Brain Region Binding of the D_{2/3} Agonist [¹¹C]-(+)-PHNO and the D_{2/3} Antagonist [¹¹C]Raclopride in Healthy Humans

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Abstract: The D₂ receptors exist in either the high- or low-affinity state with respect to agonists, and while agonists bind preferentially to the high-affinity state, antagonists do not distinguish between the two states. [¹¹C]-(+)-PHNO is a PET D₂ agonist radioligand and therefore provides a preferential measure of the D₂^{high} receptors. In contrast, [¹¹C]raclopride is an antagonist radioligand and thus binds with equal affinity to the D₂ high- and low-affinity states. The aim was to compare the brain uptake, distribution and binding characteristics between [¹¹C]-(+)-PHNO and [¹¹C]raclopride in volunteers using a within-subject design. Both radioligands accumulated in brain areas rich in D₂/D₃-receptors. However, [¹¹C]-(+)-PHNO showed preferential uptake in the ventral striatum and globus pallidus, while [¹¹C]raclopride showed preferential uptake in the dorsal striatum. Mean binding potentials were higher in the putamen (4.3 vs. 2.8) and caudate (3.4 vs. 2.1) for [¹¹C]raclopride, equal in the ventral-striatum (3.4 vs. 3.3), and higher in the globus pallidus for [¹¹C]-(+)-PHNO (1.8 vs. 3.3). Moreover [¹¹C]-(+)-PHNO kinetics in the globus pallidus showed a slower washout than other regions. One explanation for the preferential binding of [¹¹C]-(+)-PHNO in the globus pallidus and ventral-striatum could be the presence of a greater proportion of high- vs. low-affinity receptors in these areas. Alternatively, the observed distribution could also be explained by a preferential binding of D₃-over-D₂ with [¹¹C]-(+)-PHNO. This differential binding of agonist vs. antagonist radioligand, especially in the critically important region of the limbic striatum/pallidum, offers new avenues to investigate the role of the dopamine system in health and disease. *Hum Brain Mapp* 29:400–410, 2008. ©2007 Wiley-Liss, Inc.

Key words: positron emission tomography; dopamine; globus pallidus; ventral striatum; substantia nigra; caudate; putamen; PET Scan; HRRT

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INTRODUCTION

Dopamine neurotransmission has a central role in normal behavior, as well as in the pathophysiology of several disorders such as schizophrenia, addictions and Parkinson's disease [Grace, 2002; Nieoullon and Coquerel, 2003]. Dopamine's downstream effects are mediated through G protein-coupled receptors (GPCR) [Missale et al., 1998], and these receptors exist in two different states depending on the amount of GDP or GTP associated with the relevant G proteins: a state of high affinity (D_2^{high}) and a state of low affinity (D_2^{low}) for dopamine [Sibley et al., 1993]. The high-affinity state is believed to be the functionally active one, as it mediates the activation of the second-messenger cascade on the postsynaptic membrane [George et al., 1985; Leff, 1995]. An imbalance of the normal proportion of D_2^{high} and D_2^{low} is present in many animal models of psychosis [Seeman et al., 2002, 2005b, 2006a] and it has been proposed to be present in some disorders such as schizophrenia, addictions and Parkinson's disease [Seeman et al., 2002, 2005b, 2006a]. In vitro studies to measure D_2^{high} vs D_2^{low} can be done using a variety of techniques; these include competition assays between antagonist radiotracers and agonist ligands in the presence and in the absence of nonhydrolysable GTP analogs, the latter converting the receptor from its high-affinity state to its low-affinity state [De Keyser et al., 1985; De Lean et al., 1980; Seeman et al., 2002, 2005b]. However, none of these methods are possible in humans in vivo; moreover, the only feasible method in humans to distinguish the high- vs. low-affinity states is to use agonist radioligands with an imaging modality such as positron emission tomography (PET) or single photon emission computed tomography.

Progress in this field was recently made with the development of D_2 -agonist radioligands such as [¹¹C]-N-propylnorapomorphine ([¹¹C]-NPA) and [¹¹C]-M-N-propylnorapomorphine ([¹¹C]-MNPA), which both showed satisfactory brain signals in vivo using PET in nonhuman primates [Hwang et al., 2000, 2004; Narendran et al., 2004, 2005]. However, these ligands exhibit a low signal-to-noise ratio and as yet no human data has been reported.

