac{\int_0^T m dt}{m(T)}
\]

In the following, we applied two linear plots derived from equation (9) to estimate the time of onset of steady state, and we subsequently applied the Logan plot11 to estimate \( V_T \). To determine the onset of steady state, we first applied the linearized model described by Reith et al.,12 where the \( K_1 \) is directly obtained from the slope, and \( k_2^-1 \) from the y-intercept,

\[
\frac{1}{\Theta(T)} = K_1 \frac{1}{V_{app}(T)} - k_2^-1
\]

We assumed that steady state is present at the time interval, when the best linear fit is obtained with equation (13). To confirm this assumption, we applied a second linearized solution as presented by Gjedde et al.,13

\[
V_{app}(T) = -\frac{1}{K_2} K_{app}(T) + V_r,
\]

where \( k_2^-1 \) is determined from the reciprocal value of the slope. Finally, we used the range of times consistent with steady state to estimate \( V_T \) from the slope of the Logan plot,

\[
\Theta(T) = V_r \frac{1}{K_{app}(T)} \left( \frac{1}{k_2^-1} \right)
\]

**Binding potential relative to non-displaceable uptake.** The binding potential relative to non-displaceable uptake, \( BP_{ND} \), can be calculated in terms of \( V_T \) when the non-displaceable partition volume, \( V_{ND} \) is known. To compute the \( V_T \) of \([11C]\)yohimbine in rat brain, we applied the linearized binding equation known as the Inhibition plot, which is derived from two measures of receptor occupancy at baseline and upon pharmacological challenge. The \( BP_{ND} \) is a parameter that represents the ratio of bound to free quantities of tracer in the free solution in brain tissue.14 The relative receptor availability equals the ratio of the binding potentials at inhibition (\( BP_{ND}(i) \)) and baseline (\( BP_{ND}(b) \)),

\[
1 - s = \frac{BP_{ND}(i)}{BP_{ND}(b)}
\]

where \( 1-s \) expresses the fraction of receptors available for binding in the presence of a competitor, and \( s \) is the fraction of receptors actually occupied by the competitor. The \( BP_{ND} \) can also be expressed in terms of the distribution volumes, such that,

\[
BP_{ND}(b) = \frac{V_{ND}(b) - V_{ND}(i)}{V_{ND}(b)}
\]

and

\[
BP_{ND}(i) = \frac{V_{ND}(i) - V_{ND}(b)}{V_{ND}(i)}
\]
where $V_T$ is the total volume of distribution, of which $V_{ND}$ is the non-displaceable volume of distribution, and the subscripts (b) and (i) denote the baseline and challenge conditions. When the $V_{ND}$ value remains the same at an amphetamine challenge, compared with baseline ($V_{ND(b)} = V_{ND(i)} = V_{ND}$), combination of equations (16 to 18) can be reduced to yield the relative receptor availability in terms of the volumes, $$1 - s \equiv \frac{V_{T(b)} - V_{ND}}{V_{T(i)} - V_{ND}} \quad (19)$$

from which the $V_{ND}$ value can be obtained to yield the binding potential, $$BP_{ND} = \frac{V_T}{V_{ND}} - 1 \quad (20)$$

Three linearized versions of the relative receptor availability (equation (19)) previously were derived to obtain the $V_{ND}$ value by linear regression, including the Inhibition plot, the Saturation (or Lassen) plot, and the Occupancy plot.\textsuperscript{16} Gjedde et al.\textsuperscript{10,15} linearized equation (19) in the form of the Inhibition plot that yields the inhibited binding volume, $V_{TB(i)}$, as a function of the baseline volume, $V_{TB(b)}$:

$$V_{TB(i)} = (1 - s)V_{TB(b)} + sV_{ND} \quad (21)$$

where the estimate of $V_{TB}$ is the intercept of the linear regression line and the line of identity. Lassen et al.\textsuperscript{10} linearized equation (19) in the form of the Saturation plot that yields the baseline volume of distribution, $V_{TB(b)}$:

$$V_{TB(b)} = \frac{1}{s}V_T = V_{ND} \quad (22)$$

from which $V_{ND}$ can be obtained as theordinate intercept of the linear regression. Cunningham et al.\textsuperscript{16} derived the third linearization, commonly known as the Occupancy plot, to yield the difference between the volumes of distribution at baseline relative to the challenge condition, $\Delta V_T$, and $V_{ND}$ can be obtained from the x-intercept:

$$\Delta V_T = sV_{TB(b)} - sV_{ND} \quad (23)$$

where $V_{ND}$ is obtained as the abscissa intercept. To use any of the linearized plots, at least two consecutive PET recordings with two different levels of receptor occupancy are required. For the Inhibition plot, unlike the Saturation and Occupancy plots, the dependent ($V_{TB(i)}$) and independent ($V_{TB(b)}$) variables are not intermixed, such that the fractional receptor availability and $V_{ND}$ are obtained directly from the volumes of distribution. As the three linearizations are derived from the same original relative receptor availability formulation (equation (19)), they are all based on the common fundamental assumptions that there are different brain regions with different receptor densities ($B_{max}$), that the $B_{max}$ values remain unchanged in the challenge condition, and that receptor affinity and non-displaceable binding volumes have the same values in all the regions and remain constant upon challenge.

**Plasma-free fraction, $f_p$.** A further essential prerequisite for the linearizations of relative receptor availability (equation (19)) is that the value of $V_{ND}$ must remain the same at baseline and inhibition, which entails that the free available concentration of radiotracer in the plasma, $f_p$, at equilibrium must remain constant at baseline to challenge condition. This is because a change in $f_p$ will consequently introduce changes in $V_{ND}$. Amphetamine is known to not alter the binding of yohimbine to peripheral α2 receptors on platelets, as reported for humans.\textsuperscript{17} Therefore, we performed plasma ultrafiltration to test the hypothesis that amphetamine does not affect plasma protein binding as reflected in measures of the plasma-free fraction, in support of the assumption underlying the Inhibition plot that the values of $V_{ND}$ are unchanged.

