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First Human Evidence of *d*-Amphetamine Induced Displacement of a $D_{2/3}$ Agonist Radioligand: A [^{11}C]-(+)-PHNO Positron Emission Tomography Study

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Imaging the competition between $D_{2/3}$ radioligands and endogenous dopamine is so far the only way to measure dopamine release in the living human brain. The dopamine D_2 receptor exists in a high (D_2^{high}) and a low-affinity state for dopamine. Under physiological conditions, dopamine is expected to bind to D_2^{high} only. [^{11}C]-(+)-4-propyl-9-hydroxynaphthoxazine ((+)-PHNO) is the first $D_{2/3}$ agonist radioligand for positron emission tomography (PET) imaging in humans. Since [^{11}C]-(+)-PHNO is expected to bind preferentially to D_2^{high}, it should be particularly vulnerable to competition with endogenous dopamine. Nine healthy subjects participated in two PET scans, one after administration of *d*-amphetamine and one after placebo. [^{11}C]-(+)-PHNO PET test re-test variability was determined in 11 healthy subjects. Binding potentials (BPs) were calculated for caudate, putamen, ventral striatum, and globus pallidus. *d*-Amphetamine led to a significant decrease of [^{11}C]-(+)-PHNO BPs in caudate (−13.2%), putamen (−20.8%), and ventral striatum (−24.9%), but not in globus pallidus (−6.5%). *d*-Amphetamine-induced displacement correlated with serum *d*-amphetamine levels in all regions but caudate. This is the first report on competition between endogenous dopamine and a $D_{2/3}$ agonist radioligand in humans. [^{11}C]-(+)-PHNO PET might be a superior measure for release of endogenous dopamine than PET employing conventional $D_{2/3}$ antagonist radioligands.
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INTRODUCTION

Measuring changes in radioligand binding caused by fluctuations in synaptic dopamine is at present the only way to draw inference on changes in dopamine concentrations in the living human brain. Reductions in radioligand binding to dopamine $D_{2/3}$ receptors measured with positron emission tomography (PET) or single photon emission computer tomography (SPECT) have been shown after several behavioral (Koepp *et al*, 1998; de la Fuente-Fernandez *et al*, 2002; Pruessner *et al*, 2004; Zald *et al*, 2004; Volkow *et al*, 2006) and pharmacological manipulations (Farde *et al*, 1992; Volkow *et al*, 1994; Tedroff *et al*, 1996; de la Fuente-Fernandez *et al*, 2004), which raise endogenous dopamine levels. In particular, the well-replicated finding of greater reductions in [^{11}C]-raclopride and [^{123}I]IBZM binding after *d*-amphetamine administration in patients with schizophrenia when compared to healthy controls (Laruelle *et al*, 1996, 1999; Breier *et al*, 1997; Abi-Dargham *et al*, 1998) has generated considerable interest in this research strategy. A critical shortcoming of these studies is the ceiling-effect found with conventional antagonist-radiotracers (Laruelle, 2000), and although competitive inhibition of radioligand binding at the $D_{2/3}$ receptor by dopamine is believed to be one of the main mechanisms underlying reductions in radioligand-binding, the exact nature of the process is still not fully elucidated (for review see Ginovart, 2005).

It is well established *in vitro* that D_2 receptors exist in two interconvertible affinity states for their natural agonist dopamine, the high-affinity state (D_2^{high}; K_d for dopamine

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1.5 ± 0.2 nM) and the low-affinity state (D_2^{low}; K_d for dopamine in the micromolar range; Sibley *et al*, 1982). Since D_2^{high} mediates signal transduction at the postsynaptic neuron, the high-affinity state is believed to be the functionally important one (Zahniser and Molinoff, 1978; George *et al*, 1985; Leff, 1995). As a result of the large difference in K_d values between D_2^{high} and D_2^{low}, under physiological conditions, dopamine is expected to bind to D_2^{high} only. So far, all $D_{2/3}$ radioligands used in human PET or SPECT studies are radio-labelled antagonists, which do not differentiate between D_2^{high} and D_2^{low}. Several research groups have recently reported on development and experimental use of newly developed $D_{2/3}$ agonist radioligands (Zijlstra *et al*, 1993a, b; Shi *et al*, 1999, 2004; Hwang *et al*, 2000; Mukherjee *et al*, 2000, 2004; Finnema *et al*, 2005; Narendran *et al*, 2004). Similar to dopamine, $D_{2/3}$ agonist radioligands are expected to bind mainly to D_2^{high}. As a consequence, they should be particularly sensitive to competition with endogenous dopamine. This has recently been confirmed in experiments in pigs (Cumming *et al*, 2003), rodents (Cumming *et al*, 2002), non-human primates (Narendran *et al*, 2004; Seneca *et al*, 2006), and cats (Ginovart *et al*, 2006a).

(+)-4-Propyl-9-hydroxynaphthoxazine, (+)-(PHNO), is a full agonist at $D_{2/3}$ receptors (Brown *et al*, 1997). Labelled with carbon-11 (Wilson *et al*, 2005), it is a PET ligand with excellent signal-to noise ratio and favorable kinetics for PET imaging in humans (Ginovart *et al*, 2006b; Willeit *et al*, 2006). As [^{11}C]-(+)-PHNO is expected to bind to D_2^{high} only, in theory, all binding sites should be vulnerable to competition with endogenous dopamine, and its binding should be reduced to a considerably larger extent than binding of an antagonist-radioligand by pretreatment with *d*-amphetamine, a potent releaser of central nervous dopamine. In a PET study performed in cats, Ginovart *et al* (2006a) have shown not only that [^{11}C]-(+)-PHNO is more vulnerable towards *d*-amphetamine effects than the $D_{2/3}$ antagonist radioligand [^{11}C]raclopride, but also that [^{11}C]-(+)-PHNO binding is reduced to an even larger extent than binding of the D_2 agonist ligand [^{11}C]NPA.

In this study, we aimed to investigate the effects of endogenous dopamine on striatal [^{11}C]-(+)-PHNO binding in healthy human subjects undergoing two [^{11}C]-(+)-PHNO PET scans, one at placebo conditions and another one after pretreatment with *d*-amphetamine. Test–retest variability of [^{11}C]-(+)-PHNO PET was determined in an independent study sample.

MATERIALS AND METHODS

Study Protocol

This study consisted of two parts. Part one was designed to determine test–retest reliability of [^{11}C]-(+)-PHNO PET imaging, part two investigated the effects of *d*-amphetamine on [^{11}C]-(+)-PHNO binding. For the test-retest protocol, subjects underwent two [^{11}C]-(+)-PHNO PET scans at drug-naive conditions at least 1 week apart from each other. For the *d*-amphetamine protocol, subjects underwent one [^{11}C]-(+)-PHNO PET scan 2 h after oral intake of *d*-amphetamine, and another [^{11}C]-(+)-PHNO PET scan after oral intake of a placebo. Conditions were randomly counterbalanced, scans took place at least 1 week apart from each other.

