A Comparative Evaluation of the Dopamine D_{2/3} Agonist Radiotracer [¹¹C](–)-N-Propyl-norapomorphine and Antagonist [¹¹C]Raclopride to Measure Amphetamine-Induced Dopamine Release in the Human Striatum

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ABSTRACT

(–)-N-Propyl-norapomorphine (NPA) is a full dopamine D_{2/3} receptor agonist, and [¹¹C]NPA is a suitable radiotracer to image D_{2/3} receptors configured in a state of high affinity for agonists with positron emission tomography (PET). In this study, the vulnerability of the in vivo binding of [¹¹C]NPA to acute fluctuation in synaptic dopamine was assessed with PET in healthy humans and compared with that of the reference D_{2/3} receptor antagonist radiotracer [¹¹C]raclopride. Ten subjects (eight females and two males) were studied on two separate days, a minimum of 1 week apart, both with [¹¹C]raclopride and [¹¹C]NPA at baseline and after the administration of 0.5 mg · kg^{–1} oral *d*-amphetamine. Kinetic modeling with an arterial input function was used to derive the binding potential relative to nonspecific uptake (BP_{ND}) in the ventral striatum (VST), cau-

date (CAD), and putamen (PUT). [¹¹C]Raclopride BP_{ND} was significantly reduced by 9.7 ± 4.4, 8.4 ± 4.2, and 14.7 ± 4.8% after amphetamine administration in the VST, CAD, and PUT. [¹¹C]NPA BP_{ND} was also reduced significantly, by 16.0 ± 7.0, 16.1 ± 6.1, and 21.9 ± 4.9% after the same dose of amphetamine in the VST, CAD, and PUT. Although these results suggest that [¹¹C]NPA is more vulnerable to endogenous competition by dopamine compared with [¹¹C]raclopride by a factor of 1.49 to 1.90, the same data for a related outcome measure, binding potential relative to plasma concentration, was not significant. Nevertheless, these data add to the growing literature that suggests D_{2/3} agonist radiotracers are more vulnerable to endogenous competition by dopamine than existing D_{2/3} antagonist radiotracers.

PET studies comparing D_{2/3} (hereafter referred to as D₂) agonist and antagonist radiotracers with respect to their vulnerability to endogenous competition by dopamine suggest that the agonist radiotracers such as [¹¹C]NPA (Narendran et al., 2004), [¹¹C]2-methoxy-N-propyl-norapomorphine (Seneca et al., 2006), and [¹¹C]PHNO (Ginovart et al., 2006) are more displaceable than the antagonist radiotracer [¹¹C]raclopride after an acute amphetamine challenge. This

increased vulnerability to endogenous competition by dopamine for D₂ agonist radiotracers has been attributed to the fact that agonists but not antagonists distinguish between G protein-coupled and uncoupled high- and low-affinity D₂ receptor states in vivo (Zahniser and Molinoff, 1978; George et al., 1985). Because the endogenous agonist dopamine competes only at G protein-coupled D₂ receptors, which are the same sites that the agonist radiotracers bind with preference, a relatively larger fraction of the in vivo binding of agonist radiotracers is vulnerable to endogenous competition by dopamine. In contrast, a smaller fraction of the in vivo binding of antagonist radiotracers is vulnerable to endogenous competition by dopamine because it binds to both high- and low-affinity states with equal affinity.

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ABBREVIATIONS: PET, positron emission tomography; NPA, (–)-N-propyl-norapomorphine; PHNO, (+)-4-propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9-ol; C_L, plasma clearance; f_p, plasma free fraction; VST, ventral striatum; CAD, caudate; PUT, putamen; STR, whole striatum; V_{ND}, tissue distribution volume; BP_p, binding potential relative to plasma concentration; BP_{ND}, binding potential relative to nonspecific uptake; V_T, distribution volume; RM ANOVA, repeated measures analysis of variance; DA, dopamine; f_{ND}, nondisplaceable free fraction in the brain.

