Estimating the effect of endogenous dopamine on baseline [^{11}C]-(+)-PHNO binding in the human brain

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Abstract

Endogenous dopamine (DA) levels at dopamine $D_{2/3}$ receptors ($D_{2/3}R$) have been quantified in the living human brain using the agonist radiotracer [^{11}C]-(+)-PHNO. As an agonist radiotracer, [^{11}C]-(+)-PHNO is more sensitive to endogenous DA levels than antagonist radiotracers. We sought to determine the proportion of the variance in baseline [^{11}C]-(+)-PHNO binding to $D_{2/3}R$ s which can be accounted for by variation in endogenous DA levels. This was done by computing the Pearson's coefficient for the correlation between baseline binding potential (BP_{ND}) and the change in BP_{ND} after acute DA depletion, using previously published data. All correlations were inverse, and the proportion of the variance in baseline [^{11}C]-(+)-PHNO BP_{ND} that can be accounted for by variation in endogenous DA levels across the striatal subregions ranged from 42–59%. These results indicate that lower baseline values of [^{11}C]-(+)-PHNO BP_{ND} reflect greater stimulation by endogenous DA. To further validate this interpretation, we sought to examine whether these data could be used to estimate the dissociation constant (K_d) of DA at $D_{2/3}R$. In line with previous in vitro work, we estimated the in vivo K_d of DA to be around 20 nM. In summary, the agonist radiotracer [^{11}C]-(+)-PHNO can detect the impact of endogenous DA levels at $D_{2/3}R$ in the living human brain from a single baseline scan, and may be more sensitive to this impact than other commonly employed radiotracers.

Keywords

AMPT; dopamine; PET; [^{11}C]-(+)-PHNO; $D_{2/3}R$

1| INTRODUCTION

Over the past 30 years, positron emission tomography (PET) has been used in conjunction with specific radioligands to quantify protein targets and neurochemicals in the living human brain (Gunn, Slifstein, Searle, & Price, 2015). In 1983, the availability of dopamine (DA) $D_{2/3}$ receptors ($D_{2/3}R$) was quantified for the first time in the striatum of living humans using [^{11}C]-N-methylspiperone (Wagner et al., 1983). Notably, an earlier attempt in 1979 using [^{11}C]-chlorpromazine failed (Comar et al., 1979). Amidst the ensuing attempts to quantify $D_{2/3}R$ in vivo, it became apparent that certain radiotracers – the substituted benzamides [^{11}C]-raclopride and [^{123}I]-IBZM–could compete with endogenous DA for binding to $D_{2/3}R$ (Laruelle, 2012). For example, unlike the butyrophenone ligands N-[3H]-methylspiperone and [3H]-spiperone, it was found that binding of [3H]-raclopride was reduced by the presence of DA in human and rat striata (Kohler, Hall, Ogren, & Gawell, 1985; Seeman, Guan, & Niznik, 1989). This led to the inference that a portion of potential binding sites for striatal $D_{2/3}R$ were unavailable to [^{11}C]-raclopride in vivo due to the occupancy of those sites by endogenous DA (Seeman, 1988, 1992; Seeman, Niznik, & Guan, 1989; 1990).

The idea that this property could be utilized to quantify synaptic DA levels at $D_{2/3}R$ in vivo appeared as early as 1984 (Friedman, DeJesus, Revenaugh, & Dinerstein, 1984), and led to the proposal of the occupancy model (Laruelle, 2000; Laruelle et al., 1997). Simply, the model predicts that challenges which increase synaptic DA should reduce radiotracer binding to $D_{2/3}R$, while challenges that reduce synaptic DA should increase binding to $D_{2/3}R$. Thus, changes in radiotracer binding from a baseline state could be used to quantify DA release and endogenous DA levels at $D_{2/3}R$, respectively. Using the catecholamine depleting agent alpha-methyl-paratyrosine (α -MPT), baseline endogenous DA levels at $D_{2/3}R$ were quantified for the first time in the striatum of healthy humans using [^{123}I]-IBZM (Laruelle et al., 1997). This was soon followed by studies validating the use of [^{11}C]-raclopride to quantify endogenous DA levels at striatal $D_{2/3}R$ in healthy humans (Verhoeff et al., 2001, 2002, 2003). Using this DA depletion method, invaluable insights have been gained regarding the alteration of endogenous DA in neuropsychiatric disorders. For example, it was discovered that persons with schizophrenia have greater endogenous DA levels at striatal $D_{2/3}R$ compared with healthy controls (Abi-Dargham et al., 2000), specifically in the dorsal caudate (Kegeles et al., 2010). Moreover, not all persons at clinical high-risk for schizophrenia may demonstrate this increase in endogenous striatal DA levels (Bloemen et al., 2013; De Koning et al., 2014), and it is still under investigation whether endogenous DA levels change in the face of chronic stable-antipsychotic treatment (Caravaggio, Nakajima, et al., 2015). Finally, this method has also been used to demonstrate that persons with cocaine-addiction have both reduced $D_{2/3}R$ expression and reduced endogenous DA levels at these receptors throughout the striatum (Martinez et al., 2009).