Study Subjects and Safety Procedures

This study has been approved by the local Ethics Committee and the Canadian Ministry of Health, Therapeutic Products Research Department. Twenty-three healthy volunteers (nine females, 14 males; mean age: 33 ± 9 years, range: 18–49 years) were recruited by advertisements or word of mouth. Written informed consent was obtained after full explanation of study procedures and risks. Routine blood and urine tests, an electrocardiogram (ECG) and a physical exam were performed before inclusion. Psychiatric disorders were assessed using the MINI-Plus structured interview (Sheehan *et al*, 1998). Subjects with serious or unstable medical or neurological conditions, axis-one psychiatric diagnoses, substance abuse other than caffeine or nicotine within 6 months before baseline visit were not included into the study. On a day of a PET examination, smokers ($n = 5$) were asked to consume no more than their usual amount of cigarettes, and all participants were asked to abstain from alcohol intake 24 h before PET scans, and from caffeine-containing beverages 12 h before scans. A standardized light breakfast was served before *d*-amphetamine/placebo intake.

Standard urine tests for psychotropic substances were performed at inclusion and before PET scans. Pregnancy was excluded using serum HCG analysis at inclusion and standard urine pregnancy tests before each scan. Blood pressure measurements and continuous ECG monitoring were performed during all scans. Participants had a physical exam and standard ECGs immediately after scans. To document safety of [^{11}C]-(+)-PHNO PET procedures, the first 12 subjects underwent an additional physical exam, ECG, routine blood and urine analysis the day after PET scans.

Eleven subjects (one female) completed the test–retest protocol, nine subjects (five females) the *d*-amphetamine protocol. Three subjects (all females) dropped out during or after the first [^{11}C]-(+)-PHNO PET scan (two because of nausea, one subject had moved away).

d-Amphetamine Administration

Two hours before radiotracer injection, participants were administered either two or three capsules containing *d*-amphetamine (Dexedrine® tablets, Glaxo Smith Kline, Mississauga, ON) or an equal number of identical capsules containing inactive lactose-powder. Participants and research personnel were blind to the content of the capsules. According to body weight, 25, 30, or 35 mg *d*-amphetamine were administered, resulting in a dose of 0.38–0.45 mg/kg body weight (mean ± SD dose: 27.8 ± 3.02 mg; mean ± SD dose per kg body weight: 0.42 ± 0.02 mg). Five millilitres of blood were drawn immediately before PET scans, centrifuged, and stored at −80°C for determination of serum *d*-amphetamine levels.

Subjective drug effects were measured using the Drug Effects Questionnaire (DEQ; Justice and de Wit, 2000) and stimulant-subscales of the Subjective States Questionnaire (SSQ; White *et al*, 2002). Both scales are visual analog-scales previously shown to be sensitive to *d*-amphetamine effects

(Justice and de Wit, 2000; White *et al*, 2002). Scales were administered before *d*-amphetamine/placebo intake and 60 and 230 min thereafter. Heart rate and blood pressure were measured at 15, 30, 60, 90, and 180 min after *d*-amphetamine administration, after tracer injection, and after PET scans.

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

Radiosynthesis of [^{11}C]-(+)-PHNO has been described in detail elsewhere (Wilson *et al*, 2005). Briefly, [^{11}C]propionyl chloride was reacted with 9-hydroxynaphthoxazine to generate a [^{11}C]amide, which was subsequently reduced by lithium aluminium hydride. Purification by HPLC and formulation gave radiochemically pure [^{11}C]-(+)-PHNO as a sterile, pyrogen-free solution suitable for human studies.

Image Acquisition

All PET images were acquired on a CPS-HRRT high-resolution neuro-PET camera system (Siemens Medical Imaging, Knoxville, TN) with an in-plane resolution of approximately 2.8 mm full-width at half-maximum (FWHM). Participants were scanned in supine position using a custom-made thermoplastic facemask together with a head-fixation system (Tru-Scan Imaging, Annapolis). Transmission scans were acquired before emission scans using a single photon point source, ^{137}Cs ($T = 30.2$ years, $E\gamma = 662$ keV) and used for attenuation correction. A saline solution of 355.2 ± 44 MBq [^{11}C]-(+)-PHNO with a specific activity at time of injection of 42.65 ± 13.2 GBq/μmol was injected as a bolus into an intravenous line placed in an antecubital vein. The line was flushed with 10 ml saline immediately after tracer injection and subsequently removed. Emission data were acquired in list mode over 90 min, raw data were reconstructed by filtered-back projection to yield dynamic images with 15 1-min frames and 15 5-min frames. Proton-density (PD) magnetic resonance images (MRIs) were obtained on a General Electric Medical System Signa 1.5T MRI scanner (General Electric Medical Systems, Milwaukee, WI).

Image Analysis

All PET images were analyzed using the in-house automated image analysis software ROMI. Exact procedures used in ROMI are described elsewhere (Rusjan *et al*, 2006a). In brief, a PD-MRI template in Montreal Neurologic Institute/International Consortium for Brain Mapping (MNI/ICBM) standard brain space was co-registered to PD-MRI images using nonlinear iterative co-registration algorithms implemented in SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). Transformation matrices were then applied to a standardized template in MNI/ICBM space containing predefined regions of interest (ROIs) for caudate (CAU), putamen (PUT), ventral striatum (VST), globus pallidus (GP), and cortical cerebellum (CER). Definition of the VST in the template followed the method of Mawlawi *et al* (2001). After spatial co-registration to PD-MRIs, the template was refined using gray matter probability-maps obtained from MRIs using SPM2. Since GP is imaged with a tone halfway between gray and white matter structures, a special algorithm using a predefined volume for GP (Spinks *et al*, 2005) was applied to refine the GP-ROI (Rusjan *et al*, 2006b). PD-MRIs were co-registered to summed PET images, the spatial transformation matrix was then applied to the refined ROIs. Data from both hemispheres were pooled to obtain average radioactivity concentrations in the volumes of interest. Regional radioactivity was determined for each frame, corrected for decay, and plotted *vs* time to obtain time–activity curves (TACs). The simplified reference tissue model (SRTM; Lammertsma *et al*, 1996) was applied to derive binding potentials (BPs) for each region of interest using PMOD software (Version 2.6.1; PMOD Technologies Ltd, Zurich, Switzerland). Cortical cerebellum served as reference region since it is virtually devoid of dopamine D_2 and D_3 receptors in humans (Camps *et al*, 1989; Hall *et al*, 1996; Levant, 1998). The use of the SRTM with a cerebellar input function has recently been validated using kinetic modelling and shown to provide adequate quantification of $D_{2/3}$ receptors with [^{11}C]-(+)-PHNO in humans (Ginovart *et al*, 2006b).