The concentrations of tracer free in plasma, $C_{FT}$, or freely dissolved in tissue water, $C_{RT}$, can be expressed in terms of total concentration of the tracer in plasma, $C_T$, and non-displaceable concentration in tissue, $C_{ND}$, and the free fractions in plasma, $f_p$, and in brain tissue, $f_{Tr}$:

$$C_{FT} = f_pC_T \quad (24)$$

$$C_{RT} = f_{Tr}C_{ND} \quad (25)$$

The volume of non-displaceable tracer is defined as ratio of the concentration of non-displaceable tracer in tissue, $C_{ND}$, and the total plasma concentration, $C_T$:

$$V_{ND} = \frac{C_{ND}}{C_T} \quad (26)$$

where $V_T$ is the physical volume of distribution of the tracer in the solvent in which $C_{ND}$ is measured. The combination of equations (24 to 26) gives:

$$V_{ND} = \frac{f_p C_{RT}}{f_{Tr}C_{ND}} \quad (27)$$

Hence, $V_{ND}$ is defined as the product of a physical volume of distribution and two unitless ratios, including the ratio $f_p/f_{Tr}$, which is determined by nonspecific binding in plasma and brain, and the second ratio $C_{RT}/C_{ND}$ that depends on the properties of the BBB. When it is assumed that a radioligand penetrates the BBB by passive diffusion, the concentrations of radioligand will assume a steady state depending on the relative solubilities on the two sides of the BBB. In this case, equation (27) can be reduced to:

$$V_{ND} = \frac{f_p}{f_{Tr}} \quad (28)$$

It follows that the linearizations derived from the relative receptor availability can only be applied with validity when $f_p$ of the radioligand is unchanged from the baseline to the challenge condition. Therefore, plasma ultrafiltration was performed in this work to determine [11C]-yohimbine $f_p$ to confirm that it remains constant across the two conditions to consolidate the basic assumptions applicable for Inhibition plot.

**Animal experiments**

All the animal experiments were conducted under humane conditions with an approval from the Danish Animal Experiments Inspectorate and in accordance with the guidelines set by the Danish Ministry of Food, Agriculture, and Fisheries in agreement with European Legislation for the protection of vertebrate animals used for experimental and other scientific purposes. Animals were housed two per cage under a 12/12 hours light/dark cycles, and with ad libitum access to food and water.

Initially, six Sprague Dawley rats (250 to 300 g) were subjected to dual PET sessions with [11C]-yohimbine at baseline and after amphetamine challenge. One animal was excluded because of difficulties with the intravenous catheter, which led to amphetamine administration through the femoral artery and resulted in a different kinetic profile, which was not comparable with the animals that received intravenous amphetamine.

A second subset of experiments (four Sprague Dawley rats) was designed to measure the free available fraction of [11C]-yohimbine in plasma at the baseline and post-amphetamine condition. Anesthesia was induced in a chamber filled with 2% isoflurane and maintained throughout the studies with a mask for delivery of isoflurane (1.8 to 2.0%). Physiologic parameters were monitored during all dynamic recordings. We catheterized the femoral artery for blood sampling and the tail vein for tracer injection and amphetamine administration. At the end of the experiments, the animals were anesthetized with intravenous pentobarbital (100 mg/kg) before decapitation.

**Positron emission tomography data acquisition**

Each animal underwent two consecutive PET acquisitions with [11C]-yohimbine of 90 minutes on the same day, first at baseline and then followed by amphetamine challenge (2 mg/kg intravenously). Approximately 20 minutes after baseline recording, we administered 2 mg/kg amphetamine as a bolus intravenous injection. The second [11C]-Yohimbine PET acquisition began 5 to 10 minutes after this administration. Time-activity curves of [11C]-Yohimbine uptake were normalized to the plasma integral to enable the comparison of dynamic data among individual acquisitions and among animals, as normalization to the plasma integral corrects for the effects of bioavailability factors, such as the body weight, peripheral washout, and radioactivity dose.

Initial studies in rats demonstrated that yohimbine is a p-glycoprotein substrate at the BBB, which restricts the transfer of [11C]-Yohimbine to the brain. Thus, cyclosporine (50 mg/kg), a nonspecific p-glycoprotein inhibitor, was administered through the tail vein 30 minutes before tracer administration to facilitate the delivery of [11C]-Yohimbine into the brain. Cyclosporine pretreatment was only done once before the first imaging session, as cyclosporine has a long half-life of more than 10 hours in both rat brain and peripheral tissue, when treated with doses higher than 30 mg/kg.\textsuperscript{18}

Animals were placed prone in the aperture of the small animal tomograph (microPET R4, CTI Concorde, Knoxville, TN, USA) and the head was immobilized with a custom-built Plexiglas head holder, allowing reproducible and comparable positioning of all animals. The spatial resolution
the time parametric maps.

Using Logan plot with arterial plasma input. We used the open-source software, threedimensional Slicer (4.3.1 22599), to create surface brain models processed from the MRI atlas of the rat brain. The anatomic location of the coronal, horizontal, and sagittal slices of the rat brain was superimposed on an MRI atlas (Figure 3A), showing an abundant distribution of the activity in the unfiltered phosphate buffer (\(A_{\text{phosphate}}\)) to the filtered buffer (\(A_{\text{filtered}}\)).

\[
y = \frac{A_{\text{phosphate}}}{A_{\text{filtered}}} = \frac{A_{\text{plasma}}}{A_{\text{filtered}}}
\]  

The plasma-free fraction, \(f_p\), was calculated as the ratio of the activity of the filtered plasma \(A_{\text{plasma}}\) to the unfiltered plasma \(A_{\text{plasma, u}}\), multiplied by the correction factor, \(y\), for recovery,

\[
f_p = \frac{A_{\text{plasma}}}{A_{\text{filtered}}} = \frac{A_{\text{plasma}}}{A_{\text{plasma, u}}} \cdot y.
\]

Image processing

Dynamic PET acquisitions were processed using Montreal Neurological Institute MINC software. We reconstructed the photon attenuation correction of dynamic recordings by three-dimensional-filtered back projection, resulting in a 128×128×63 matrix. Summed emission recordings were manually registered to a digital magnetic resonance imaging (MRI) atlas of the rat brain, using the program Register (Montreal Neurological Institute) with nine degrees of freedom. The dynamic emission recordings were registered in the stereotaxic space. Using the program Display, masks of volumes of interest, including frontal cortex, striatum, thalamus, pons, and cerebellum were drawn on the MRI atlas according to the stereotaxic atlas of the rat brain. The volume of interest masks were then used to extract time–activity curves from the resampled dynamic data sets. We obtained the total volumes of distribution \(V_{T0}\) and \(V_{T1}\) with the Logan analysis of the time–activity curves for each region, and the corresponding plasma activity curve as input.\(^1\) Using radio-HPLC, no radioactive metabolites were detected in plasma samples (data from two animals), similar to the previously reported absence of yohimbine metabolites in pig plasma.\(^5\)

Thus, we assumed that plasma input was not influenced by plasma metabolites. Parametric maps of \(V_1\) were processed by voxel-wise normalization using Logan plot with arterial plasma input. We used the open-source software, threedimensional Slicer (4.3.1 22599), to create surface brain models processed from the MRI atlas of the rat brain to illustrate the anatomic location of the coronal, horizontal, and sagittal slices of the parametric maps.

