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Selectivity of probes for PET imaging of dopamine D3 receptors

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Abstract

Dopamine D3 receptors have key roles in behavioral reward, addiction, Parkinson's disease, and schizophrenia, and there is interest in studying their role in these disorders using PET. However, current PET radiotracers for studying D3 receptors in humans all bind to both D2 and D3 due to similarities between the two receptors. Selective D2 and D3 radioligands would aid investigation of the differences between D2 and D3 circuitry in the central nervous system. While there are currently *in vitro* measures of ligand D3/D2 selectivity, there is a need for an *in vivo* PET measure of D3/D2 selectivity. This review discusses current PET imaging of dopamine D2/D3 receptors and proposes methodology for quantitating *in vivo* selectivity of probes for PET imaging of dopamine D3 receptors.

Keywords

dopamine; D3 receptors; PET radiotracers

1. Introduction

Dopaminergic pathways in the human brain are integral for behavioral reward, motivation, emotion, and movement coordination. Dysfunction of these circuits has been implicated in many neurological disorders including drug and alcohol dependence, Parkinson's disease, schizophrenia, and attention deficit hyperactivity disorder (ADHD) [1–7]. Dopamine receptors are divided into two major types, D1-like (D1 and D5) and D2-like (D2, D3, and D4) [8], with the D2 family of receptors often targeted by medications that are used clinically to treat neuropsychiatric disorders [3].

The cloning of the dopamine D3 receptor by Sokoloff et al. in 1990 [9] has proven to be an important milestone in dopamine receptor pharmacology. For example, the observation that typical antipsychotics have a higher *in vitro* binding affinity for D2 versus D3 receptors whereas atypical antipsychotics displayed a similar affinity for both receptor subtypes [9]

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Conflicts of Interest: none

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implicated the D3 receptor as a target for drug development. The high localization of the D3 receptor in limbic structures in brain [9–12] and that dopamine had a much higher affinity for the D3 versus D2 receptor [9], further implicated its role in other neuropsychiatric disorders. However, the high sequence homology in the transmembrane domains (~80%) [2, 3, 13], the region thought to bind dopamine and other small molecules, presented a serious challenge towards developing compounds having a high selectivity for the D3 receptor.

A second advance came with the discovery that conformationally-flexible benzamide analogs having an *N*-phenylpiperazine moiety possessed good affinity and modest selectivity for D3 versus D2 receptors [3, 14–16]. This observation spawned a significant effort by medicinal chemists to develop antagonists having both a high D3 affinity and excellent selectivity versus D2 receptors [2, 17]. The high selectivity of the resultant compounds was attributed to the binding of the benzamide fragment to a “secondary binding site” which was initially identified through *in silico* modeling studies of the D3 and D2 receptors and later confirmed from the X ray crystal structure of the human D3 receptor with eticlopride, a high affinity D2/D3 antagonist, bound to the orthosteric binding site [18–20]. The crystal structure has led to a further identification of D3 ligands through computational screening of large compound libraries [21].

The availability of D3-selective ligands has enabled study of the distinct roles of D2 and D3 receptors both *in vivo* and *in vitro*. For example, behavioral pharmacology studies have indicated that the D3 receptor plays an important role in mediating the reinforcing effects of psychostimulants such as cocaine [17, 22]. Several *in vivo* studies of the efficacy of D3 receptor-targeting treatments for schizophrenia and addiction are now underway [3, 23]. Furthermore, *in vitro* autoradiography studies in control human brain sections and diffuse Lewy body disease (DLBD) including both dementia with Lewy bodies and Parkinson disease dementia revealed an increase in D3 receptor density in striatal regions but no change in D2 receptors [24], and the D3 receptor is thought to play a key role in mediating L-DOPA-induced dyskinesia [24–26].

