

# The Effect of Antipsychotics on the High-Affinity State of D<sub>2</sub> and D<sub>3</sub> Receptors

## A Positron Emission Tomography Study With [<sup>11</sup>C]-(+)-PHNO

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**Context:** Most antipsychotics are thought to have an effect on D<sub>2</sub> and D<sub>3</sub> receptors. The development of carbon 11-labeled (+)-4-propyl-9-hydroxynaphthoxazine ([<sup>11</sup>C]-(+)-PHNO), the first agonist radioligand with higher affinity for D<sub>3</sub> than D<sub>2</sub> receptors, allows one to differentiate the effects of antipsychotics on high-affinity vs low-affinity sites of the D<sub>2</sub> receptor and on D<sub>3</sub> vs D<sub>2</sub> receptor subtypes.

**Objectives:** To examine the effects of antipsychotics (clozapine, risperidone, or olanzapine) on the high- vs high- + low-affinity sites of the D<sub>2</sub> and D<sub>3</sub> receptors by comparing the [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride binding in the D<sub>3</sub> receptor-rich (globus pallidus and ventral striatum) and D<sub>2</sub> receptor-rich (caudate and putamen) regions.

**Design:** Two sequential studies with different participants and appropriate controls were performed. The first compared the occupancy produced by 3 antipsychotics as measured with [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride. The second was a double-blind, placebo-controlled experiment to compare the effect of pramipexole (a D<sub>3</sub> receptor-preferring agonist) vs placebo on the increased [<sup>11</sup>C]-(+)-PHNO signal observed in the globus pallidus of patients.

**Setting:** Positron Emission Tomography Centre, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

**Participants:** Twenty-three patients with schizophrenia and 23 healthy controls.

**Main Outcome Measures:** Antipsychotic occupancies as measured with [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride.

**Results:** The antipsychotic-treated patients showed high occupancies with both [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride in the dorsal striatum, with [<sup>11</sup>C]raclopride occupancies about 20% higher. Most strikingly, patients did not show any occupancy with [<sup>11</sup>C]-(+)-PHNO in the globus pallidus as compared with normal controls or with their own scans using [<sup>11</sup>C]raclopride. This unblocked [<sup>11</sup>C]-(+)-PHNO signal was displaced by a single dose of pramipexole.

**Conclusions:** Antipsychotics block both the high- and low-affinity states of the D<sub>2</sub> receptors across the brain, but antipsychotic treatment does not block the [<sup>11</sup>C]-(+)-PHNO signal in the D<sub>3</sub> receptor-rich regions, despite the ongoing D<sub>2</sub> receptor blockade. This [<sup>11</sup>C]-(+)-PHNO signal in regions such as the globus pallidus seems increased despite antipsychotic treatment and is displaceable by a D<sub>3</sub> receptor-preferring agonist. The radiotracer [<sup>11</sup>C]-(+)-PHNO and the data open up new avenues for exploring the potential therapeutic significance of the D<sub>3</sub> receptor in schizophrenia.

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**U**NDERSTANDING THE ACTION of antipsychotic drugs has been at the heart of 5 decades of schizophrenia research.<sup>1,2</sup> The clinical efficacy and adverse effects of antipsychotics have been consistently related to the level of in vivo occupancy at the D<sub>2</sub> receptors.<sup>3-9</sup> Previous studies proposed that the occupancy required for clinical efficacy is in the range of 60%, while the occurrence of adverse effects has been observed at higher than 80% occupancy.<sup>3-9</sup> All these studies have been performed with positron emission tomography

(PET) or single-photon emission tomography radioligands that are antagonists for the dopamine D<sub>2</sub>/D<sub>3</sub> receptors: carbon 11-labeled raclopride ([<sup>11</sup>C]raclopride), [<sup>11</sup>C]FLB ([O-methyl-11C]-(-)-5-bromo-N-((1-ethyl-2-pyrrolidinyl)methyl)-2,3-dimethoxybenzamide), fluor 18-labeled fallypride, or iodine 123-labeled iodobenzamide.<sup>10-12</sup> However, since these radioligands have similar affinity for the D<sub>2</sub> and D<sub>3</sub> receptors, one cannot infer selective effects of antipsychotics on D<sub>2</sub> vs D<sub>3</sub> receptors; thus, the differential effects of antipsychotics on D<sub>2</sub> vs D<sub>3</sub> receptors remain unexplored in patients.

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## CLINICAL SAMPLE

The high-affinity state of the D<sub>2</sub> receptor is believed to be the functionally important one, as it mediates the activation of the second-messenger cascade on the postsynaptic membrane after the endogenous agonist has bound to the receptor.<sup>13,14</sup> What makes the high-affinity state of the D<sub>2</sub> receptors particularly interesting is a series of experiments that have shown an increase in the number of D<sub>2</sub> receptors in the high-affinity state in several animal models of psychosis<sup>15</sup> and after long-term antipsychotic exposure,<sup>15,16</sup> suggesting that the high-affinity state of the D<sub>2</sub> receptor is involved in the illness and its treatment. While these 2 states have been convincingly demonstrated for the D<sub>2</sub> receptor, the existence of the G-protein coupled and uncoupled states of the D<sub>3</sub> receptors is controversial<sup>17-19</sup> because of the inability of the G-protein analogs (eg, GppNHp) to shift it from the high- to the low-affinity state.<sup>20</sup> Since all previous PET imaging studies have used antagonist radioligands, which do not distinguish between the high- and low-affinity states of the receptors, it remains unknown how antipsychotics bind to these 2 states *in vivo*.