To validate the automated image analysis software for [^{11}C]-(+)-PHNO, 12 PET scans of six participants were analyzed using ROMI software and in a conventional manual way. For manual analysis, MRI scans were co-registered to PET scans using Analyze 5.0 software (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). Regions of Interest for CAU, PUT, VST, GP, and CER were drawn on PD-MRI images and subsequently transferred onto PET images. Typically, five axial PET slices were used for CER (around the outermost border of cerebellar cortex, sparing midline structures), 7–8 axial slices for CAU and PUT, and 6–7 axial slices for GP. Delineation of the VST followed the method described by Mawlawi *et al* (2001).

For an analysis of *d*-amphetamine effects without *a priori* anatomical hypothesis, parametric maps of *d*-amphetamine and placebo scans were constructed using PMOD software and subsequently analyzed using SPM2. For each scan, the SRTM was applied voxelwise using CER as reference region to create parametric maps with a voxel size of $2 \times 2 \times 2$ mm (x–y–z). A template was constructed using a mean image of naïve BP maps. Individual parametric maps were spatially normalized to the template by Nearest Neighbor interpolation algorithm. Effects of *d*-amphetamine administration were assessed voxel-wise using paired *t*-test procedures implemented in SPM2.

Statistical Analysis

Differences in the magnitude of change between placebo and *d*-amphetamine and between scan one and scan two in *d*-amphetamine/placebo and test/re-test parts of the study were analyzed using repeated measures analysis of variance (RM-ANOVA). Binding potentials in the four ROIs were the dependent variables, the repetition factor was termed 'condition' (ie, *d*-amphetamine *vs* placebo and scan one *vs* scan two). The respective study part (*d*-amphetamine/placebo *vs* test/retest) was the between-subject variable. The significance of the 'study part*condition' interactions are reported. Paired-samples *t*-tests (two-tailed) were used for *post hoc* comparisons. Correlations between *d*-amphetamine plasma levels and *d*-amphetamine-induced reductions in [^{11}C]-(+)-PHNO BPs, and correlations between results obtained with ROMI software and manual image analysis

were analyzed using Pearson Product Moment correlations. Paired-samples *t*-tests (two-tailed) were used to analyze subjective and physiological effects of *d*-amphetamine.

Test-retest variability was calculated as $[(BP_{scan\ one} - BP_{scan\ two})/BP_{scan\ one} \times 100]$, *d*-amphetamine-induced reductions in [^{11}C]-(+)-PHNO BPs were calculated as the percentage reduction in BP obtained after drug treatment when compared to placebo $[(BP_{placebo} - BP_{d\text{-amphetamine}})/BP_{placebo} \times 100]$.

All tests were performed using the statistical software package SPSS, Release 12.0.1 (SPSS Inc., Chicago, IL).

RESULTS

Physiological Effects/Safety of [^{11}C]-(+)-PHNO

Injection of [^{11}C]-(+)-PHNO did not lead to any significant changes in blood pressure, heart rate, or ECG at any time in the study. Similarly, there were no relevant findings in physical or neurological exams or in routine blood and urine analyses during the study. However, as described previously (Willeit *et al*, 2006), participants described mild and self-limited (duration 2–3 min) side effects (slight nausea or abdominal sensations of warmth) in one-third of the scans (14 of 43 scans). One subject wished to interrupt the scan; another one had a single episode of vomiting and did not reassume scanning thereafter because of data loss. Full data sets were acquired in 20 subjects.

Physiological and Subjective Effects of *d*-Amphetamine Administration

In good agreement with previous data [http://us.gsk.com/products/assets/us_dexedrine.pdf], administration of *d*-amphetamine resulted in serum levels of 53.2 ± 24.4 ng/ml (range: 11.6–73.8 ng/ml) two hours post *d*-amphetamine intake (p.i.), that is, immediately before the PET scan. There was a significant increase in systolic (baseline: 115 ± 12 mm Hg; peak 90 min p.i.: 132 ± 23 mm Hg) and diastolic (baseline: 73 ± 7 mm Hg; peak 60 min p.i.: 77 ± 13 mm Hg) blood pressure and in DEQ-ratings (baseline: 5.2 ± 1.8; 60 min p.i.: 6.6 ± 2.6; 230 min p.i.: 8.3 ± 8.4) after *d*-amphetamine intake. Heart rate and SSQ did not differ significantly between conditions.

Imaging Results

Binding potentials obtained with the automated image analysis software ROMI and conventional manual analysis showed excellent correlations in all investigated ROIs: CAU: $r = 0.973$, $p < 0.001$; PUT: $r = 0.993$, $p < 0.001$; VST: $r = 0.966$, $p < 0.001$; GP: $r = 0.916$, $p < 0.001$.

Indicating a lack of blood-flow effects on the free and non-specific tracer compartment, TACs obtained in *d*-amphetamine and placebo scans for the reference region CER were congruent (Figure 1). As described in detail elsewhere (Ginovart *et al*, 2006b; Willeit *et al*, 2006), tracer