Nevertheless, a limitation of the aforementioned agonist-antagonist comparison studies is the fact that they were conducted in anesthetized and not awake animals, which might not reflect the behavior of these agonist radiotracers in conscious human studies. This issue was raised in an *ex vivo* rat study in which the amphetamine-induced displacement of the agonist radiotracers [^{11}C]PHNO and [^{11}C]NPA was no different from that observed for the antagonist radiotracer [^{11}C]raclopride under unanesthetized conditions (McCormick et al., 2008). Also consistent with this observation is a nonhuman primate PET study in which methamphetamine-induced displacement of the agonist [^{11}C]2-methoxy-*N*-propyl-norapomorphine was significantly greater in the anesthetized as opposed to the awake condition (Ohba et al., 2009). However, both of these studies had methodological issues that complicate the interpretation of their results. For example, the *ex vivo* rodent study administered nontracer doses of [^{11}C]PHNO and evaluated the amphetamine-induced displacement only at a single time point. These factors may have led to a lower displacement of the agonist after amphetamine and thereby affected the agonist-antagonist comparison. In the nonhuman primate study, no parallel evaluations of amphetamine-induced displacement of a D_2 antagonist radiotracer were performed in the same animals under awake and anesthetized conditions. Thus, a true comparison of the agonist and antagonist displacements in the same animals under both the anesthetized and awake conditions was not provided. Despite the limitations these results raise the possibility that D_2 agonist radiotracers may not offer any significant advantage over D_2 antagonist radiotracers in the study of amphetamine-induced dopamine transmission under unanesthetized conditions. This has important implications for the use of D_2 agonist radiotracers in human research, which is almost always conducted in conscious subjects.

To date only one published study has evaluated the displacement of a D_2 agonist radiotracer after amphetamine challenge in humans (Willeit et al., 2008). In this study, the D_2 agonist radiotracer [^{11}C]PHNO was displaceable in the caudate (−13.2%), putamen (−20.8%), and ventral striatum (−24.9%) but not globus pallidus (−6.5%) after the administration of 0.5 mg · kg^{−1} oral amphetamine. Because this study did not measure the magnitude of displacement of the D_2 antagonist radiotracer [^{11}C]raclopride in the same subjects, it was not possible to ascertain from this data set whether D_2 agonist radiotracers are superior tools to measure amphetamine-induced dopamine release in humans. To address this question we evaluated the *in vivo* binding characteristics of the D_2 agonist radiotracer [^{11}C]NPA and the reference antagonist radiotracer [^{11}C]raclopride in the same healthy human subjects before and after an acute amphetamine challenge.

Materials and Methods

General Design. The study was approved by the Institutional Review Board of the University of Pittsburgh. In total, 40 PET scans were acquired for this study in 10 healthy control subjects over 20 experimental sessions. Each experimental session included two PET scans: a baseline scan and a postamphetamine scan with the same radiotracer. All subjects returned for a second experimental session in a minimum of 1 week (but no longer than 3 weeks) identical to the first, but with the other radiotracer (a total of four scans per subject).

The sequence of the radiotracers was counterbalanced across subjects to prevent bias in the between-radiotracer comparison. Five subjects received [^{11}C]raclopride scans during the first experimental session and the remaining five received [^{11}C]NPA scans during the first experimental session. The postamphetamine scan occurred 3 h after the administration of 0.5 mg · kg^{−1} oral *d*-amphetamine.

PET Protocol. The radiolabeling of [^{11}C]NPA and [^{11}C]raclopride was performed by using previously published procedures (Halldin et al., 1991; Hwang et al., 2000). PET outcome measures described in the article are consistent with the recommended consensus nomenclature for *in vivo* imaging of reversibly binding radioligands (Innis et al., 2007).

Imaging experiments were conducted on an ECAT EXACT HR+ camera (Siemens, Knoxville, TN) consistent with previously described image acquisition protocols (Narendran et al., 2009a). In brief, after completion of a transmission scan (~10 min) for attenuation correction of the emission data, subjects received either an intravenous injection of [^{11}C]raclopride or [^{11}C]NPA as a bolus over 20 s. Based on previous reports, the maximal injected mass for [^{11}C]raclopride and [^{11}C]NPA was restricted to 6 and 2 µg, respectively (Mawlawi et al., 2001; Narendran et al., 2009a) to be at tracer dose (less than 5% receptor occupancy). Emission data were collected for 60 min. The postamphetamine scan was performed 3 h after the administration of 0.5 mg · kg^{−1} oral amphetamine.