Recently, our group has developed carbon 11-labeled (+)-4-propyl-9-hydroxynaphthoxazine ([<sup>11</sup>C]-(+)-PHNO), a D<sub>2</sub>/D<sub>3</sub> receptor agonist radiotracer for use in humans.<sup>21</sup> [<sup>11</sup>C]-(+)-PHNO binds with nanomolar affinity to D<sub>2</sub> and D<sub>3</sub> receptors, allowing for a prominent signal from the striatum, globus pallidus (GP), and substantia nigra.<sup>18,21,22</sup> Because [<sup>11</sup>C]-(+)-PHNO is an agonist radiotracer, it binds preferentially to the high-affinity state of the receptor.<sup>13,14</sup> In addition, [<sup>11</sup>C]-(+)-PHNO shows more than 10-fold higher *in vitro* affinity for the D<sub>3</sub> receptor than the D<sub>2</sub> receptor.<sup>18,23</sup> This preferential *in vitro* binding to the D<sub>3</sub> receptor has been confirmed *ex vivo* in rodents<sup>24</sup> and *in vivo* in nonhuman primates and humans<sup>25,26</sup> in the D<sub>3</sub> receptor-rich regions like the GP, where D<sub>3</sub> receptor binding is thought to account for about 80% of the [<sup>11</sup>C]-(+)-PHNO signal. This is in contrast to the signal in the dorsal striatum (caudate and putamen), which is 10% to 40% in the D<sub>3</sub> receptor.<sup>27,28</sup> Consequently, the GP can be used as a preferential D<sub>3</sub> receptor binding region and the dorsal striatum, as a preferential D<sub>2</sub> receptor region.<sup>21,24-26,28-30</sup> Therefore, the concomitant use of [<sup>11</sup>C]-(+)-PHNO and a non-specific D<sub>2</sub>/D<sub>3</sub> receptor antagonist radiotracer like [<sup>11</sup>C]raclopride provides an opportunity to study the differential effects of antipsychotics on high- vs high- + low-affinity states by comparing [<sup>11</sup>C]-(+)-PHNO vs [<sup>11</sup>C]raclopride and of D<sub>3</sub> vs D<sub>2</sub> receptors by comparing the caudate and putamen vs the GP in the same subject.

The aim of this study was to compare the effects of 3 commonly used antipsychotic drugs (clozapine, risperidone, and olanzapine) on the D<sub>2</sub> and D<sub>3</sub> receptors as measured with [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride. While the 2 radiotracers gave relatively similar occupancies in the caudate and putamen, they showed widely divergent results in the GP, where no occupancy was found with [<sup>11</sup>C]-(+)-PHNO. To understand this discrepancy further, and to confirm the D<sub>3</sub> receptor of [<sup>11</sup>C]-(+)-PHNO signal in the GP, we challenged a second set of patients with a single dose of pramipexole dihydrochloride, a preferential D<sub>3</sub> receptor agonist, to obtain displacement (reduction) of the increased binding in the GP and corroborate the D<sub>3</sub> receptor nature of this signal.

This study was approved by the local research ethics board and by Health Canada. Forty-six subjects were included in the following groups: (1) patients receiving long-term antipsychotic treatment (n=20: clozapine, n=7; olanzapine, n=7; and risperidone, n=6); (2) patients receiving long-term risperidone treatment studied before and after a pramipexole-placebo challenge (n=3); and (3) healthy controls (n=23).

The participants provided written informed consent after the study procedures and risks were explained. They were excluded if they had a current diagnosis of substance abuse or dependence at screening, had a positive result in urine drug screen at enrollment or before any of the PET scans, had a history of clinically significant physical illness, were pregnant or lactating at screening, or had positive urine pregnancy test results before PET scans or metal implants that would preclude magnetic resonance imaging.

The patients were evaluated before each PET scan with the Clinical Global Impression scale, Positive and Negative Syndrome Scale, Simpson-Angus Scale, and Barnes Akathisia Scale in addition to the variables recorded during the initial assessment that are detailed in **Table 1**.

## STUDY GROUPS

## Antipsychotic-Treated Patients

Twenty patients with either schizophrenia, schizophreniform, or schizoaffective disorder corroborated with the Mini-International Neuropsychiatric Interview Plus<sup>31</sup> underwent PET scanning twice on separate days, 1 day apart. The age range for inclusion was 18 to 50 years. The patients were receiving clozapine, olanzapine, or risperidone as antipsychotic monotherapy at a steady clinical dose for more than 4 weeks without use of depot antipsychotic drugs during the previous 6 months. The first scan was performed with [<sup>11</sup>C]-(+)-PHNO and the second, with [<sup>11</sup>C]raclopride. The PET scans were acquired at the expected serum peak time of the last antipsychotic dose (2, 6, and 3 hours for risperidone, olanzapine, and clozapine, respectively). Blood samples for the quantification of antipsychotic and prolactin plasma levels were obtained before and after each PET scan. The reported plasma levels were the mean of both measures.

## Patients With Pramipexole Challenge

Three patients treated long-term with risperidone and with similar characteristics to the antipsychotic-treated patients were scanned twice with [<sup>11</sup>C]-(+)-PHNO at least 2 days apart. The PET scans were timed 12 hours after the last oral dose of risperidone. Two hours before each PET scan, the subjects received a single oral dose of 0.5 mg of pramipexole dihydrochloride or placebo.

## Healthy Controls

Twenty-three healthy controls (17 male, 6 female; mean [SD] age, 34.5 [8.38] years) were recruited through local advertisement. Each was scanned with [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride. Psychiatric disorders were excluded using the Mini-International Neuropsychiatric Interview Plus.<sup>31</sup> The age range for inclusion was 18 to 50 years. Subjects with any medical or neurological conditions or with Axis I psychiatric diagnoses were

**Table 1. Characteristics of the Long-term-Treated Patients Group and Equalized Healthy Controls**

|                                   | Mean (SD) [Range]          |                     | Comparison                           |
|-----------------------------------|----------------------------|---------------------|--------------------------------------|
|                                   | Long-term-Treated Patients | Healthy Controls    |                                      |
| Age, y                            | 37.6 (7.3) [27-50]         | 34.57 (8.3) [20-50] | $t = -1.25$ ; $P = .21$              |
| Sex, No. (M/F)                    | 11/9                       | 17/6                | Mann Whitney $U = 186.5$ ; $P = .20$ |
| Diagnosis, No.                    |                            |                     |                                      |
| Schizophrenia                     | 19                         |                     |                                      |
| Schizophreniform                  | 1                          |                     |                                      |
| Age at onset, y                   | 25.2 (6.68)                |                     |                                      |
| Acute episodes                    | 4.90 (7.05)                |                     |                                      |
| Hospitalizations                  | 3.25 (4.20)                |                     |                                      |
| Previous neuroleptic exposure, mo | 112.80 (105.07)            |                     |                                      |
| Time with current neuroleptic, mo | 46.35 (32.05)              |                     |                                      |
| Current treatment, mg/d           |                            |                     |                                      |
| Olanzapine (n=7)                  | 17.86 (7.83)               |                     |                                      |
| Clozapine (n=7)                   | 328.5 (149.60)             |                     |                                      |
| Risperidone (n=6)                 | 2.79 (1.99)                |                     |                                      |

for inclusion was 19 to 50 years. 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 nicotine within 6 months prior to their baseline visit were not included. Participants were asked to consume no more than their usual amount of coffee (and if smokers, cigarettes) the day of PET examinations and to abstain from alcohol intake 24 hours before PET scans. Standard urine tests for psychotropic substances were performed at inclusion and immediately before each PET scan. Pregnancy was excluded using serum analysis at inclusion and urine pregnancy tests before each scan.