[¹¹C]-(+)-4-Propyl-9-hydroxynaphthoxazine (henceforth [¹¹C]-(+)-PHNO) is a D_2/D_3 agonist PET radioligand [Wilson et al., 2005]. It shows high affinity and selectivity for the D_2 receptor with the expected agonist binding profile ($K_i = 0.2\text{--}0.5$ nM at the D_2^{high} -affinity state), which is >10-fold lower than that for the D_2^{low} -affinity state [Freedman et al., 1994; Madras et al., 1988; Seeman et al., 1993]. The pre-clinical data with PET studies done in cats has shown that [¹¹C]-(+)-PHNO displays higher striatum-to-cerebellum ratio than [¹¹C]-NPA; it is specific to D_2/D_3 -receptors and is more sensitive to the dopamine-releasing effect of amphetamine than [¹¹C]raclopride [Ginovart et al., 2006]. This radiotracer has been recently used in PET humans studies, showing specific [¹¹C]-(+)-PHNO binding and delineating brain structures known to contain D_2/D_3 -receptors such as the caudate, the putamen, the ventral

striatum, the globus pallidus and the substantia nigra [Willeit et al., 2006b]. The suitability of [¹¹C]-(+)-PHNO for research on the D_2/D_3 receptors could be confirmed by the observations that tissue data can be described using standard kinetic procedures and that simplified quantitative methods, using the cerebellum as reference, provide reliable estimates of D_2/D_3 binding parameters [Ginovart et al., in press].

Thus, while [¹¹C]-(+)-PHNO provides a reliable and replaceable measure of the D_2/D_3 receptors, how this compares to the measure of these receptors as obtained with the classical D_2 antagonist radioligand, [¹¹C]raclopride, is not known. However, preliminary results contrasting [¹¹C]-(+)-PHNO and [¹¹C]raclopride suggested a preferential binding by [¹¹C]-(+)-PHNO in the globus pallidus, ventral striatum and substantia nigra [Willeit et al., 2006b].

D_2/D_3 binding as measured by [¹¹C]raclopride is heterogeneous within the striatal regions with higher binding in the dorsal striatum than in the ventral striatum [Drevets et al., 1999, 2001; Frank et al., 2005; Martinez et al., 2003; Mawlawi et al., 2001]. This regional heterogeneity agrees with the D_2 total receptor densities shown in human post-mortem studies [Camps et al., 1989; De Keyser et al., 1988; Gurevich and Joyce, 1999; Joyce et al., 1991; Lahti et al., 1995; Murray et al., 1994].

The aims of the present study were to describe and compare, in healthy volunteers, the regional distribution of brain uptake characteristics of [¹¹C]-(+)-PHNO and [¹¹C]raclopride, using a within-subject design. To this end, images of the radiotracer uptake were normalized to standard uptake values. Brain maps of the distribution concentration of each radioligand were then generated. Regional differences in binding characteristics between the radiotracers were evaluated by statistical comparison of BP maps [Gunn et al., 1997], and by comparison of BPs obtained with a region of interest (ROI) analysis.

MATERIALS AND METHODS

Study Subjects

This study has been approved by the local Ethics Committee and the Canadian Ministry of Health, Therapeutic Products Research Department. Twelve healthy volunteers (11 males, 1 female; mean age 32.7 (SD 7.8) years) were recruited by advertisement. Written informed consent was obtained after full explanation of the study procedures and risks. Psychiatric disorders were excluded using the MINI-Plus structured interview [Sheehan et al., 1998]. Subjects with serious or unstable medical or neurological conditions or with axis I psychiatric diagnoses were not included into the study. Likewise, subjects with substance abuse other than caffeine or nicotine within 6 months prior to their baseline visit were not included. Also, routine blood and urine tests were performed during the inclusion assessment. Participants were asked to consume no more than their usual amount of coffee (and if smokers, ciga-

rettes) the day of PET examinations, and to abstain from alcohol intake 24 h before PET scans.

Standard urine tests for psychotropic substances were performed at inclusion and immediately before PET scans. Pregnancy was excluded, using serum HCG analysis at inclusion and standard urine pregnancy tests before each scan.

Study Protocol

This was an open nonrandomized study in twelve healthy volunteers. After confirming their eligibility for the study, the subjects were scheduled for two PET scans. For six of these subjects both PET scans were on the same day, at 2 h intervals, counterbalanced for [^{11}C]raclopride and [^{11}C](+)-PHNO. The PET scans for the remaining six subjects were on two separate days, at least 24 h apart.