Input Function Measurement. After radiotracer injection, arterial samples were collected manually approximately every 6 s for the first 2 min and thereafter at longer intervals. In total, 35 samples were obtained per scan. After centrifugation, plasma was collected in 200-µl aliquots, and activities were counted in a gamma counter. To determine the plasma activity representing unmetabolized parent compound for [^{11}C]raclopride (collected at 8, 12, 20, 30, 40, 50, and 60 min) and [^{11}C]NPA (collected at 4, 8, 12, 16, 20, 40, and 50 min), seven samples were further processed by using high-performance liquid chromatographic methods described previously for both the radiotracers (Mawlawi et al., 2001; Narendran et al., 2009a).

For [^{11}C]raclopride, the seven measured parent fractions were fitted by using a sum of two exponentials (Narendran et al., 2004). For [^{11}C]NPA the parent fractions were fitted to a Hill plot model (Narendran et al., 2009a). The input function was then calculated as the product of total counts and interpolated parent fraction at each time point. The measured input function values were fitted to a sum of three exponentials from the time of peak plasma activity, and the fitted values were used as the input to the kinetic analysis. The clearance of the parent compound (C_L , expressed in liters per hour) was calculated as the ratio of the injected dose to the area under the curve of the input function (Abi-Dargham et al., 1994). The determination of the plasma free fraction (f_p) for both [^{11}C]raclopride and [^{11}C]NPA was performed by using ultrafiltration units (Gandelman et al., 1994).

In the postamphetamine condition, amphetamine plasma levels were measured in three arterial samples obtained at 0, 30, and 60 min relative to the PET scan as described previously (Reimer et al., 1993). These data ensured that differences in plasma amphetamine concentration did not bias the radiotracer comparison.

Image Analysis. A three-dimensional spoiled gradient recalled sequence magnetic resonance image was acquired by using a Signa 1.5T magnetic resonance imaging scanner (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for coregistration of the PET data. PET data were reconstructed using filtered back-projection and standard corrections applied that included those for photon attenuation, scatter, and radioactive decay. Reconstructed image files were then processed with MEDx image analysis software (Sensor Systems, Inc., Sterling, VA) and SPM2 software (www.fil.ion.ucl.ac.uk/spm). Frame-to-frame motion correction for head movement and magnetic resonance-PET image alignment were performed by using a mutual information algorithm implemented in SPM2 software.

Time-activity curves were generated for the three anatomical subdivisions of the human striatum: ventral striatum (VST), caudate

(CAD, which included both the precommissural and postcommissural caudate), and putamen (PUT, which included both precommissural and postcommissural putamen) using criteria outlined in Martinez et al. (2003). In addition, a whole striatum region (STR) was derived as the weighted average of the VST, CAD, and PUT. The cerebellum was subsampled in 15 consecutive coronal magnetic resonance imaging slices caudal to the cerebellar peduncle and used as a reference region using previously described methods (Narendran et al., 2009b).

For bilateral regions, right and left values were averaged. The contribution of plasma total activity to the regional activity was calculated by assuming a 5% blood volume in the regions of interest (Mintun et al., 1984), and tissue activities were calculated as the total regional activities minus the plasma contribution.

Derivation of Radiotracer Binding Parameters. The three outcome measures provided are reference tissue distribution volume (V_{ND} , milliliters per cubic centimeter), regions of interest binding potential relative to plasma concentration (BP_P , milliliters per cubic centimeter), and binding potential relative to nonspecific uptake (BP_{ND} , unitless). The definitions of these outcome measures are outlined in Innis et al. (2007).

The amphetamine-induced change in BP_{ND} was calculated as the difference between BP_{ND} measured in the postamphetamine condition ($BP_{ND\ AMPH}$) and BP_{ND} measured in the baseline condition on that day ($BP_{ND\ BASE}$) and is expressed as a percentage of $BP_{ND\ BASE}$:

$$\Delta BP_{ND} = 100 \times \frac{BP_{ND\ AMPH} - BP_{ND\ BASE}}{BP_{ND\ BASE}} \quad (1)$$

ΔBP_{ND} is generally preferred to ΔBP_P (which is derived using eq. 1 and substituting BP_P for BP_{ND}) in clinical studies to measure the effect of amphetamine, because the test/retest reproducibility of BP_{ND} is typically better than that of BP_P . In this article, we include the amphetamine-induced changes in both of these outcome measures. In addition, we report the effect of amphetamine on plasma clearance (C_L), f_p , and V_{ND} expressed relative to the preamphetamine value measured the same day.