#### [<sup>11</sup>C]-(+)-PHNO AND [<sup>11</sup>C]RACLOPRIDE SYNTHESIS

The radiosynthesis of [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride has been described in detail elsewhere.<sup>32,33</sup>

#### PET IMAGING

Studies were performed using a high-resolution brain PET camera system (HRRT; Siemens Molecular Imaging, Knoxville, Tennessee), described in detail elsewhere.<sup>25,34,35</sup> After being placed on the scanning bed, a mean (SD) of 330 (55) MBq (to convert to curies, divide by 37 000) (range, 200-407 MBq) with a specific activity of 39 000 (11 360) MBq/μmol (range, 17 940-64 000 MBq/μmol) of [<sup>11</sup>C]-(+)-PHNO was injected as a bolus followed by a flush with 2 mL of saline into an intravenous catheter placed in an antecubital vein. Scanning data were acquired for 90 minutes after the injection. Once scanning was completed, the data were reframed into 30 frames (1-15 of 1-minute duration and 16-30 of 5-minute duration). The mean (SD) [<sup>11</sup>C]raclopride dose was 370 (23) MBq (range, 329-440 MBq), with a specific activity of 42 200 (14 300) MBq/μmol (range, 14 400-72 150 MBq/μmol) injected as a bolus immediately followed by a flush with 2 mL of saline. The total scanning time was 60 minutes. The [<sup>11</sup>C]raclopride list mode data were reframed into 28 frames (1-5 of 1-minute duration, 6-25 of 2-minute duration, and 26-28 of 5-minute duration).

#### MAGNETIC RESONANCE IMAGING

Subjects underwent a proton density image (echo time = 17 milliseconds; repetition time = 6000 milliseconds; field of view = 22 cm, 2-dimensional, 256 × 256; slice thickness, 2 mm; number of excitations = 2), acquired on a 1.5-T Signa scanner (General Electric Medical Systems, Milwaukee, Wisconsin). These images were used for the analysis of the PET scans.

#### IMAGE ANALYSIS

The procedure for the analysis of the [<sup>11</sup>C]raclopride and [<sup>11</sup>C]-(+)-PHNO PET images was performed as previously described.<sup>25,34,35</sup> The quantitative estimate of binding was estimated using the Simplified Reference Tissue Method (SRTM),<sup>36</sup> with the cerebellar cortex as the reference region. This method has been validated to reliably estimate the nondisplaceable binding potential (BP<sub>ND</sub>), which compares the concentration of radioligand in the receptor-rich region with the receptor-free region,<sup>37</sup> for both [<sup>11</sup>C]raclopride<sup>38</sup> and [<sup>11</sup>C]-(+)-PHNO.<sup>30</sup> In addition, to assure the robustness of our findings, we analyzed the data using 3 additional methods: the interval ratio,<sup>39</sup> the multilinear reference tissue model (MRTM), and the multilinear reference tissue model 2 (MRTM2).<sup>40</sup> We included the interval method as an intuitive approach based on the shape of the time activity curves, and the MRTM and MRTM2 were included because, in contrast to SRTM, they are linearized reference tissue methods that are resistant to noise.<sup>40</sup> The times for the interval method were caudate and putamen, 840 to 1800 seconds; ventral striatum, 900 to 3000 seconds; and GP, 2100 to 4200 seconds. These intervals showed the lowest variation in our controls (<5%). The BP<sub>ND</sub> was estimated using PMOD v2.7 software (PMOD Technologies Ltd, Zurich, Switzerland). In addition, PMOD provided for SRTM, MRTM, and MRTM2 the coefficients of variation of the standard error of the parameter identifiability (called "standard error" of the compartmental fit).

Parametric voxel-wise BP<sub>ND</sub> maps from the dynamic images in native space were generated according to the method of Gunn et al,<sup>41</sup> with the cerebellum as the reference region, and as implemented in PMOD v2.7 software. The

BP<sub>ND</sub> map images were spatially normalized into Montreal Neurological Institute brain space by nearest neighbor interpolation and with a voxel size fixed at 2 × 2 × 2 mm using the SPM2 software.<sup>42</sup> The normalized images were smoothed with a gaussian filter in each coordinate direction, with a kernel of 4 mm.

Receptor occupancy was defined as the percentage of reduction of the BP<sub>ND</sub> from a baseline state to the treated state. To calculate receptor occupancy, a measure of baseline BP<sub>ND</sub> in the drug-free (untreated) state was necessary. Since we could not obtain baseline measures of D<sub>2</sub> receptors for the antipsychotic-treated patients within our current design, we used estimates of BP<sub>ND</sub> from age- and sex-matched controls derived from healthy volunteers, using the slope and intercept for each region of interest obtained from the linear regression of BP<sub>ND</sub> and age for each sex (eTable 1, <http://www.archgenpsychiatry.com>). Also, as an additional analysis, the data from drug-free patients<sup>43</sup> were pooled with the healthy controls data to estimate the percentage of receptor occupancy and did not modify the occupancies as estimated with healthy controls (analysis presented). The occupancies reported in the results correspond to those obtained with SRTM unless otherwise indicated. The occupancies were estimated with the following formula:

$$\% \text{ Occupancy} = \frac{BP_{ND} \text{ Baseline Estimate} - BP_{ND} \text{ Patient}}{BP_{ND} \text{ Baseline Estimate}} \times 100\%.$$

The percentage of BP<sub>ND</sub> change after the pramipexole challenge was estimated considering as baseline the PET scan with placebo.

$$\% BP_{ND} \text{ Change} = \frac{BP_{ND} \text{ Placebo} - BP_{ND} \text{ After Pramipexole}}{BP_{ND} \text{ Placebo}} \times 100\%$$

Nonlinear regression analysis was applied to correlate the plasma level of clozapine, olanzapine, or risperidone with the respective occupancy (SRTM). The data were fitted to a rectangular hyperbola (1-site binding model) described by the equation:

$$\text{Occupancy} = \frac{X \times \text{Antipsychotic (Plasma Level)}}{\text{Antipsychotic (Plasma Level)} + ED_{50}}$$

where *ED*<sub>50</sub> represents the plasma level of antipsychotics resulting in 50% receptor occupancy and *X* corresponds to the ceiling receptor's occupancy. In the models, *X* was fixed to 100 and *ED*<sub>50</sub> > 0.