Determination of plasma-free fraction, \(f_p\)

\([11\text{C}]\text{yohimbine}\) \(f_p\) had to be determined because the \(V_{T0}\) value estimated from the inhibition plot is based on the assumption that the value remains constant from baseline to inhibition, implying that \([11\text{C}]\text{yohimbine}\) \(f_p\) is the same in the two conditions. The amount of blood required for adequate determination would have been too large to be obtained in the same animals that underwent PET in addition to the sampling of the plasma input curve, as blood loss may affect the estimates of kinetic parameters. Thus, we used a separate group of four rats, treated in the same manner as the rats that underwent PET acquisition. Administration of cyclosporine (50 mg/kg) was done through an intravenous catheter in the tail vein, and we catheterized the femoral artery for blood sampling. Blood from the baseline condition was collected 30 minutes after cyclosporine treatment, and then, to obtain blood from the challenge condition, we administered amphetamine (2 mg/kg intravenously) and sampled after 30 minutes. To generate plasma, the blood was centrifuged 20 minutes at 4 °C. With slight modifications, the ultrafiltration was conducted as previously described by Gandelman et al.\(^12\) We used Centrifree® centrifugal filter device (Millipore, Bedford, MA, USA) with a molecular cutoff of 30 kDa for all the samples. Each sample unit of 1000 μL rat plasma was spiked with 50 μL \([11\text{C}]\text{yohimbine}\) tracer. After 10-minute incubation at room temperature, aliquots of 50 μL of this solution were used to measure the total activity (unfiltered plasma). For triplicate measurement of the filtered plasma, the remaining volume was divided into three ultrafiltration devices and centrifuged for 20 minutes using a centrifuge with a fixed angle rotor at 1,000 g. After filtration, we removed aliquots of 50 μL to assess the activity in protein-free plasma. The activity of filtered plasma was determined in triplicates. All the samples were counted in a gamma device (Packard Cobra Gamma Counter, Model DS003). Nonspecific binding of the tracer to the ultrafiltration device was recovered by performing the same measurements with a phosphate-buffered solution. The correction factor, \(y\), was determined as the ratio of the activity of the unfiltered phosphate buffer (\(A_{\text{phosphate}}\)) to the filtered buffer (\(A_{\text{filtered}}\)).

Statistics

We used repeated measures two-way ANOVA at \(P < 0.05\) to determine whether pharmacological challenge with amphetamine significantly altered the regional \(BP_{\text{ND}}\) of \([11\text{C}]\text{yohimbine}\). For the plasma-free fraction, nonparametric test and paired t-test were applied to test whether the free fraction after amphetamine challenge significantly differed from baseline condition. Paired t-test was used to determine whether \(V_1\) estimates obtained from the Logan plot were significantly different when dynamic data were fitted at 6 to 28 minutes compared with 28 to 85 minutes.

RESULTS

\([11\text{C}]\text{yohimbine}\) clearance declined robustly in response to amphetamine in all examined regions, compared with the baseline condition, presented as regional time–activity curves, normalized to the plasma integral in Figures 1A–1E. The reduced clearance reveals the sensitivity of \([11\text{C}]\text{yohimbine}\) binding to the release of endogenous monoamines. Time–activity curves were normalized to the corresponding plasma integral to enable accurate comparability among animals, as the plasma integral directly reflects the administered quantity of radiotracer, which depends on the individually injected radioactive dose, the body weight, and the extent of peripheral washout. As shown in Figure 1F, the exponentially time-interpolated plasma activity curves were similar in shape and magnitude, implying that the difference of binding in brain tissue post amphetamine was not due to differences of input function, tracer delivery, or other bioavailability factors.

The steady-state period was determined by identifying the interval, in which the transfer coefficient estimates, \(k_1\) and \(k_2\), remained constant. Figure 2A displays the linear solution presented by Reith et al.\(^1\) in which \(k_1\) was obtained from the slope and \(k_2\) from the ordinate intercept, indicating steady-state approximation at the time interval from 6 to 28 minutes, where \(k_1\) is constant. A second linear solution\(^1\) yielded \(k_2^*\) as the reciprocal value of the slope, as presented in Figure 2B. Together, the two linear plots confirmed that steady state is present in the time interval of 6 to 28 minutes. We estimated the regional \(V_1\) from Logan plots with arterial plasma input, fitted in the time interval of 6 to 28 minutes, as exemplified in Figure 2C. All estimates of the steady-state parameters are listed in Table 1A. The clearance \(k_1\) decreased in response to amphetamine, indicating a decrease in tracer uptake in brain tissue in the challenge condition relative to baseline. Likewise, the rate constant \(k_2^*\) calculated from equation (5) declined post amphetamine, consistent with \([11\text{C}]\text{yohimbine}\) being displaced by release of endogenous monoamines. In contrast, the \(k_1^*\) estimates were unchanged in two regions, including frontal cortex and striatum. The \(m/m\) ratio was negligible \(\pm 1\%\) in this period (listed in Table 1B), as verification that the negligible magnitude of the \(m/m\) ratio coincides with the linearity of equation (9). Notably, \(k_2^*\) values estimated from the linearization of Reith et al\(^1\) are similar to those estimated from the solution presented by Gjedde et al.\(^13\) and the \(V_1\) values obtained from this plot are equal to the estimates from the Logan plot.\(^1\)

Figure 3 displays the parametric maps of \([11\text{C}]\text{yohimbine}\) \(V_1\) superimposed on an MRI atlas (Figure 3A), showing an abundant distribution of the activity in the area of interest. The time parametric maps are presented by Reith et al.\(^1\) and the regional estimates are listed in Table 1A. The \(BP_{\text{ND}}\) value estimated from the Logan plot.\(^1\)

\begin{equation}
y = \frac{A_{\text{plasma}}}{A_{\text{filtered}}} = \frac{A_{\text{plasma}}}{A_{\text{plasma, u}}} \cdot y.
\end{equation}

\(V_1\) was calculated as the ratio of the activity of the filtered plasma \(A_{\text{plasma}}\) to the unfiltered plasma \(A_{\text{plasma, u}}\), multiplied by the correction factor, \(y\), for recovery,
Figure 1. (A–F) Regional time–activity curves of $[{^{11}}C]$yohimbine uptake. Time–activity curves of $[{^{11}}C]$yohimbine from each volume of interest are normalized to the respective plasma input integrals (A–E). The displacement by amphetamine was uniform in all VOIs, as shown by the reduction of ~ 34% of the area under the curves of amphetamine (green curve), relative to baseline (black curve). The error bands represent the s.e.m. of five animals. The time-interpolated plasma activity curves (F) at baseline (black curve) and post-amphetamine (green curve) are similar in profile and magnitude, which excludes the possibility that the observed effect is because of differences in the dose of administered radiotracer. CEREB, cerebellum; FC, frontal cortex; STR, striatum; THA, thalamus; VOI, volume of interest.
distribution throughout the brain at baseline condition (Figure 3B) and a marked decrease upon amphetamine challenge (Figure 3C) because of competition with released monoamines.