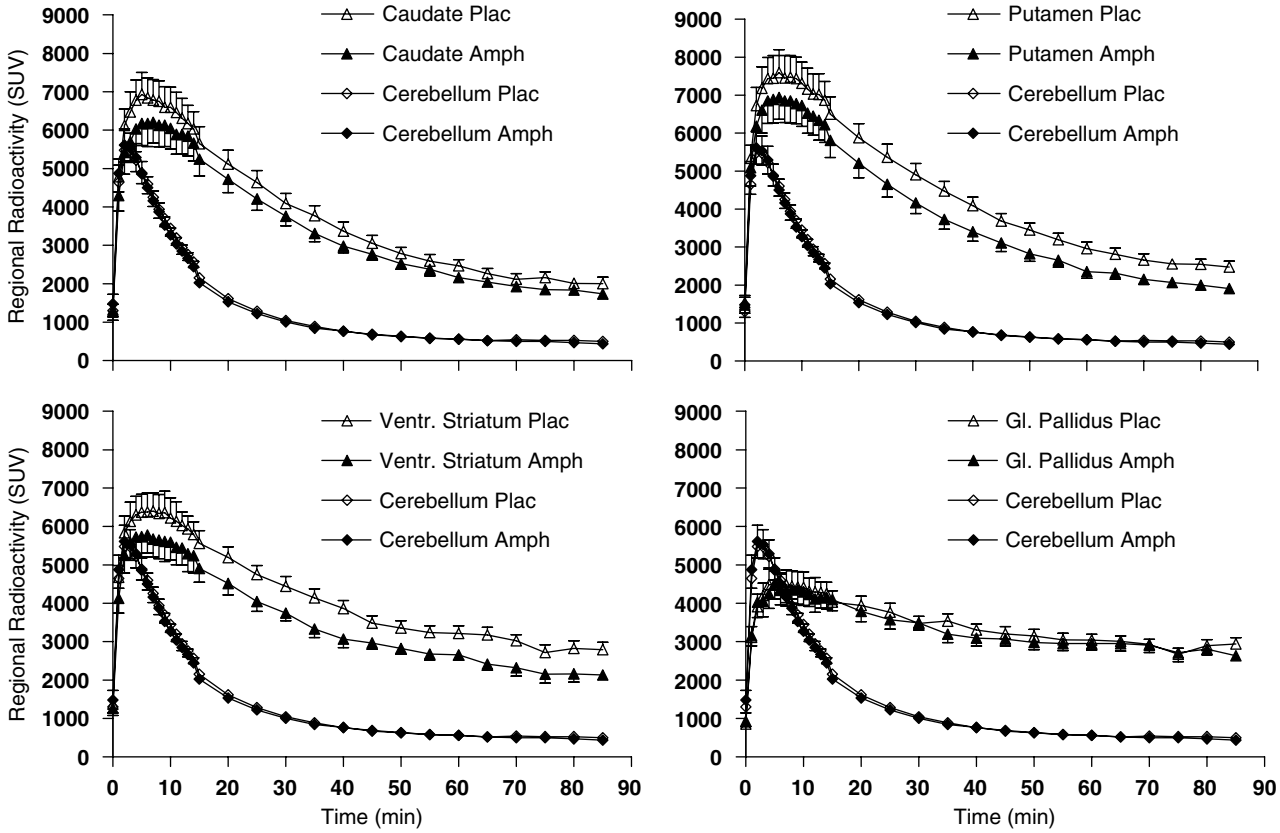

Figure 1 Time–activity curves (mean ± SEM) for caudate, putamen, ventral striatum, and globus pallidus 2 h after oral intake of placebo (Plac; empty triangles) or *d*-amphetamine (Amph; filled triangles). *Y*-axis represents standardized uptake values (SUV; calculated as: regional radioactivity concentration/(injected radioactivity/body weight) for [^{11}C]-(+)-PHNO. The congruent curves in cerebellum (empty diamonds: placebo; filled diamonds: *d*-amphetamine) indicate that administration of *d*-amphetamine did not have relevant influence on free and non-specific [^{11}C]-(+)-PHNO binding in this study.

kinetics in GP differed from those in CAU, PUT, and VST: TACs peaked at a lower level and showed a slower washout.

In the test–retest group, there were no significant differences in [^{11}C](+)-PHNO BPs between scan one and scan two in any of the ROIs (paired *t*-test; Table 1). Using absolute, that is, unsigned values of the differences between scan one and scan two, test–retest variability of [^{11}C]-(+)-PHNO BPs was 8.7±8% for CAU, 9.9±8% for PUT, 18.6±19% for VST, and 21.3±16% for GP, respectively.

There was a significant decrease in [^{11}C]-(+)-PHNO BPs in *d*-amphetamine scans *vs* placebo (Table 1, Figure 2) in CAU, PUT, and VST. Changes in GP did not reach level of significance. Indicating significant differences for within-subject changes in [^{11}C]-(+)-PHNO BPs between *d*-amphetamine/placebo and the test/re–test part of the study, the interaction term 'condition*study-part' was significant in all ROIs with exception of GP: CAU: $F(1)=6.248$, $p=0.022$; PUT: $F(1)=11.637$, $p=0.003$; VST: $F(1)=5.501$, $p=0.031$; GP: $F(1)=1.125$, $p=0.303$.

Serum *d*-amphetamine levels correlated significantly with *d*-amphetamine induced reductions in [^{11}C]-(+)-PHNO BPs in all ROIs but the CAU (CAU: $r=-0.0116$, $p=0.77$;

Table 1 [^{11}C]-(+)-PHNO Binding Potentials (BPs) Obtained in Healthy Control Subjects after Oral Ingestion of d-Amphetamine or Placebo ($n=9$) and under Test-Retest Conditions ($n=11$)

	Caudate	Putamen	Ventral striatum	Globus pallidus
Placebo	1.95±0.3	2.54±0.2	2.63±0.4	2.90±0.7
d-Amphetamine	1.69±0.3	2.01±0.3	1.96±0.4	2.62±0.5
Two-tailed *p*[a]	0.001	<0.001	0.001	0.224
Relative change[b]	−13.2±7%	−20.8±9%	−24.9±13%	−6.5±24%
Test	2.06±0.3	2.88±0.6	3.40±1.4	3.94±1.1
Re-test	2.07±0.4	2.90±0.6	3.34±0.8	3.75±1.3
Two-tailed *p*[a]	0.939	0.877	0.813	0.614
Relative change[b]	1.6±12%	3.0±12%	−7.0±27%	−2.1±28%

[a]Paired *t*-test.
[b]Calculated as [(BP placebo−BP d-amphetamine)/BP placebo × 100] and [(BP scan 1−BP scan 2)/BP scan 1 × 100].