## STATISTICAL ANALYSIS

The analysis was made using SPSS (version 12.0; SPSS, Chicago, Illinois). Variables were presented as mean (standard deviation). Demographic and clinical characteristics were compared between patients and controls by using the Mann-Whitney *U* test for categorical data and independent-sample *t* tests for continuous data. Analysis of variance with a post hoc paired *t* test was performed to compare the clinical variables and plasma levels between PET scan days and to compare the occupancies between radiotracers (<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]raclopride) in regions of interest. Also, Pearson product-moment correlations were estimated between clinical variables and radioligand occupancies and also between

**Table 2. Comparison of Occupancies Between [<sup>11</sup>C]-(+)-PHNO and [<sup>11</sup>C]Raclopride in the Group of Patients Treated Long-term**

|                | Mean (SD), %                |                              | Comparison <sup>a</sup> |
|----------------|-----------------------------|------------------------------|-------------------------|
|                | [ <sup>11</sup> C]-(+)-PHNO | [ <sup>11</sup> C]Raclopride |                         |
| Caudate (mean) | 53.33 (5.18)                | 71.48 (10.28)                | <i>P</i> < .001         |
| Clozapine      | 55.20 (14.92)               | 65.68 (10.11)                |                         |
| Olanzapine     | 50.18 (19.24)               | 76.82 (8.29)                 |                         |
| Risperidone    | 54.84 (11.99)               | 72.06 (10.45)                |                         |
| Putamen (mean) | 41.69 (15.69)               | 69.06 (11.99)                | <i>P</i> < .001         |
| Clozapine      | 41.03 (12.34)               | 59.44 (11.48)                |                         |
| Olanzapine     | 43.56 (22.04)               | 76.46 (7.84)                 |                         |
| Risperidone    | 40.27 (12.75)               | 71.65 (10.04)                |                         |
| VS (mean)      | 17.32 (34.88)               | 72.27 (11.34)                | <i>P</i> < .001         |
| Clozapine      | 26.47 (25.66)               | 63.31 (10.55)                |                         |
| Olanzapine     | 11.21 (20.88)               | 78.31 (7.24)                 |                         |
| Risperidone    | 13.80 (55.92)               | 75.69 (10.72)                |                         |
| GP (mean)      | -70.74 (86.52)              | 59.03 (14.20)                | <i>P</i> < .001         |
| Clozapine      | -100.89 (130.66)            | 47.64 (15.11)                |                         |
| Olanzapine     | -68.26 (28.45)              | 64.67 (10.21)                |                         |
| Risperidone    | -38.46 (66.80)              | 65.76 (9.31)                 |                         |

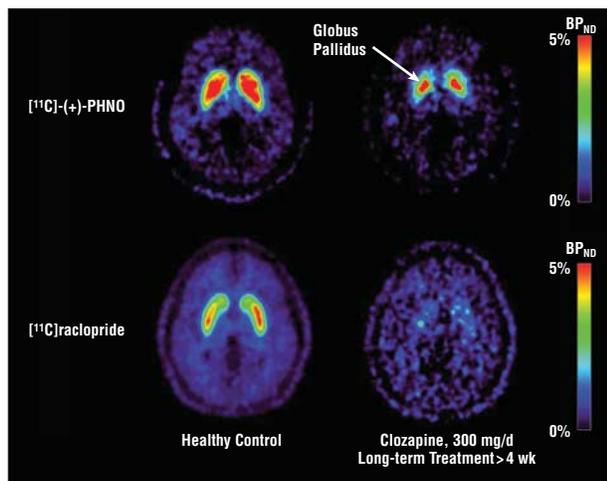
Abbreviations: [<sup>11</sup>C]raclopride, carbon 11-labeled raclopride; [<sup>11</sup>C]-(+)-PHNO, carbon 11-labeled (+)-4-propyl-9-hydroxynaphthoxazine; GP, globus pallidus; VS, ventral striatum.  
<sup>a</sup>*F*<sub>1,136</sub> = 36.59; *P* < .001.

the BP<sub>ND</sub> obtained by SRTM and the other methods. The significance was always assumed at *P* < .05.

## RESULTS

### LONG-TERM-TREATED PATIENTS IN COMPARISON WITH HEALTHY CONTROLS

The patients treated long-term with olanzapine, clozapine, or risperidone had mean (SD) occupancies with [<sup>11</sup>C]raclopride of 71% (10%) and 69% (12%) in the caudate and putamen, respectively. The mean (SD) occupancies with [<sup>11</sup>C]-(+)-PHNO were lower: 53% (5%) and 41% (15%) in the caudate and putamen (*t*<sub>19</sub> = -5.15; *P* < .001 and *t*<sub>19</sub> = -7.16; *P* < .001), respectively. The most striking results were in the D<sub>3</sub> receptor-rich region (GP) wherein [<sup>11</sup>C]raclopride showed mean (SD) occupancies of 59% (14%). In contrast, the BP<sub>ND</sub> as measured by [<sup>11</sup>C]-(+)-PHNO was higher rather than lower in antipsychotic-treated patients compared with healthy controls, with binding values in the GP (mean [SD]) 70% (86%) higher than the estimated baseline (unmedicated) measure using healthy controls (*t*<sub>19</sub> = -6.96; *P* < .001). The mean (SD) occupancy with [<sup>11</sup>C]raclopride was 72% (11%) in the ventral striatum, while with [<sup>11</sup>C]-(+)-PHNO, this was 17% (34%) (*t*<sub>19</sub> = -6.04; *P* < .001), which is between that observed in the dorsal striatum (caudate and putamen) and the GP (**Table 2**) (**Figures 1, 2, and 3**). To confirm the reliability of this finding across different analytic methods, the [<sup>11</sup>C]-(+)-PHNO occupancies were obtained using BP<sub>ND</sub> derived by the interval ratio, MRTM, and MRTM2 methods. The results with all the methods showed higher BP<sub>ND</sub> in patients taking antipsychotics than controls in the GP (eTable 2).



**Figure 1.** Representative voxel-wise nondisplaceable binding potential ( $BP_{ND}$ ) maps of carbon 11-labeled (+)-4-propyl-9-hydroxynaphthoxazine ( $[^{11}C]$ -(+)-PHNO) and  $[^{11}C]$ raclopride in a patient with schizophrenia treated long-term with clozapine (300 mg/d) and a sex- and age-matched healthy control. Note the complete occupancy in the  $[^{11}C]$ raclopride patient scan in contrast to the high signal in the globus pallidus of the  $[^{11}C]$ -(+)-PHNO scan. The images are in Montreal Neurological Institute space and shown in axial projection.

