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In Vivo Binding of Antipsychotics to D_3 and D_2 Receptors: A PET Study in Baboons with [^{11}C]-(+)-PHNO

Ragy R Girgis*[1,2], Xiaoyan Xu[1,2], Nobumi Miyake[1,2], Balu Easwaramoorthy[1,2], Roger N Gunn[3,4], Eugenii A Rabiner[3,4], Anissa Abi-Dargham[1,2,5] and Mark Slifstein[1,2]

[1]Department of Psychiatry, Columbia University, New York, NY, USA; [2]New York State Psychiatric Institute, New York, NY, USA; [3]GlaxoSmithKline Clinical Imaging Centre, Hammersmith Hospital, London, UK; [4]Department of Neurosciences, Imperial College, London, UK; [5]Department of Radiology, Columbia University, New York, NY, USA

Measuring the *in vivo* occupancy of antipsychotic drugs at dopamine D_2 and D_3 receptors separately has been difficult because of the lack of selective radiotracers. The recently developed [^{11}C]-(+)-PHNO is D_3-preferring, allowing estimates of the relative D_2 and D_3 binding of antipsychotic drugs. We used positron emission tomography (PET) imaging in baboons with [^{11}C]-(+)-PHNO to examine the binding of clozapine and haloperidol to D_2 and D_3 receptors. Four animals were scanned with dynamically acquired PET and arterial plasma input functions. Test and retest scans were acquired in single scanning sessions for three subjects to assess the reproducibility of [^{11}C]-(+)-PHNO scans. Four additional scans were acquired in each of three subjects following single doses of antipsychotic drugs (clozapine 0.5534 mg/kg, haloperidol 0.0109 mg/kg, two administrations per drug per subject) and compared with baseline scans. The percent change in binding (ΔBP_{ND}) following challenges with antipsychotic drugs was measured. A regression model, based on published values of regional D_2 and D_3 fractions of [^{11}C]-(+)-PHNO BP_{ND} in six brain regions, was used to infer occupancy at D_2 and D_3 receptors. BP_{ND} following antipsychotic challenge decreased in all regions. Estimated D_2:D_3 selectivity was 2.38 for haloperidol and 5.25 for clozapine, similar to published *in vitro* values for haloperidol (3.03), but slightly higher for clozapine (2.82). These data suggest that acute doses of clozapine and haloperidol bind to D_3 receptors *in vivo*, and that the lack of D_3 occupancy by antipsychotics observed in some recent imaging studies may be because of other phenomena.
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INTRODUCTION

Historically, research into the dopaminergic aspects of the mechanism of function of antipsychotic medications has focused on antagonism of the dopamine-2 (D_2) receptor (Creese *et al*, 1976; Seeman and Lee, 1975). Dopamine-3 (D_3) receptors belong to the same class of receptors as D_2, and there is evidence they may have a role in several neuropsychiatric disorders, including addiction, Parkinson's disease, and schizophrenia (Sokoloff *et al*, 2006). In particular, therapeutic agents targeted towards the D_3 receptor may hold promise as novel antipsychotic agents. Until recently, direct *in vivo* evidence of D_3 receptor dysfunction in neuropsychiatric populations has not been possible to obtain because of the lack of a positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging agent that can suitably distinguish between dopamine D_3 and D_2 binding. Hence, the potential contribution of D_3 receptor blockade to antipsychotic efficacy has been difficult to assess.

[^{11}C]-(+)-PHNO is a recently developed dopamine $D_{2/3}$ radioligand (Wilson *et al*, 2005) that is strongly D_3 preferring (Narendran *et al*, 2006). *In vivo* imaging in non-human primates and mouse autoradiography paradigms have demonstrated that PHNO binding in the midbrain dopamine nuclei (substantia nigra, ventral tegmental area, SN/VTA) is almost completely due to D_3 binding (Rabiner *et al*, 2009). [^{11}C]-(+)-PHNO binding also displays vulnerability to competition by selective D_3 agents in other D_3-rich brain regions such as globus pallidus (GP), and ventral striatum (VST) (Narendran *et al*, 2006; Rabiner *et al*, 2009; Searle *et al*, 2010). These findings suggest that the D_3-preferring ligand [^{11}C]-(+)-PHNO can be used to ascertain the extent of D_3 binding by antipsychotics.

Extensive preclinical data suggest that many antipsychotic medications bind to D_2 receptors with moderately higher affinity than they do to D_3 receptors (Table 1) (Levant, 1997; Schotte *et al*, 1996). On the basis of these *in vitro* selectivity ratios, predicted occupancy of D_3 at therapeutic doses would be lower than D_2 occupancy, but still non-negligible

*Correspondence: Dr RR Girgis, New York State Psychiatric Institute, Unit 31, 1051 Riverside Dr., New York, NY 10032, USA, Tel: +212 543 5055, Fax: + 212 568 6171, E-mail: rg2290@columbia.edu
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Table 1 *In Vitro* Estimates of D_2/D_3 Selectivity of Antipsychotics and the Predicted Occupancy at D_3 Receptors at Clinically Effective Levels of D_2 Receptor Blockade Based on Previous Reports (Levant, 1997; Schotte *et al*, 1996)

Antipsychotic	No. of studies	$D_2:D_3$ Selectivity ± SD	D_2 Occupancy	Predicted D_3 occupancy
Clozapine	9	2.82 ± 2.01	60%	35%
Haloperidol	11	3.03 ± 4.42	75%	50%
Risperidone	3	4.9 ± 3.71	75%	38%
Olanzapine	1	3.18	75%	49%
Amisulpride 230 mg	3	2.29 ± 0.58	67%	47%
Amisulpride 670 mg	3	2.29 ± 0.58	80%	64%
Raclopride	3	1.16 ± 0.53	—	—

D_2, and predicted D_3, occupancies are for illustrative purposes and are intended to reflect the normal therapeutic range for each compound. The calculation suggests that D_3 occupancy should range from 1/2 to 2/3 of D_2 occupancy at these levels of D_2 occupancy.