The $V_T$ values from the solution presented by Logan et al.\textsuperscript{11} were used in the Inhibition plot, shown in Figure 4A. The $V_T$ values after amphetamine were plotted as a function of baseline, which produced a $V_{ND}$ value of 0.286 ± 0.136 mL/cm$^3$. The slope of the Inhibition plot represents the fraction of unoccupied receptors (1−s), which declined with the amphetamine challenge compared with baseline, as expected after the release of endogenous ligands. A reduction of receptor availability of 23 to 56% occurred in response to amphetamine, supporting yohimbine’s sensitivity to endogenous transmitters. The absolute values of receptor occupancy, $s$, exerted by endogenous monoamines are shown in Figure 4A for individual animals.

Figure 4B shows the binding potentials calculated in terms of the known $V_{ND}$ value. At baseline, the $BP_{ND}$ was greatest in the thalamus (5.84 ± 0.95) and striatum (5.82 ± 1.09), followed in descending order by frontal cortex (5.22 ± 0.99), pons (5.10 ± 0.85), and cerebellum (4.02 ± 0.66).

We determined values of $f_p$ by plasma ultrafiltration in a separate group of animals, treated in the same manner as those that underwent dynamic imaging. We found a baseline $f_p$ of 0.23 ± 0.03 that did not significantly differ from $f_p$ at post-amphetamine of 0.23 ± 0.02 ($P > 0.87$). Individual values of $f_p$ are listed in Table 2. The result of the statistical test of plasma-free fractions was not at variance with the null hypothesis of no difference, as shown with a nonparametric as well as a $t$-test. The unchanged plasma-free fraction supports constant $V_{ND}$ values from baseline to amphetamine challenge and confirms the assumption of no change underlying the Inhibition plot.

**DISCUSSION**

In this work, we used the Inhibition plot to evaluate $[^{11}C]$-yohimbine as a marker of monoaminergic competition at the a2R in brain. First, we determined the steady-state interval by using two linear plots to show that the transfer coefficients $K_1$ and $K_2$ are constant at 6 to 28 minutes. Correspondingly, the $m_1/m$ ratio was negligible $\approx 1\%$ in this period, confirming that the tracer exchange across BBB followed the linear relationship predicted by equation (9). Interestingly, the magnitude of $k_2$ estimates obtained from the linearized plot presented by Reith et al.\textsuperscript{12} were similar to the estimates from Gjedde et al.\textsuperscript{13} suggesting that the estimates obtained from different linear solutions approximate the same parameter values when estimated at steady state. The magnitude of the blood–brain clearance $K_1$ declined in all the examined regions in response to amphetamine, whereas the values of $k_2$ remained constant in particular regions (frontal cortex and striatum), indicating the opposite effects of reduced clearance and increased binding on the magnitude of this rate constant.

Calculations of $k_2$ using equation (5) verified that the magnitude of this rate constant $k_2$ declined with the magnitude of the clearance $K_1$ post amphetamine. The suggestion of reduced blood flow in striatum and frontal cortex in response to amphetamine in this study is consistent with prior reports of low striatal blood flow in monkeys and rats in response to amphetamine administration,\textsuperscript{20} and reduced cortical blood flow in response to noradrenaline release after locus coeruleus stimulation.\textsuperscript{21}

Figure 2. (A–C) Determination of time of steady-state onset. Typical examples of regression to linearizations of the one-compartment model to determine the steady-state interval of acquisition data. First, the times when $K_1$ was constant were identified by applying the linear model described by Reith et al.\textsuperscript{12} (A), in which $K_1$ was directly estimated as the slope. The equilibrium period was 6 to 28 minutes, which was further confirmed with the linear plot described by Gjedde et al.\textsuperscript{13} (B), where $k_2$ is obtained as the reciprocal of the slope. Ultimately, the steady-state interval was applied also to the Logan plot\textsuperscript{11} to estimate the equilibrium volume of distribution, $V_{ND}$ (C). CEREB, cerebellum; FC, frontal cortex; STR, striatum; THA, thalamus.
Table 1. Regional kinetic parameters at baseline and with amphetamine

| Region | Baseline | AMPH | Mean ± s.d. | Mean ± s.d. |
|--------|----------|------|-------------|-------------|
| FC     | 0.28     | 0.18 | 0.06 ± 0.08 | 0.05 ± 0.07 |
| STR    | 0.29     | 0.18 | 0.10 ± 0.06 | 0.09 ± 0.06 |
| PONS   | 0.26     | 0.12 | 0.32 ± 0.23 | 0.30 ± 0.22 |
| CEREB  | 0.61     | 0.50 | 0.26 ± 0.24 | 0.26 ± 0.24 |
| Post-synaptically | 0.21 | 0.22 | 0.20 ± 0.04 | 0.21 ± 0.05 |
| SC     | 0.19     | 0.18 | 0.20 ± 0.04 | 0.19 ± 0.05 |
| THA    | 0.26     | 0.20 | 0.20 ± 0.05 | 0.20 ± 0.06 |
| CEREB  | 0.25     | 0.20 | 0.31 ± 0.09 | 0.31 ± 0.06 |