The clinical variables and the serum plasma level of the antipsychotics and metabolites were not different between the  $[^{11}C]$ -(+)-PHNO and  $[^{11}C]$ raclopride PET scan days. There were no significant correlations between the estimated occupancies and clinical measures (Positive and Negative Syndrome Scale, Simpson-Angus Scale, Barnes Akathisia Scale, Clinical Global Impression scale) ( $P > .05$ ). The nonlinear regression analysis results between antipsychotic plasma levels and respective occupancy (SRTM) are presented in eTable 3. We did not obtain a significant relationship between plasma levels and the degree of  $BP_{ND}$  elevation or occupancy in the GP and ventral striatum with  $[^{11}C]$ -(+)-PHNO.

#### DISPLACEMENT OF ELEVATED BINDING BY THE PRAMIPEXOLE CHALLENGE

The patients included in the pramipexole challenge were receiving long-term treatment with risperidone (mean [SD] dose, 5.2 [1.3] mg/d) and, as expected, showed a mean (SD) 40% (33%) occupancy in the caudate and 41% (20%) in the putamen and a 64% (18%) elevation of  $BP_{ND}$  in the GP, as described in the previous group of subjects. Two hours after the administration of an oral dose of 0.5 mg of pramipexole dihydrochloride, the patients showed almost no change in the caudate (mean [SD], 0.8% [11%]), putamen (mean [SD], -0.8% [7.6%]), and ventral striatum (mean [SD], 7.5% [2%])  $BP_{ND}$  but showed a mean (SD) 45% (8%) reliable decrease in the signal derived from the GP ( $BP_{ND}$  from mean [SD], 5.3% [1.0%] to 2.8% [0.1%]) (**Figure 4**).

#### COMMENT

To our knowledge, this is the first study to compare the antipsychotic occupancies as measured by an agonist and an antagonist radiotracer and provides the first evi-

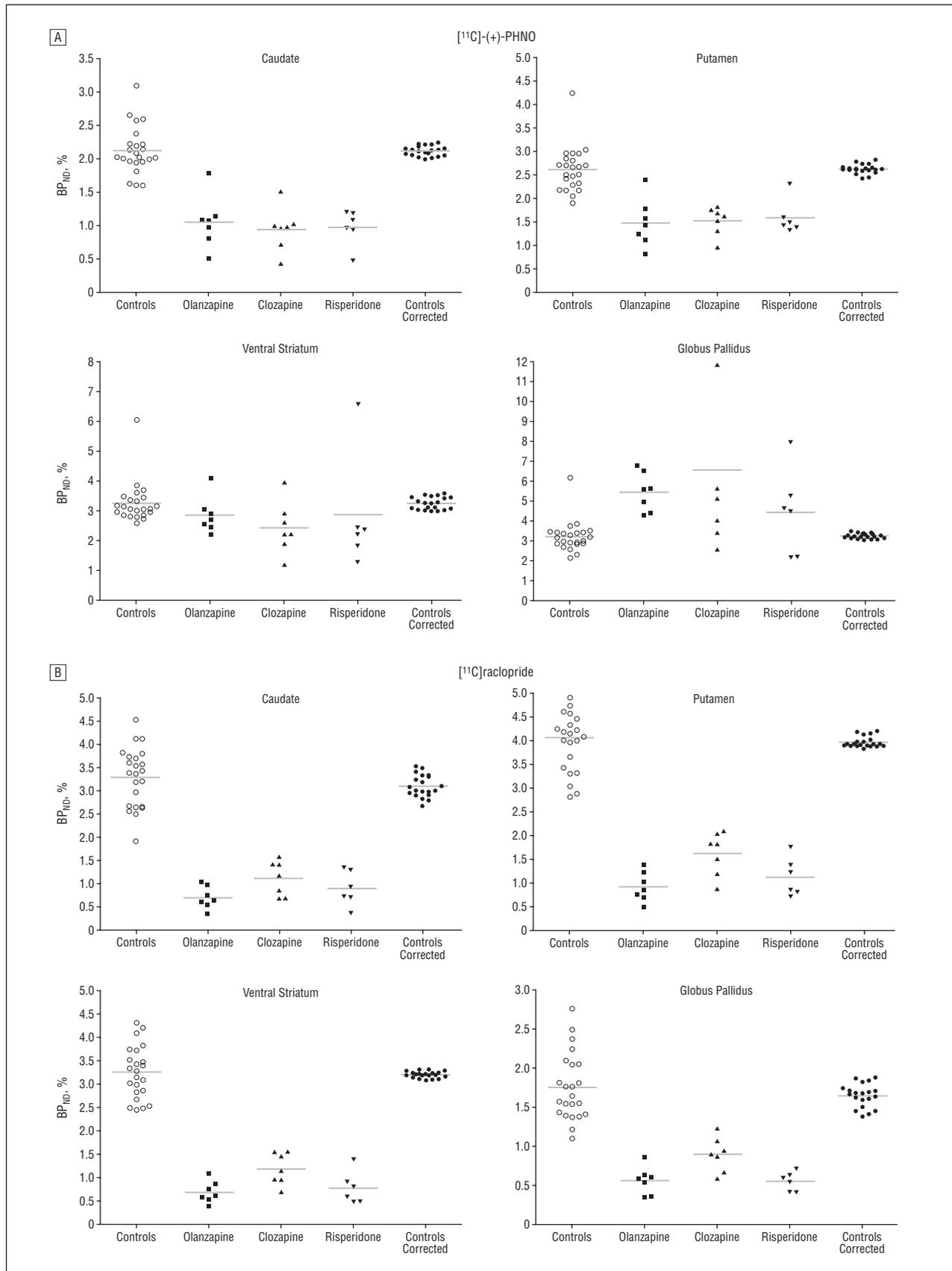
dence of the effects of antipsychotics on the low- and the high-affinity states of the dopamine receptors, as well as providing insights into the differential effect of the drugs on  $D_3$  and  $D_2$  receptors. Our results indicate that while antipsychotics do block both the low-affinity and the high-affinity states of the  $D_2$  receptors as estimated by  $[^{11}C]$ -(+)-PHNO and  $[^{11}C]$ raclopride, antipsychotics do not block the  $D_3$  receptors as estimated by  $[^{11}C]$ -(+)-PHNO in the  $D_3$  receptor-rich regions (GP and ventral striatum). The data with pramipexole, a  $D_3$  receptor-preferring agonist, show that the signal with  $[^{11}C]$ -(+)-PHNO in the  $D_3$  receptor-rich regions is displaceable and supports a  $D_3$  receptor basis for this signal.