[^{11}C](+)-PHNO and [^{11}C]raclopride Synthesis

The radiosynthesis of [^{11}C](+)-PHNO has been described in detail elsewhere [Wilson et al., 2005]. Briefly, [^{11}C]propionyl chloride was reacted with 9-hydroxynaphthoxazine to generate a [^{11}C]amide which was subsequently reduced by lithium aluminium hydride. Purification by HPLC and formulation gave radiochemically pure [^{11}C](+)-PHNO as a sterile, pyrogen-free solution suitable for human studies. [^{11}C]raclopride was synthesized as previously described by methylation of the desmethyl precursor using [^{11}C]methyl iodide [Ehrin et al., 1985; Wilson et al., 2000].

Positron Emission Tomography Imaging

Studies were performed using a high resolution head-dedicated-PET camera system, CPS-HRRT (Siemens Medical Imaging, Knoxville, TN), which measures radioactivity in 207 brain slices with a thickness of 1.2 mm each. This camera system consists of 8 panel detectors, each panel being composed of 117 phoswich detectors, arranged 13 in the axial plane by 9 in the radial plane. The in-plane resolution of the scanner was approximately 2.8 mm full-width-at-half-maximum (FWHM). Transmission scans were acquired using a ^{137}Cs ($T_2 = 30.2$ yr, $E_\gamma = 662$ KeV) single photon point source to provide attenuation-correction, and the emission data were acquired in list mode. The raw data were reconstructed by filtered-back projection. A custom-fitted thermoplastic mask (Tru-Scan Imaging, Annapolis) was made for each subject and used with a head fixation system during PET scans to avoid any movement during the acquisition. After being placed on the scanning table, 359 ± 37 MBq (9.7 ± 1 mCi, range: 7.4–11.4 mCi) with a specific activity of 1213 ± 278 mCi/ μmol (injected mass: 1.9 ± 0.5 μg ; range: 1.9–2.6 μg) of [^{11}C](+)-PHNO was injected as a bolus followed by a flush with 2 mL saline into an intravenous line placed in an antecubital vein. Scanning time was 90 min in list mode, and then 30 frames were defined: 1 to

15 of 1 min duration and 16–30 of 5 min duration. The [^{11}C]raclopride dosage was 370 ± 26 MBq (10 ± 0.7 mCi) (range: 8.99–11.0) with a specific activity of 1124 ± 433 mCi/ μmol (injected mass: 4.4 ± 2.6 μg ; range: 2.1–9.7 μg), injected as a bolus immediately followed by a flush with 2 mL saline. The total scanning time was 60 min in list mode, and then 28 frames were defined: 1 to 5 of 1 min, 6–25 of 2 min and 26–28 of 5 min.

MRI Imaging

Subjects undertook a proton density image (TE = 17, TR = 6000, FOV = 22 cm 2D, 256×256 , slice thickness of 2 mm, NEX = 2) acquired on a 1.5T Signa-GE scanner. These images were used for the analysis of the PET scans.

Image Analysis

Time activity curves (TACs) from regions of interest (ROIs) (caudate, putamen, globus pallidus, substantia nigra, cerebellar cortex (hereafter referred to as cerebellum) [Rusjan et al., 2006a,b] and ventral striatum [Mawlawi et al., 2001], were obtained from the dynamic [^{11}C](+)-PHNO or [^{11}C]raclopride PET images in native space with reference to co-registered MRI image. The co-registration of the MRI to the PET space image was done using the normalized mutual information algorithm [Studholme et al., 1997] as implemented in SPM2 (SPM2, Wellcome Department of Cognitive Neurology, London; <http://www.fil.ion.ucl.ac.uk/spm>). The TACs were obtained using an in-house software for semi-automated generation of ROIs [Rusjan et al., 2006a]. To obtain a quantitative estimate of binding, the TACs were analyzed using the Simplified Reference Tissue Method (SRTM) [Lammertsma and Hume, 1996] with the cerebellum as reference region. This method has been validated to **reliable estimate** the BP for both [^{11}C]raclopride [Lammertsma et al., 1996] and [^{11}C](+)-PHNO [Ginovart et al., in press]. The BPs were estimated using the PMOD v2.7 software [PMOD Technologies, Zurich, Switzerland]. SRTM provides an estimate of the BP of the radiotracer, which is proportional to the more fundamental parameters of receptor density (B_{max}) and dissociation constant (K_d) [$\text{BP} = f_2 B_{\text{max}}/K_d$] (f_2 , free fraction in the nonspecific distribution). Our previously estimated [^{11}C]raclopride test–retest scan reliability from 13 healthy controls, did not show significant differences between BPs of scan one and scan two in any of the ROIs. The absolute variability was 10.5% for caudate, 9.7% for putamen, 11.6% for ventral striatum, and 16.5% for the globus pallidus. The test–retest scan reliability for [^{11}C](+)-PHNO from 9 healthy controls, did not show significant differences between scan one and scan two in any of the ROIs. The absolute variability was 8.7% for caudate, 9.9% for putamen, 18.6% for ventral striatum, and 21.3% for globus pallidus [Willeit et al., 2006a].