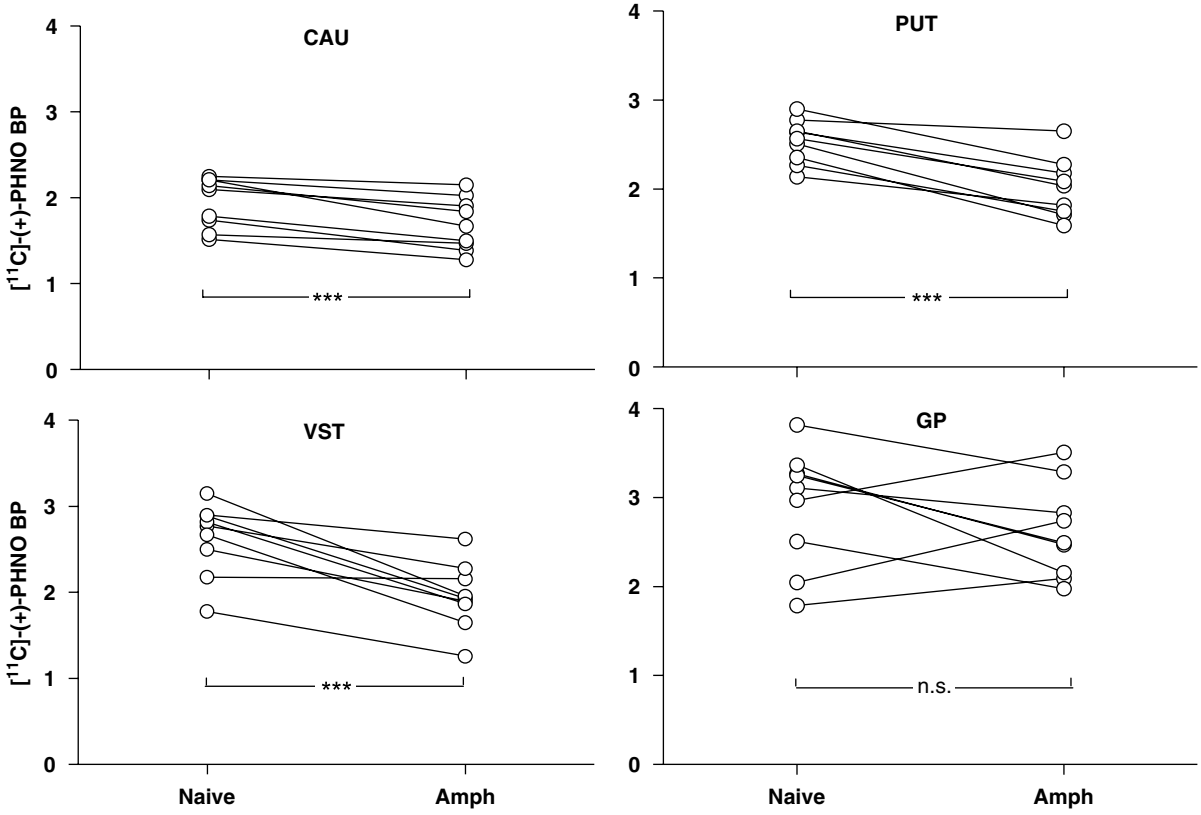

Figure 2 [^{11}C]-(+)-PHNO binding potentials (BPs) in healthy subjects for the regions of interest caudate (CAU), putamen (PUT), ventral striatum (VST), and globus pallidus (GP) 2 h after intake of placebo capsules or capsules containing 27.8±3 mg (mean±SD) *d*-amphetamine. ***Two-tailed $p\leqslant 0.001$; n.s.: two-tailed $p=0.224$.

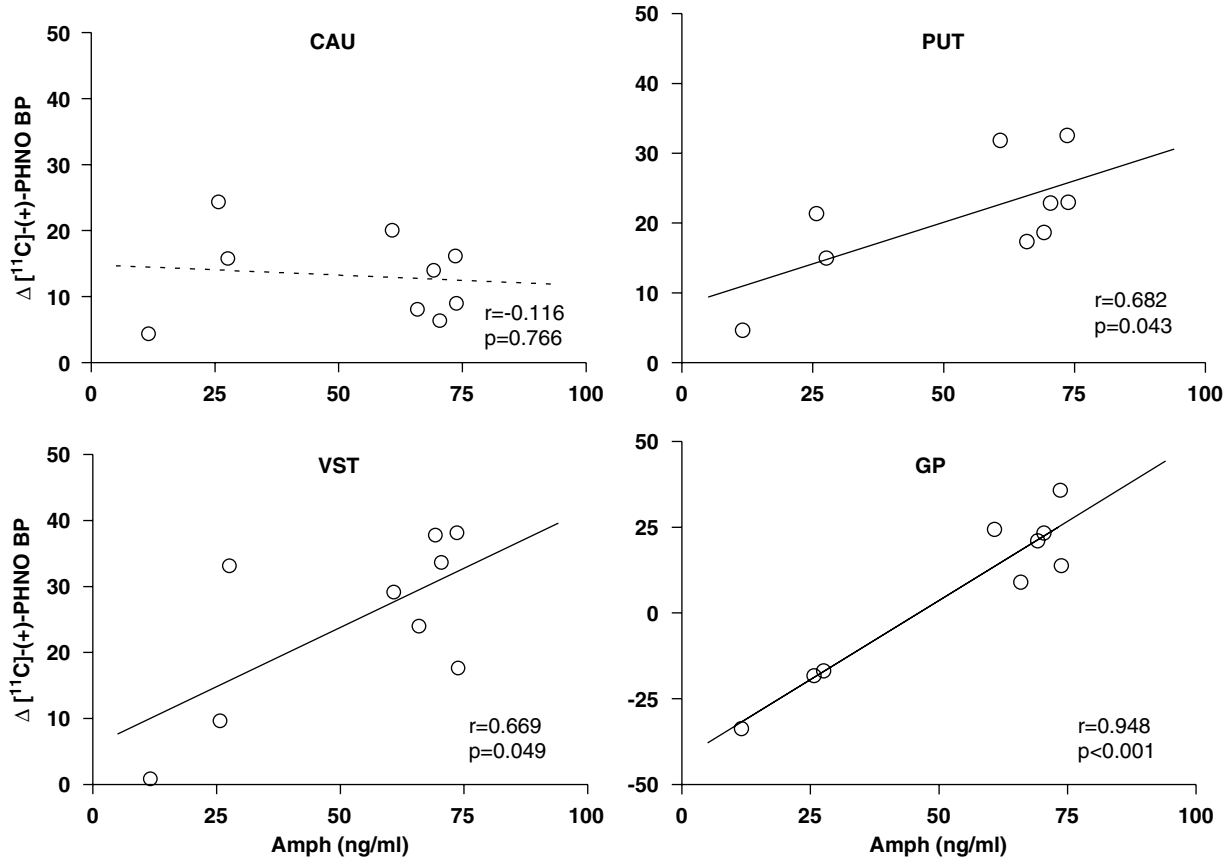

Figure 3 Correlations between serum *d*-amphetamine levels (Amph) and reductions in [^{11}C]-(+)-PHNO binding potentials (Δ[^{11}C]-(+)-PHNO BPs) calculated as ((BP placebo−BP *d*-amphetamine)/BP placebo) × 100. Note scaling of the *Y* axis in scatter-plot for GP. CAU, caudate; PUT, putamen; VST, ventral striatum; GP, globus pallidus.

PUT: $r=0.682$, $p=0.043$; VST: $r=0.669$, $p=0.049$; GP: $r=0.948$, $p<0.001$). Notably, an increase rather than a decrease in post-*d*-amphetamine [^{11}C]-(+)-PHNO BPs in the GP measured in the three subjects with lowest serum *d*-amphetamine levels contributed visibly (Figure 3) to the high correlation coefficient found in this ROI.

