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The Dopamine D_2 Receptors in High-Affinity State and D_3 Receptors in Schizophrenia: A Clinical [^{11}C]-(+)-PHNO PET Study

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The dopamine D_2 receptors exist in two states: a high-affinity state (D_2^{high}) that is linked to second messenger systems, is responsible for functional effects, and exhibits high affinity for agonists; and a low-affinity state that is functionally inert and exhibits lower affinity for agonists. The dopamine D_3 receptors have high-affinity for agonist (eg dopamine) and the existence of the two affinity states is controversial. Although preclinical studies in animal models of psychosis have shown a selective increase of D_2^{high} as the common pathway to psychosis, the D_3 has been suggested to be involved in the pathophysiology of psychosis. We report the first study of the D_2^{high} and D_3 in schizophrenia using the novel PET radiotracer, [^{11}C]-(+)-PHNO. We recruited 13 patients with schizophrenia-spectrum disorder amidst an acute psychotic episode, drug free for at least 2 weeks, and 13 age–sex-matched healthy controls. The binding potential no-displaceable (BP_{ND}) was examine in the main regions of interest (caudate, putamen, ventral striatum, globus pallidus, substantia nigra, and anterior thalamus) and in a voxel-wise analysis. The BP_{ND} between patients and controls was not different in any of the regions. The voxel-wise analysis did not reveal any difference and no correlations were found between the BP_{ND} and positive and negative syndrome scale subscales. Our results do not find support for the hypothesis linking psychosis to a selective increase in D_2^{high} and/or D_3 in schizophrenia. It is possible that receptors with high affinity are not accessible by [^{11}C]-(+)-PHNO because they are occupied by endogenous dopamine, a possibility that can be ruled out in future experiments.
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INTRODUCTION

It is well established that the dopamine $D_{2/3}$ receptors are the target of most antipsychotic drugs, though it remains unclear whether the $D_{2/3}$ receptors are involved in the pathophysiology of psychosis. An important recent development has been the evidence from preclinical models indicating that the 'high-affinity state' of the dopamine D_2 receptors (D_2^{high}) is elevated in psychosis (Seeman *et al*, 2005b). The dopamine D_2 and D_3 receptors are members of the G-protein-coupled receptors (GPCR) family. The D_2 receptor exists in two inter-convertible states: a G-protein-coupled state which has a high affinity for agonist binding and is responsible for the functional effects of dopamine; and a G-protein-uncoupled state which is a functionally inert state with a low-affinity for dopamine. On the other hand, the existence of the G-protein-coupled and uncoupled states of the D_3 receptors is controversial (Freedman *et al*, 1994; Seeman *et al*, 2005a; Sokoloff *et al*, 1992), due to the inability of the G-protein analogs (eg guanilylimidodiphosphate, GppNHp) to shift it from the high- to the low-affinity state (Vanhauwe *et al*, 2000). Nevertheless, the D_3 receptor has at least 20-fold higher affinity than D_2 for dopamine (Freedman *et al*, 1994; Seeman *et al*, 2005a; Sokoloff *et al*, 1992).

Data from over a dozen preclinical animal models of psychosis (eg amphetamine sensitization, phencyclidine sensitization, ethanol withdrawal, hippocampal lesion) (Seeman *et al*, 2005b) suggests that there is a selective elevation of high-affinity states in psychosis. Though, the method of distinguishing high- from low-affinity states *in vitro* involves the addition of high concentrations of G-protein analogs, a method that is not feasible in humans. Molecular imaging techniques such as positron emission

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tomography (PET) allow for the *in vivo* study of brain receptors; but, until recently, the available radioligands for $D_{2/3}$ receptors (eg [^{11}C]-raclopride, [^{18}F]-fallypride) were all 'antagonist' radioligands which would not distinguish between the high- and low-affinity states of the receptors. Studying the high-affinity state of the receptor directly in patients requires the use of an 'agonist' radioligand.

Recently, our group has developed [^{11}C]-(+)-PHNO ([^{11}C]-(+)-4-propyl-9-hydroxynaphthoxazine) (Wilson *et al*, 2005), a $D_{2/3}$ agonist radiotracer for use in humans. [^{11}C]-(+)-PHNO binds with nanomolar affinity to D_2 and D_3 receptors allowing for a prominent signal from the striatum, globus pallidus (GP), substantia nigra (SN), and anterior thalamus (Freedman *et al*, 1994; Graff-Guerrero *et al*, 2008; Narendran *et al*, 2006; Seeman *et al*, 1993; Willeit *et al*, 2006). It shows at least three critical differences compared to antagonist radiotracers. First, unlike antagonist radiotracers which do not distinguish between the low- and high-affinity states, [^{11}C]-(+)-PHNO is assumed to bind preferentially to the high-affinity states of the receptors (Ginovart *et al*, 2006; Seeman *et al*, 2007).