While efforts have been made to elucidate how baseline $D_{2/3}R$ availability is related to DA synthesis capacity (Ito et al., 2011), it is still unclear to what extent endogenous DA levels influence $D_{2/3}R$ availability at baseline. It has been suggested that the correlation between baseline binding to $D_{2/3}R$ and the change in binding after DA depletion can be used to estimate the magnitude of this influence (Kegeles, Martinez, Slifstein, Laruelle, & Abi-

Dargham, 2014). This correlation would indicate the proportion of the variance in baseline $D_{2/3}R$ availability, which can be accounted for by endogenous DA levels (r^2). Prima facie, this seems to provide a reasonable estimate of a radioligands' sensitivity to endogenous DA at baseline. It does so subject to several potential limitations of the α -MPT paradigm, which include that the level of DA depletion is not equivalent across subjects, and that 100% DA depletion is not achievable.

Notably, D_2R exist in two distinct conformational states for agonists: a state of high affinity (D_2^{High}) and a state of low affinity (D_2^{Low} ; George et al., 1985; Seeman, 2010; Sibley, D Lean, & Creese, 1982a). In the simplest conceptualization, when D_2R are coupled to their intracellular G-protein they are in a high-affinity state, promoting the binding of agonists like DA. When these agonists bind, the G-protein uncouples via guanosine-5'-triphosphate (GTP), initiating an intracellular cascade of second messenger events. Once the G-protein is detached from the D_2R , the D_2R is in a low-affinity state for DA; DA will prefer to bind again when the G-protein is recycled and re-coupled (Seeman, 2011). Thus, it has been demonstrated in vitro that D_2^{High} is the functional state of D_2R in the anterior pituitary (George et al., 1985; McDonald, Sibley, Kilpatrick, & Caron, 1984; Sibley et al., 1982) and the striatum (Hamblin, Leff, & Creese, 1984; Richfield, Young, & Penney, 1986; Starke, Reimann, Zumstein, & Hertting, 1978). The consequence of this is that antagonists for D_2R should bind with equal affinity to D_2^{High} and D_2^{Low} sites, while agonists like endogenous DA are expected to compete for binding at D_2^{High} sites but not D_2^{Low} sites. Thus, in theory agonist radiotracers for D_2R —such as [^{11}C]NPA (Narendran et al., 2004), [^{11}C]MNPA (Finnema et al., 2005), and [^{11}C]-(+)-PHNO (Willeit et al., 2006)—should be more vulnerable to competition by endogenous DA, and label a smaller proportion of the total D_2R pool than antagonist radiotracers. For example, in calf caudate membranes agonists show 50 times more affinity for [3H]-DA binding sites over [3H]-haloperidol binding sites; antagonists display about 100 times greater affinity for [3H]-haloperidol binding sites versus [3H]-DA sites (Creese Burt, D. R., & Snyder, 1975). Thus, quantifying endogenous DA levels with an agonist radiotracer may offer a more sensitive and physiologically meaningful measure compared with the use of an antagonist.