Derivation of [¹¹C]raclopride and [¹¹C]NPA distribution volume (V_T) was performed by using kinetic analysis and the arterial input function. A one- and two-tissue compartment model was used to describe the kinetics in the cerebellum and striatal subdivisions for [¹¹C]raclopride (Lammertsma et al., 1996). A two-tissue compartment model was used to describe both the cerebellar and striatal kinetics for [¹¹C]NPA (Narendran et al., 2009a).

Statistical Analysis. The effect of amphetamine on the outcome measures was evaluated for each tracer by using repeated measures (RM) ANOVA, with the outcome measure as the dependent variable, the baseline and postamphetamine conditions as the repeated condition, and the region as the cofactor ($n = 3$; VST, CAD, and PUT). The significance levels of the condition and the dose $\times$ region interaction are reported. In addition, post hoc comparisons between baseline and postamphetamine conditions in the individual regions of

interest were evaluated with paired t tests ($n = 4$; VST, CAD, PUT, and STR).

When a significant effect of amphetamine was observed for at least one of the two tracers, a second-level analysis was performed to test for between-tracer difference in this amphetamine effect. This evaluation was performed by using RM ANOVA, with the amphetamine effect on the outcome measure as the dependent variable (ΔBP_{ND} or ΔBP_P), the tracer as the repeated condition, and the region as the cofactor. The significance levels of the tracer condition and the region $\times$ condition interaction are reported. A two-tailed $p = 0.05$ was selected as the significance level all statistical tests.

Results

Demographics

Ten subjects (two males and eight females; one African American and nine whites) participated in the study. The mean age of the subjects was 28 ± 9 years (range, 19–50). All 10 subjects who participated in the study were nonsmokers.

Baseline Scan Parameters: Injected Dose and Mass

The mean injected dose, mass, and specific activity at the time of injection for the baseline and postamphetamine condition for radiotracers [¹¹C]raclopride and [¹¹C]NPA are listed in Table 1. No significant differences were observed between the baseline and postamphetamine condition in injected radiation dose and injected mass for [¹¹C]raclopride and [¹¹C]NPA.

Plasma Analysis

Clearance. Under baseline conditions, [¹¹C]NPA plasma C_L was significantly faster than [¹¹C]raclopride plasma C_L (RM ANOVA, $p < 0.001$). Amphetamine did not significantly alter the plasma C_L for [¹¹C]raclopride or [¹¹C]NPA (Table 1).

Free Fraction in Plasma. Under baseline conditions, [¹¹C]raclopride f_p was significantly higher than [¹¹C]NPA f_p (RM ANOVA, $p < 0.02$). Amphetamine did not significantly alter f_p for [¹¹C]raclopride or [¹¹C]NPA (Table 1).

Amphetamine Plasma Levels. No significant differences in the amphetamine plasma levels were observed between the postamphetamine [¹¹C]raclopride and [¹¹C]NPA scans (RM ANOVA, $p = 0.20$). The amphetamine plasma levels were relatively stable throughout the duration of the post-amphetamine scan for both radioligands (Table 1).

Regions of Interest Volumes

The mean volumes of the ventral striatum, caudate, and putamen were 2039 ± 582 , 5535 ± 514 , and 8337 ± 851 mm³.

TABLE 1
Baseline scan parameters and plasma analysis ($n = 10$ subjects)