Second, (+)-PHNO has a higher *in vitro* and *in vivo* affinity for D_3 than for D_2 receptors. *In vitro*, Freedman *et al* (1994) and Parker *et al* (2006) reported that (+)-PHNO has more than 10-fold higher affinity for D_3 than for D_2 using conventional *in vitro* techniques, though Seeman *et al* (2005a) reported that (+)-PHNO has a higher affinity for the D_2^{high} than D_3^{high}) using *in vitro* analysis in the presence of GppNHp. *In vivo*, Narendran *et al* (2006) suggested that [^{11}C]-(+)-PHNO has fourfold higher preference for D_3 than D_2^{high}—a finding supported by studies in nonhuman primates showing that the [^{11}C]-(+)-PHNO signal in the GP and midbrain binding is selectively displaceable with the D_3 preferential agonist (BP897) and antagonist (SB-277011) (Narendran *et al*, 2006; Rabiner *et al*, 2007b, 2008). Finally, studies in healthy humans show that the [^{11}C]-(+)-PHNO signal in the GP is preferentially displaceable with the D_3 antagonist (ABT925) (Abi-Saab *et al*, 2008; Graff Guerrero *et al*, 2008). Thus, across species, methods and techniques, [^{11}C]-(+)-PHNO shows a modest higher affinity for D_3 over D_2.

Finally, [^{11}C]-(+)-PHNO has shown higher sensitivity for amphetamine displacement than [^{11}C]-raclopride in anesthetized cats (Ginovart *et al*, 2006). However, this higher sensitivity was not found in an *ex vivo* dissection study in awake rodents (McCormick *et al*, 2008), though it is suggested by the study in awake and healthy humans (Willeit *et al*, 2008). The cause of this discrepancy between studies is still elusive, though the cat and human studies suggest that [^{11}C]-(+)-PHNO binds preferentially to the high-affinity states of the dopamine receptor.

The development of [^{11}C]-(+)-PHNO has finally made it possible to test the hypothesis with regards to elevated high-affinity states in schizophrenia. Moreover, as [^{11}C]-(+)-PHNO provides a robust binding from the D_3-rich areas in the brain (GP and SN), for the first time it has provided an opportunity to explore directly the suggested involvement of the dopamine D_3 receptors in schizophrenia (Griffon *et al*, 1995; Gurevich *et al*, 1997; Joyce and Gurevich, 1999).

The current study was aimed to compare the [^{11}C]-(+)-PHNO binding in drug-free patients with schizophrenia and appropriate controls to test the D_2 high-affinity states hypothesis and to compare the dopamine D_3 binding between patients with acute psychosis and controls.

MATERIALS AND METHODS

Clinical Sample

This study has been approved by the local Research Ethics Board and by Health Canada. Twenty-six subjects were included: 13 drug-free patients and 13 healthy controls. The participants provided written informed consents after the study procedures and risks were explained. They were excluded if they had a current diagnosis of substance abuse or dependence at screening, positive result in urine drug screen at enrollment or before any of the PET scans, history of clinically significant physical illness, pregnant or lactating women at screening or positive urine pregnancy test before PET scans, or metal implants that would preclude the MRI scan.

The patients were evaluated before PET scan with the clinical global impression (CGI) and positive and negative syndrome scale (PANSS).

The patients had the diagnosis of schizophrenia or schizophreniform disorder corroborated with the MINI-Plus structured interview (Sheehan *et al*, 1998). The age range for inclusion was from 18 to 50 years old. The patients were drug free for at least 2 weeks and were excluded if they had been exposed to depot antipsychotic in the past 2 years; had a minimum PANSS total score of 55, score of >3 on at least two PANSS psychosis items (P1, P2, P3, P5, or P6) or >4 on one psychosis item; and CGI severity score $\geqslant 4$ (moderately ill).