(Table 1). However, D_3-binding levels by antipsychotics were called into question by a recent study by Graff-Guerrero *et al* (2009), which used [^{11}C]-(+)-PHNO to investigate *in vivo* binding of antipsychotics to D_3. Patients with schizophrenia on stable, chronically-administered doses of clozapine (CLZ), risperidone, or olanzapine showed no decrease or even increased [^{11}C]-(+)-PHNO binding in GP and modest decrease in VST (17 ± 35%) when compared with 20 healthy control subjects at baseline, whereas binding in the D_2-dominated dorsal striatum was considerably reduced compared with controls. Specific binding of the D_2-preferring tracer [^{11}C] raclopride, which in VST and GP is predominantly because of D_2, was substantially reduced in all measured brain regions in patients compared with controls. These results suggested either that antipsychotics do not bind D_3 receptors *in vivo*, there is a change in the availability of D_3 receptors following chronic administration of drugs (that is, upregulation) or a change in the affinity of [^{11}C]-(+)-PHNO to D_3 receptors in schizophrenia. Another study from the same research group used *ex vivo* and *in vitro* [^{3}H]-(+)-PHNO binding in rat brain and found that acute doses of CLZ, olanzapine, haloperidol (HAL), and risperidone demonstrated lower selectivity for the D_3 compared with D_2 receptors *ex vivo* than *in vitro* (McCormick *et al*, 2010).

Determining the degree of antipsychotic binding to the D_3 receptor, if any, has important implications for the development of novel treatments for schizophrenia. Given the discrepancies between the older *in vitro* literature and recent *ex* and *in vivo* reports about the binding of antipsychotic medications to the D_3 receptor and the importance of identifying whether or not, and to what degree, antipsychotic medications bind to D_3 receptors, we undertook to examine the acute binding of antipsychotics to D_3 receptors in the *in vivo* setting. We also had concern, however, that because of the much higher affinity of [^{11}C]-(+)-PHNO for D_3 compared with D_2 receptors (Beaver *et al*, 2009), there was a potential for our results to be confounded by mass-carryover effects at D_3, but not D_2, receptors. Therefore, we conducted this study in two phases. First, we performed test-retest scans in three anesthetized baboons to ascertain if there would be mass-carryover effects when two [^{11}C]-(+)-PHNO scans were conducted in a single scanning session. Next, we performed an acute dose study in anesthetized baboons with two commonly used and prototypical antipsychotic agents, CLZ, and HAL. We measured the binding of [^{11}C]-(+)-PHNO in subcortical brain regions before and after administration of CLZ and HAL. We then estimated individual D_2 and D_3 receptor occupancies, as well as the $D_2:D_3$ receptor selectivity of these medications using a model based on all of the data.

Table 2 Study Design and Number of Scans per Subject in each Study

Subject	Test-retest study		Antipsychotic study		
	Test	Retest	Baseline	CLZ	HAL
A	1	1	—	—	—
B	1[a]	1	3	2	2
C	1[a]	1	3	2	2
D	—	—	2	2	2

[a]The Test scans from the test-retest Study for these two subjects were included among the baseline scans in the Antipsychotic Study.
For subjects A, B, and C, the test and retest scans were performed on the same day (that is, the same scanning session). All other scans were obtained on separate days (that is, single scan sessions). Each subject was given at least 12 days in between scanning sessions, and no subject was scanned more than three times in a month.

SUBJECTS AND METHODS

Subjects

Three adult male baboons were scanned in the test-retest study (baboons A, B, and C, *papio Anubis*, 20.1 ± 4.8 kg) and antipsychotic studies (baboons B, C, and D, *papio anubis*, 23.9 ± 2.4 kg; Table 2). A minimum of 12 days was allowed between each experimental day for each baboon and no baboon was studied more than three times per month. Study procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of Columbia University and the New York State Psychiatric Institute.

Test-Retest Study

To detect a potential mass-carryover effect at the D_3 site, three baboons were scanned at baseline and then 172 ± 36 min later (retest condition 52 ± 36 min after the end of the test scan; Table 2). The percent change in binding potential (see Data Analysis, below), ΔBP_{ND}, was used as an outcome measure to assess reproducibility. This signed formulation was used rather than a measure of variability because the direction of change across conditions was the quantity of interest.