	[¹¹ C]Raclopride		[¹¹ C]NPA	
	Baseline	After Amphetamine	Baseline	After Amphetamine
Injected dose (mCi)	8.4 ± 0.3	7.8 ± 0.9	8.2 ± 0.3	8.4 ± 0.3
Specific activity (Ci/mmol)	1574 ± 476	1723 ± 770	2270 ± 491	2018 ± 520
Injected mass (μ g)	2.0 ± 0.7	1.9 ± 1.0	1.1 ± 0.3	1.3 ± 0.3
Plasma free fraction (f_p , %)	11.6 ± 3.2	11.5 ± 3.1	9.3 ± 2.0	9.4 ± 2.2
Nondisplaceable free fraction (f_{ND} , %)	28.4 ± 5.9	30.2 ± 5.5	2.9 ± 0.9	3.0 ± 0.9
Clearance (l/h)	11.7 ± 5.0	12.4 ± 2.9	118.5 ± 17.5	129.4 ± 25.1
Cerebellum V_T (ml $\cdot$ cm ⁻³)	0.41 ± 0.07	$0.38 \pm 0.08^*$	3.31 ± 0.77	3.20 ± 0.86
Plasma amphetamine (0 min, ng $\cdot$ ml ⁻¹)		65.9 ± 12.7		73.9 ± 5.4
Plasma amphetamine (30 min, ng $\cdot$ ml ⁻¹)		61.8 ± 11.2		69.7 ± 8.2
Plasma amphetamine (60 min, ng $\cdot$ ml ⁻¹)		61.1 ± 11.5		67.0 ± 8.2

* $p < 0.05$, paired t test, baseline compared with postamphetamine condition; plasma amphetamine times are relative to the postamphetamine PET scan.

The mean volumes of the whole striatum and subsampled cerebellum were $15,912 \pm 1313$ and $20,397 \pm 3071$ mm³.

Reference Region Analysis

Cerebellum Distribution Volume ($V_{T\text{ CER}}$ or V_{ND}). Under baseline conditions, [¹¹C]NPA V_{ND} was significantly higher than [¹¹C]raclopride V_{ND} (RM ANOVA, $p < 0.001$). Amphetamine led to a statistically significant decrease in [¹¹C]raclopride V_{ND} ($-7.2 \pm -7.4\%$; RM ANOVA, $p = 0.01$). No such amphetamine-induced decrease was observed for [¹¹C]NPA V_{ND} ($-3.3 \pm 11.0\%$; RM ANOVA, $p = 0.39$).

Region of Interest Analysis: Binding Potential BP_{ND}

Table 2 lists the values of [¹¹C]raclopride and [¹¹C]NPA BP_{ND} under baseline and postamphetamine conditions. Under baseline conditions, [¹¹C]raclopride BP_{ND} was significantly higher than [¹¹C]NPA BP_{ND} (RM ANOVA, BP_{ND} as dependent variable; tracer factor, $p < 0.001$; tracer $\times$ region interaction, $p < 0.001$).

Amphetamine produced a statistically significant decrease in [¹¹C]raclopride BP_{ND} in the striatal subdivisions (RM ANOVA, ΔBP_{ND} as dependent variable; tracer factor, $p = 0.004$; tracer $\times$ region interaction, $p < 0.001$).

Amphetamine produced a statistically significant decrease in [¹¹C]NPA BP_{ND} in the striatal subdivisions (RM ANOVA, ΔBP_{ND} as dependent variable; tracer factor, $p < 0.001$; tracer $\times$ region interaction, $p = 0.004$).

When both radiotracers were included in the analysis, the amphetamine-induced change in [¹¹C]NPA ΔBP_{ND} was significantly higher than [¹¹C]raclopride ΔBP_{ND} (RM ANOVA, ΔBP_{ND} as dependent variable; tracer factor, $p = 0.003$; tracer $\times$ region interaction, $p = 0.80$).

Region of Interest Analysis: Binding Potential BP_P

Table 3 lists the values of [¹¹C]raclopride and [¹¹C]NPA BP_P under baseline and postamphetamine conditions. Under baseline conditions, [¹¹C]NPA BP_P was significantly higher than [¹¹C]raclopride BP_P (RM ANOVA, BP_P as dependent

variable; tracer factor, $p < 0.001$; tracer $\times$ region interaction, $p < 0.001$). Although this observation appears contradictory to that reported in the previous section with BP_{ND} , it is consistent with the higher nonspecific binding (V_{ND}) of [¹¹C]NPA relative to [¹¹C]raclopride, because the variable BP_{ND} is derived as the ratio of BP_P to V_{ND} . Thus, a higher BP_P for [¹¹C]NPA does not indicate a higher signal-to-noise ratio in tissue compared with [¹¹C]raclopride.