We have recently employed the α -MPT PET paradigm to estimate endogenous DA in humans using the agonist radiotracer [^{11}C]-(+)-PHNO (Caravaggio, Raitsin, et al., 2014). This study and others, including competition studies in humans and non-human primates, suggest that agonist radiotracers may be 1.4–1.6 times more sensitive to changes in endogenous DA levels than antagonist radiotracers like [^{11}C]-raclopride (Laruelle, 2012; Narendran et al., 2010; Seneca et al., 2006; Shotbolt et al., 2012). Reanalyzing our previously published α -MPT dataset, we sought to use this correlation approach to estimate the influence of endogenous DA on the baseline $D_{2/3}R$ availability of [^{11}C]-(+)-PHNO. In addition, we used this dataset to estimate free synaptic DA concentration (nM) which in turn was used to estimate the concentration of DA required to occupy half of the striatal $D_{2/3}R$ (i.e., Kd). This was compared to the in vitro literature to validate the estimate of intrasynaptic DA. These results are useful for interpreting the impact of endogenous DA on

baseline measurements with [^{11}C]-(+)-PHNO and contribute to our conceptual understanding of the PET occupancy model (Laruelle, 2012).

2 | METHODS

2.1 | α -MPT [^{11}C]-(+)-PHNO PET data

For this investigation, we reanalyzed previously published data using [^{11}C]-(+)-PHNO to estimate endogenous DA levels in the brains of healthy humans using the α -MPT paradigm (Caravaggio, Raitsin, et al., 2014). Ten healthy subjects (4 female; mean age = 29.1 ± 8.4) participated in the study, providing [^{11}C]-(+)-PHNO scans at baseline and after an acute DA depletion challenge. Depletion was achieved via oral administration of α -MPT (64 mg/kg). The maximal inhibition of tyrosine hydroxylase (and thus DA synthesis) with α -MPT has been observed to be ~80% (Engelman, Jequier, Udenfriend, & Sjoerdsma, 1968; Laruelle et al., 1997). Thus all α -MPT-PET studies underestimate their quantification of DA levels accordingly. Despite this underestimation, this method is still sensitive enough to elucidate differences in endogenous DA levels between healthy controls and persons with schizophrenia. While there are differences in the rate of α -MPT metabolism across individuals (Engelman et al., 1968), dosing regimens of α -MPT aim to achieve maximal tyrosine hydroxylase inhibition during PET scans. Notably, individual differences in plasma levels of α -MPT have not been found to be correlated with changes in BP_{ND} . Full details regarding the DA depletion and PET scanning protocols can be found in the parent study (Caravaggio et al., 2014). We only analyzed data from those regions of interest (ROIs) where [^{11}C]-(+)-PHNO BP_{ND} reliably increased after DA depletion. Thus, the substantia nigra (SN)/ventral tegmental area (VTA), hypothalamus, and ventral pallidum ROIs were excluded. The unique nature of these ROIs with regards to [^{11}C]-(+)-PHNO, as well as potential explanations for their paradoxical binding, has been discussed in detail elsewhere (Caravaggio et al., 2014). Briefly, based on the results of animal studies combining the use of microdialysis and PET, it has been argued that the α -MPT-PET paradigm can only quantify DA levels at synaptic $\text{D}_{2/3}\text{R}$, not extrasynaptic $\text{D}_{2/3}\text{R}$ (Breier et al., 1997; Kim & Han, 2009; Laruelle, 2000). Similarly to the retina (Hirasawa, Contini, & Raviola, 2015), there is no synaptic DA release into the SN (Bergquist & Nissbrandt, 2005), nor into the VTA (Adell & Artigas, 2004; Kalivas & Duffy, 1991; Omelchenko & Sesack, 2009). Rather, DA is stored and released extrasynaptically from the dendrites of the SN/VTA (Adell & Artigas, 2004; Cheramy, Leviel, & Glowinski, 1981; Geffen, Jessell, Cuello, & Iversen, 1976; Jaffe, Marty, Schulte, & Chow, 1998; Korf, Zielesman, M., & Westerink, 1976; Nieoullon, Cheramy, & Glowinski, 1977; Wassef, Berod, & Sotelo, 1981). Consistently, no significant change in [^{11}C]-(+)-PHNO BP_{ND} to extrasynaptic D_3R in the SN/VTA was observed after DA depletion with α -MPT (Caravaggio et al., 2014). However, one study did observe a significant increase in $\text{D}_{2/3}\text{R}$ BP_{ND} in the SN/VTA after α -MPT using [^{18}F]-fallypride (Riccardi et al., 2008). This was not confirmed in another study employing a larger sized sample (Cropley et al., 2008). Despite these discrepancies in the literature, the SN/VTA ROI for [^{11}C]-(+)-PHNO was excluded from our current analysis due to our observation of no change in BP_{ND} to extrasynaptic D_3R after α -MPT, consistent with findings from microdialysis-PET experiments. For the hypothalamus and ventral pallidum, baseline and post BP_{ND} 's could not be reliably calculated for all subjects. Despite following