The healthy controls were recruited through local advertisement. Psychiatric disorders were excluded using the MINI-Plus structured interview (Sheehan *et al*, 1998). The age range for inclusion was from 18 to 50 years old. Subjects with any medical or neurological conditions or with axis I psychiatric diagnoses were excluded from the study. Similarly, subjects with substance abuse (other than smoking) within 6 months before their baseline visit were not included. Participants were asked to consume no more than their usual amount of coffee (and if smokers, cigarettes) on the day of PET examination, 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 scan. Pregnancy was excluded using serum analysis at inclusion and urine pregnancy tests before each scan.

[^{11}C]-(+)-PHNO 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 is subsequently reduced by lithium aluminum hydride. Purification by HPLC and formulation gave radiochemically pure [^{11}C]-(+)-PHNO as a sterile, pyrogen-free solution suitable for human studies.

Positron Emission Tomography Imaging

Studies were performed using a high-resolution brain PET camera system, HRRT (Siemens Molecular Imaging, Knoxville, TN), which measures radioactivity in 207 brain slices with an interslice distance of 1.2 mm. The in-plane resolution of the scanner is approximately 2.8 mm full-width-at-half-maximum. Transmission scans were acquired using a ^{137}Cesium single photon point source to provide attenuation correction. The emission data were acquired with a head fixation system during PET scans to avoid movement during the acquisition. After being placed on the scanning table, a total of 329 ± 59 MBq (8.9 ± 1.6 mCi) with a specific activity of 955.9 ± 295 mCi/μmol (range: 407–1790 mCi/μmol) and a mass of 2.33 ± 0.39 μg (range: 1.2–3.3 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 data were acquired for 90 min after the injection. Once scanning was completed, the data were reframed into 30 frames (1–15 of 1 min duration and 16–30 of 5 min duration). From the 26 participants in this study, one healthy control experienced transient mild nausea. This was presented few minutes ($\sim$10 min) after [^{11}C]-(+)-PHNO injections and lasted less than 5 min. No interruption of the PET procedures was required due to this adverse effect of the radiotracer.

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 scanner (General Electric Medical Systems, Milwaukee, WI). These images were used for the analysis of the PET scans.

Image Analysis

The time activity curves (TACs) from the regions of interest (ROIs) caudate, putamen, ventral striatum (VS), GP, SN, and cerebellar cortex were obtained from the dynamic [^{11}C]-(+)-PHNO PET images in native space with reference to co-registered MRI image as previously described (Graff-Guerrero *et al*, 2008; Rusjan *et al*, 2006; Willeit *et al*, 2008). The ROI in the anterior thalamus was drawn manually using the Analyze Software Version 6.0 (AnalyzeDirect, Lenexa, Kansas) in the anterior part of the thalamus as observed in the first axial projection where putamen and thalamus can be shown. The co-registration of the MRI to the PET space image was carried out using the normalized mutual information algorithm as implemented in SPM2 (SPM2, Welcome Department of Cognitive Neurology, London; http://www.fil.ion.ucl.ac.uk/spm).

The TACs were obtained using a in-house software for semi-automated generation of ROIs (Rusjan *et al*, 2006). The quantitative estimate of binding was estimated using the simplified reference tissue method (Lammertsma and Hume, 1996) with the cerebellar cortex as reference region. This method has been validated to reliable estimate the binding potential no-displaceable (BP_{ND}), which compares the concentration of radioligand in the receptor-rich region to the receptor-free region (Innis *et al*, 2007), and has been validated for use with [^{11}C]-(+)-PHNO (Ginovart *et al*, 2007). The BP_{ND}s were estimated using the PMOD v2.7 software (PMOD Technologies Ltd, Zurich, Switzerland).

Parametric voxel-wise BP_{ND} maps from the dynamic images in native space were generated according to the method of Gunn *et al* (1997) with the cerebellum as reference region and as implemented in PMOD v2.7 software (PMOD Technologies Ltd). The BP_{ND} map images were spatially normalized into the Montreal Neurological Institute brain space by nearest neighbor interpolation and with a voxel size fixed in $2 \times 2 \times 2$ mm using the SPM2 software (Friston, 1995). The normalized images were smoothed with a Gaussian filter in each coordinate direction with a kernel of 4 mm.