Binding Potential Maps

Overall appearance of parametric BP maps was similar to summated [^{11}C]-(+)-PHNO PET images, dopamine $D_{2/3}$ receptor rich regions were clearly delineated. Highest [^{11}C]-(+)-PHNO BPs were found in GP and the ventral portion of the neostriatum. The region with statistically most significant *d*-amphetamine-induced displacement of [^{11}C]-(+)-PHNO binding was a bilateral cluster located in the medio-ventral portion of the striatum (Figure 4). Peak voxels were identified at MNI coordinates $x=-14$, $y=16$, $z=-6$ ($T=12.52$, $p_{\text{uncorrected}}<0.001$), $x=4$, $y=14$, $z=-8$ ($T=12.01$, $p_{\text{uncorrected}}<0.001$), and $x=18$, $y=16$, $z=-8$ ($T=11.71$, $p_{\text{uncorrected}}<0.001$). The cluster followed the contours of the putamen in dorso-caudal direction (Figures 4 and 5).

DISCUSSION

This study is, to our knowledge, the first to demonstrate *in vivo* competition of endogenous dopamine with the new $D_{2/3}$ agonist radioligand [^{11}C]-(+)-PHNO in humans. Administration of *d*-amphetamine led to a significant reduction in [^{11}C]-(+)-PHNO BPs in neostriatal ROIs. Largest reductions were found in the VST (Figure 2), a brain region that is particularly sensitive for the actions of psychostimulants and critical for their reinforcing properties (Di Chiara, 1999; Kiyatkin and Brown, 2003; Wise, 2004; Sellings *et al*, 2006). This result was confirmed in a voxel-wise analysis of parametric BP maps. The specificity of the measured signal-change is supported by significant correlations between changes in [^{11}C]-(+)-PHNO BPs and serum *d*-amphetamine levels in all ROIs but CAU (Figure 3). Congruent TACs derived in the reference region CER during the *d*-amphetamine and placebo condition (Figure 1) show that changes in free and non-specific tracer compartments did not contribute significantly to the findings.

This being the first study on $D_{2/3}$ agonist displacement in humans, the present results will be discussed in light of human experiments using $D_{2/3}$ antagonist radioligands and data employing agonist radioligands in animals. Several studies have examined the effect of *d*-amphetamine administration on $D_{2/3}$ antagonist radioligand binding in humans (Breier *et al*, 1997; Farde *et al*, 1992; Laruelle *et al*, 1995; Abi-Dargham *et al*, 1998; Cardenas *et al*, 2004; Riccardi *et al*, 2006). Owing to methodological differences such as *d*-amphetamine administration route (most studies used intravenous *d*-amphetamine administration), tracer administration (eg bolus *vs* bolus/constant infusion), control

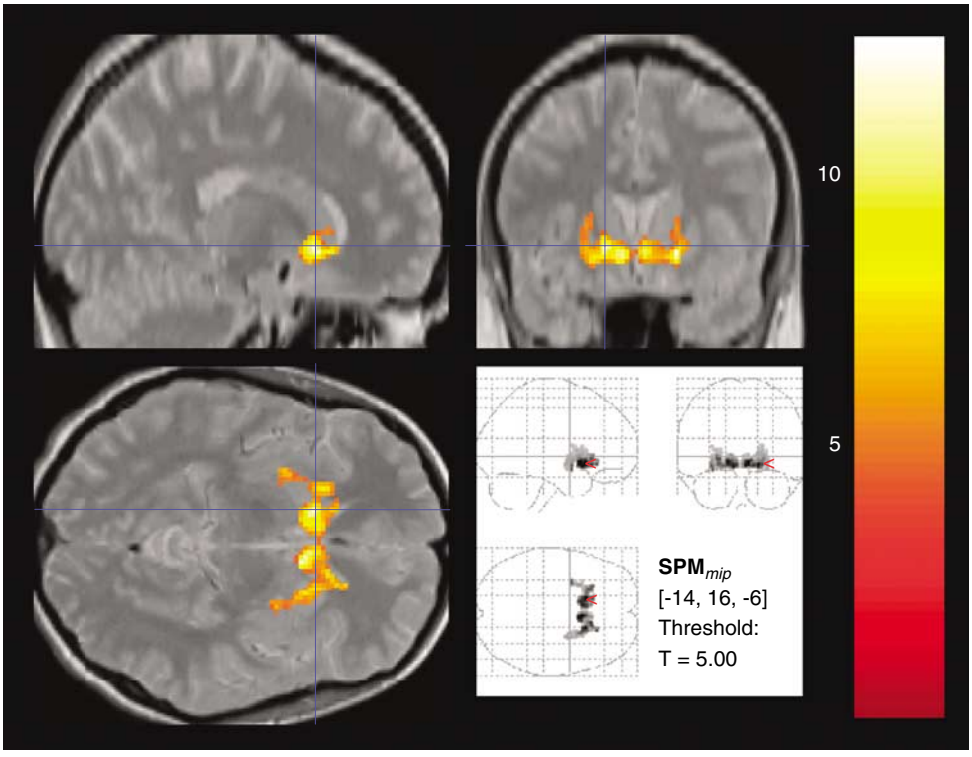

Figure 4 Statistical parametric map showing areas of significant *d*-amphetamine induced reductions (paired samples *t*-test) in [^{11}C]-(+)-PHNO binding potentials. Most significant displacement is found in a bilateral area located in the ventral striatum.

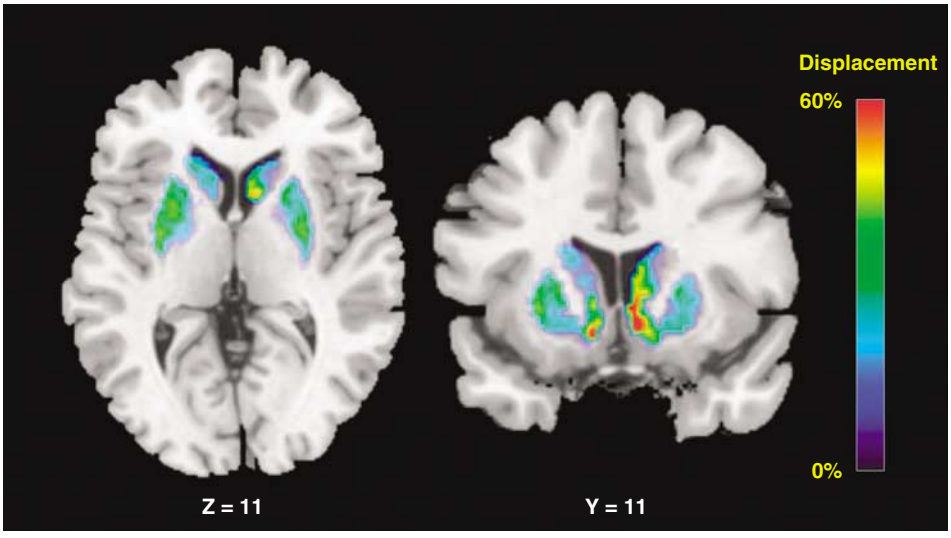

Figure 5 Colored areas show percent decrease in [^{11}C]-(+)-PHNO binding potentials (BPs) in *d*-amphetamine scans as compared to placebo scans. Image represents mean image of nine individual subtraction images calculated as ((BPmap placebo−BPmap *d*-amphetamine)/BPmap placebo) × 100.