the ROI segmentation guidelines of Tziortzi and colleagues (Tziortzi et al., 2011), for some subjects we observed poor SRTM model fitting (associated with noise in the time-activity curve) and no washout of the signal. While it is unclear why in our hands this occurred, it is reassuring to note that our reported BP_{ND} values in those subjects for which it was possible are in accordance with previous reports (Tziortzi et al., 2011). Coupled with a lack of test-retest reliability data for these regions (Gallezot, Zheng, et al., 2014; Lee et al., 2013; Willeit et al., 2008), these ROIs were excluded from the current analysis. Finally, it has been demonstrated that the acute DA depletion induced by the α -MPT-PET paradigm is not enough to induce a significant up-regulation in the number of striatal $D_{2/3}R$ in rodents (Laruelle et al., 1997). This is assumed to also be true for humans. In turn, given this acute DA depletion and lack of change in receptor expression, it is also unlikely to alter the affinity of $D_{2/3}R$ for agonists (Todd, Carl, Harmon, O'malley, & Perlmutter, 1996).

2.2 | Estimates of endogenous DA levels at $D_{2/3}R$

We examine three measures that estimate endogenous DA levels at baseline in the vicinity of the $D_{2/3}R$. These are computed from the change in [^{11}C]-(+)-PHNO BP_{ND} induced by DA depletion with α -MPT in the following ways.

2.2.1 | The “ α -MPT effect”—The term “ α -MPT effect” has been used (Abi-Dargham et al., 2000) to denote the percent change in baseline radiotracer binding resulting from DA depletion: $BP_{ND} = -100 * (\text{Depletion } BP_{ND} - \text{Baseline } BP_{ND}) / \text{Baseline } BP_{ND}$. The relationship of BP_{ND} to endogenous DA at baseline is based on the occupancy model, in which endogenous DA competes with the binding of radiotracers like [^{11}C]-(+)-PHNO for $D_{2/3}R$ at baseline (Laruelle, 2000; Laruelle et al., 1997; Verhoeff et al., 2001). It is assumed by this model that, (a) baseline $D_{2/3}R$ BP_{ND} is confounded by endogenous DA, such that higher concentration of DA results in lower $D_{2/3}R$ BP_{ND} , (b) $D_{2/3}R$ BP_{ND} under depletion more accurately reflects the true status of $D_{2/3}R$, and, (c) the percent increase in $D_{2/3}R$ BP_{ND} after DA depletion, BP_{ND} , is linearly proportional to the baseline DA concentration at $D_{2/3}R$, provided the process of DA depletion does not change the number and affinity of the $D_{2/3}R$. Thus, BP_{ND} , under appropriate assumptions, is considered a quantitative index of baseline endogenous DA levels at $D_{2/3}R$ (Verhoeff et al., 2001).

2.2.2 | $D_{2/3}R$ occupancy by DA—The estimate of $D_{2/3}R$ occupancy by DA is calculated as a percent change in radiotracer binding induced by DA depletion, relative to the depletion BP_{ND} : $\%Occ = 100 * (\text{Depletion } BP_{ND} - \text{Baseline } BP_{ND}) / \text{Depletion } BP_{ND}$. Since 100% DA depletion cannot be achieved with oral α -MPT, the true receptor occupancy by DA is underestimated in such paradigms. This metric has been used in some studies in lieu of calculating BP_{ND} (Martinez et al., 2009).