The Parametric voxelwise BP maps from the dynamic images in native space were generated according to the

Figure 1.

Brain distribution of [¹¹C]-(+)-PHNO and [¹¹C]raclopride shown as mean standard uptake value (SUV) images. Arrows indicate, in axial views, the substantia nigra (1), globus pallidus (2) and ventral striatum (3). The lower threshold for the images corresponds to the mean SUV activity in the cerebellum. The sagittal view indicates the levels of the axial (1 and 2) and coronal (3) projections. The images correspond to the average of the twelve healthy controls.

method of Gunn et al. [1997] with the cerebellum as reference region and as implemented in PMOD v2.7 software (PMOD Technologies, Zurich, Switzerland). The BP map images were spatially normalized into the Montreal Neurological Institute (MNI) brain space by Nearest Neighbor

interpolation and with a voxel size fixed in $2 \times 2 \times 2 \text{ mm}^3$ using the SPM2 software [Friston, 1995, 1996] (SPM2, Wellcome Department of Cognitive Neurology, London; <http://www.fil.ion.ucl.ac.uk/spm>). For analysis of the uptake distribution with these two radiotracers we generated “concentration distribution maps” [Rosa-Neto et al., 2004]. These distribution maps were calculated from the voxelwise BP maps [Gunn et al., 1997] spatially normalized into the MNI brain space. The images were proportionally scaled to the mean striatal (caudate, putamen, ventral striatum, and globus pallidus) value as 100% for each radiotracer and overlay over the MNI T1 template. Additionally, images of the radiotracer uptake distribution were converted from nCi/cc to standard uptake values (SUV) images spatially normalized into MNI space. The SUV images were generated with the following formula: $\text{SUV} = [\text{Mean radioactivity image (nCi/cc)}] / [\text{injected radiotracer dose (mCi)} / \text{body weight (Kg)}]$. The images are shown with a fixed lower threshold that corresponds to the mean SUV in the cerebellum.

Statistical analysis

The comparison between [¹¹C]-(+)-PHNO and [¹¹C]raclopride BP maps was performed with a paired-*T* test using SPM2. We reported areas only if they met the joint criteria of: (a) $P < 0.001$; (b) an extent ≥ 10 voxels; and (c) in the ROIs of caudate, putamen, globus pallidus, ventral striatum, and substantia nigra. Additionally, the statistically significant a priori regions were then corrected for multiple comparisons using the false discovery rate [FDR] approach [Genovese et al., 2002].

Figure 2.

Binding concentration distribution maps showing the percentage of striatal uptake distribution with [¹¹C]-(+)-PHNO and [¹¹C]raclopride. The percentage images correspond to axial projections rendered over a T1 template in the MNI space. The

images correspond to the average of the twelve healthy controls. Z, corresponds to the millimeters above (+) or below (−) the anterior commissure in the AC-PC plane.

Figure 3.

Time activity curves (TAC) showing $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$ (a) and $[^{11}\text{C}]\text{raclopride}$ (b) binding kinetics in the caudate, putamen, ventral striatum, globus pallidus, cerebellum and substantia nigra (only in $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$). The TACs correspond to the average of the twelve healthy controls. Y axes represent standard uptake values.

The ROI analysis was made using the Statistical Program for the Social Sciences (version 12.0; SPSS, Chicago, IL). BPs were presented as mean $\pm$ standard deviation (SD). Analysis of variance with Bonferroni correction for multiple comparisons as a post-hoc were performed to compare the BPs between radiotracers per ROI (caudate, putamen, globus pallidus, ventral striatum, and substantia nigra), and BPs between ROIs per radiotracer ($[^{11}\text{C}]\text{raclopride}$ and $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$). The significance was assumed at $P < 0.05$.

RESULTS

Brain Distribution of Radiotracers

Both the $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$ and the $[^{11}\text{C}]\text{raclopride}$ PET images showed a high uptake in brain areas rich in D_2/D_3 receptors. The radiotracers' distributions are shown in the mean SUV-images (Fig. 1), the uptake for both radiotracers were high in the caudate and in the putamen. The ventral striatum uptake was more ventrally extended with $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$, the globus pallidus uptake was low with $[^{11}\text{C}]\text{raclopride}$, and the uptake in the substantia nigra were only seen with $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$. Moreover, there was slight uptake in the thalamus area; however, it can not be reliable quantified.