PET is a functional imaging modality that is often used to image receptor availability and function, with D2-like receptors being the most comprehensively studied receptors in the central nervous system. However, the ability to investigate the unique roles of D2-like receptors in dopaminergic pathways has been limited by current radiotracers binding to both D2 and D3 receptors. Reports of PET imaging of a D3-selective radiotracer in nonhuman primates [27] coupled with identification of five distinct scaffolds for high affinity D3-selective ligands [28–32] motivate this review of the selectivity of probes for PET imaging of dopamine D3 receptors and proposed method for assessing the *in vivo* selectivity of D3-targeting radioligands.

2. Current PET imaging of dopamine D3 receptors in humans

There are four radiotracers that have been largely used in human PET imaging studies that primarily target only D2-like receptors: 1) [¹¹C]raclopride [33]; 2) [¹¹C]FLB 457 [34]; 3) [¹⁸F]fallypride [35]; and 4) [¹¹C]-(+)-PHNO [36]. [¹¹C]Raclopride, a non-selective dopamine D2/D3 antagonist initially reported by the group at the Karolinska Institute in the

mid-1980s, is the most established [37, 38] of the four. [^{11}C]FLB 457, a non-selective dopamine D2/D3 antagonist, has a higher signal-to-background uptake in extrastriatal regions than [^{11}C]raclopride [34]. However, in 2011 a D2/D3 blocking study with aripiprazole reported [^{11}C]FLB 457 has significant specific binding in the cerebellum reference region and advised caution when interpreting results calculated using popular, blood-sample-free, reference tissue methods [39]. [^{18}F]Fallypride, a non-selective dopamine D2/D3 antagonist developed about a decade later than [^{11}C]raclopride, has a low level of nonspecific binding and has the advantage of having a longer 110 min half-life of fluorine-18. Consequently, [^{18}F]fallypride is the preferred radiotracer for imaging extrastriatal D2-like receptors [35, 40, 41]. [^{11}C]Raclopride has an affinity for D2 and D3 receptors in the low nM range whereas [^{18}F]fallypride has a sub-nM affinity for both receptors [42] as shown in Figure 1. Therefore, PET studies with [^{11}C]raclopride and [^{18}F]fallypride provide a measurement of the D2/D3 binding potential [43–46]. [^{11}C]Raclopride PET brain imaging proved instrumental in understanding D2/D3 receptor changes that occur in cocaine dependency, nicotine use, and Parkinson's disease [47, 48]. Furthermore, the availability of these tracers permitted dynamic visualization and measurement of dopamine release in response to a given challenge such as smoking or methylphenidate [49, 50]. More recently, such challenges have been visualized as dynamic movies [51]. Both [^{11}C]raclopride and [^{18}F]fallypride have been used to determine receptor occupancy of antipsychotics used in the treatment of schizophrenia [52, 53].

[^{11}C]-(+)-PHNO has been described as a full agonist at both D2 and D3 receptors [54, 55]. [^{11}C]-(+)-PHNO was initially developed as a PET radiotracer for imaging the high affinity state of the D2 receptor (i.e., $^{\text{high}}\text{D2}$) as a means of measuring synaptic dopamine changes in response to pharmacologic challenge [56, 57]. Subsequent studies revealed that there was no difference in the displacement of [^{11}C]-(+)-PHNO and [^3H]raclopride by dopamine antagonists and agonists; these data led the investigators to conclude that PHNO was a nonselective D2/D3 ligand, and questioned the existence of the $^{\text{high}}\text{D2}$ in vivo [58, 59]. PET imaging studies in humans revealed that the regional distribution of [^{11}C]-(+)-PHNO was different from [^{11}C]raclopride [60]. Since [^{11}C]-(+)-PHNO is thought to have a higher D3 affinity than [^{11}C]raclopride, these results were explained by [^{11}C]-(+)-PHNO labeling a higher population of D3 receptors, and subsequently led to the description of [^{11}C]-(+)-PHNO as a D3-preferring radioligand [54, 61]. Like [^{11}C]raclopride and [^{18}F]fallypride, [^{11}C]-(+)-PHNO has been used to determine the in vivo receptor occupancy of drugs [62]. Because of the overlap with D2, investigators using [^{11}C]-(+)-PHNO have used independent component analysis to help tease out the D3 signal [63, 64]. This approach remains sub-optimal due to co-expression of both D3 and D2 dopamine receptor subtype in the same brain regions [65].