Antipsychotic Study

Baseline [^{11}C]-(+)-PHNO scans were acquired for each subject. Two baboons underwent three baseline scans and one baboon underwent two baseline scans (Table 2). The average of each subject's baseline data was used for comparison with the drug challenge data. For challenge scans, each baboon received CLZ (0.5534 mg/kg) or HAL (0.0109 mg/kg), dissolved in 10 ml of saline vehicle. These doses were selected to attain target occupancies of 50% (CLZ) or 75% (HAL) in dorsal striatum where binding is predominantly to the D_2 receptors. Challenges were administered manually as a five-minute intravenous (iv) bolus, 15 min before the scan. Each subject underwent two scans following each drug. In all, 20 scans were acquired (eight baseline scans plus three subjects * two drugs * two post-challenge scans per drug).

Radiochemistry

[^{11}C]-(+)-PHNO was prepared as described by Wilson *et al* (2005), with some modifications (Rabiner *et al*, 2009).

PET Procedures

Fasted animals were initially immobilized with ketamine (10 mg per kg i.m.). Anesthesia during preparation and scans was maintained with 2% isoflurane via endotracheal tube. An iv catheter was inserted for radioligand administration, drug administration and hydration. An arterial catheter was placed in a femoral artery for arterial blood sampling and continuous blood pressure monitoring. Vital signs (blood pressure, pulse, EKG, temperature, pulse oximetry, and respiration) were monitored continuously using a patient monitoring system (DataScope Corp, Paramus, NJ) and remained within normal parameter ranges during all studies that did not include the administration of CLZ. Subjects' blood pressures did decrease by ~20% during administration of CLZ. In all cases, hemodynamic parameters normalized with gentle administration of normal saline. No subject demonstrated other abnormalities in vital signs or EKG or required resuscitative measures. Temperature was maintained at 37°C with a heated water blanket. All scanning was performed on an HR + scanner (Siemens, Knoxville, TN), operating in 3D mode. The animal's head was placed in the center of the field of view. A 10-min transmission scan was acquired before tracer injection for attenuation correction. [^{11}C]-(+)-PHNO was administered as a 30 s iv bolus. Emission data were binned into a sequence of frames of increasing length. Each scan included 22 frames (4×15 s, 2×1 min, 4×2 min, 2×5 min, and 10×10 min). Total scan durations were 121 min. PET data were corrected for attenuation, scatter, and randoms and reconstructed using filtered back-projection with a Shepp filter (cutoff 0.5 cycles/ ray).

Arterial plasma samples were collected using an automated sampling system during the first 4 min (11 samples), and manually thereafter at longer intervals. A total of 22 arterial samples were collected for input function measurement. Six additional samples were collected during each scan for HPLC analysis of the unmetabolized fraction of radiotracer. Aliquots were also removed from six of the obtained samples for analysis of CLZ and HAL plasma levels.

Data Analysis

Plasma data. Arterial plasma input functions were formed as described previously (Abi-Dargham *et al*, 2000). Briefly, centrifugation was performed to separate plasma from whole blood and activity in plasma was counted on a gamma counter (Wallac 1480, Perkin-Elmer, Boston MA). The unmetabolized fraction of radioligand was measured in the six samples taken for this analysis by HPLC using a fraction collection method. The measured fractions were fitted to a sum of two exponential functions. Whole-blood activity was multiplied by this parent-fraction curve to obtain an empirical estimate of the unmetabolized radiotracer concentration in arterial plasma. The empirical curve was fitted to a sum of three exponentials starting at the time of peak concentration. This modeled curve was used as the input function for compartment analysis. Peripheral clearance of radiotracer (L/hr) was computed as the injected activity divided by the area under the curve of the input function. Additionally, fp, the fraction of unmetabolized radiotracer not bound to plasma proteins, was measured with an ultrafiltration technique as previously described (Ekelund *et al*, 2007). For fp measurement, a small quantity of [^{11}C]-(+)-PHNO was added to a sample of arterial plasma collected before radiotracer administration.

PET data. Decay-corrected reconstructed data were co-registered to T1 weighted anatomical MRIs acquired for each animal, using maximization of mutual information as implemented in the SPM2 software environment (Friston *et al*, 1995). Regions of interest (ROIs) were drawn on the MRIs and transferred to the co-registered PET images. ROIs included putamen (PUT), caudate (CAU), VST, GP, SN/VTA, and thalamus (THA). Time activity curves were generated from the mean activity in each region and each frame. Data were fitted using a two-tissue compartment model (2TC) with arterial plasma input, with cerebellum as reference region. The total distribution volume (VT) was computed in each region including cerebellum, and BP_{ND}, the binding potential relative to the non-displaceable compartment (Innis *et al*, 2007), was computed as:

$$\frac{V_T(ROI)}{V_T(CER)} - 1 \quad (1)$$

For the antipsychotic study, combined D_2 and D_3 receptor occupancy by CLZ and HAL was estimated as $-\Delta BP_{ND}$, where ΔBP_{ND} is the percent change in BP_{ND} across

Table 3 The Fractions of [^{11}C]-(+)-PHNO BP_{ND} Attributable to D_3 in Each Region as Reported in Table III of Rabiner *et al* (2009)

Region	f_{D_3} (%)
SN/VTA	95
Thalamus	88
GP	72
VST	49
Caudate	23
Putamen	8

Abbreviations: GP, globus pallidus; SN/VTA, substantia nigra/ventral tegmental area; VST, ventral striatum.
This data was used in the regression model (Equation (3)) to calculate $D_2:D_3$ binding ratios for clozapine and haloperidol.