The major new finding in our study is that the  $[^{11}C]$ -(+)-PHNO binding in the GP and ventral striatum is not blocked by long-term antipsychotic exposure in patients with schizophrenia. Our group recently reported that the  $[^{11}C]$ -(+)-PHNO binding is not different between drug-free patients with schizophrenia and age- and sex-matched healthy controls in any brain region.<sup>43</sup>

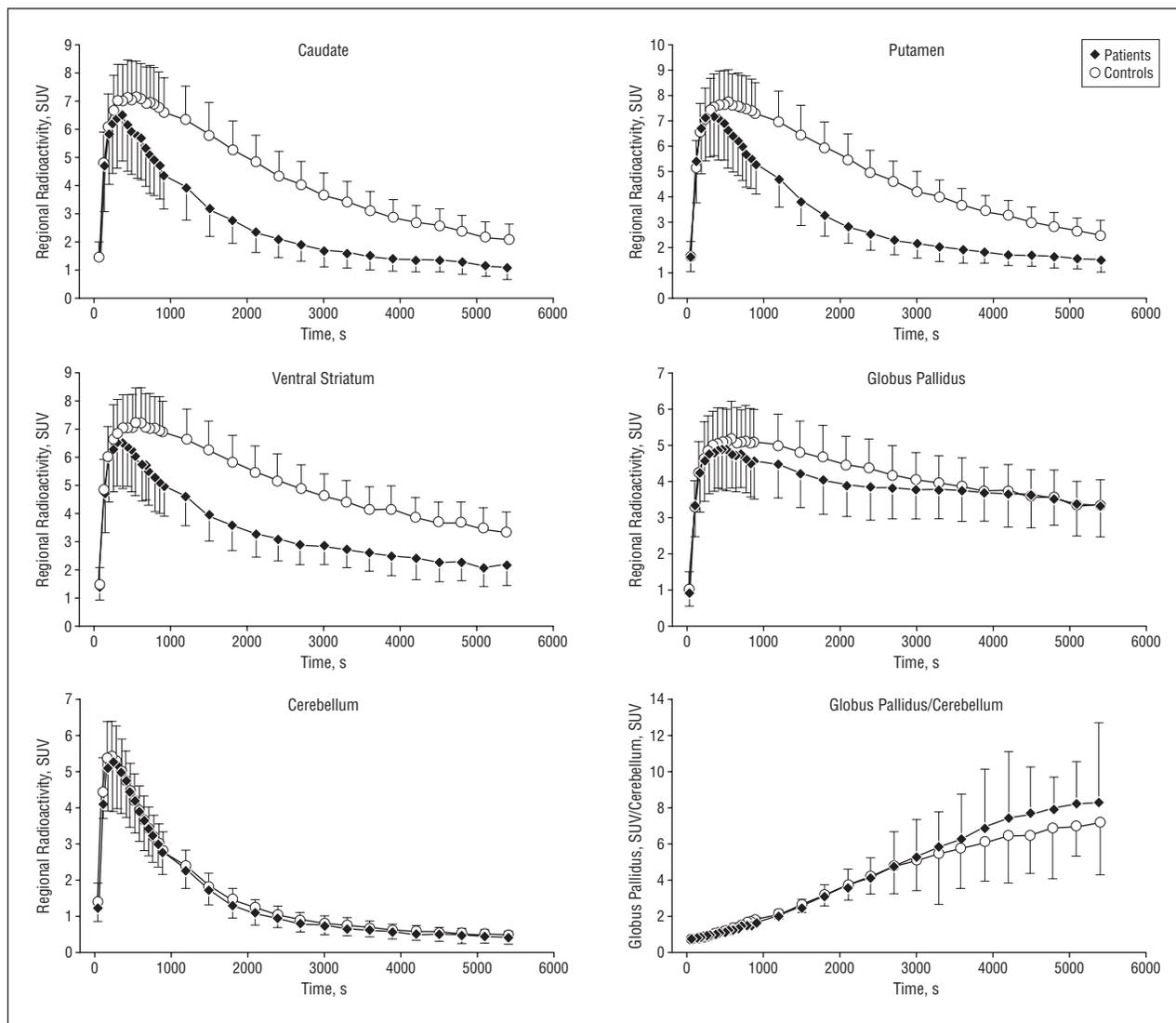
What makes the  $D_3$  receptors particularly interesting is their preferential distribution in the limbic regions and their rigid configuration in high-affinity states for dopamine.<sup>17,18</sup> This configuration may imply that in vivo this receptor is occupied by endogenous dopamine for longer periods, suggesting a special role for  $D_3$  receptors in mediating tonic (background) dopamine transmission.<sup>44</sup> As a result of this functional distribution and sensitivity to background dopamine levels, small changes in their number may have important functional implications, especially for neuropsychiatry disorders.

Several converging lines of evidence suggest that our results likely reflect the  $D_3$  receptor binding signal of the radioligand  $[^{11}C]$ -(+)-PHNO as confirmed by the following: (1) The main distribution of the increased  $BP_{ND}$  was within the  $D_3$  receptor-rich area (mainly GP) where  $[^{11}C]$ -(+)-PHNO has shown preferential distribution and selectivity in vitro and in vivo.<sup>18,23-26</sup> (2) Studies in  $D_3$  receptor knockout mice confirm that tritium-labeled ( $^3H$ )-(+)-PHNO binding in this region is  $D_3$  receptor related.<sup>29</sup> (3) Studies in nonhuman primates have shown that the  $[^{11}C]$ -(+)-PHNO signal in GP binding is preferentially displaced with the preferential  $D_3$  receptor agonist (BP897)<sup>26</sup> and antagonist SB277011.<sup>28</sup> (4) In normal human volunteers, we have recently shown that the  $[^{11}C]$ -(+)-PHNO signal in GP binding is preferentially displaced after a single dose of a  $D_3$  receptor antagonist (ABT925)<sup>27</sup> and partially displaced with the  $D_2/D_3$  receptor antagonist haloperidol.<sup>21</sup> (5) Finally, most importantly, the  $[^{11}C]$ -(+)-PHNO binding in patients exposed to risperidone long-term was displaced by a challenge with the preferential  $D_3$  receptor agonist pramipexole. Thus, it seems reasonable to suggest that patients treated long-term with antipsychotics show an unblocked signal in the GP that could be best understood as  $D_3$  receptor binding.