PET imaging of human uptake of what may be the first D3-selective radiotracer [^{18}F]fluortripride ([^{18}F]FTP) [27] are under way, but no results have been published to date and therefore human uptake results of this new radiotracer are too preliminary to be included in this review.

3. Assessing selectivity of radiotracers between dopamine D2 and D3 receptors

As stated above, D2 and D3 receptors have an 80% sequence homology within the seven transmembrane regions that contain the ligand binding regions [2, 3, 13], which has made it difficult to develop a D3-selective radiotracer. Dopamine D3/D2 selectivity has been defined by MacDonald et al. as the antilogarithm of the difference between D3 and D2 pK_i values [66]. This definition of D3/D2 selectivity is equivalent to the ratio of a ligand's D2 K_i value to its D3 K_i value that has been reported by other groups [67]. While the MacDonald et al. *in vitro* D3/D2 selectivity metric or equivalent is widely reported when discussing new D3-targeting ligands, this measure may not be optimal for assessing D3/D2 selectivity *in vivo* due to complications caused by receptor occupancy from endogenous dopamine. This is discussed in greater detail in the following subsections.

3.1. Metrics for quantitating radiotracer selectivity of D3 over D2 receptors

The currently used D2/D3 radiotracers of [¹⁸F]fallypride [35], [¹¹C]-(+)-PHNO [36], and [¹¹C]raclopride [33] each have different degrees of D2 to D3 receptor selectivity with [¹¹C]-(+)-PHNO often described as a “D3-preferring” [28, 54, 61, 63, 68, 69] PET tracer. A commonly reported *in vitro* measure of a radioligand's relative D2/D3 affinity for these two dopamine receptors is the ratio of receptor affinities in Figure 1 (D2/D3 K_i ratios of 0.1, 0.4, and 0.5, respectively). However, using these *in vitro* D2/D3 K_i ratios would suggest [¹¹C]raclopride is similar to [¹¹C]-(+)-PHNO, which is not supported by amphetamine challenge studies in humans, which suggested that [¹¹C]-(+)-PHNO's higher selectivity for the D3 receptor accounted for its higher sensitivity than [¹¹C]raclopride to fluctuations in extracellular dopamine levels [28]. Investigators of [¹¹C]-(+)-PHNO have proposed *in vivo* D3/D2 selectivity methodologies for assessing the percentage of D3- relative to D2-receptor binding in regional [¹¹C]-(+)-PHNO BP_{ND} values based on blocking studies with unlabeled (+)-PHNO in nonhuman primates (n=2) [70] and with the D3-selective blocker GSK598809 in humans (n=19) [63]. Both the Tziortzi et al. and Gallezot et al. methods estimate that 100% of [¹¹C]-(+)-PHNO BP_{ND} is due to occupying D3 receptors in the substantia nigra while 0% of [¹¹C]-(+)-PHNO BP_{ND} is due to binding to D3 receptors in putamen [63, 70]. The [¹¹C]-(+)-PHNO methods' *in vivo* estimates of 100% and 0% binding to D3-receptors (or 0% and 100% binding to D2-receptors) in the substantia nigra and putamen, respectively, do not correspond to *in vitro* measures of 78% and 33% for the substantia nigra and putamen from relative D2 and D3 receptors densities in the human brain from autoradiography [65]. These discrepancies between reported *in vitro* D2/D3 binding affinities and *in vivo* observations may be due to differences in the densities of receptors *in vitro* (B_{max}) and the densities of receptors available to bind radioligands *in vivo* (B_{avail}). Therefore, another metric is needed to assess the relative B_{avail} of D2 over D3 receptors that can still be understood in terms of *in vitro* measures of B_{max}. Ideally, this *in vivo* measure of selectivity would make use of readily available PET imaging measures in the literature (e.g. binding potentials, BP), not require the D3-receptor blocking study results used by the [¹¹C]-(+)-PHNO D3/D2 selectivity methods, and be a unitless ratio to minimize differences in experimental methods between studies. We propose the following selectivity ratio, S_{D2:D3}, for assessing the relative selectivity of radiotracers for D2 and D3 receptors:

$$S_{D2:D3} = \frac{B_{avail,1}}{B_{avail,2}} = \frac{\frac{BP_{ND,1}K_D}{f_{ND}}}{\frac{BP_{ND,2}K_D}{f_{ND}}} \quad \text{Equation 1}$$

where $B_{avail,1}$ is the density of receptors available for radiotracer binding in region 1, BP_{ND} is the nondisplaceable uptake binding potential, K_D is the radioligand equilibrium dissociation constant, and f_{ND} is the free fraction in nondisplaceable compartment as defined by Innis et al. [71] If the K_D and f_{ND} are assumed to be equal in the two regions in Equation 1, the equation simplifies to:

$$S_{D2:D3} = \frac{BP_{ND,1}}{BP_{ND,2}} \quad \text{Equation 2}$$

3.2. Region selection for quantitating radiotracer in vivo D3:D2 selectivity

When selecting reference regions for $S_{D2:D3}$, we recommend choosing one region where D2 receptor density is higher than D3 and a second region in which D3 receptor density is higher than D2 to magnify the differences in D2:D3 selectivity between compared radiotracers. An autoradiography study measuring the density of D2 and D3 receptors in different human brain regions (Figure 2) was used to guide selection of optimal regions for Equation 2. In this study, the densities of D2 receptors were at least 1.7-fold higher than for D3 receptors in the caudate, putamen, and nucleus accumbens [65] while the thalamus, substantia nigra, and red nucleus had at least a 3-fold higher density of D3 receptors to D2 receptors [65]. Out of these two sets of candidate regions, the putamen and thalamus were selected as regions one and two in Equation 2. The putamen was selected due to its high D2:D3 ratios (Table 1 and Figure 2B), as well as the consistency of its D2:D3 ratio between pre- and postcommissural locations. PET imaging measures from the putamen are subject to less partial volume error than that of the smaller nucleus accumbens and the caudate which is adjacent to the lateral ventricles. The thalamus was selected as a representative D3-rich region due to it possessing the highest D3:D2 ratio of any brain region reported [65] (Figure 2B) and, since it is much larger than the substantia nigra and red nucleus regions, PET measures from this region are subject to less partial volume error. For these reasons, the putamen and thalamus were used as the reference regions in Equation 2 to calculate $S_{D2:D3}$ in the following section.

3.3. Comparing radiotracer D2:D3 selectivity in vivo to autoradiography

Using B_{max} densities of D2 and D3 receptors in the human brain from autoradiography [65], one can calculate the theoretical $S_{D2:D3}$ of a perfectly D2-selective, D3-selective, and nonselective radiotracer in the absence of any *in vivo* receptor regulation. When using the putamen and thalamus as regions one and two, respectively, a D2-selective radiotracer will have a $S_{D2:D3}$ of 19.8, a D3-selective ligand will have a $S_{D2:D3}$ of 1.2, and an equipotent, nonselective D2/D3 radiotracer will have a $S_{D2:D3}$ of 3.2 (Table 1). This indicates D3-