The concentration distribution maps of $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$ and $[^{11}\text{C}]\text{raclopride}$ are shown in Figure 2. The $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$ distribution map showed a greater percentage of

uptake in the ventral striatum and the globus pallidus than in the dorsal striatum; conversely the $[^{11}\text{C}]\text{raclopride}$ maps showed a greater percentage uptake in the dorsal striatum.

ROI and Time Activity Curves Analysis

The TAC of $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$ (Fig. 3a) showed a rapid brain uptake with the radioactivity counts peaking around at 2 min in the cerebellum and substantia nigra and at 8 min in the caudate, putamen, ventral striatum and globus pallidus. The rank order for the peak uptake of radioactivity was: putamen > caudate and ventral striatum > cerebellum > substantia nigra > globus pallidus. The washout of radioactivity was distinctly slower in the globus pallidus.

The TAC of $[^{11}\text{C}]\text{raclopride}$ (Fig. 3b) showed a rapid brain uptake, with radioactivity counts peaking approximately at 1 min in the cerebellum, and at 12 min in the caudate, putamen, ventral striatum, and globus pallidus. The rank order for the peak uptake of radioactivity was: putamen > caudate and ventral striatum > globus pallidus > cerebellum. The washout was fastest in the cerebellum and radioactivity decreased at similar rate in the other regions.

The most striking differences in the TACs between both radiotracers were the kinetics observed in the globus pallidus and in the ventral striatum. The $[^{11}\text{C}]\text{-}(+)\text{-PHNO}$ signal in the globus pallidus showed a very slow washout in

Figure 4.

Binding Potential (BP) for [¹¹C]-(+)-PHNO and [¹¹C]raclopride in each ROI. The BPs correspond to the mean of twelve healthy controls. Error bars represent standard deviation. Significant differences are presented between radiotracers ($F_{[9,110]} = 29.5$, $P < 0.001$) per ROI. (* $P < 0.01$, Bonferroni corrected). SRTM, Simplified reference tissue model.

comparison to the caudate and the putamen. Furthermore, the [¹¹C]-(+)-PHNO signal in the ventral striatum also exhibited a slow washout at a rate between that of the globus pallidus and the caudate and the putamen signals. In contrast, the globus pallidus and ventral striatum TACs of [¹¹C]raclopride exhibited the same shape as the caudate and putamen.

The BP values for [¹¹C]-(+)-PHNO ranged from 2.0 to 3.43 in the ROI analysis, and the analysis of variance showed differences between ROIs ($F_{[4,55]} = 7.9$, $P < 0.001$); the lowest to highest values are as follow: substantia nigra < caudate < putamen < globus pallidus < ventral striatum. The BP for [¹¹C]raclopride ranged from 1.88 to 4.32 with differences between ROIs ($F_{[4,55]} = 103.2$, $P < 0.001$); the lowest to highest BPs are globus pallidus < ventral striatum < caudate < putamen. The [¹¹C]-(+)-PHNO BPs

were statistically higher in the ventral striatum and globus pallidus (mean ± SD; 3.4 ± 0.9 and 3.4 ± 1.0) than in the caudate and substantia nigra (2.1 ± 0.4 and 2.0 ± 1.1) (Bonferroni corrected, $P < 0.005$); the putamen (2.8 ± 0.6) did not show significant difference with any ROI. Conversely the BP's from the [¹¹C]raclopride scans were higher in the caudate, putamen and ventral striatum vs. globus pallidus (3.4 ± 0.6 , 4.3 ± 0.7 , 3.4 ± 0.5 vs. 1.9 ± 0.4) ($P < 0.001$); the putamen was higher than the caudate, ventral striatum, and substantia nigra (0.52 ± 0.1) ($P < 0.001$); and the substantia nigra were lower than all the ROIs ($P < 0.001$) (Fig. 4). The [¹¹C]raclopride BPs in the substantia nigra was 0.5 ± 0.1 , though not surprisingly the signal was extremely noisy.

The comparison of BPs values between radiotracers ($F_{[9,110]} = 29.5$, $P < 0.001$) per ROI showed that the BP's from [¹¹C]raclopride were higher than [¹¹C]-(+)-PHNO in the caudate ($P < 0.01$) and putamen ($P < 0.01$). Conversely, the BP's from [¹¹C]-(+)-PHNO were higher than [¹¹C]raclopride in the globus pallidus ($P < 0.01$) and substantia nigra ($P < 0.01$). The BPs from the ventral striatum did not show difference between both radiotracers (Fig. 4).