Statistical Analysis

The analysis was made using the Statistical Program for the Social Sciences (version 15.0; SPSS, Chicago, IL). Variables were presented as mean ± standard deviation (SD). Demographic and clinical characteristics were compared between patients and controls by using Mann–Whitney *U*-test (gender) on categorical data and independent sample *t*-tests on continuous data (age). Analysis of variance with Bonferroni correction for multiple comparisons as a *post hoc* was performed to compare the BP_{ND}s between drug-free patients and sex- and age-matched healthy controls per ROI (caudate, putamen, VS, GP, SN, and anterior thalamus). Pearson's product moment correlations between PANSS (total and subscales) and BP_{ND}s were estimated. Repeated measures analysis of variance was performed to compare the standardized radioactivity in the cerebellum (within subjects factor) between drug-free patients and sex- and age-matched healthy controls (between subjects factor). The significance was always assumed at $p < 0.05$.

The voxel-wise comparison between BP_{ND} maps, of drug-free patients and sex- and age-matched healthy controls, was performed with a two sample *t*-tests as implemented in SPM2. We reported areas only if they met the joint criteria of (a) p(uncorrected) < 0.001; (b) an extent $\geqslant 10$ voxels; and (c) in the caudate, putamen, GP, VS, midbrain, and anterior thalamus. Additionally, the statistically significant *a priori* regions would be corrected for multiple comparisons using the false discovery rate (FDR) approach.

RESULTS

The characteristics of the patients and controls are summarized in Table 1. There were no differences between groups in age (drug-free patients mean = 25.8 ± SD = 5.9 years; healthy controls = 26.8 ± 6.4 years, $t = -0.40$, $p = 0.69$) and in gender (drug-free patients = 9 men; healthy controls = 9 men; Mann–Whitney *U*-test = 84.5, $p = 1.0$). No differences emerged between drug-free patients and age- and sex-matched healthy controls in the BP_{ND} in any of the ROI ($F(6,19) = 0.671$, $p = 0.674$). The BP_{ND} (mean ± SD) in the caudate for controls was 2.1 ± 0.2 and for patients was 2.2 ± 0.4 ($p = 0.52$, pair-wise Bonferroni corrected); putamen: controls = 2.6 ± 0.3 and patients = 2.6 ± 0.2 ($p = 0.50$); VS: controls = 3.1 ± 0.4 and patients = 3.2 ± 0.7 ($p = 0.63$); GP: controls = 2.9 ± 0.6 and patients = 2.7 ± 0.5 ($p = 0.51$); SN: controls = 1.5 ± 0.6 and patients = 1.3 ± 0.4 ($p = 0.33$);

Table 1 Characteristics of the Drug-Free Patients Group and Age- and Sex-Matched Healthy Controls

	Drug-free patients mean (± SD)	Healthy controls mean (± SD)	Comparison
Age (years)	25.85 (5.9)	26.85 (6.4)	$t = -0.40$, $p = 0.69$
Gender (male/female)	9/4	9/4	*U*-MW = 84.5, $p = 1.0$
Smokers (yes/no)	3/10	NA	
Diagnosis			
Schizophrenia	10		
Schizophreniform	3		
Age onset	23.8 (6.7)		
Acute episodes	0.75 (0.62)		
Hospitalizations	0.23 (0.59)		
PANSS			
Positive	20.9 (7.14)		
Negative	14.0 (6.42)		
General	39.9 (5.40)		
Previous neuroleptic exposure (months)	3 patients 4.0 (11.58)		

U-MW, *U* of Mann–Whitney; NA, data not available; PANSS, positive and negative syndrome scale.

thalamus: controls = 1.2 ± 0.2 and patients = 1.1 ± 0.3 ($p = 0.43$) (Figure 1). This result was corroborated by the voxel-wise comparison of the BP_{ND} maps between drug-free patients and controls which did not show any differences (Figure 2). Moreover, the BP_{ND} did not correlate with the severity of the illness as measured by the PANSS even before correction for multiple comparisons (Table 2).

DISCUSSION

This study represents the first comprehensive investigation of the dopamine D_2 receptors in the high-affinity state (D_2^{high}) and the D_3 receptors in patients. Contrary to our expectation based on preclinical data, we did not find evidence for elevated D_2^{high} in untreated patients with schizophrenia or schizophreniform disorder during acute psychosis.

The high-affinity state hypothesis has been sustained in many preclinical models of psychosis (Seeman *et al*, 2005b) and has been indirectly suggested by dopamine depletion studies in patients (Abi-Dargham *et al*, 2000). Our results in drug-free patients failed to support this hypothesis. Although the reason for this discrepancy between predictions from *in vitro* data and findings in patients is not clear, several issues need to be considered.