Table 1B. m/m ratios

| Midframe time | Typical example | Baseline | AMPH |
|---------------|----------------|----------|------|
| (minute)      | Baseline | AMPH | Mean ± s.d. | Mean ± s.d. |
| 1.13          | 0.017    | 0.025 | 0.014 ± 0.006 | 0.021 ± 0.003 |
| 1.38          | 0.016    | 0.023 | 0.014 ± 0.005 | 0.020 ± 0.003 |
| 1.63          | 0.015    | 0.021 | 0.013 ± 0.005 | 0.018 ± 0.002 |
| 1.88          | 0.014    | 0.020 | 0.012 ± 0.004 | 0.018 ± 0.002 |
| 2.25          | 0.012    | 0.018 | 0.011 ± 0.004 | 0.016 ± 0.002 |
| 2.75          | 0.011    | 0.016 | 0.010 ± 0.003 | 0.015 ± 0.002 |
| 3.25          | 0.010    | 0.014 | 0.009 ± 0.003 | 0.014 ± 0.002 |
| 3.75          | 0.009    | 0.013 | 0.009 ± 0.002 | 0.013 ± 0.001 |
| 4.5           | 0.008    | 0.012 | 0.008 ± 0.002 | 0.012 ± 0.002 |
| 5.5           | 0.007    | 0.011 | 0.007 ± 0.002 | 0.011 ± 0.002 |
| 7             | 0.006    | 0.010 | 0.006 ± 0.002 | 0.010 ± 0.002 |
| 9             | 0.005    | 0.009 | 0.006 ± 0.002 | 0.009 ± 0.002 |
| 12.5          | 0.004    | 0.008 | 0.005 ± 0.001 | 0.008 ± 0.002 |
| 17.5          | 0.004    | 0.008 | 0.005 ± 0.001 | 0.008 ± 0.002 |
| 22.5          | 0.004    | 0.008 | 0.005 ± 0.001 | 0.007 ± 0.002 |
| 27.5          | 0.004    | 0.008 | 0.005 ± 0.001 | 0.007 ± 0.002 |
| 35            | 0.004    | 0.008 | 0.005 ± 0.001 | 0.007 ± 0.002 |
| 45            | 0.005    | 0.008 | 0.005 ± 0.001 | 0.007 ± 0.002 |
| 55            | 0.005    | 0.008 | 0.006 ± 0.001 | 0.007 ± 0.002 |
| 65            | 0.005    | 0.008 | 0.006 ± 0.001 | 0.007 ± 0.002 |
| 75            | 0.006    | 0.008 | 0.006 ± 0.001 | 0.007 ± 0.002 |
| 85            | 0.006    | 0.008 | 0.007 ± 0.002 | 0.007 ± 0.002 |

Although no radioactive metabolites were detected in plasma, the two linear plots used to estimate $K_1$ and $K_2$ revealed that the results may have been influenced by metabolites at times later than 28 minutes, as exemplified by the curvatures seen in the plots of Figures 5A and 5B after 28 minutes of circulation. The curvature was not immediately apparent in the Logan plots, as shown in Figure 5C, but the slopes are significantly different when obtained for the two intervals before and after 28 minutes of circulation (P < 0.05, data from five animals). Here, the global baseline $V_1$ estimate at 6 to 28 minutes averaged 1.71 ± 0.10 mL/cm³, compared with the global estimate of 1.58 ± 0.07 mL/cm³ at 28 to 85 minutes. The curvatures suggest that the steady state is affected by plasma metabolites at times later than 28 minutes, perhaps explaining the well-known underestimation by the Logan plot of the value of $V_1$, as reported previously.27 The comparison of the early and late Logan slopes demonstrates that departures from steady state may be difficult to detect with this plot towards the end of circulation times with the increased spacing of the last points. The curvatures and the decline of the $V_1$ estimates, associated with a possible end of steady state, may indicate the presence of low concentrations of plasma metabolites below the limit of detection by HPLC, possibly related to the negligible amounts of tracer in small animals, the limited plasma volume in rodents, and a low level itself of the plasma metabolites. However, the effect was circumvented by identification of times of preserved steady state before the effective appearance of plasma metabolites.

We have demonstrated that [11C]yohimbine binding across the brain varied as expected from previous binding assays in rat brain in vitro with [3H]yohimbine, in which a high level of
Binding was found throughout the brain. Regions with the greatest [3H]yohimbine binding included striatum, cerebral cortex, hypothalamus, hippocampus, cerebellum, and pons. These findings agree with the present in vivo data with the same rank order of magnitudes of [11C]yohimbine binding. In the rat central nervous system, the expression of α2R has three subtypes, including α2A, α2B, and α2C, which are classified on the basis of the pharmacological characteristics and molecular cloning. In rats, the central α2R are regionally heterogeneous. It is known from in situ hybridization of α2R gene expression that the cerebral cortex, hippocampus, thalamic nuclei, and cerebellum predominantly express both α2A and α2C subtypes, while the basal ganglia express α2C, and the α2A subtype is expressed exclusively but weakly in the thalamus. Further binding assays in human brains have shown that α2A and α2C in the caudate nucleus, based on [11C]yohimbine binding that was displaced by oxymetazoline and prazosine, both drugs known to bind with high affinity for α2A and α2C, respectively. Consistent with these findings, in vitro autoradiography of rat brain with the two α2R antagonists, [3H]rauwolscine and [3H]idazoxan, revealed binding to sites in striatum that was displaced by noradrenergic but not dopaminergic ligands, which further supports the existence of striatal α2C and α2A sites in rat brain.

Figure 3. (A–C) Parametric maps of [11C]yohimbine V_T. Representative parametric maps of [11C]yohimbine V_T were superimposed on an average MRI rat atlas (A), shown in horizontal (left column), coronal (middle column), and sagittal planes (right column). The magnitude of V_T was obtained from the Logan plot with voxel-wise normalization to plasma input and linear regression in the steady-state interval of 6 to 28 minutes. V_T remained uniformly high throughout the brain at baseline (B) and declined markedly with acute amphetamine challenge (C), suggesting that [11C]yohimbine binding is sensitive to the elevated level of monoamines. MRI, magnetic resonance imaging.