The results indicate a higher  $BP_{ND}$  in the GP with  $[^{11}C]$ -(+)-PHNO that could reflect either an increase in the available number of  $D_3$  receptors in the region ( $B_{max}$ ), an increase in the apparent affinity ( $K_d$ ), or a reference tissue-based quantitation artifact (something we discuss further later). A treatment-induced increase in  $D_3$  receptor



**Figure 2.** Nondisplaceable binding potential ( $BP_{ND}$ ) of carbon 11-labeled (+)-4-propyl-9-hydroxynaphthoxazine ( $[^{11}C]$ -(+)-PHNO (A) and  $[^{11}C]$ raclopride (B) estimated by the simplified reference tissue model of every region of interest. The  $BP_{ND}$  corresponds to controls, controls corrected (estimated sex- and age-corrected by linear regression), and patients treated with clozapine, olanzapine, and risperidone. Horizontal lines indicate the mean  $BP_{ND}$  from each region of interest.



**Figure 3.** Average time activity curves of patients (n=20) and controls (n=23) for every region of interest. Error bars correspond to standard deviations. SUV indicates standard uptake value.

binding may be explained by changes induced by antipsychotics on a variety of intracellular mechanisms leading to an increase in  $B_{max}$ .<sup>45-47</sup> However, the preclinical data on this matter are equivocal. Some studies have reported an increase in  $D_3$  receptor messenger RNA,<sup>48-50</sup> but other studies have found no change in  $D_3$  receptor messenger RNA or  $D_3$  receptor labeling.<sup>51-55</sup> Additionally, a postmortem study<sup>54</sup> in patients with schizophrenia treated long-term did not find any difference in the total number and affinity (dissociation constant) of  $D_3$  receptors. At present, both an increase in the  $B_{max}$  or  $K_d$  remain possible and only further experimentation will choose between them.

The occupancies in the  $D_2$  receptor-rich areas (caudate and putamen) were always lower with [<sup>11</sup>C]-(+)-PHNO (Table 2). A simple explanation for the difference in occupancy could be that antipsychotics show less affinity for the high- vs the low-affinity states of the  $D_2$  receptors, and since [<sup>11</sup>C]-(+)-PHNO is assumed to label only the former, the 2 radioligands may result in different occupancies. This assumption has been indirectly sustained by the higher sensitivity of [<sup>11</sup>C]-(+)-PHNO for amphet-

amine displacement than [<sup>11</sup>C]raclopride in anesthetized cats.<sup>56</sup> However, this higher sensitivity was not replicated in an ex vivo dissection study in awake rodents,<sup>57</sup> though it is suggested by the study in awake and healthy humans.<sup>35</sup> The cause of this discrepancy between studies is still elusive, though the cat and human studies suggest that [<sup>11</sup>C]-(+)-PHNO binds preferentially to the high-affinity states of the dopamine receptor.

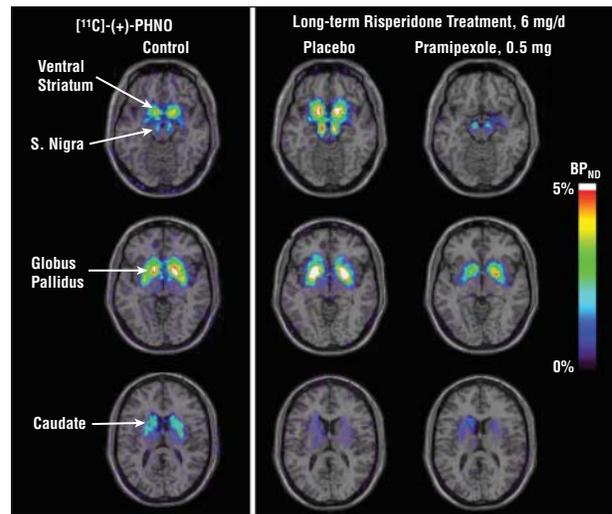
The explanation of less affinity for the high- vs the low-affinity states seems to be unlikely, as studies in vitro have generally shown that the current antipsychotics have similar affinities for the high- and the low-affinity states.<sup>58</sup> In addition, an ex vivo study in rodents using [<sup>11</sup>C]-(+)-PHNO and [<sup>3</sup>H]raclopride confirmed the in vitro results, indicating that affinities ( $ED_{50}$ ) for haloperidol and clozapine are the same with both radiotracers.<sup>57</sup>

On the other hand, the more likely explanation is that the lower [<sup>11</sup>C]-(+)-PHNO mean occupancies are related to the signal from [<sup>11</sup>C]-(+)-PHNO having a higher  $D_3$  vs  $D_2$  receptor contribution as compared with the signal from [<sup>11</sup>C]raclopride. It has been shown that the rela-

tive D<sub>3</sub> vs D<sub>2</sub> receptor density as observed in humans in vitro and in nonhuman primates is caudate less than putamen less than ventral striatum less than GP.<sup>28,59-61</sup> This is well supported by recent data from Rabiner et al,<sup>28</sup> who demonstrated that the D<sub>3</sub> receptor contribution of [<sup>11</sup>C]-(+)-PHNO in the dorsal striatum in nonhuman primates is between 8% and 21% using the selective D<sub>3</sub> receptor antagonist SB277011. In addition, the [<sup>11</sup>C]-(+)-PHNO signal in the dorsal striatum can be blocked up to 13% to 47% by BP897 and ABT925<sup>26,27</sup> in nonhuman primates and humans, suggesting D<sub>3</sub> receptor availability in this region. The final possibility is that while the use of reference tissue-based quantifications have been validated for both [<sup>11</sup>C]raclopride<sup>38</sup> and [<sup>11</sup>C]-(+)-PHNO,<sup>30</sup> they do not reflect absolute parameters—something we discuss further later. Nonetheless, on comparing these 3 possibilities, we favor the differential D<sub>3</sub> vs D<sub>2</sub> receptor as the preferred explanation as it explains not only the finding in the dorsal striatum, but the differentials in the other regions and is consistent with our main explanation for the GP.