BP Maps Comparison

The SPM2 comparison of the [¹¹C]-(+)-PHNO BP maps and [¹¹C]raclopride BP maps revealed a statistically higher BP in the globus pallidus region for [¹¹C]-(+)-PHNO. In contrast, [¹¹C]raclopride showed a statistically higher BP in the dorsal caudate and dorsal putamen (Table I).

DISCUSSION

This study compares in humans the differential distribution of a D₂/D₃ agonist radiotracer ([¹¹C]-(+)-PHNO) versus an antagonist radiotracer ([¹¹C]raclopride). We observed two striking findings: (a) [¹¹C]-(+)-PHNO showed preferential uptake in the ventral striatum and the globus pallidus, while [¹¹C]raclopride showed preferential distribution in the dorsal striatum; (b) the TAC of the globus

TABLE I. Statistical parametric mapping results (SPM2) showing clusters of voxels having differences (paired-t-test) in the BP values among [¹¹C]-(+)-PHNO and [¹¹C]raclopride

	X	Y	Z	Cluster size	T-value	P-uncorrected	P-corrected
[¹¹C]-(+)-PHNO > [¹¹C]raclopride							
Left Globus Pallidus	-18	-2	-10	17	9.25	<0.001	0.005
Right Globus Pallidus	8	2	-5	23	8.35	<0.001	0.006
[¹¹C]raclopride > [¹¹C]-(+)-PHNO							
Left Putamen	-26	4	0	47	8.64	<0.001	0.005
Right Putamen	24	4	0	14	7.83	<0.001	0.005
Right Putamen	30	-10	4	35	7.56	<0.001	0.005
Right Caudate	14	6	12	13	7.06	<0.001	0.007

Coordinates are in the MNI brain space. T-values correspond to the maximal voxel of each cluster. P-corrected corresponds to false discovery rate (FDR). Cluster size, corresponds to the number of voxels in the cluster. Voxel size = $2 \times 2 \times 2$ mm³.

pallidus displayed a unique shape with a lower peak and slower washout than the other regions. We consider that these two findings arise from a differential distribution of D_2^{high} vs. D_2^{low} in the striatum, and/or a differential distribution of D_2 vs. D_3 receptors. We discuss these two hypotheses below:

According to the two-state model and the extended three-state model of receptor activation [Leff, 1995; Scaramellini and Leff, 1998], it is expected that an agonist such as [^{11}C](+)-PHNO will bind preferentially to the high-affinity state of the receptor and an antagonist such as [^{11}C]raclopride will bind to the total (high + low) of D_2 [George et al., 1985; Leff, 1995; Seeman et al., 2002]. If this is so, the regional brain distribution of [^{11}C](+)-PHNO is anticipated to be restricted to those areas labeled by an antagonist radiotracers such as [^{11}C]raclopride, though they may differ in relative strength depending upon the proportional distribution of D_2^{high} vs. D_2^{low} . Our results agree with this model because both radiotracers significantly bind to the caudate, the putamen, the ventral striatum and the globus pallidus. The only seeming discrepancy is that [^{11}C](+)-PHNO shows a reliable signal from the substantia nigra, while [^{11}C]raclopride signal is extremely low. However, other antagonist D_2/D_3 radiotracers such as [^{18}F]fallypride [Kessler et al., 2006] and [^{11}C]FLB 457 [Okubo et al., 1999] do show higher uptake in this region.

[^{11}C](+)-PHNO shows an anteroventral preference in its distribution in the striatum, in contrast to [^{11}C]raclopride which has a postero-dorsal preference. This pattern of distribution has been recently observed in baboons; where Narendran et al. [2006] reported a [^{11}C](+)-PHNO uptake of 196% in the ventral striatum and 146% in the globus pallidus relative to the binding within all of the striatum, providing further empirical support for these findings. One reason for this may be a higher availability of D_2^{high} in the globus pallidus, the ventral striatum and in the substantia nigra—an idea that is supported by the finding of De Keyser et al. [1985] who demonstrated, in-vitro, that the D_2 -like receptors in the human membranes of the globus pallidus are all guanine-nucleotide-insensitive (D_2^{high}), on the other hand, only the 15–20% of the D_2 -like receptors in the caudate and in the putamen are guanine-nucleotide-insensitive. This finding, however, can also be explained by a preferential distribution of D_3 over D_2 in the globus pallidus because D_3 are also guanine-nucleotide insensitive (although it has been found that guanilylimidodiphosphate fully converted the high-affinity state of the human cloned D_3 receptor into its low-affinity state; Seeman et al., to be published). If, however, [^{11}C](+)-PHNO vs. [^{11}C]raclopride differences were all attributed to the high vs. low difference, one would expect to observe them with all agonist radiotracers. However, Narendran et al. [2006] failed to find such regional differences between [^{11}C]NPA and [^{11}C]raclopride in the baboon brain. The latter finding makes it unlikely that the high vs. low explanation is the sole cause for this differential distribution, and suggests that another parallel difference between [^{11}C](+)-

PHNO and [^{11}C]raclopride may be contributing to this differential.