First, the methods of measuring the high-affinity state *in vitro* and in patients are very different. The *in vitro* studies include competition assays between antagonist radiotracers and agonist ligands in the presence of non-hydrolysable GTP analogs (GppNHp), the latter converting the receptor from its high-affinity state to its low-affinity state (De Lean *et al*, 1980; Seeman *et al*, 2002, 2005b). This differs from the approach in humans which involves a direct study of the high-affinity states with an agonist radioligand.

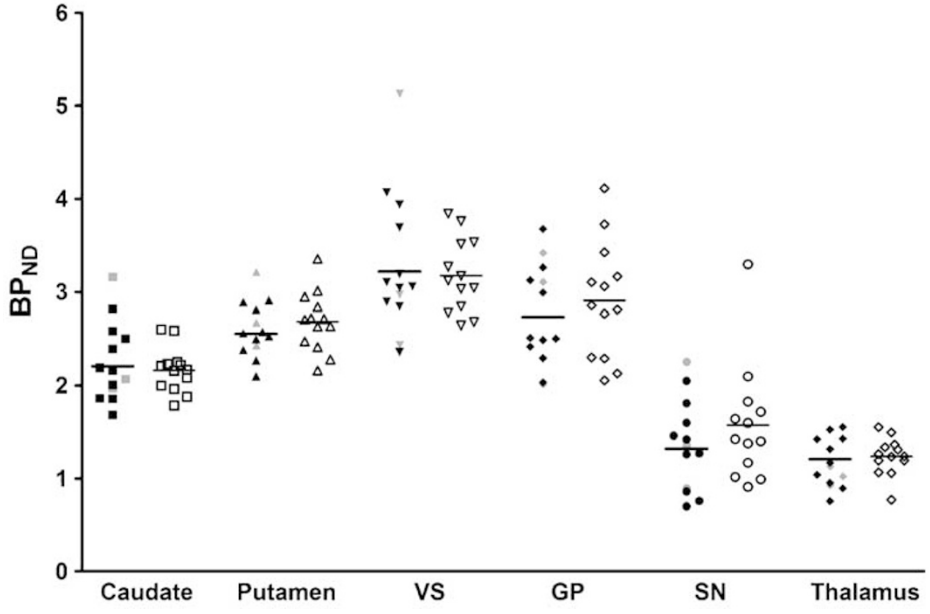

Figure 1 Comparison of the [^{11}C]-(+)-PHNO binding potential no-displaceables (BP_{ND}s) of every region of interests (ROI) between drug-free patients and age- and sex-matched healthy controls. Black symbols indicate drug-naive patients, grey symbols indicate drug-free patients, and white symbols indicate healthy controls. Horizontal lines indicate mean BP_{ND} from each ROI. VS, ventral striatum; GP, globus pallidus; SN, substantia nigra.