Figure 4. (A–B) The Inhibition plot and [11C]yohimbine binding potentials, BPND. Steady-state volumes of distribution, V_T, obtained from the Logan plot applied to the inhibition plot to yield the reference volume, VND (A). The regional volumes of distribution in response to pharmacological challenge, V_T(i), of each animal were plotted as a function of the volume of distribution at baseline, V_T(b). The dashed line, representing the linear regression of the averaged baseline data (n = 5), also represents the line of identity. The solid lines are the linear regressions of amphetamine challenge against baseline in individual animals (n = 5). Each point represents the volume of distribution in a VOI, and the linear regressions are based on five VOIs, including frontal cortex (FC), striatum (STR), thalamus (THA), pons (PO), and cerebellum (CEREB). The slope of the linear regressions of amphetamine challenge, (1 − s), is the receptor availability, shown here as the result of competition with endogenous transmitters released by amphetamine. The estimate of VND was obtained from the shared intercept of the linear regressions with the baseline curve, with a value of 0.286 ± 0.136 mL/cm³. The VND estimated from the Inhibition plot was used to calculate the regional binding potentials, BPND, presented in B. Values of BPND were highest in the thalamus and striatum, followed in descending order by, frontal cortex, pons, and cerebellum. BPND declined significantly in response to acute amphetamine (black bars) (P < 0.05), compared with baseline (white bars) across all brain regions. Regional BPND from individual animals are presented with a line that connects the values at baseline and post-amphetamine to indicate the pharmacological effect in each animal. VOI, volume of interest.
In contrast, [11C]yohimbine is not specific to any of these subtypes and thus labels α2R without discriminating among subtypes. The combination of known α2R subtype expression in vitro and [11C]yohimbine PET in vivo is a valuable tool of prediction of which α2R subtype possibly may be implicated in a particular condition. Especially noteworthy is the finding that in vivo striatal [11C]yohimbine binding significantly exceeds that of the frontal cortex, because striatum is known to receive generous dopaminergic innervation via nigrostriatal afferents but very little noradrenergic input.28 The discrepancy between α2R distribution and noradrenergic innervation is in agreement with previous autoradiography of the rat brain, for example, demonstrating more numerous binding sites of the α2R antagonist [3H]-rauwolscine in striatum than in cerebral cortex.29 In light of the known regional distribution of α2R subtypes in rat brain, the observed striatal [11C]yohimbine binding may be ascribed to the labeling of both α2C and α2A sites.

Of course, the functional role of α2R in a region that has limited noradrenergic input28 is not immediately evident. However, interaction between other monoamines and these receptors possibly means that α2R in striatum has a role in, and responds to, dopaminergic neurotransmission. This interpretation is supported by evidence from work in zebra finches, in which dopamine binds to α2R, although with considerably lower affinity than for noradrenaline.30 Further studies have shown that the affinity of α2C-R for dopamine was higher than that of α2AR for noradrenaline in rat striatum, suggesting that dopamine may be the preferred neurotransmitter of the striatal α2CR.31 In the light of this evidence in support of homology between noradrenergic and dopaminergic receptors, it is likely that the displacement of [11C]yohimbine binding in striatum with amphetamine is because of competition with dopamine released from dopaminergic terminals in the presence of minimal noradrenergic input. We observed significantly displaced [11C]yohimbine binding after amphetamine in regions of minimal dopaminergic input, including frontal cortex, thalamus, pons, and cerebellum, suggesting that this displacement may be ascribed to competition with endogenous noradrenaline in these brain areas, where the distribution of noradrenergic projections and α2R are known to coincide.28,29

Figure 5. (A–C) Linear fitting of acquisition data from 28 to 85 minutes in comparison with 6 to 28 minutes. The comparison of the regression results of the periods of 6 to 28 minutes (dashed curves) and the later time points of 28 to 85 minutes (solid curves), to illustrate the differential properties of the three linearized versions of one-compartment model. The linearizations described by Reith et al (A) and by Gjedde et al (B), respectively, show that data at times later than 28 minutes are nonlinear, indicating probable influence from radioactive metabolites, which is less visible on the Logan plot (C).

Table 2. Plasma-free fractions at baseline and with amphetamine

| Animal | Baseline | Post-amphetamine |
|--------|----------|------------------|
|        | fp       | fp               |
| 1      | 0.30     | 0.20             |
| 2      | 0.21     | 0.27             |
| 3      | 0.16     | 0.21             |
| 4      | 0.23     | 0.25             |
| Mean ± s.e.m. | 0.23 ± 0.03 | 0.23 ± 0.02 |

No significant differences of plasma-free fractions at baseline and with amphetamine challenge, as measured with plasma ultrafiltration (n = 4, P = 0.87). This confirms that the same VND estimate can be used for baseline and in response to amphetamine, as required by the Inhibition plot. The bold entries are made to improve the delivery of information so that the mean values can easily be separated from the standard error values.
This study successfully yielded estimated receptor availability or $BP_{ND}$ of $[^{11}C]$yohimbine in rat brain in the absence of a reference region. When the $BP_{ND}$ declined significantly in response to the amphetamine challenge, the Inhibition plot yield $V_{ND}$ value that must be known to calculate the $BP_{ND}$ of radiotracers with displaceable accumulation in all parts of the brain. Similar findings were reported in recent PET studies in pigs, where $[^{11}C]$yohimbine binding was significantly displaced by competition with endogenous transmitters, as shown by a decline of $V_{T}$ in response to acute amphetamine treatment, although the binding potential was not calculated because the magnitude of $V_{ND}$ varied among the few subjects studied.

In contrast to the significant decline of $[^{11}C]$yohimbine $BP_{ND}$ found in present study, an ex vivo study in rat brain showed that the binding of $[^{11}H]$RX821002, a highly selective $\alpha_2$R antagonist, declined only in three cortical regions (frontopolar, anterior cingulate, and frontal parietal cortices) in response to amphetamine, with no changes observed in other cortical or subcortical structures. On the basis of previous characterizations of RX821002 selectivity with equal affinity by $\alpha_2\delta$R and $\alpha_2\beta$R and a threefold lower affinity by the $\alpha_2\delta$ subtype, in parallel with the presence of $\alpha_2\delta$ and $\alpha_2\beta$ subtypes in both cerebral cortex and cerebellum, and the rich noradrenergic input from locus coeruleus, we argue that the choice of cerebellum as reference would be at variance with the presence of displaceable binding sites in cerebellum, which would lead to the underestimation of $BP_{ND}$.