2.2.3 | Free synaptic DA concentration—It has been suggested that the free synaptic DA concentration (F_{DA}) (nM) can be estimated from α -MPT PET studies using the following formula: $F_{DA} = K_i(a - 1)$, where “ a ” is Depletion BP_{ND} /Baseline BP_{ND} , and K_i is the intrinsic affinity of DA to compete with the radiotracer (Abi-Dargham et al., 2000; Laruelle et al., 1997). The use of the above formula to calculate F_{DA} requires several assumptions, which are to some extent violated in α -MPT studies. These include the

assumptions of, (a) achieving 100% DA depletion with α -MPT and (b) competitive inhibition between DA and the radiotracer occurring at only one receptor site (Laruelle et al., 1997). Moreover, since K_i is unknown in vivo, an in vitro estimate must be used. Thus, the formula assumes similar K_i values in vitro and in vivo. Moreover, the necessary K_i values are often not available from human postmortem tissue. Notably, the two AMPT [^{123}I]-IBZM studies, which attempted to estimate striatal F_{DA} using this formula did so using K_i values from rodent striata. Moreover, K_i in vitro differs based on the presence or absence of NaCl, and it is unclear which values are most appropriately used (Laruelle et al., 1997). Despite the limitations inherent to making assumptions about in vivo conditions from in vitro data, the above formula is currently the only one available in the literature for calculating F_{DA} from α -MPT PET data. Nevertheless, important and consistent observations have been made using this calculation of F_{DA} in healthy persons and persons with schizophrenia (Abi-Dargham et al., 2000; Laruelle, 2012; Laruelle et al., 1997). Ultimately, the usefulness of this quantification will be evidenced by its ability to produce consistent findings with the larger body of literature, and make accurate predictions for future investigations.

2.3 | Validation by estimating the kd for DA at D_{2/3}R *in vivo*

In theory, the relationship between the fraction of radiotracer bound to receptors does not increase continuously with decreasing competition from endogenous DA. Rather, it approaches an asymptotic limit, which has been simulated and described mathematically (Friedman et al., 1984). For further details on the mathematical equations describing the competition of two ligands for one receptor site, please see the work of Friedman et al. (1984). Assuming that the concentration of free synaptic DA remains constant during a respective PET scan, and then the relationship between free synaptic DA and the amount of DA bound to receptors should also approach an asymptotic limit, and be nonlinear. The relationship between the free synaptic DA concentration (F_{DA}) and receptor occupancy (%Occ) can be viewed as analogous to an in vitro saturation binding plot. These plots (known as a rectangular hyperbola, binding isotherm, or saturation binding curves) describe the binding of a ligand to a receptor as a function of increasing ligand concentration at equilibrium. The concentration of the ligand is plotted on the x-axis and the amount of specific-binding to receptors is plotted on the y-axis, with the half-maximal point of the curve signifying the equilibrium dissociation constant (Kd). Kd here signifies the concentration of ligand that occupies half the binding sites at equilibrium. This can be described using the following equation: $= \frac{B_{\text{max}} * X}{K_d + X}$; where X denotes the concentration of ligand and Y denotes specific binding. For a hypothetical saturation binding curve, please see Figure 1. Notably, Y is initially zero, and increases until it reaches the value of B_{max} . Despite the PET experiments not being conducted at equilibrium, by plotting the natural variation in the free synaptic DA concentration (x-axis) versus receptor occupancy (y-axis) across people, this may provide an estimate of Kd for DA at D₂R and D₃R in vivo in humans. These estimates can then be compared to in vitro values reported in the literature. Notably, in vitro the fraction of a ligand bound to receptors does not increase linearly with increasing concentrations of a ligand, but rather reaches an asymptotic limit (Friedman et al., 1984).

3 | RESULTS

3.1 | Relationship of baseline BP_{ND} with endogenous DA levels

The relationships between baseline BP_{ND} and three measures of baseline endogenous DA levels (BP_{ND}, %Occ, and F_{DA}) are presented in Table 1. R^2 -values in this table signify the proportion of the variance in baseline [¹¹C]-(+)-PHNO BP_{ND} which can be accounted for by endogenous DA estimated by these three quantities respectively. Notably, persons with lower [¹¹C]-(+)-PHNO BP_{ND} to D_{2/3}R at baseline demonstrated greater levels of endogenous DA occupying these receptors, and vice-versa. Thus, lower baseline values of [¹¹C]-(+)-PHNO BP_{ND} reflect greater stimulation by endogenous DA, and vice-versa Plasma levels of α-MPT (27 h) were not significantly correlated with endogenous DA levels or receptor occupancy as measured with [¹¹C]-(+)-PHNO (data not shown).