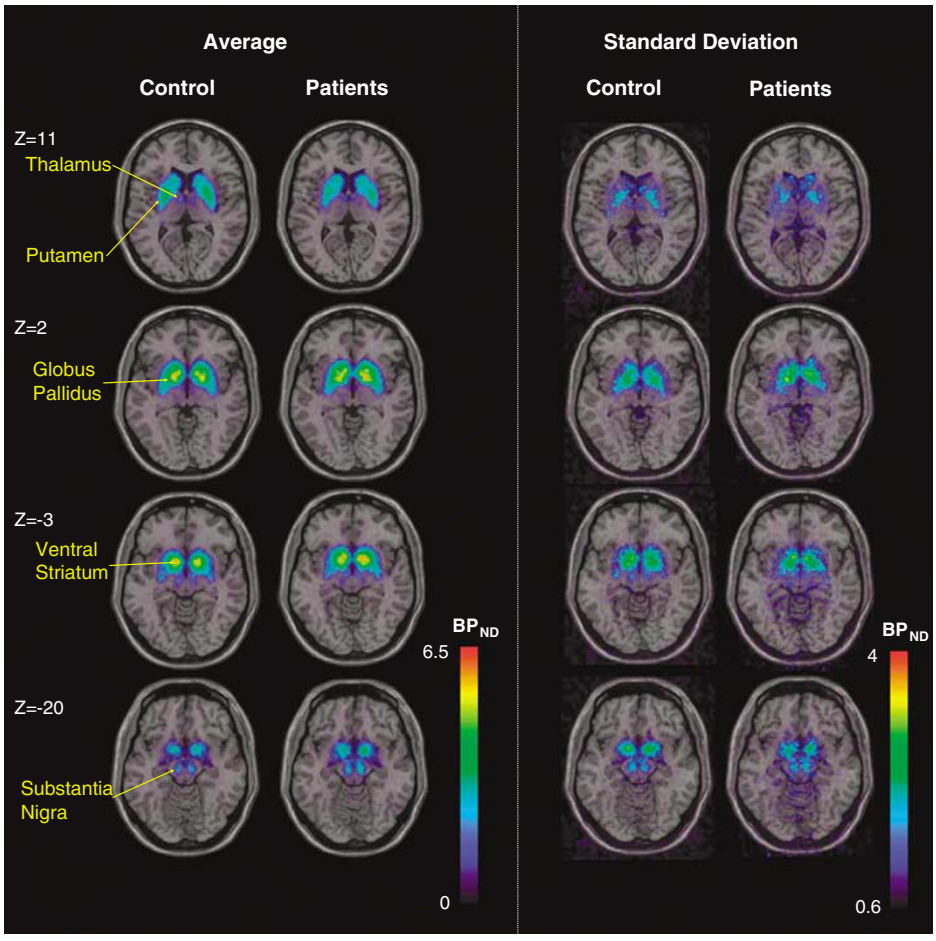

Figure 2 [^{11}C]-(+)-PHNO mean and standard deviation binding potential no-displaceable (BP_{ND}) maps of drug-free patients with schizophrenia ($n = 13$) and age- and sex-matched healthy controls ($n = 13$) illustrating the similitude in binding between controls and patients. The BP_{ND} maps images correspond to axial projections overlay in a T1 template in the Montreal Neurological Institute (MNI) space. The images correspond to 13 patients and 13 controls. Z corresponds to the millimeters above (+) or below (−) the anterior commissure in the AC–PC plane.

Table 2 Pearson's Product Moment Correlations Between [^{11}C]-(+)-PHNO BP_{ND} and Positive and Negative Syndrome Scale

PANSS subscales	Caudate	Putamen	VS	GP	SN	Anterior thalamus
Positive						
r	0.18	0.11	0.22	0.45	0.32	0.23
p	0.5	0.7	0.4	0.1	0.2	0.4
Negative						
r	0.01	0.06	−0.13	−0.23	0.11	−0.12
p	0.9	0.8	0.6	0.4	0.7	0.6
General						
r	0.09	0.05	0.03	−0.19	−0.31	−0.48
p	0.7	0.8	0.9	0.5	0.2	0.09
Total						
r	0.12	0.11	0.06	0.05	0.10	−0.13
p	0.6	0.7	0.8	0.8	0.7	0.6

VS, ventral striatum; GP, globus pallidus; SN, substantia nigra; BP_{ND}; binding potential no-displaceable.

Second, there is ongoing debate as to the measurability of high-affinity state *in vivo*: eg Sibley *et al* (1983) identified both high- and low-affinity binding sites in membrane cells, but only low-affinity sites on the same intact cells. However, Seeman (2008) identified the high-affinity binding sites on intact cells with [^{3}H]domperidone, but not with [^{3}H]spiperone or [^{3}H]raclopride. Moreover, one PET study in nonhuman primates with [^{11}C]-NPA (D_2 agonist) and [^{11}C]-raclopride ($D_{2/3}$ antagonist) through a Scatchard plot analysis was able to differentiate the density of receptors (Bmax) in the high- *vs* high + low-affinity state (Narendran *et al*, 2005); although the same approach in cats with [^{11}C]-(+)-PHNO and [^{11}C]-raclopride did not find any difference in the Bmax between the two radiotracers (Ginovart *et al*, 2006). On the other hand, studies in anesthetized cats (Ginovart *et al*, 2006), anesthetized nonhuman primates (Narendran *et al*, 2004; Seneca *et al*, 2006), and awake humans (Willeit *et al*, 2008) showed greater displacement with an agonist radiotracer ([^{11}C]-NPA, [^{11}C]-MNPA, [^{11}C]-(+)-PHNO) than with an antagonist radiotracer in response to an amphetamine challenge. But one *ex vivo* study in awake rodents did not find any difference in amphetamine displacement between [^{11}C]-(+)-PHNO and [^{11}C]-raclopride (McCormick *et al*, 2008). These studies suggest that although agonist radiotracers do show a distinct binding profile *in vivo*, the exact difference varies as a function of the ligand and the tissue/animal characteristics.