Cyclosporine administration was used in this study to block $p$-glycoprotein action at the BBB, the influence of potential metabolic interactions of cyclosporine, yohimbine, and amphetamine must be weighed. It has been reported that the half-life of cyclosporine is dose-dependent, with a duration above 10 hours when treated with doses higher than 30 mg/kg in rats. The dose used in this study (50 mg/kg) may saturate metabolic enzymes and compromise the metabolic degradation of yohimbine and amphetamine. Although direct pharmacological interaction between cyclosporine and yohimbine has not been described, interaction of enzymatic metabolism is unlikely because degradation is catalyzed by different rate-limiting enzymes. Metabolism of cyclosporine is primarily catalyzed by CYP3A enzyme, while amphetamine primarily is metabolized by CYP2D6, such that the pharmacological action of amphetamine is not influenced by interaction with cyclosporine metabolism. However, cyclosporine is essentially lipophilic, and reports in humans have indicated that 98.5% of cyclosporine is bound to plasma proteins, which may reduce the protein-binding capacity for yohimbine and amphetamine. Although it cannot be excluded that cyclosporine may potentiate the bioavailability of yohimbine and amphetamine, this potential interference would not compromise the binding properties of yohimbine to $\alpha_2$R, nor the sensitivity to (endogenous) monoamines in this work. The decline of yohimbine binding with the amphetamine challenge can be attributed to factors other than monoamine release, including a decrease of the pharmacological action of cyclosporine, which was administered as a single dose (50 mg/kg). However, the long half-life of cyclosporine made it reasonable for us to assume constant pharmacological action throughout the study. In contrast, yohimbine is peripherally metabolized by oxidation to 11-hydroxy-yohimbine and 10-hydroxy-yohimbine, as catalyzed by the CYP2D6 and CYP3A4 enzymes that degrade amphetamine and cyclosporine. It is possible that the metabolism of yohimbine is competitively suppressed to a greater extent with amphetamine administration than at baseline. However, this potential difference of yohimbine metabolism in the two conditions has minimal influence on the results because the dynamic data from both conditions were analyzed at times of apparent steady state before as discussed above. Central metabolism of yohimbine may also have an impact on the binding profile, but the expression of CYP450 enzymes in brain tissue is only 1% of the expression in liver, in support of the claim that peripheral metabolism is more dominant than central metabolism.

As PET in humans generally is conducted without anesthesia, the effects of anesthetics in animals must be considered carefully when we use the present findings to predict the results that would be obtained in future human PET studies of noradrenergic neurotransmission. The use of isoflurane in the present study is a potential confound for two reasons. First, compared with the awake condition, isoflurane evokes a substantial three- to fourfold increase of noradrenaline in the rat preoptic area. However, as the duration of the microdialysis was only 50 minutes, it is not a simple matter to predict whether noradrenaline release remains stable or declines with the prolonged anesthesia used in the present study. When we consider the possibility that the effect of isoflurane on the level of noradrenaline could be constant, we would expect an underestimation of the $BP_{ND}$ in both the baseline and inhibition states, compared with an awake state. Isoflurane has been shown to enhance the pharmacological actions of amphetamine and potentiate the release of monoamines, which has affected the binding of certain radioligands of dopamine receptors. Thus, although the possibility that isoflurane may influence the magnitude of noradrenaline release and interfere with $[^{11}C]$yohimbine binding cannot be excluded, we argue that the binding properties of $[^{11}C]$yohimbine and its sensitivity to endogenous noradrenaline is not compromised by persistently elevated noradrenaline.

Altered $\alpha_2$R expression has been reported in a spectrum of brain disorders, including postmortem analysis that demonstrated a marked increase of $\alpha_2$R in the brains of suicide victims. In future investigations, $[^{11}C]$yohimbine can be used to determine $\alpha_2$R density in vivo. We suggest at least two consecutive PET radio-tracer acquisitions at high, followed by low, specific activity, with Eadie–Hofstee or Scatchard plot analysis of binding to quantify receptor density and affinity.

In summary, $[^{11}C]$yohimbine can be used to visualize binding to $\alpha_2$R, and the binding is sensitive to noradrenaline and other monoamine release. The Inhibition plot yields an estimate of the $V_{ND}$ of $[^{11}C]$yohimbine that overcomes the absence of a reference region of non-displaceable uptake. Unlike the Logan plot, the linearized plots of Reith et al. and Gjedde et al. assist in the identification of the onset and termination of true steady state of the dynamic acquisition data when plasma metabolism may interfere with the maintenance of steady state. In the future, the use of $[^{11}C]$yohimbine with PET can be beneficial to in vivo investigation of $\alpha_2$R density and affinity, and noradrenaline and other monoamine release in physiologic and pathologic states of the brain.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Garcia-Sevilla JA, Escriba PV, Ozaita A, La Harpe R, Walzer C, Eytan A et al. Up-regulation of immunolabeled alpha2A-adrenoceptors, Gi coupling proteins, and regulatory receptor kinases in the prefrontal cortex of depressed suicides. J Neurochem 1999; 72: 282–291.
2 Szot P, White SS, Greenup JL, Leverenz JB, Peskind ER, Raskind MA. Compensatory changes in the noradrenergic nervous system in the locus ceruleus and hippocampus of postmortem subjects with Alzheimer’s disease and dementia with Lewy bodies. J Neurosci 2006; 26: 467–478.
3 Tam SW, Worcel M, Wylie M. Yohimbine: a clinical review. Pharmacol Ther 2001; 91: 215–243.
4 Millan MJ, Newman-Tancredi A, Audinot V, Cussac D, Lejeune F, Nicolas JP et al. Agonist and antagonist actions of yohimbine as compared to fluperoxan at alpha-(2)-adrenergic receptors (ARs), serotonin (5-HT)(1A), 5-HT1B, 5-HT1D and
dopamine D-2 and D-3 receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states. Synapse 2000; 35: 79–95.

5. Jakobsen S, Pedersen K, Smith DF, Jensen SB, Munk OL, Cumming P. Detection of alpha2-adrenergic receptors in brain of living pig with 11C-yohimbine. J Nucl Med 2006; 47: 2008–2015.

6. Landau AM, Doudet DJ, Jakobsen S. Amphetamine challenge decreases yohimbine binding to alpha2 adreceptors in Landrace pig brain. Psychopharmacology (Berlin) 2012; 222: 155–163.

7. Logan J, Volkow ND, Fowler JS, Wang GJ, Dewey SL, Macgregor R et al. Effects of blood-flow on [C-11]raclopride binding in the brain - model simulations and kinetic-analysis of pet data. J Cereb Blood Flow Metab 1994; 14: 995–1010.

8. Gjedde A, Gjedde A, Gee A, Smith D. Basic CNS drug transport and binding kinetics. Marcel Dekker: New York, NY, USA, 2000; 225–277.

9. Gjedde A, Wong DF. Receptor occupancy in absence of reference region. Neuroimage 2000; 11: 548.

10. Jones SR, Gainetdinov RR, Wightman RM, Caron MG. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci 1998; 18: 1979–1986.

11. Logan J, Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ et al. Graphical analysis of reversible radioligand binding from time-activity measurements applied to [N-11C-methyl](-)-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 1990; 10: 740–747.

12. Reith J, Dyve S, Kuwabara H, Guttman M, Diksic M, Gjedde A. Blood-brain transfer and metabolism of 6-[18F]fluoro-L-dopa in rat. J Cereb Blood Flow Metab 1990; 10: 707–719.