conditions in each ROI,

$$\Delta BP_{ND} = \frac{BP_{ND}(\text{Drug condition})}{BP_{ND}(\text{Average baseline})} - 1 \quad (2)$$

To estimate the $D_2:D_3$ selectivity of CLZ and HAL, data from all ROIs were fitted to a linear model that used previously established regional fractions of the D_2 and D_3 contributions to total [^{11}C]-(+)-PHNO BP_{ND} in anesthetized baboons in each of the regions:

$$BP_{ND}(\text{drug condition}) = BP_{ND}(\text{baseline}) \times [f_{D_3}(1 - occ(D_3)) + f_{D_3}(1 - occ(D_2))] \quad (3)$$

where fD_3 and fD_2 are the fractions of [^{11}C]-(+)-PHNO BP_{ND} attributable to each receptor type (Table 3) (Rabiner *et al*, 2009), occ(*) is the drug occupancy at each receptor type. The selectivity for D_2 *vs* D_3 was then computed from the estimated occupancies as

$$ED_{50}(D_3)/ED_{50}(D_2) = [occ(D_2)/(1 - occ(D_2))]/[occ(D_3)/(1 - occ(D_3))] \quad (4)$$

where ED_{50}(*) is the estimated dose at which 50% of receptor type * is occupied by drug. Equation (4) follows from the rearrangement of the equilibrium mass action law for reversibly binding ligands (Equation (5))

$$Occ(*) = \frac{Dose}{Dose + ED_{50}(*)} \quad (5)$$

RESULTS

Test-Retest Study

The scan parameters for the test-retest study are shown in Table 4. There were no significant differences between the baseline and retest conditions on any scan parameter. Regional BP_{ND} and ΔBP_{ND} values are presented in Table 5. One test value in GP was excluded because of unstable parameter estimates. The mean retest BP_{ND} was lower than the test BP_{ND} by 20% or more in all D_3-rich regions (GP, VST, THA, SN/VTA), reaching statistical significance in VST, but was within 7% of test BP_{ND} in dorsal striatum (PUT and CAU). These results indicated that in D_3-rich regions, [^{11}C]-(+)-PHNO binding potential decreased purely from an order effect, suggesting the possibility that use of pre- and post- antipsychotic challenge scans from the same scanning session would introduce a quantification artifact. Therefore, in the subsequent antipsychotic occupancy study, only one antipsychotic challenge scan was acquired per session without baseline, thereby avoiding any possible mass carryover or other order effect from scanning twice in the same session. To measure the change across conditions, BP_{ND} obtained from each antipsychotic challenge scan was individually compared with an average of previous baseline scans for the same subject, maintaining the within-subject design of this study.

Table 4 Scan Parameters (Mean ± SD) Across Three Baboons of [^{11}C]-(+)-PHNO for Test-Retest Study[a]

Parameter	Baseline	Retest
n	3	3
Injected activity (mCi)	2.47 ± 0.74	2.24 ± 1.23
Injected mass (μg)	1.01 ± 0.39	0.79 ± 0.18
f_P	0.45 ± 0.04	0.44 ± 0.06
Clearance (L/h)	49.8 ± 4.2	51.5 ± 19.8
V_T cerebellum (ml/g)	5.28 ± 0.91	6.17 ± 0.80

[a]No significant differences on any parameter.

Table 5 [^{11}C]-(+)-PHNO BP_{ND} and $-\Delta BP_{ND}$ (%) (Mean ± SD) Values Across Three Baboons for Test-Retest Study

Region	Baseline BP_{ND} ($n = 3$)	Retest BP_{ND} ($n = 3$)	$-\Delta BP_{ND}$ (%)	*p*
Putamen	2.59 ± 0.33	2.57 ± 0.37	1 ± 12	0.91
Caudate	2.33 ± 0.38	2.16 ± 0.40	7 ± 11	0.34
VST	4.10 ± 0.49	3.25 ± 0.80	22 ± 9	0.04
GP	5.77 ± 2.77	3.32 ± 0.56	42 ± 18	0.33
SN/VTA	1.31 ± 0.23	0.89 ± 0.30	29 ± 32	0.23
Thalamus	1.08 ± 0.20	0.75 ± 0.12	29 ± 16	0.11

Abbreviations: GP, globus pallidus; SN/VTA, substantia nigra/ventral tegmental area; VST, ventral striatum.

Antipsychotic Study

The scan parameters for the Antipsychotic Study are shown in Table 6. There were no significant differences on any scan parameter across all conditions.

Plasma input functions and time activity curves from several brain regions, averaged across all scans before and after administration of 0.5534 mg/kg of CLZ and 0.0109 mg/kg of HAL, are shown in Figure 1a and b. Representative BP_{ND} maps from one subject across all conditions are shown in Figure 2. Regional BP_{ND} values at baseline and after administration of CLZ and HAL are shown in Table 7. Both CLZ and HAL decreased [^{11}C]-(+)-PHNO to an extent that was close to the target level in the dorsal striatum (44% decrease for CLZ, 70% decrease for HAL, Table 7).