Finally, it is theoretically possible that an increase in the D_2^{high} and/or D_3 is indeed present in acute psychotic state in schizophrenia, but this may be masked by the presence of abnormal high levels of dopamine in the synaptic cleft. The abnormal high levels of dopamine in psychosis has been indirectly shown after an amphetamine challenge (Abi-Dargham *et al*, 1998), but the dopamine striatal synthesis capacity has reliably been found higher than in controls (Dao-Castellana *et al*, 1997; Hietala *et al*, 1999; Huttunen *et al*, 2007; Reith *et al*, 1994).

Moreover, if the density of receptors configured in the high-affinity state is extremely high as suggested in normal conditions ($\sim$80%) (Narendran *et al*, 2005), the difference between patients and controls may be below the resolution of the PET technique. One approach to uncover the differences and to test this hypothesis would require depleting dopamine in patients before imaging studies (Abi-Dargham *et al*, 2000). We did not deplete dopamine levels and thus cannot definitively rule out a change in D_2^{high}/D_3 receptors which may have been cancelled by the ambient increase in endogenous dopamine. This is not just a theoretical concern, because studies by Abi-Dargham *et al* (2000) have shown that patients not only have higher baseline occupancy of dopamine receptors by dopamine, but also that when they are depleted of dopamine, they reveal a higher number of absolute D_2 receptors (Abi-Dargham *et al*, 2000). Thus, a study depleting dopamine would be very critical in definitively resolving this issue.

The ROI and voxel-wise analyses did not reveal any difference in the D_3-rich areas (GP and SN) between patients and controls. These results disagree with a previous postmortem study (Gurevich *et al*, 1997), including drug-free patients with schizophrenia, describing twofold increase in the total number of D_3 receptors. The reason of this discrepancy with our results is unclear. But, differences in the sample characteristics between postmortem studies and our data such as age (old *vs* young), length of illness (years *vs* months), and previous exposure to antipsychotic medications (Graff-Guerrero *et al*, 2007) may help to explain the discrepancy on the results.

The current study also provides the first insight to the status of the D_3 receptors in the schizophrenia, as the [^{11}C]-(+)-PHNO signal in the SN is predominantly related to D_3 (Graff Guerrero *et al*, 2008; Rabiner *et al*, 2007a, b). The SN (midbrain) is important because it is the origin of the nigrostriatal dopaminergic system and the D_3 receptors in the SN are autoreceptors (Diaz *et al*, 2000). Thus alterations in these receptors could contribute to the hyperactivity of this midbrain dopamine system which is believed to play an important role in the genesis of schizophrenic symptoms (Murray *et al*, 2008). However, we do not find support for an alteration of the D_3 in the SN.

Another issue to consider is that the [^{11}C]-(+)-PHNO signal reflects its binding to both D_2^{high} and D_3 receptor populations. The contribution of each receptor to the regional binding varies according to the numbers of each receptor sub-type in the region and to the affinity of the radioligand for that receptor sub-type. Although the precise contribution of D_2^{high} and D_3 to each ROI is not fully mapped, studies in rodents, baboons, and humans estimate that the vast majority of the [^{11}C]-(+)-PHNO binding in the GP (68–89%) and in the midbrain SN (91–100%) corresponds to D_3. On the other hand, the estimation of [^{11}C]-(+)-PHNO binding in the dorsal striatum that corresponds to D_3 is between 8 and 50% (Graff Guerrero *et al*, 2008; Rabiner *et al*, 2007a, b). These proportions suggest that [^{11}C]-(+)-PHNO binding in the GP and SN is a reasonable proxy for the estimation of D_3 receptors in those regions, although the dorsal striatum should be understood as a mixture of predominantly D_2^{high} with some contributions from D_3. However, this limitation in radioligand selectivity should not take away our empirical finding that we find no difference in the [^{11}C]-(+)-PHNO BP_{ND} in drug-free patients with schizophrenia *vs* controls. Further, our inference that there is no substantial difference in either D_2^{high} or D_3 is also secure because a change in either of these should have led to an increase in the overall BP_{ND} and we do not observe that. There is of course the hypothetical possibility that a simultaneous increase in D_2^{high} coupled with a decrease in D_3 (or vice versa) could hide a true finding. But, there is no data in the literature that suggest such possibility.