13. Gjedde A, Gee A, Smith D. Basic CNS drug transport and binding kinetics in vivo. Begley DJ, Bradbury MW, Kreuter J (eds). The Blood–Brain Barrier and Drug Delivery to the CNS. Marcel Dekker: New York, NY, USA, 2000; 225–242.

14. Innis RB, Cunningham VI, Delforge J, Fujita M, Gjedde A, Gunn RN et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 2007; 27: 1533–1539.

15. Lassen NA, Bartenstein PA, Lammertsma AA, Prevett MC, Turton DR, Luthra SK et al. Benzodiazepine receptor quantification in vivo in humans using 11C-flumazenil and PET: application of the steady-state principle. J Cereb Blood Flow Metab 1995; 15: 152–162.

16. Cunningham VI, Rabener EA, Silfvenius M, Laruelle M, Gunn RN. Measuring drug occupancy in the absence of a reference region: the Lassen plot re-visited. J Cereb Blood Flow Metab 2010; 30: 46–50.

17. Shekim WO, Bylund DB, Hodges K, Glaser R, Ray-Prenger C, Oetting G. Platelet alpha 2-adrenergic receptor binding and the effects of d-amphetamine in boys with attention deficit hyperactivity disorder. Neuropsychopharmacology 1994; 9: 120–124.

18. Tanaka C, Kawai R, Rowland M. Dose-dependent pharmacokinetics of cyclazocine A in rats: events in tissues. Drug Metab Dispos 2000; 28: 582–589.

19. Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB. Evaluation of ultrafiltration for the free-fraction determination of single photon emission computed tomography (SPECT) radiotracers: beta-CIT, IIB, and iomazenil. J Pharm Sci 1994; 83: 1014–1019.

20. Lavyn MH, Koltun WA, Clement JA, Rosene DL, Picken KS, Zervas NT et al. Decrease in neostriatal blood flow after D-amphetamine administration or electrical stimulation of the substantia nigra. Brain Res 1977; 135: 77–86.

21. Rachle ME, Hartman BK, Eichling JO, Sharpe LG. Central noradrenergic regulation of cerebral blood flow and vascular permeability. Proc Nutl Acad Sci USA 1975; 72: 3726–3730.

22. Zhou Y, Ye W, Brasic JR, Wong DF. Multi-graphical analysis of dynamic PET. Neuroimage 2010; 49: 2947–2957.

23. Rout O, Quennessy MC, Schwartz J. Characteristics of the [H-3]-labeled yohimbine binding on rat-brain alpha 2-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol 1982; 321: 253–259.

24. Bylund DB. Subtypes of alpha 2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol Sci 1988; 9: 356–361.

25. Scheinin M, Lomasney JW, Hayden-Hixson DM, Schambra UB, Canor MG, Lefkowitz RJ et al. Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. Brain Res Mol Brain Res 1994; 21: 133–149.

26. Ordway GA, Jaconetta SM, Halaris AE. Characterization of subtypes of alpha 2-adrenoceptors in the human brain. J Pharmacol Exp Ther 1993; 264: 967–976.

27. Boyajian CL, Leslie FM. Pharmacological evidence for alpha 2-adrenoceptor heterogeneity: differential binding properties of [3H]rauwolscine and [3H]idazoxan in rat brain. J Pharmacol Exp Ther 1987; 241: 1092–1098.

28. Eschenko O, Evrad HC, Neves RM, Beyerlein M, Murayama Y, Logothetis NK. Tracing of noradrenergic projections using manganese-enhanced MRI. Neuroimage 2012; 59: 3252–3265.

29. Boyajian CL, Loughlin SE, Leslie FM. Anatomical evidence for alpha 2-adrenoceptor heterogeneity: differential autoradiographic distributions of [3H]rauwolscine and [3H]idazoxan in rat brain. J Pharmacol Exp Ther 1987; 241: 1079–1091.

30. Cornil CA, Castelino CB, Ball GF. Dopamine binds to alpha(2)-adrenergic receptors in the song control system of zebra finches (Taeniopygia guttata). J Chem Neuroanat 2008; 35: 202–215.

31. Zhang W, Klimke V, Farley JT, Zhu MY, Ordway GA. alpha2C adrenoceptors inhibit adenyl cyclase in mouse striatum: potential activation by dopamine. J Pharmacol Exp Ther 1999; 289: 1286–1292.

32. Tyacke RJ, Robinson ES, Lailds MD, Hume SP, Hudson AL, Nutt DJ. Estimation of endogenous noradrenaline release in rat brain in vivo using [3H]RX 821002. Synapse 2005; 55: 126–132.

33. O’Rourke MB, Blaxall HS, Iversen LJ, Bylund DB. Characterization of [3H]RX821002 binding to alpha 2-adrenergic receptor subtypes. J Pharmacol Exp Ther 1994; 268: 1362–1367.

34. Watkins PB. The role of cytochromes P-450 in cyclosporine metabolism. J Am Acad Dermatol 1990; 23: 1301–1309, discussion 1309–1311.

35. Kraemer T, Maurer HH. Toxicokinetics of amphetamines: metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine, and their N-alkyl derivatives. Ther Drug Monit 2002; 24: 277–289.

36. Yang H, Elmqist WF. The binding of cyclosporin A to human plasma: an in vitro microdialysis study. Pharm Res 1996; 13: 622–627.

37. Le Corre P, Parmer RJ, Kailasam MT, Kennedy BP, Skaar TP, Ho H et al. Human sympathetic activation by alpha2-adrenergic blockade with yohimbine: bimodal, epistatic influence of cytochrome P450-mediated drug metabolism. Clin Pharmacol Ther 2004; 76: 139–153.

38. Warner M, Kohler C, Hansson T, Gustafsson JA. Regional distribution of cytochrome P-450 in the rat brain: spectral quantitation and contribution of P-450(b,e) and P-450(c,d). J Neurochem 1988; 50: 1057–1069.

39. Anzawa N, Kushikata T, Ohkawa H, Yoshida H, Kubota T, Matsuki A. Increased noradrenaline release from rat preoptic area during and after sevoflurane and isoflurane anesthesia. Can J Anaesth 2001; 48: 462–465.

40. McCormick PN, Ginovart N, Wilson AA. Isoflurane anaesthesia differentially affects the amphetamine sensitivity of agonist and antagonist D2/D3 position emission tomography radiotracers: implications for in vivo imaging of dopamine release. Mol Imaging Biol 2011; 13: 737–746.