Table 6 Scan Parameters (Mean ± SD) Across Three Baboons of [^{11}C]-(+)-PHNO for Antipsychotic Study[a]

Parameter	Baseline	Clozapine challenge	Haloperidol challenge
n	8	6	6
Injected activity (mCi)	2.81 ± 0.63	2.52 ± 0.47	2.38 ± 0.44
Injected mass (μg)	0.73 ± 0.22	0.92 ± 0.07	0.90 ± 0.07
fP	0.43 ± 0.05	0.46 ± 0.04	0.45 ± 0.04
Clearance (L/h)	57.7 ± 14.6	57.8 ± 20.6	61.0 ± 21.1
V_{ND} (ml/g)	5.69 ± 1.36	5.46 ± 1.30	5.01 ± 0.95

[a]No significant differences between any baseline-Clozapine, baseline-Haloperidol, and Clozapine-Haloperidol conditions.

Regression Model-Based Estimates for $D_2:D_3$ Selectivity and Occupancy

Using the model from Equation (3) and the *in vivo* data acquired in this study, CLZ *in vivo* occupancy (±95% confidence interval) was 47% (±14%) at D_2 and 15% (±12%) at D_3. HAL *in vivo* occupancy was 76% (±18%) at D_2 and 57% (±14%) at D_3. ED_{50} values were computed based on the administered doses and these occupancy estimates. ED_{50} values for CLZ were 0.6135 mg/kg at D_2 and 3.2216 mg/kg at D_3. HAL ED_{50} values were 0.0035 mg/kg at D_2 and 0.0083 mg/kg at D_3.

The $D_2:D_3$ selectivities of CLZ and HAL were calculated, based on these occupancy estimates. *In vivo*, HAL selectivity was very similar to *in vitro* predictions (2.38 *in vivo* compared with 3.03 ± 4.42, $n=11$ *in vitro* studies). *In vivo* selectivity for CLZ was approximately one SD higher than the mean of nine *in vitro* studies (5.25 *in vivo* compared with 2.82 ± 2.01 *in vitro*).

DISCUSSION

This study used [^{11}C]-(+)-PHNO to investigate whether acute doses of the antipsychotic medications CLZ and HAL bind *in vivo* to the D_3 receptor and to estimate their $D_2:D_3$ receptor selectivities. We observed that acute doses of HAL bind to D_3 receptors within the range predicted by *in vitro* data, and that CLZ does bind to D_3 receptors at the lower end of the range predicted by *in vitro* data. ΔBP_{ND} in dorsal striatum (PUT, CAU), which is attributable mostly to D_2 occupancy, was similar to the clinical range for both drugs (that is, ~45% for CLZ and ~70% for HAL). ΔBP_{ND} was lower in D_3-rich regions, but measurable, as most clearly illustrated in the SN/VTA region, where [^{11}C]-(+)-PHNO specific binding is almost exclusively due to D_3 receptor binding (Narendran *et al*, 2006; Rabiner *et al*, 2009; Searle *et al*, 2010). Our results differ from those of Graff-Guerrero *et al* and McCormick *et al* in some important ways. We observed decreased binding of [^{11}C]-(+)-PHNO in GP following CLZ, consistent with D_3 binding by CLZ, whereas Graff-Guerrero *et al* observed no decrease (relative to matched controls) or even increased [^{11}C]-(+)-PHNO binding in GP. We observed robust binding of HAL to D_3, whereas McCormick *et al* observed negligible binding of HAL to D_3 when administered *in vivo* and measured *ex vivo* in rats.

Graff-Guerrero *et al* reported that chronically administered olanzapine, risperidone, and CLZ in patients with schizophrenia did not occupy D_3, and that in some cases paradoxical increases in radioligand binding were observed (Graff-Guerrero *et al*, 2009). The data we report in this study show that both CLZ and HAL do bind to D_3 receptors when administered acutely to anesthetized primates; this suggests that the observation in patients could be attributable to some other phenomenon such as upregulation of receptors following chronic administration, as discussed by the authors. The regulation of D_3 expression has been shown to be distinct from that of D_2 in a number of ways (Sokoloff *et al*, 2006), and currently there is insufficient evidence either corroborating or ruling out the upregulation scenario. Another difference between the studies is the use of anesthesia in this study. Although it has been shown that isoflurane anesthesia can cause alterations in PET measurements of stimulant-induced dopamine release (Tsukada *et al*, 2002), we are not aware of studies that have examined the effect of anesthesia on PET measurements of receptor occupancy by antipsychotic drugs, but this remains a possibility we cannot rule out. Additionally, our selectivity estimate used the assumption that the fractions of [^{11}C]-(+)-PHNO binding to D_2 and D_3 receptors in this cohort were similar to those measured in our previous studies with D_3-selective agents. Although we have found these parameters to be consistent across subjects, we did not measure them directly in this study.