conditions (placebo *vs* no intervention), ROI delineation and differences in scanner equipment and sample composition, results of these studies are not directly comparable to the present ones. However, reductions compared to baseline described after similar doses of *d*-amphetamine and similar post-intake scanning intervals lie generally between approximately 10 and 15% for the whole striatum (Breier *et al*, 1997; Cardenas *et al*, 2004; Farde *et al*, 1992; Laruelle *et al*, 1997; Abi-Dargham *et al*, 1998). Average reductions in [^{11}C]raclopride BPs described in five studies giving separate figures for the VST (Drevets *et al*, 2001; Leyton *et al*, 2002; Martinez *et al*, 2005; Munro *et al*, 2006; Oswald *et al*, 2005) are approximately 13% for VST, 6% for CAU, and 11% for PUT. A study performed at our PET Centre using a similar dose of oral *d*-amphetamine and the same post-intake scanning interval found reductions of 13% in striatal [^{11}C]raclopride BPs (Cardenas *et al*, 2004). However, this latter study reported considerably higher serum *d*-amphetamine levels and it used a different PET scanning system. A recent study using a similar dose of oral *d*-amphetamine and the antagonist $D_{2/3}$ radioligand [^{18}F]fallypride found reductions of 5.6, 11.2, and 6.6% in CAU, PUT, and VST, respectively

(Riccardi *et al*, 2006), reductions that are sensibly smaller than the 13.2, 20.8, and 24.9% reductions found in these corresponding structures with [^{11}C]-(+)-PHNO. A notable aspect of our results is that [^{11}C]-(+)-PHNO BPs measured in placebo scans were consistently lower than the ones in the test–retest condition. One possible explanation for this result is enhanced competition with endogenous dopamine, released in expectation of a real drug, a finding that has already been described using [^{11}C]raclopride and PET (de la Fuente-Fernandez *et al*, 2002). However, a direct comparison of [^{11}C]-(+)-PHNO with [^{11}C]raclopride, possibly employing a within-subject design, will help to quantify the difference between agonist and antagonist imaging in competition experiments. All together, it seems that reductions measured with [^{11}C]-(+)-PHNO in our present study are somewhat larger than the ones generally seen with $D_{2/3}$ antagonist radioligands.

Several animal PET studies have recently reported on the effects of *d*-amphetamine on *in vivo* $D_{2/3}$ agonist radioligand binding in the brain. Experiments have been carried out using [^{11}C]NPA (Narendran *et al*, 2004), [^{11}C]MNPA (Seneca *et al*, 2006), and [^{11}C]-(+)-PHNO (Galineau *et al*, 2006; Ginovart *et al*, 2006a; Wilson *et al*, 2005; Narendran *et al*, 2006). Some of the studies used parallel imaging with the antagonist radioligand [^{11}C]raclopride (Narendran *et al*, 2004, 2006; Seneca *et al*, 2006; Ginovart *et al*, 2006a). These studies show clearly that the *d*-amphetamine-induced displacement of agonist radioligand binding is one to two thirds larger than that which is measured with [^{11}C]raclopride. A head-to-head comparison of *d*-amphetamine induced displacement in cats showed an extrapolated maximal reduction of 68% for [^{11}C]raclopride BPs as compared to 96% for [^{11}C]-(+)-PHNO BPs (Ginovart *et al*, 2006a), and direct comparisons between [^{11}C]NPA and [^{11}C]-(+)-PHNO show that the latter ligand is more sensitive to endogenous dopamine than the former one (Ginovart *et al*, 2006a). Somewhat in contrast to these findings are results obtained in rats (Wilson *et al*, 2005) that show maximal reductions of 38% in [^{11}C]-(+)-PHNO binding even with high doses of *d*-amphetamine. One important difference of this study to the aforementioned experiments is that it was performed in un-anesthetized animals. A preliminary report (McCormick *et al*, 2006) shows that administration of volatile anaesthetics enhances the effect of *d*-amphetamine on [^{11}C]-(+)-PHNO binding. In sum, animal evidence shows clearly that [^{11}C]-(+)-PHNO is more vulnerable to competition with endogenous dopamine than [^{11}C]raclopride, and it suggests that, besides higher doses of *d*-amphetamine administered to animals, anesthesia may in part explain the greater magnitude of [^{11}C]-(+)-PHNO displacement observed in animal studies as compared to the present study.

A peculiarity of [^{11}C]-(+)-PHNO PET is the high BPs measured in GP. Tracer kinetics in GP proved to be different from those in neo-striatal regions, and no significant post-amphetamine reductions were found in the GP. Peak-uptake in GP was lower than in VST, CAU or PUT, and radioligand-washout considerably slower (Figure 1). Since equilibrium is reached later in the GP and since it is more sustained, activity measured in GP throughout the later part of the scanning session contributes substantially more to BP measures derived with SRTM than late-scan activity in neo-striatal ROIs (Ginovart *et al*, 2006b). As a direct consequence, the more noisy late parts of TACs increase variability of BP measurements in the GP. As shown in Figure 1, peak uptake in VST was slightly lower than in CAU and PUT. Although not to the extent seen in GP, tracer washout from VST seemed to be relatively slow, leading to a more extended equilibrium with greater weight of the late, more 'noisy' parts of the TACs in VST as well. This might explain in part the high variability observed in GP and VST, and it may have contributed to the lack of significant post-*d*-amphetamine reductions in the GP. On the other hand, three individuals with very low *d*-amphetamine levels showed an increase rather than a decrease in post-*d*-amphetamine [^{11}C]-(+)-PHNO BPs in GP (Figure 2). As seen easily in Figure 3, this increase contributed substantially to the highly significant correlation between serum *d*-amphetamine-levels and [^{11}C]-(+)-PHNO BPs in GP. As of yet, it is unclear whether this finding is caused by any real physiological processes in response to low-dose *d*-amphetamine, or whether this is a spurious finding relating to the high test-retest variability of [^{11}C]-(+)-PHNO PET in the GP. However, a recent study in baboons (Narendran *et al*, 2006) shows prominent (60%) *d*-amphetamine induced displacement of [^{11}C]-(+)-PHNO also in GP. It might be worth noting that in the present human study, displacement in GP was significant as well ($21.9 \pm 9\%$, $t(5) = -5.254$, $p = 0.003$) if the three subjects displaying serum *d*-amphetamine levels more than two SE below the mean were not included into the analysis.