Amphetamine produced a statistically significant decrease in [¹¹C]raclopride BP_P in the striatal subdivisions (RM ANOVA, ΔBP_P as dependent variable; tracer factor, $p = 0.024$; tracer $\times$ region interaction, $p < 0.001$).

Amphetamine produced a statistically significant decrease in [¹¹C]NPA BP_P in the striatal subdivisions (RM ANOVA, ΔBP_P as dependent variable; tracer factor, $p = 0.049$; tracer $\times$ region interaction, $p = 0.011$).

When both radiotracers were included in the analysis, the amphetamine-induced change in [¹¹C]NPA ΔBP_P was not significantly higher than [¹¹C]raclopride ΔBP_P (RM ANOVA, ΔBP_{ND} as dependent variable; tracer factor, $p = 0.50$).

Amphetamine-induced decreases in [¹¹C]raclopride or [¹¹C]NPA BP_P and BP_{ND} in the striatal regions of interest were not significantly correlated with the plasma amphetamine levels achieved during the scan (Pearson correlation coefficient, $p > 0.05$). In addition, the order of the sequence (first or second) in which subjects received the radiotracer had no significant effect on amphetamine-induced reduction in BP_{ND} or BP_P for [¹¹C]raclopride or [¹¹C]NPA (RM ANOVA, $p > 0.05$).

Discussion

The data from this comparison study raise two relevant questions regarding D₂ agonist radiotracers.

Are D₂ Agonist Radiotracers Superior Tools to Measure Amphetamine-Induced DA Release in Humans?

The primary objective of this study was to compare the effect of amphetamine on [¹¹C]raclopride and [¹¹C]NPA in vivo specific binding in healthy human subjects. This was under-

TABLE 2

Effect of amphetamine on [¹¹C]raclopride and [¹¹C]NPA BP_{ND}

Values are mean $\pm$ S.D.; $n = 10$ subjects.

Region	¹¹ C]Raclopride BP_{ND}		Difference	¹¹ C]NPA BP_{ND}		Difference	Δ Ratio, NPA/Raclopride
	Baseline	After Amphetamine		Baseline	After Amphetamine		
			%				
Ventral striatum	2.43 ± 0.25	2.19 ± 0.25	$-9.7 \pm 4.4^*$	1.04 ± 0.10	0.88 ± 0.12	$-16.0 \pm 7.0^*$	1.64
Caudate	2.49 ± 0.23	2.28 ± 0.20	$-8.4 \pm 4.2^*$	0.87 ± 0.10	0.73 ± 0.11	$-16.1 \pm 6.1^*$	1.90
Putamen	3.29 ± 0.28	2.80 ± 0.21	$-14.7 \pm 4.8^*$	1.25 ± 0.10	0.97 ± 0.10	$-21.9 \pm 4.9^*$	1.49
Striatum	2.91 ± 0.24	2.54 ± 0.19	$-12.3 \pm 4.4^*$	1.09 ± 0.09	0.88 ± 0.09	$-19.6 \pm 4.3^*$	1.59

* $P < 0.001$, paired t test.

TABLE 3

Effect of amphetamine on [¹¹C]raclopride and [¹¹C]NPA BP_P

Values are mean $\pm$ S.D.; $n = 10$ subjects.

Region	¹¹ C]Raclopride BP_P			¹¹ C]NPA BP_P			Δ Ratio, NPA/Raclopride
	Baseline	After Amphetamine	Difference	Baseline	After Amphetamine	Difference	
			%			%	
Ventral striatum	0.98 ± 0.16	0.83 ± 0.20	$-16.1 \pm 9.5^*$	3.42 ± 0.75	2.82 ± 0.87	$-18.4 \pm 12.9^*$	1.14
Caudate	1.01 ± 0.16	0.86 ± 0.18	$-14.9 \pm 9.4^*$	2.86 ± 0.61	2.35 ± 0.64	$-18.4 \pm 13.7^*$	1.23
Putamen	1.33 ± 0.19	1.06 ± 0.19	$-20.7 \pm 8.9^*$	4.09 ± 0.84	3.11 ± 0.84	$-24.4 \pm 9.4^*$	1.18
Striatum	1.18 ± 0.17	0.96 ± 0.18	$-18.5 \pm 9.0^*$	3.58 ± 0.76	2.80 ± 0.75	$-22.1 \pm 10.5^*$	1.19