However, there are other methodological differences between the studies that could offer a simpler explanation for the differing conclusions. We used arterial plasma-based kinetic models following 120 min of emission data, while in the Graff–Guerrero study the authors used reference region analysis (SRTM, Lammertsma and Hume, 1996) following 90 min of data acquisition. The arterial-based method is a direct implementation of the equations of the 2TC model, whereas the SRTM algorithm is derived from the 2TC equations with additional simplifying assumptions. Although these two methods give correlated estimates of BP_{ND} for many ligands, they are not identical, and can be quite different in cases where the data do not conform well to the simplifying assumptions of SRTM (Slifstein *et al*, 2000). We measured modest decreases in GP [^{11}C]-(+)-PHNO binding following CLZ (21 ± 14%) that still, however, included a component of binding of CLZ to D_3. It is possible that these relatively modest decreases were difficult to detect with shorter scans and SRTM in this brain region where [^{11}C]-(+)-PHNO has complex kinetics, and especially slow washout. To test this, we reexamined our data under three additional conditions: the same analysis as before with data truncated to 90 min, and SRTM with both 90 and 120-min data. With the plasma-based approach, baseline binding measures were nearly identical at both durations in the D_2-dominated dorsal striatum (90 min $BP_{ND}=100\pm5\%$ of 120 min BP_{ND} in caudate and 103 ± 2% of 120 min BP_{ND} in putamen), whereas in the D_3-rich regions the binding measures tended to be decreased following truncation (90 min $BP_{ND}=94\pm8\%$ of 120 min BP_{ND} in GP, 92 ± 7% in VST, 91 ± 4% in thalamus, 93 ± 4% in SN/VTA). With 120 min SRTM BP_{ND}, the ratio to plasma-based BP_{ND} decreased as a function of binding magnitude (Figure 3), and this effect was even more