While both [^{11}C](+)-PHNO and [^{11}C]raclopride are specific for the D_2 -like family of receptors, they show significant differences in their affinity to the D_2 vs. D_3 subtypes. The raclopride affinities for D_2 and D_3 are approximately the same (1.5–1.6 nM and 1.2–2.1 nM, respectively) [Malmberg et al., 1994; Seeman, 2001]; therefore, it is a radioligand for brain imaging with and equal preference for D_2/D_3 . On the other hand, while Freedman et al. [1994] and Parker et al. [2006] found that (+)-PHNO has more than ten-fold higher affinity for D_3 than of D_2 , recent data by Seeman et al. [2005a] report (+)-PHNO K_i values of 0.6 nM for D_3^{high} and 0.24 nM for D_2^{high} , indicating that (+)-PHNO has a three-fold greater affinity at the high-affinity state of D_2 than at the high-affinity state of D_3 . Therefore, because of the approximately similar affinities of (+)-PHNO at the high-affinity states of D_2 and D_3 , this raises the possibility that the anteroventral preferential binding of [^{11}C](+)-PHNO may reflect some preferential binding to the D_3 receptors, considering that one-third of the human ventral striatum D_2 -like receptors are D_3 [Seeman et al., 2006b]. This possibility is also supported by studies done in rodents that show that [^3H](+)-PHNO intensely labeled D_3 rich regions such as the Islands of Calleja in vitro [Nobrega and Seeman, 1994]. This result has been recently replicated ex vivo and extended by showing the [^{11}C](+)-PHNO binding to the ventral regions of what is displaceable by D_3 antagonist SB277011 [Nobrega, 2006; personal communication]. Furthermore a recent in vivo PET study in baboons also demonstrated that the globus pallidus signal of [^{11}C](+)-PHNO can selectively be blocked with a D_3 partial agonist (BP897) [Narendran et al., 2006]. These in-vitro, ex-vivo, and in-vivo data in animals are in favor of a preferential affinity of [^{11}C](+)-PHNO for D_3 over D_2 —and as we discuss below may account for the differential distributions of [^{11}C](+)-PHNO and [^{11}C]raclopride.

The post mortem brain distribution of the D_3 receptors in the human brain [Gurevich and Joyce, 1999; Murray et al., 1994] agrees with the preferential globus pallidus and ventral striatum uptake shown in our results with [^{11}C](+)-PHNO. The ratio of the relative densities of D_3 over D_2 shows a pattern of globus pallidus > ventral striatum > putamen > caudate [Gurevich and Joyce, 1999; Murray et al., 1994], and these ratios correlate with the percentage of regional binding, relative to the whole striatum, obtained with [^{11}C](+)-PHNO ($r \sim 0.6$). While, the percentage of regional binding, relative to the whole striatum obtained with [^{11}C]raclopride does not correlate with these ratios ($r \sim -0.1$), it does correlate with the total amount of D_2 and D_3 receptors ($r \sim 0.94$). On the whole, these figures suggest that the signal of [^{11}C](+)-PHNO represents the binding of a mixed population of D_2 and D_3 , with preference to D_3 in the globus pallidus and the ventral striatum. The binding of [^3H](+)-PHNO in vitro has been recently reported in human post-mortem tissue [Seeman et al., 2006b]. This post-mortem study shows that both radio-

tracers label D₂ in the dorsal striatum, while in the ventral striatum and the globus pallidus, the radiotracers label a mixed population composed of D₂ and D₃ [Seeman et al., 2006b]. Thus several streams of converging evidence point to the fact that the relative (to [¹¹C]raclopride) preferential distribution of [¹¹C]-(+)-PHNO in the globus pallidus may reflect, at least in part, its preferential binding to D₃ receptors in this region. Definitive evidence will require the displacement of this signal by a D₃ specific compound in humans.