selectivity and $S_{D2:D3}$ values are inversely proportional when using these two reference regions. Studies employing [^{18}F]fallypride, [^{11}C]raclopride, [^{11}C]-(+)-PHNO, and [^{11}C]FLB 457 have average $S_{D2:D3}$ values ($\pm$ standard deviation) of 10.3 ± 2.0 , 7.1 ± 1.1 , 6.0 ± 1.0 , and 4.1 respectively (Table 2), which suggests [^{11}C]-(+)-PHNO is more D3-perfering than [^{11}C]raclopride and in agreement with the amphetamine challenge study where [^{11}C]-(+)-PHNO binding was more sensitive than [^{11}C]raclopride to fluctuations of dopamine [28]. However, based on the autoradiography $S_{D2:D3}$ values, [^{18}F]fallypride, [^{11}C]raclopride, [^{11}C]-(+)-PHNO, and [^{11}C]FLB 457 are all nonselective D2/D3 radioligands, with a preference for the D2 receptor, since the $S_{D2:D3}$ for each radiotracer for all is between 3.2 and 19.8. In contrast to the widely used [^{18}F]fallypride, [^{11}C]raclopride, [^{11}C]-(+)-PHNO, and [^{11}C]FLB 457 radiotracers, the D3-selective radiotracer [^{18}F]fluortripride ([^{18}F]FTP) [27] has a $S_{D2:D3}$ of 0.91 (Table 1) for nonhuman primates, which is close to the predicted ratio of 1.2 for D3-selective radiotracers from autoradiography data [65] in the second row of Table 1.

3.4. Regional differences in D3 receptor roles

D3 receptors may fulfill different dopaminergic functions depending on receptor location within a neuron. Presynaptic D3 receptors (i.e. autoreceptors) generally provide a negative feedback mechanism for reducing the release of dopamine [72] while postsynaptic D3 receptors bind dopamine as part of dopaminergic signal transduction. The signal from D3 probe uptake may be a combination of binding to both pre- and postsynaptic D3 receptors, but regulation of these receptors may differ by brain region due to factors such as dopaminergic neuronal loss [24]. Figure 3 illustrates how D3 probe uptake can be higher in striatal regions (Figure 3A, 3F) while being lower in the substantia nigra (Figure 3B, 3G) when comparing subjects with diffuse Lewy body disease (DLBD) to age matched controls. Regional differences in D3 probe uptake in Figure 3 highlight the need to determine D3 receptor uptake in every region of interest rather than using one region such as the substantia nigra as an index of brain D3 levels [73] and assuming this index allows estimation of relative D3 levels in other areas containing more equal densities of D2 and D3 receptors.

4. Future work and challenges

4.1 D3-targeting medications and preclinical imaging of D3 receptor selective PET radiotracers

Many groups are actively pursuing D3-selective medications with some success *in vivo* but there are currently no D3-selective treatments approved by the FDA for clinical use. Le Foll and colleagues used the D3 receptor antagonist SB-277011-A to mediate short nicotine-seeking behavior in rats [74]. ABT-925, a D3 antagonist, demonstrated some success in humans at attenuating schizophrenic symptoms that was affected by the S9G genotype, a single nucleotide polymorphism implicated in altering the D3 receptor affinity for dopamine [75]. GSK598809, another D3 receptor antagonist, has shown promise at mitigating abnormal reward seeking behavior in drug and alcohol dependence as well as eating behavior in humans [76–78].

Studies of [^3H]FTP *in vitro* show that [^3H]FTP is specific for the D3 receptor and does not label D2 receptors [79]. These studies found affinity of [^3H]FTP for *D2 receptors* was too low to measure a K_D value in Scatchard studies with the cloned D2 receptor. To confirm that [$^3\text{H}/^{18}\text{F}$]FTP does not label D2 receptors, a series of direct binding experiments using increasing amounts of HEK-293 cell membranes expressing human hD2 receptors in the presence of a constant amount of hD3 dopamine receptors expressed in HEK-293 cells and a constant concentration of radioligand [79]. These studies were performed using [^3H]FTP and two radioligands which bind to D2 and D3 dopamine receptors with essentially equal affinity: a) [^{125}I]IABN (pM affinity at D2 and D3 receptors) and b) [^3H]raclopride (Figure 4) [79]. (+)-Butaclamol was used to define nonspecific binding. Increased specific binding was observed when increasing amounts of D2 dopamine receptors were added to the binding assay when the nonselective radioligands [^{125}I]IABN (Figure 4A) and [^3H]raclopride (Figure 4B) were used. However, specific binding did not increase when [^3H]FTP was used (Figure 4C). This confirmed that [$^3\text{H}/^{18}\text{F}$]FTP is specific for D3 receptors and does not label D2 receptors. Subsequent studies in nonhuman primates showed high uptake in D3-rich regions such as the thalamus [27] and first-in-human studies of [^{18}F]FTP uptake are underway. One complication of [^{18}F]FTP which could limit its utility in translational imaging studies is its high affinity for the serotonin 5-HT1A receptor [13], which is expressed in high density in human brain [80] and could overwhelm the signal from the D3 receptor.