3.2 | K_d for DA at D_{2/3}R

Our estimate of F_{DA}, the free synaptic DA concentration in each ROI, used only those subjects whose BP_{ND} in a given ROI increased after DA depletion. Nine subjects provided data for the caudate, putamen, and GP ROIs, and 10 subjects provided data for the VS. To date, no study has published K_i values for DA to inhibit [³H]-(+)-PHNO binding in human striata. In canine striata, two K_i values have been reported: 4.2 (–NaCl) and 23 (+NaCl; Seeman et al., 1993). Using a K_i of 23, the F_{DA} was estimated to be the following: VS 8.34 ± 2.71 nM, Caudate 9.13 ± 4.45 nM, Putamen 8.57 ± 3.87 nM, GP 2.78 ± 1.77 nM. Using these data, we used F_{DA} to estimate the K_d for DA at D_{2/3}R in each ROI, using the saturation binding curve equation (see Figure 2). The calculated K_d values ranged from 22 to 24 nM.

4 | DISCUSSION

The PET α-MPT paradigm is currently the optimal method for quantifying endogenous DA levels in the living human brain—providing the most direct evidence for dopaminergic abnormalities in several neuropsychiatric disorders such as schizophrenia and drug addiction (Abi-Dargham et al., 2000; Kegeles et al., 2010; Martinez et al., 2009). Here, we show that these data can also provide valuable information regarding the interpretation of dopaminergic radiotracer binding during baseline conditions. Specifically, α-MPT PET data can inform to what extent a radiotracer for D_{2/3}R is sensitive to endogenous DA at baseline. Re-analyzing previously published data (Caravaggio et al., 2014), we report for [¹¹C]-(+)-PHNO the proportion of the variance in baseline BP_{ND}, which can be accounted for by endogenous DA. This ranged from ~40 to 60% across striatal ROIs. Indeed as previously suggested (Caravaggio et al., 2014, 2015a; Laruelle, 2012; Shotbolt et al., 2012), [¹¹C]-(+)-PHNO is highly sensitive to changes in endogenous DA levels, and individual differences at baseline. This sensitivity is likely much greater than that of other commonly used radiotracers like [¹¹C]-raclopride, and may explain some of the differential findings observed between agonist and antagonist radiotracers, even within the same persons (Caravaggio et al., 2015b; Narendran et al., 2011). Using this dataset, we also estimated the free synaptic DA concentration (nM), and in turn the K_d value for DA at D_{2/3}R, in the living human brain. Several in vitro studies suggest that the K_d of [³H]-DA for striatal D_{2/3}R is

about 20 nM (Burt, Creese, & Snyder, 1976; Creese, Burt, & Snyder, 1976; Durdagi, Salmas, Stein, Yurtsever, & Seeman, 2015; Komiskey, Bossart, Miller, & Patil, 1978). For example, in rodent striata it has been reported that DA has a K_d of 21.69 nM for the high affinity state and 5.64 nM for the low affinity state (Komiskey et al., 1978). These values are in line with those K_d values estimated here (22–24 nM), adding more proof of concept to the measurement of endogenous DA in vivo using PET- α -MPT.

The increased sensitivity of [^{11}C]-(+)-PHNO to changes in endogenous DA confers several advantages. For instance, it can quantify small changes in DA release which may not be captured by antagonists such as [^{11}C]-raclopride. For example, in non-human primates the same dose of nicotine (0.1 mg/kg) could significantly reduce the BP_{ND} of [^{11}C]-(+)-PHNO, but not [^{11}C]-raclopride (Gallezot, Kloczynski, et al., 2014). Thus, [^{11}C]-(+)-PHNO could reliably quantify the small increase in DA release induced by nicotine administration, while [^{11}C]-raclopride could not. Given that PET studies are usually of high cost and thus employ small sample sizes, use of [^{11}C]-(+)-PHNO lends the practical advantage of being able to detect smaller effect sized changes in DA, thus conferring greater statistical power in smaller samples (Ko et al., 2011). Thus, use of the α -MPT-PET paradigm in conjunction with [^{11}C]-(+)-PHNO may allow for a better detection of abnormal DA levels in neuropsychiatric diseases. For example, while reduced endogenous mesolimbic DA is often cited as a pathophysiological marker of depression (Salamone et al., 2016; Treadway and Zald, 2011), no differences in baseline [^{11}C]-raclopride BP_{ND} have been observed (Hirvonen et al., 2008; Meyer et al., 2006; Montgomery et al., 2007). Moreover, the α -MPT-PET paradigm has never been employed in persons with depression using a radiotracer for $\text{D}_{2/3}\text{R}$ (Bremner et al., 2003). Thus, the use of [^{11}C]-(+)-PHNO may elucidate the potential abnormalities in endogenous mesolimbic DA in persons with depression, which [^{11}C]-raclopride could not detect; at baseline and after a DA depletion challenge. This would ultimately vindicate the DA hypothesis of depression, which heretofore has yet to be directly confirmed by in vivo brain imaging.