Our study has several limitations but none that we think detract from the main conclusion herein. Since we did not have data on the drug-free state of our antipsychotic-treated patients, we had to derive occupancy levels by reference to a control population. While less than ideal, this is the most common approach in the literature,<sup>62</sup> and since we have recently shown that there are no differences between healthy controls and drug-free patients with schizophrenia,<sup>43</sup> it is unlikely to have biased our results. Another limitation is that the smoking status of the participants was not recorded. This limitation may be important in light of the effect of cigarette consumption, craving, and its mood or hedonic effect on the dopamine system<sup>63-65</sup> and that patients with schizophrenia are more likely to be smokers than the general population.<sup>66</sup> However, the overall effect of smoking or nicotine exposure on the BP<sub>ND</sub> is null or small (about 10%). While Brody et al<sup>63</sup> reported a mean (SD) reduction of 8.5% (1.5%) in BP<sub>ND</sub> with [<sup>11</sup>C]raclopride, Barrett et al<sup>64</sup> and Montgomery et al<sup>65</sup> did not find any change in BP<sub>ND</sub> with [<sup>11</sup>C]raclopride. To our knowledge, this effect has never been explored with an agonist radiotracer.

The lack of arterial samples to perform a full kinetic analysis may also be a limitation of our study. A full kinetic analysis would allow direct estimation of the BP in the regions of interest without the potential bias induced by specific binding in the reference region and/or by changes in the kinetics of the signal of interest. However, it is of some reassurance to know that in the current study the cerebellar uptake values in patients and controls were not different (Figure 3) ( $F_{1,41}=0.29$ ;  $P=.59$ ). The SRTM approach to estimate BP<sub>ND</sub> with the cerebellum as a reference has a good correlation with the BP as estimated with a full kinetic analysis and it was validated in unmedicated controls for both radiotracers.<sup>30,38</sup> This approach has been used repeatedly with reliable results with [<sup>11</sup>C]raclopride in patients with schizophrenia taking antipsychotics.<sup>3-7,62</sup> However, to our knowledge, the current study is the first with [<sup>11</sup>C]-(+)-PHNO in patients taking antipsychotics and some limitations should be considered. To examine the de-



**Figure 4.** Representative voxel-wise nondisplaceable binding potential (BP<sub>ND</sub>) maps of carbon 11-labeled (+)-4-propyl-9-hydroxynaphthoxazine ([<sup>11</sup>C]-(+)-PHNO) from a patient with schizophrenia treated long-term with risperidone (6 mg/d) and a sex- and age-matched healthy control. The images illustrate a decrease of the BP<sub>ND</sub> in the globus pallidus, ventral striatum, and substantia nigra (S. nigra) after a pramipexole dihydrochloride dose, without a reliable change in the dorsal striatum (caudate). The BP<sub>ND</sub> maps are overlaid on a T1-weighted magnetic resonance imaging template in Montreal Neurological Institute space and showed in axial projection.

pendence of these findings on any one method, we additionally estimated the BP<sub>ND</sub> with the interval ratio, MRTM, and MRTM2 methods. Across methods, the BP<sub>ND</sub> in the GP of patients treated long-term was always higher than controls (though the extent of the increase varied, 17%, 42%, and 41%, respectively) (eTable 2), even with the MRTM and MRTM2 methods, which showed lower coefficients of variance (eTable 4). However, the visual inspection of the time activity curves from the GP (Figure 3) suggests that while the initial part of the curve is reduced compared with controls (probably secondary to D<sub>2</sub> receptor block), the latest part (likely D<sub>3</sub> receptor related) is similar to controls. These effects result in a flat curve during the 90 minutes of scanning time, which provides the kinetic model with limited information about the off rate (k<sub>4</sub>) to properly estimate k<sub>3</sub>/k<sub>4</sub>.

An increase of the scanning time, to obtain enough off-rate data, would overcome this limitation in kinetic modeling. However, the short half-life of <sup>11</sup>C would provide noisy data after 90 minutes and the agonist property of [<sup>11</sup>C]-(+)-PHNO may make a bolus infusion difficult to implement. Thus, while we can be reasonably certain that the current antipsychotics do not induce a decrease of the [<sup>11</sup>C]-(+)-PHNO binding in the D<sub>3</sub> receptor-rich areas in schizophrenia, whether there is a true upregulation (ie, an increase in D<sub>3</sub> receptor number) should be regarded as a possibility that may require more data to confirm.

We used pramipexole to displace the signal in the D<sub>3</sub> receptor-rich areas of the patients treated long-term. Pramipexole is an agonist with an approximately 10-fold preference for D<sub>3</sub> receptors over D<sub>2</sub> receptors.<sup>19,67,68</sup> We were aware of the limited D<sub>3</sub> receptor:D<sub>2</sub> receptor selectivity of pramipexole, but it was the only medication with a high D<sub>3</sub> receptor selectivity that was available for us to use in patients. In addition, in vitro studies suggested that the doses

administered to our patients were still in the D<sub>3</sub> receptor selectivity range<sup>19,67,68</sup> and this was confirmed post hoc in our study by the selective displacement showed in the GP and not in the dorsal striatum. As better and more selective D<sub>3</sub> receptor agents become available, it may become possible to definitively confirm what we propose herein. Finally, the patients were cross-sectionally chosen to meet the inclusion criteria of this study based on the drug that they were receiving, and their treatment was not prospectively controlled. This was not necessary for the current study and, hence, was not done. While confirming the results with 3 different antipsychotics and 4 modeling methods makes us confident of our finding, the clinical implications of this unblocked [<sup>11</sup>C]-(+)-PHNO signal remain to be elucidated.

To our knowledge, our study provides the first in vivo evidence for the effect of antipsychotics on the D<sub>3</sub> receptor and suggests that long-term antipsychotic treatment does not decrease [<sup>11</sup>C]-(+)-PHNO binding in the D<sub>3</sub> receptor-rich regions, though it clearly blocks the signal in the D<sub>2</sub> receptor-rich regions. This has several important implications. It introduces the application of the D<sub>2</sub>/D<sub>3</sub> receptor agonist radioligand [<sup>11</sup>C]-(+)-PHNO for the study of schizophrenia and antipsychotics; shows that current antipsychotics impact the D<sub>2</sub> receptors as measured by an agonist (high-affinity state) and an antagonist (high- + low-affinity state) radiotracer; suggests that current antipsychotics affect D<sub>3</sub> receptors in a manner distinct from D<sub>2</sub> receptors; and opens up the potential for future investigations of the role of antipsychotics in the D<sub>3</sub> receptor system.

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**Author Contributions:** Drs Graff-Guerrero, Shammi, Houle, and Kapur had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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**Additional Information:** The eTables are available at <http://www.archgenpsychiatry.com>.

**Additional Contributions:** Armando Garcia, BSc, Winston Stableford, BSc, Min Wong, BSc, Alvina Ng, BSc, Terry Bell, DiplTech, Ted Harris-Brandts, PEng, and Peter Bloomfield, MSc, provided technical assistance.

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