* $p < 0.001$, paired t tests.

taken in response to recent investigations suggesting that the results of previous comparison studies showing greater vulnerability of the *in vivo* binding of D₂ agonists relative to D₂ antagonist radiotracers in anesthetized animals may not be valid in humans (see Introduction; Tsukada et al., 2000; McCormick et al., 2008; Ohba et al., 2009). Thus, we performed the above-mentioned experiments in the same human subjects under carefully controlled conditions and in a counterbalanced sequence. Unfortunately, the results of this study did not allow for definitive conclusions to be drawn for or against the superiority of D₂ agonist radiotracers to measure dopamine transmission.

Under the experimental conditions of this study, the amphetamine-induced increase in synaptic dopamine in the striatal subdivisions resulted in a 49 to 90% larger decrease in [¹¹C]NPA BP_{ND} compared with [¹¹C]raclopride BP_{ND}. Consistent with this result was the comparison of ΔBP_P for these radioligands, which suggested a 14 to 23% larger decrease for [¹¹C]NPA BP_P relative to [¹¹C]raclopride BP_P; however, this decrease was not statistically significant. The discordance between significance level of the findings for BP_{ND} and BP_P was caused by the reduction in [¹¹C]raclopride V_{ND} in the cerebellum after the administration of amphetamine. This reduction in V_{ND} led to a poorly correlated ΔBP_{ND} and ΔBP_P for [¹¹C]raclopride. For example, the mean striatal displacement of [¹¹C]raclopride after amphetamine was 12% for BP_{ND} and 19% for BP_P. In contrast, the amphetamine-induced displacement of [¹¹C]NPA in the striatum was a nearly identical, -20 and -22%, for BP_{ND} and BP_P, respectively. A decrease in V_{ND} in a study that evaluates amphetamine-induced dopamine release is a problem because it can underestimate the true effect of amphetamine, more so when ΔBP_{ND} rather than ΔBP_P is used as an outcome measure. Nevertheless, this issue is rarely discussed in depth in the clinical PET literature in which the measured [¹¹C]raclopride cerebellum V_{ND} is lower more often than not after an acute amphetamine (or methylphenidate) challenge (Table 4). Thus, a question that is critical to the interpretation of these studies is whether ΔBP_{ND} or ΔBP_P is the preferred outcome measure for amphetamine-induced DA release studies. BP_{ND} is the ratio of the specific (BP_P) to nondisplaceable (V_{ND}) distribution volumes at equilibrium (Innis et al., 2007). Compared with BP_P, which is the ratio of specific binding to plasma parent concentration at equilibrium, BP_{ND} is less vulnerable to experimental errors associated with the measurement of the input function and is associated with lower test-retest variability (Mawlawi et al., 2001; Narendran et al., 2009a). In addition, BP_{ND} can be derived without a plasma input function using reference tissue methods, thereby eliminating

the need for an arterial line, which can be uncomfortable for the research volunteers. Thus, ΔBP_{ND} is typically preferred over ΔBP_P and often reported as the sole outcome measure in clinical PET studies that measure amphetamine-induced DA transmission in humans. Nevertheless, to exclusively ascribe changes in BP_{ND} to changes in receptor parameters ($B_{\max}/K_D$) implies that nondisplaceable free fraction in the brain (f_{ND} , derived as f_P/V_{ND}) is not affected by the experimental factors under study (Innis et al., 2007), which is a reasonable assumption in a within-subject study design. Similarly, the use of ΔBP_P as defined here implies that f_P is invariant (Innis et al., 2007). In this study, f_{ND} and f_P for both the agonist and antagonist radiotracer were unaffected by the administration of amphetamine (Table 1; note that [¹¹C]raclopride $f_{ND} = f_P/V_{ND}$ was not affected by amphetamine despite significant change in V_{ND}), thereby suggesting that both outcome measures BP_{ND} and BP_P reflect the changes in $B_{\max}/K_D$ induced by amphetamine. This is somewhat puzzling given the discrepant results observed with ΔBP_{ND} and ΔBP_P for [¹¹C]raclopride. Nevertheless, these data underscore and argue against the use of a single preferred outcome to report changes in DA release in PET studies.