In addition to D3-selective piperazine analogues [67] like the [^{18}F]FTP radiotracer [27], four alternative D3-selective receptor antagonist scaffolds have recently been proposed by the laboratories of Heidbreder [29–31] and Mach [32]. The emergence of five distinct scaffolds for D3-selective ligands suggest multiple D3-selective radiotracers may soon be available to facilitate development of D3-targeting medications and motivates development of methods, such as those proposed in section 3, to evaluate the emerging radiotracers relative selectivity for the D3.

4.2 Conclusion

Over the past three decades, PET imaging of dopamine D2/D3 receptors using [^{11}C]raclopride, [^{18}C]fallypride, and [^{11}C]-(+)-PHNO has provided important insights into normal neurobiology as well as pathophysiology of neurological disease including addiction, Parkinson's disease, ADHD, and schizophrenia. We have proposed a method for assessing the D3/D2 selectivity of radiotracers for *in vivo* applications that can be understood in terms of the traditional *in vitro* measures of receptor selectivity. Regional differences in D3 receptor roles and D2/D3 receptor densities illustrate the importance of using a D3-selective radiotracer to distinguish D3 function and avoid potentially confounding effects of region-dependent nonselective D2/D3 binding. However, there are currently no radiotracers that are indisputably selective for the D3 receptor and approved for clinical use. Early results from preclinical and early clinical trials of D3-targeting medications and PET imaging radiotracer studies are encouraging, but more research is required to evaluate efficacy in humans.

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Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
BP	binding potential
BP_{ND}	Binding Potential (Non-Displaceable)
DLBD	diffuse Lewy body disease including both dementia with Lewy bodies and Parkinson disease dementia
FTP	Fluortriopride
FLB 457	((S)-N-((1-ethyl-2-pyrrolidinyl)methyl-5-bromo-2,3-dimethoxybenzamide
[¹¹C]-(+)-PHNO	[¹¹ C]-(+)-Propyl-Hexahydro-Naphtho-Oxazin

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Highlights

1. Method to assess in vivo selectivity of ligands targeting dopamine D3 receptors
2. Understanding differences between D3 and D2 circuitry is important
3. D3 receptor probe development has been challenging due to similarities with D2

Figure 1.

Structures of Positron Emission Tomography (PET) radiotracers for imaging dopamine D3 receptors and their associated receptor affinities (K_i). The reported D2/D3 values are equivalent to Macdonald et al. D3/D2 selectivity values [66].

Figure 2.

Differential density of dopamine D2 and D3 receptors in human brain from autographic analyses with part A reproduced with permission from Figure 1 in Mach et al. [13] with labeled brain regions of caudate (CD), putamen (PU) nucleus accumbens (NA), internal capsule (IC), and thalamus (Th)) and part B adapted from data in Sun et al. [65].

Figure 3.

Quantitative autoradiographic analysis of dopamine D3 receptor density in the striatal regions and substantia nigra of subjects with diffuse Lewy body disease (DLBD) and age-matched controls. Autoradiograms show total binding of 3.54 nM [^3H]WC-10 (A, B) and nonspecific binding in the presence of 1 μM S(-)-eticlopride (C, D) in striatal regions (A, C) and substantia nigra (B, D) in DLBD and age-matched controls. [^3H]Microscale standards (ranging from 0 to 36.3 nCi/mg) were also counted (E). Quantitative analysis of the dopamine D3 receptor density (fmol/mg) in the striatal regions and substantia nigra of DLBD and age-matched controls are shown in F and G respectively. The numbers 1–4 designate the following regions: Putamen (1); Caudate (2); Nucleus Accumbens (3); Substantia Nigra (4). * $p < 0.05$ for DLBD vs. control (reproduced with permission from Figure 4 in Sun et al. [24]).