We did not record the smoking status of the healthy controls. This limitation may be important in the light of the effect of cigarette consumption, craving and its mood or hedonic effect on the dopamine system (Barrett *et al*, 2004; Brody *et al*, 2008; Montgomery *et al*, 2007), and the fact that patients with schizophrenia are more likely to be smokers than general population (de Leon and Diaz, 2005). However, given that we found no effect of smoking within our patients with schizophrenia (three smokers *vs* 10 nonsmokers) (data not shown), we think that it is unlikely that smoking alone could have obscured differences between patients and controls.

The lack of a full kinetic analysis is another potential limitation. This approach would have allowed for the direct estimation of the BP in the ROIs, without the assumption of

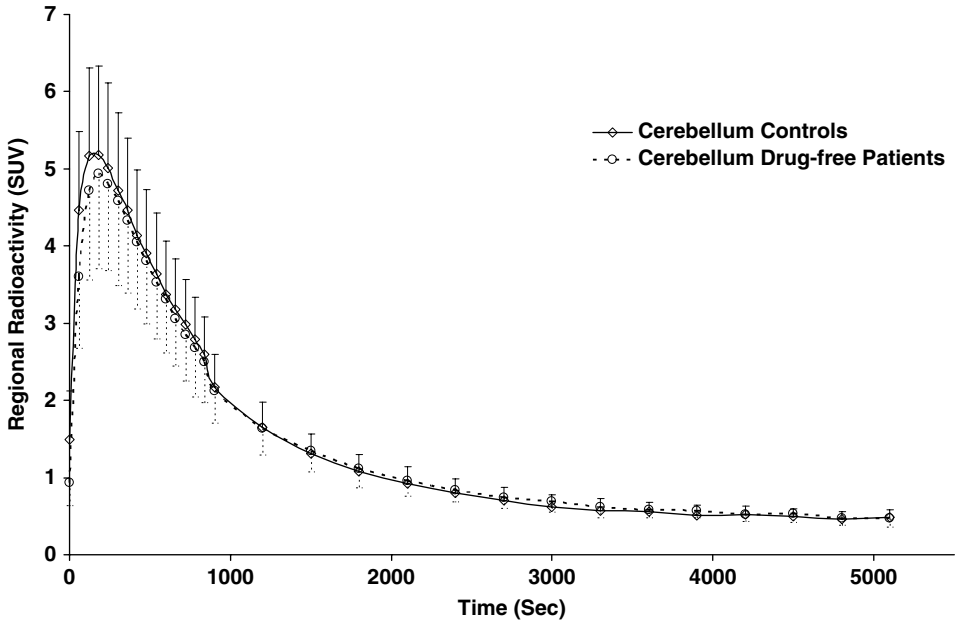

Figure 3 Time–activity curves (mean ± SD) from the cerebellum of 13 drug-free patients with schizophrenia and 13 age- and sex-matched healthy controls. *y* axis represents standardized uptake values (SUV; calculated as: regional radioactivity concentration/(injected radioactivity/body weight) for [^{11}C]-(+)-PHNO. The time–activity curves from the cerebellum illustrate that there was no difference between groups on free and nonspecific [^{11}C]-(+)-PHNO binding. Error bars correspond to standard deviation (repeated measure ANOVA, $F(1,24) = 0.34$, $p = 0.56$).

equivalent nonspecific and free fraction in the reference region. However, our data showed that the cerebellar uptake in the drug-free patients and sex- and age-matched healthy controls was not different (Figure 3; $F(1,24) = 0.34$, $p = 0.56$) and, therefore, it is unlikely that a true finding could have been obscured by differences in the reference region.

The current study represents the first effort to measure the D_2^{high} and D_3 receptors in drug-free patients with psychotic disorders (schizophrenia-spectrum disorders). Our results indicate that patients in an acute psychotic episode do not exhibit an increase of the D_2^{high} or D_3 receptors in comparison to sex- and age-matched healthy controls. We cannot rule out the possibility that any difference of the D_2^{high} or D_3 were occluded by endogenous dopamine. The next step to definitively rule out such a possibility would require studies using [^{11}C]-(+)-PHNO but in patients whose dopamine has been depleted. Until such a finding is observed, accounts should not assume a change in the D_2^{high} or D_3 in the pathophysiology of psychosis.

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DISCLOSURE/CONFLICTS OF INTEREST

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