The other striking difference between [¹¹C]-(+)-PHNO and [¹¹C]raclopride is the relatively unique shape of [¹¹C]-(+)-PHNO kinetics in the globus pallidus. The globus pallidus kinetics of [¹¹C]-(+)-PHNO showed a slower time to peak and slower wash-out when compared with other regions (e.g. caudate, the putamen) and also when compared with [¹¹C]raclopride (which showed similar kinetic shapes in all regions). The slower wash-out seen in only one ROI sustains the binding differential properties across the striatal regions, and it agrees, with the lower dissociation rate constant (k₄) of [¹¹C]-(+)-PHNO in the globus pallidus recently observed by Ginovart et al. [in press]. Such a kinetic shape suggests the presence of a relatively low density (as compared with other regions) and very high affinity receptor population in this region. As discussed above—this may represent the differential binding of [¹¹C]-(+)-PHNO either the high-affinity states of the D₂ receptor, or its preferential binding to the D₃ receptor, for which it has particularly high affinity.

The analysis of this study has the limitation that it did not include partial volume effect (PVE) correction. This correction is especially important in small ROIs with radioactivity contamination or dilution from neighborhood regions [Rousset et al., 1998]. For example, while the ventral striatum activity with [¹¹C]raclopride could be overestimated by a spill-over effect from the caudate, the substantia nigra activity with [¹¹C]-(+)-PHNO could be underestimated by a spill-in effect from the midbrain's white matter.

Another limitation concerns our general assumption (e.g., [¹¹C]-(+)-PHNO binds only D₂^{high} and [¹¹C]raclopride to D₂^{high+low}), which would predict that the total number of receptor sites observed by [¹¹C]-(+)-PHNO would be less than those observed by [¹¹C]raclopride. Since our study did not include a saturation analysis which would allow one to delineate the individual parameters $B_{\max}$ and K_d , we are unable to resolve the question in this study. While we cannot answer this question from our data—two recent studies have compared the absolute number of agonist-labeled vs. antagonist-labeled receptors in baboons and cats. Narendran et al. [2005] compared the striatal $B_{\max}$ as measured by the agonist, [¹¹C]NPA and by the antagonist, [¹¹C]raclopride in baboons. They found that the maximal density of sites for [¹¹C]NPA was 79% of the [¹¹C]raclopride sites, hence suggesting that 79% of the D₂ are in the high-affinity state. On the other hand, the striatal $B_{\max}$ in cats measured with [¹¹C]-(+)-PHNO and [¹¹C]raclopride

exhibited similar $B_{\max}$ with both radiotracers [Ginovart et al., 2006], thus suggesting that almost all the receptors were configured in the D₂^{high} state. Both these estimates of D₂^{high} proportion are rather high—most previous studies (and all of them in vitro) find that the relative proportion of D₂^{high}/D₂^{total} in brain homogenates are in the range of 40–80% [de Vries and Beart, 1986; Ferre et al., 1991; George et al., 1985; Malmberg and Mohell, 1995; Richfield et al., 1986; Richfield et al., 1989; Seeman et al., 2005b; Sibley et al., 1982; Tajuddin and Druse, 1996]. However, it is to be pointed out that these in-vitro studies differ significantly in their methods: not only they worked with homogenized tissue, but the assessment of D₂^{high}/D₂^{total} is done indirectly by adding a very high concentration of GppNHp to the assay—thereby converting the high-states into low-states. The latter in vitro method, using a guanine nucleotide to convert the high-affinity sites into low-affinity sites, did not reveal high-affinity D₂ receptors in intact anterior pituitary cells [Sibley et al., 1983]. Recent work, however, found that dopamine/[³H]spiperone competition experiments do not readily reveal D₂^{high} receptors, as compared with dopamine/[³H]domperidone competition experiments, where such high-affinity states are easily observed in both homogenized tissue [Seeman et al., 2003] and intact cells [Ko et al., 2002].

CONCLUSION

This is the first human PET study, which compares the brain uptake, distribution, and binding characteristics of a D₂ agonist radioligand ([¹¹C]-(+)-PHNO) with a D₂ antagonist radioligand ([¹¹C]raclopride). [¹¹C]-(+)-PHNO showed a preferential distribution in the globus pallidus and in the ventral striatum, while [¹¹C]raclopride showed preferential uptake in the dorsal striatum—thus providing two distinct views for the D₂/D₃ system of the human brain. Our results and several lines of evidence suggest that [¹¹C]-(+)-PHNO is a PET radioligand with a mixed binding to the D₂/D₃ receptors with possible preference for D₃. The signal seen in the dorsal striatum may correspond preferentially to D₂, while the higher signal of [¹¹C]-(+)-PHNO in the globus pallidus and in the ventral striatum likely reflects its ability to preferentially bind to the high states of the D₃ and D₂ receptors. Further understanding of the characteristics of [¹¹C]-(+)-PHNO, as well as its use in clinical populations will help to clarify the contributions of the different affinity states of the receptors (high vs. low), and different receptor subtypes (D₂ and D₃) in health and disease.

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