There are several potential limitations to the current investigation. First, our sample size was relatively small, though large enough to detect a significant change in [^{11}C]-(+)-PHNO BP_{ND} after DA depletion. While we employed a typical sample size for an α -MPT-PET study, future studies aimed at quantifying endogenous DA at $\text{D}_{2/3}\text{R}$ using α -MPT and [^{11}C]-(+)-PHNO will benefit from employing larger sample sizes. Second, it is possible that individual differences in DA synthesis capacity and/or DA clearance can affect the estimate of endogenous DA using the α -MPT paradigm. In theory, every individual is given the maximum amount of α -MPT exposure which results in 80% tyrosine hydroxylase inhibition and thus DA synthesis. However, in practice this may not always be the case for all subjects. At least for the current investigation, we employed all healthy subjects of relatively young age. Thus, we expect individual differences in DA synthesis and clearance to be minimal. Notably, this is a potential limitation shared by all α -MPT-PET studies.

Though focused on [^{11}C]-(+)-PHNO, this report provides a framework for analyses of α -MPT PET studies with other $\text{D}_{2/3}\text{R}$ radioligands such as [^{11}C]-raclopride, [^{18}F]-fallypride, and [^{11}C]-NPA to evaluate endogenous DA levels at baseline. With this approach, the impact

of endogenous DA levels on single baseline-only PET studies with these radioligands can be assessed.

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FIGURE 1.

A hypothetical plot of a typical saturation binding curve used to calculate the dissociation constant (K_D) of a ligand for its target

FIGURE 2.

The in vivo dissociation constant (K_D) for DA at $D_{2/3}R$ in each region of interest

Correlations between baseline [¹¹C]-(-)-PHNO BP_{ND} and three measures of endogenous DA levels in each region of interest

TABLE 1

Region of Interest	BP _{ND} (α-MPT effect)			%Occ (receptor occupancy by endogenous DA)			FDA (free synaptic DA concentration)		
	r-value	r ²	p values	r-value	r ²	p values	r-value	r ²	p values
Caudate	-0.77	0.59	0.01 ^b	-0.75	0.56	0.01 ^b	-0.78	0.61	0.01 ^b
Putamen	-0.77	0.59	0.009 ^c	-0.75	0.56	0.01 ^b	-0.77	0.59	0.009 ^c
Ventral Striatum	-0.65	0.42	0.04 ^b	-0.70	0.49	0.02 ^b	-0.65	0.42	0.04 ^b
Globus Pallidus	-0.33	0.11	0.35	-0.35	0.12	0.32	-0.34	0.12	0.34
Covariate: Plasma AMPT (27 h)									
Caudate	-0.67	0.45	0.05 ^a	-0.65	0.42	0.06	-0.67	0.45	0.05 ^a
Putamen	-0.68	0.46	0.04 ^b	-0.64	0.41	0.06	-0.69	0.48	0.04 ^b
Ventral Striatum	-0.81	0.66	0.008 ^c	-0.85	0.72	0.004 ^c	-0.81	0.66	0.009 ^c
Globus Pallidus	-0.32	0.10	0.40	-0.33	0.11	0.39	-0.33	0.11	0.39

^aDenotes trend level significance (two-tailed).
^bDenotes significance at $p < 0.05$, two-tailed.
^cDenotes significance at $p < 0.01$, two-tailed.