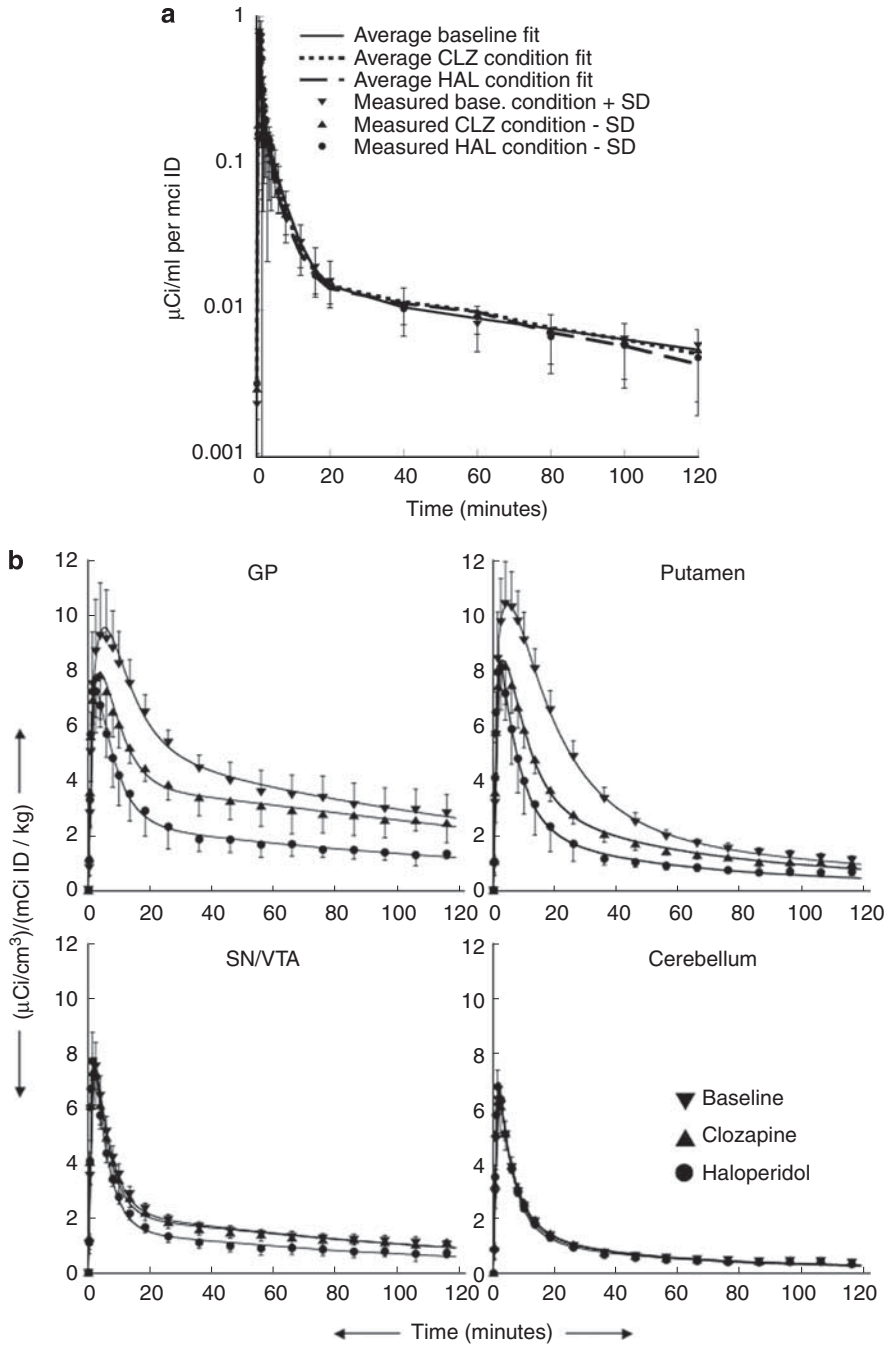

Figure 1 (a) Averaged arterial plasma concentration of [^{11}C]-(+)-PHNO under baseline (▼, $n=8$ scans), clozapine challenge (▲, $n=6$ scans) and haloperidol challenge (●, $n=6$ scans) conditions. Continuous curves (—) represent the average of the modeled fits as described in the Methods section. Data are normalized to injected activity. The graph shows that [^{11}C]-(+)-PHNO concentration in arterial plasma was unaffected by the drug challenges. (b) Averaged time activity curves from all studies under baseline (▼, $n=8$ scans), clozapine challenge (▲, $n=6$ scans) and haloperidol challenge (●, $n=6$ scans) conditions. Continuous curves (—) represent the average of the two-tissue compartment model (2TC) fits. Error bars are the SD across the measured data. Data are normalized to injected activity and body mass and are shown for a region with similar contributions from dopamine-2 (D_2) and dopamine-3 (D_3) binding (globus pallidus, (GP)), mostly D_2 binding (putamen) and mostly D_3 binding (substantia nigra/ventral tegmental area (SN/VTA)), as well as the cerebellum.

pronounced with 90-min SRTM BP_{ND} (Figure 3). When the occupancy model was applied to 120-min SRTM data, $D_2:D_3$ selectivity was 3.6 for HAL but increased to 19 for CLZ. When applied to 90-min data, selectivity was 3.7 for HAL and 49 (that is, nearly undetectable) for CLZ. Thus in these data at least, there was a pronounced qualitative difference in outcome associated with both scan duration and method of analysis, suggesting the longer duration with 2TC may be a more sensitive and reliable approach in this setting. It remains to be seen if a similar relationship is observable in data acquired in human subjects; our data in this study highlight the importance of comparing the different approaches in that setting.

In rat brains, McCormick *et al* observed that CLZ, HAL, olanzapine, and risperidone all competed robustly with [^{3}H]-(+)-PHNO binding in homogenates from a number of

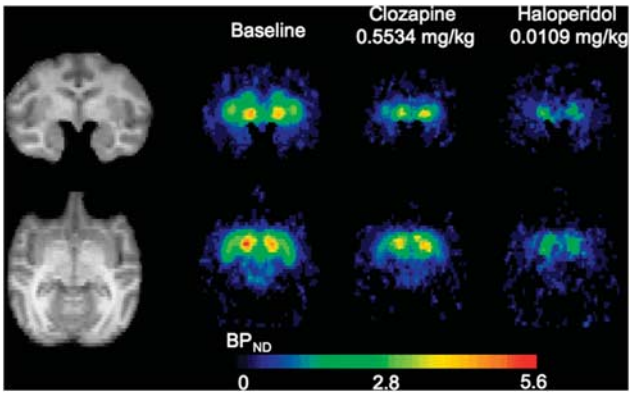

Figure 2 [^{11}C]-(+)-PHNO BP_{ND} maps in one baboon at the level of the GP across the three conditions. Images are the average of three baseline scans and two post-drug scans for each antipsychotic, and were generated at each voxel with the SRTM algorithm.

Table 7 [^{11}C]-(+)-PHNO BP_{ND} and $-\Delta BP_{ND}$ (%) (Mean ± SD) Values Across Three Baboons for Antipsychotic Study

Region	BP_{ND}			$-\Delta BP_{ND}$ (%)	
	Baseline	CLZ	HAL	CLZ	HAL
Putamen	2.41 ± 0.40	1.38 ± 0.28	0.74 ± 0.36	43 ± 11	70 ± 14
Caudate	2.37 ± 0.51	1.35 ± 0.32	0.75 ± 0.31	44 ± 10	69 ± 13
VST	3.57 ± 1.15	2.76 ± 0.74	1.18 ± 0.39	24 ± 17	66 ± 18
GP	5.54 ± 1.73	4.30 ± 0.71	2.12 ± 0.76	21 ± 14	61 ± 14
SN/VTA	1.27 ± 0.26	1.23 ± 0.33	0.77 ± 0.61	7 ± 12	39 ± 50
Thalamus	1.12 ± 0.25	0.99 ± 0.21	0.79 ± 0.86	14 ± 10	31 ± 75

Abbreviations: GP, globus pallidus; SN/VTA, substantia nigra/ventral tegmental area; VST, ventral striatum.
Baselines are averages of three scans (two subjects) or two scans (one subject). Post-drug scans are average of two scans per drug for every subject. ΔBP_{ND} for each subject is relative to the average of that subject's baseline BP_{ND}.

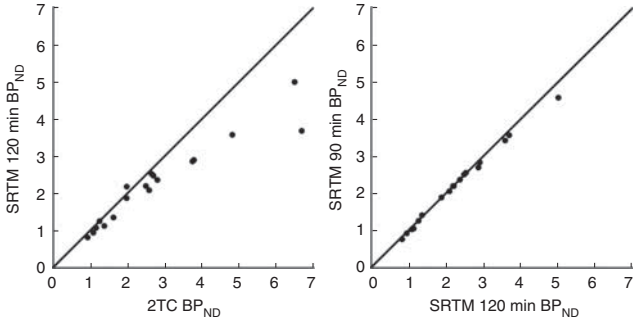

Figure 3 Scatter plots of regions of interest (ROI) analysis using SRTM. The left panel shows SRTM-derived BP_{ND} using the full 120 min of data plotted against 2TC-derived BP_{ND}. The graph shows that there is a similar proportionality between methods across moderate-binding regions, but not in high-binding regions. In the right panel, SRTM-derived data using 90-min truncated data are plotted against SRTM-derived data using 120 min of data. The graph shows the measures are virtually identical in the moderate-binding regions, but that the decrease in high-binding regions (relative to 2TC) is more pronounced with the truncated data. The solid line in each graph is the line of identity.