Figure 4.

Binding of $[^{125}\text{I}]\text{IABN}$, $[^3\text{H}]\text{raclopride}$ and $[^3\text{H}]\text{FTP}$ to hD3 receptors in the presence and absence of increasing amounts of hD2 dopamine receptors expressed in HEK-293 cells (adapted from Figure 9 in Rangel-Barajas et al. [79]).

Table 1

Dopamine D2 and D3 receptor densities from an autoradiography study [65] and *in vitro* $S_{D2:D3}$ for putamen and thalamus regions.

Dopamine receptor	Putamen B_{max} (fmol/mg)	Thalamus B_{max} (fmol/mg)	Putamen:Thalamus $S_{D2:D3}$ (unitless)
D2	79.0 [*]	4	19.8
D3	38.5 [*]	33	1.2
D2 + D3	117.5 [*]	37	3.2

^{*} Receptor density results are an average of pre- and post-commissural values.

Table 2

$S_{D2:D3}$ and BP_{ND} from putamen and thalamus regions for D2/D3 PET radiotracers ^{*}.

Radiotracer	Putamen BP_{ND} (unitless)	Thalamus BP_{ND} (unitless)	$S_{D2:D3}$ [*] (unitless)	Reference
[¹⁸ F]Fallypride (n=6, human)	27.2 [†]	3.23 [†]	8.4	Mukherjee et al. [35]
[¹⁸ F]Fallypride (n=11, human)	37.0 [†]	3.44 [†]	10.8	Kessler et al. [81]
[¹⁸ F]Fallypride (n=22, human)	34.3 [†]	2.59	13.2	Kegeles et al. [82]
[¹⁸ F]Fallypride (n=14, human)	38.3	4.58	8.4	Riccardi et al. [83]
[¹⁸ F]Fallypride (n=50, human)	21.6	2.00	10.8	Cumming et al. [84]
[¹¹ C]Raclopride (n=7, human) [‡]	4.41	0.67	6.7	Alakurtti et al. [85]
[¹¹ C]Raclopride (n=7, human) [§]	4.44	0.70	6.3	Alakurtti et al. [86]
[¹¹ C]Raclopride (n=5, human)	2.79	0.32	8.7	Black et al. [87]
[¹¹ C]Raclopride (n=10, human)	2.98	0.45	6.6	Shotbolt et al. [28]
[¹¹ C]-(+)-PHNO (n=13, human)	2.24	0.47	4.8	Shotbolt et al. [28]
[¹¹ C]-(+)-PHNO (n=19, human) ^{//}	2.37	0.39	6.1	Searle et al. [69]
[¹¹ C]-(+)-PHNO (n=19, human) ^{//}	2.19	0.37	5.9	Tziortzi et al. [63]
[¹¹ C]-(+)-PHNO (n=42, human)	2.62	0.36	7.3	Matuskey et al. [88]
[¹¹ C]FLB 457 (n=8, human)	7.82 [¶]	1.93 [¶]	4.1	Olsson et al. [89]
[¹⁸ F]FTP (n=4, preclinical NHP)	0.21	0.23	0.91	Mach et al. [27]

^{*} $S_{D2:D3}$ is the Putamen:Thalamus BP_{ND} ratio as defined by Equation 2.

[†] BP_{ND} results are the mean of subregion values for the indicated brain region.

[‡] BP_{ND} results are the mean of 2 scans conducted on the same day for a total of 14 scans.

[§] BP_{ND} results are the mean of 2 scans conducted 5 weeks apart for a total of 14 scans.

^{//} The 19 subjects in the Tziortzi et al. study may be the same subjects in the Searle et al. study.

[¶] Only BP_{ND} s calculated using an input function from arterial blood samples reported here since [¹¹C]FLB 457 was later found to have significant specific binding in the cerebellum reference region [39].