It is unknown up to date what causes the particular binding pattern of [^{11}C]-(+)-PHNO in GP and the relatively high binding to ventral striatal structures. Evidence points towards a greater role of D_3 over D_2 receptors in [^{11}C]-(+)-PHNO binding in GP, and to a greater role of D_3 in VST as compared to CAU and PUT. First, anatomical distribution of [^{11}C]-(+)-PHNO uptake, with high BPs in GP and ventral portions of the neo-striatum, regions where post-mortem studies have shown relatively high densities of D_3 receptors (Seeman *et al*, 2006; Gurevich and Joyce, 1999; Murray *et al*, 1994), is compatible with a significant contribution of D_3 receptors to the captured signal. Second, some studies point towards a higher *in vitro* (Freedman *et al*, 1994) and *in vivo* (Narendran *et al*, 2006) affinity of [^{3}H]-(+)-PHNO for D_3 over D_2 receptors. On the other hand, there is evidence suggesting that the affinity of [^{11}C]-(+)-PHNO for D_2^{high} is considerably higher than the affinity for D_3 receptors (Seeman *et al*, 2005), and in contrast to pretreatment with the partial D_3 receptor agonist BP897 in baboons (Narendran *et al*, 2006), pre–treatment with the D_3 antagonist SB-277011 did not significantly reduce [^{11}C]-(+)-PHNO binding in the cat striatum (Ginovart *et al*, 2006a). However, reductions in [^{11}C]-(+)-PHNO BPs after *d*-amphetamine in this study were largest in VST, but smallest in GP. It is thus unlikely that binding to D_3 receptors alone accounts for both observations. Other factors, such as regional differences in the amount of dopamine released, should be considered in the interpretation of this finding. In sum, while not conclusive so far, evidence points to an important contribution of D_3 receptor binding to the high BPs measured in GP.

Although competitive inhibition of radioligand binding is the hypothesis most commonly put forward to explain

reductions in radioligand binding after *d*-amphetamine administration, several observations suggest that other mechanisms such as receptor internalization (Sun *et al*, 2003) or changes in receptor affinity (Ginovart *et al*, 2004) could contribute to this effect (for review see Ginovart, 2005; Laruelle, 2000). It is thus a limitation of the present study—as of all other competition studies performed with $D_{2/3}$ antagonist radioligands in humans—that the methodology does not allow to identify the exact mechanism leading to decreased radioligand binding after *d*-amphetamine administration. Another limitation of the present study is that we did not apply correction for partial volume effects (PVEs). Since ROIs such as GP and VST are relatively small for resolutions reached by current PET scanning systems, and since they are adjacent to receptor-rich regions such as PUT, correction for PVEs can be expected to add to the reliability of *d*-amphetamine-induced BP changes in this regions. However, a recent study using [^{11}C]raclopride showed that correction for PVEs resulted in higher estimates of the *d*-amphetamine effect in the VST of healthy subjects (Martinez *et al*, 2005), suggesting that, if anything, we might have underestimated the actual *d*-amphetamine effects on [^{11}C]-(+)-PHNO BPs in the VST.

A peculiarity of this study was that we observed nausea (in one case vomiting) after injection of [^{11}C]-(+)-PHNO. Nausea and emesis are typical unwanted drug effects during treatment with dopamine agonists. Injected radioligand mass was by no means higher than what is usually administered in PET studies using antagonist radioligands. Still, the close temporal contiguity between tracer injection and nausea is suggestive for a pharmacological effect of [^{11}C]-(+)-PHNO, and, possibly owing to the presence of spare D_2^{high} states, some of the pharmacological effects of dopamine agonists, such as inhibition of prolactin secretion (Meller *et al*, 1991), have been shown to occur at low receptor occupancies. According to the tracer principle, a radioligand should not perturb the biological system it is measuring. Although nausea might be interpreted as an indication that the tracer principle was violated in the present study, evidence suggests that this is unlikely to be the case. First, estimation of central receptor occupancy during [^{11}C]-(+)-PHNO PET according to the method described by Hume *et al* (1998) results in a mean $\pm$ SD occupancy of $1.6 \pm 0.5\%$ when using an ED_{50} value of 7.7 nmol/kg as measured *in vivo* in cats (Ginovart; unpublished observation). This is similar to what has been described for [^{11}C]raclopride PET (Nordström *et al*, 1992). Second, the ED_{50} of (+)-PHNO for inducing emesis in animal experiments is two orders of magnitude smaller than the ED_{50} for inducing motor effects or stereotyped behavior (Martin *et al*, 1984). Third, therapeutic (+)-PHNO plasma levels, even at the low end, are at least three orders of magnitude higher than what is measured after a single injection of approximately 2 μg total tracer mass as used in our study (Coleman *et al*, 1990; Ginovart *et al*, 2006b). Finally, (+)-PHNO-induced emesis is readily prevented by pretreatment with peripherally acting dopamine receptor antagonists in animals (Martin *et al*, 1984; Nomoto *et al*, 1987) and humans (Grandas *et al*, 1987). In sum, nausea is most likely a peripheral effect caused by even minute doses of (+)-PHNO acting at dopamine receptors outside the blood brain barrier in the area postrema (Carpenter, 1990). Pretreatment with a peripheral dopamine receptor antagonist may be a viable strategy to avoid [^{11}C]-(+)-PHNO induced nausea in future studies.

SUMMARY

This study is the first to demonstrate *in vivo* competition between endogenous dopamine and a $D_{2/3}$ agonist radioligand in humans. With exception of GP, the study showed clear-cut *d*-amphetamine effects in all striatal ROIs, and *d*-amphetamine-induced reductions in [^{11}C]-(+)-PHNO binding were larger than those reported for $D_{2/3}$ antagonist radioligands in the literature. Our data suggest that, in spite of relatively high test-retest variability, [^{11}C]-(+)-PHNO might be a superior radioligand for investigating alterations in pre-synaptic dopamine release in patients with schizophrenia and other psychiatric disorders.

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