brain regions including the D_3-rich cerebellar lobes 9 and 10 and the ventral pallidum, but were much less competitive in the setting of *ex vivo* autoradiographic measurements of [^{3}H]-(+)-PHNO binding in rats treated with the same drugs and then killed at 1 h after radioligand injection (McCormick *et al*, 2010). In particular, it was observed that average HAL occupancy in the dorsal striatum (mostly attributable to D_2) was 80%, whereas occupancy in cerebellar lobes 9 and 10 (mostly D_3) was negligible, in contrast to our data where HAL exhibited robust D_3 binding ($D_2:D_3$ selectivity ratio $=2.38$). The CLZ results from the two studies, on the other hand, are much more consistent with each other.

In addition to species differences, the kinetic methods used in the two studies were different; the *ex vivo* occupancy estimates in the rodent study were based on tissue ratios of [^{3}H]-(+)-PHNO concentration measured at the single time point of killing across all regions, whereas the primate study used 120 min of dynamic data from each brain region. Additionally, the doses of PHNO were different between the studies. In their *ex vivo* study, McCormick *et al* injected 2 nmol of [^{3}H]-(+)-PHNO, approximately 1.8 μg/kg in a 275 g rat. In this study, we injected ~0.04 μg/kg of [^{11}C]-(+)-PHNO in baboons. The characterization of the precise limits on tracer dose for (+)-PHNO has not been definitively established, but this 45-fold difference in injected concentration suggests that the present data were less likely to have been affected by mass-dose effects, especially at D_3 receptors where (+)-PHNO affinity is 20-fold higher than at D_2 (Beaver *et al*, 2009). These facts, however, seem unlikely by themselves to explain why no binding by the high affinity drug HAL was observed in the D_3-dominated cerebellar lobes, whereas the lower affinity drug CLZ displayed moderate binding in those regions in rodents.

In this study, the $D_2:D_3$ selectivity ratio for CLZ was slightly higher than that predicted by *in vitro* literature. One possible explanation for the lower than expected *in vivo* D_3 binding of CLZ observed here following a single iv dose may be a kinetic effect related to its fast off-rate (Seeman, 2002). Kapur and Seeman measured *in vitro* on and off rates separately for a large number of antipsychotics in homogenates prepared from rat striatum and observed that there was much greater variability in k_{off} than k_{on} between drugs, and that k_{off} therefore accounted for most of the observed affinity differences between drugs (Kapur and Seeman, 2000). The ratio of CLZ k_{on} to HAL k_{on} was 1.4, whereas that of CLZ k_{off} to HAL k_{off} was 81, accounting for most of their affinity difference. These differences, and the fact that plasma clearance of CLZ and HAL following iv injection are similar (Cheng *et al*, 1988; Kudo and Ishizaki, 1999), suggest that, following a bolus administration, CLZ will dissociate from receptors in response to decreasing plasma concentration much more rapidly than HAL. Receptor occupancy measured during a radiotracer scan (combined D_2 and D_3 occupancy in this case) is a weighted average of the occupancy over the course of the scan with weight at time t proportional to the concentration of the non-displaceable component (free plus nonspecifically bound) of the radioligand concentration (Endres and Carson, 1998; Kegeles *et al*, 2008). Compared with dorsal striatum, the slower washout of [^{11}C]-(+)-PHNO from D_3-rich regions,

especially in the GP, might have caused the measured occupancy to be weighted more towards later times in the scan when CLZ occupancy may have decreased. This effect would be less pronounced for HAL because of its higher affinity and slower off-rate. The presence of this effect in these data is conjectural, but could be tested by administration of CLZ as a constant infusion rather than as a bolus.

In conclusion, we observed that acute doses of both CLZ and HAL bind to D_3 receptors *in vivo*. HAL binds to D_3 receptors within the range predicted by *in vitro* data and CLZ binds to D_3 receptors at the lower end of the range predicted by *in vitro* data. These results suggest that antipsychotic medications do bind to D_3 receptors at predicted levels when administered acutely *in vivo*, and that therefore the apparent lack of occupancy by antipsychotics, including CLZ, in D_3-rich regions when administered chronically to patients, is due to some other phenomenon. We have highlighted some of the methodological issues that might have lead to discrepancies in estimates of occupancy in D_3-rich regions between our report and previous reports. An alternative explanation that could reconcile our data and those of Graff–Guerrero *et al* would be the upregulation of D_3 receptors following chronic antipsychotic treatment. The regulation of D_3 expression has been shown to be distinct from that of D_2 in a number of ways (Sokoloff *et al*, 2006), and additional studies are required to test the upregulation scenario. Future studies should address the effects of chronic administration on expression of D_3 and also assess the binding of other commonly used antipsychotic medications to D_3 receptors. This information will allow researchers a more complete understanding of the role of the D_3 receptor in the pathophysiology and treatment of schizophrenia, and therefore has great implications for the targeted development of novel therapeutics.

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