Another question that relates to the interpretation of these [¹¹C]raclopride-[¹¹C]NPA comparison data is whether the amphetamine-induced decrease in [¹¹C]raclopride BP_{ND} was underestimated by changes in V_{ND} and, if so, by what magnitude. This issue was assessed by contrasting the ΔBP_{ND} of [¹¹C]raclopride obtained in this study with that reported for other D₂ antagonist radiotracers after the administration of a comparable 0.5 mg · kg⁻¹ dose of oral amphetamine. A review of these results in Table 5 suggests that the mean displacement of [¹¹C]raclopride BP_{ND} after amphetamine in this study is consistent with that reported in previous studies that have used the same paradigm. Based on these data, we interpret the results of the ΔBP_{ND} comparison as valid in suggesting that the agonist [¹¹C]NPA is more vulnerable to endogenous competition by dopamine compared with the antagonist [¹¹C]raclopride. Such an interpretation is also consistent with our previous report in nonhuman primates in which *in vivo* binding of [¹¹C]NPA was more displaceable than [¹¹C]raclopride by 42% after an acute amphetamine challenge. Nevertheless, future amphetamine studies comparing D₂ agonists and antagonist radiotracers in humans are necessary to confirm our interpretation of these data.

How Do the D₂ Agonist Radiotracers [¹¹C]NPA and [¹¹C]PHNO Compare as Tools to Study Amphetamine-Induced DA Release in Humans? In a previous human study, the D₂ agonist radiotracer [¹¹C]PHNO was displace-

TABLE 4
Stimulant-induced decrease in [¹¹C]raclopride V_{ND} in healthy controls

Reference	n	Stimulant Challenge	Baseline V _{ND}	Postchallenge V _{ND}	Mean Difference
				<i>ml · cm⁻³</i>	%
This study	10	Amphetamine, 0.5 mg/kg p.o.	0.41 ± 0.07	0.38 ± 0.08*	-7
Volkow et al. (1997)	23	Methylphenidate, 0.5 mg/kg i.v.	0.42 ± 0.06	0.38 ± 0.08*	-10
Wang et al. (1999)	14	Methylphenidate, 0.5 mg/kg i.v.			-13
Volkow et al. (2001)	11	Methylphenidate, 0.8 mg/kg p.o.	0.38 ± 0.05	0.36 ± 0.05	-5
Drevets et al. (2001)	6	Amphetamine, 0.3 mg/kg i.v.	0.38 ± 0.03	0.35 ± 0.03	-6
Martinez et al. (2003)	14	Amphetamine, 0.3 mg/kg i.v.	0.34 ± 0.08	0.34 ± 0.08	0
Martinez et al. (2005)	15	Amphetamine, 0.3 mg/kg i.v.	0.40 ± 0.08	0.36 ± 0.08*	-10
Martinez et al. (2007)	24	Amphetamine, 0.3 mg/kg i.v.	0.39 ± 0.07	0.36 ± 0.07*	-8

* Reported as statistically significant.

TABLE 5

Displacement of dopamine D₂ antagonist radioligands in human striatum after oral amphetamine (~0.5 mg · kg⁻¹)

Healthy Control	Radioligand	Region of Interest			
		Ventral Striatum	Caudate	Putamen	Striatum
This study	[¹¹ C]Raclopride	9.7 ± 4.4	8.4 ± 4.2	14.7 ± 4.8	12.3 ± 4.4
Ziolko et al. (2007)	[¹¹ C]Raclopride	11.3 ± 4.9	6.5 ± 5.0	14.0 ± 5.0	11.3 ± 4.9
Cárdenas et al. (2004)	[¹¹ C]Raclopride				13.3 ± 5.0
Busto et al. (2009) ^a	[¹¹ C]Raclopride				11.1 ± 3.6
Busto et al. (2009) ^b	[¹¹ C]Raclopride				14.2 ± 3.9
Riccardi et al. (2005)	[¹⁸ F]Fallypride	7.2 ± 5.3	5.6 ± 4.6	11.2 ± 4.3	
Cropley et al. (2008)	[¹⁸ F]Fallypride	8.5 ± 2.8	7.6 ± 2.7	12.3 ± 2.8	

^a Healthy control smokers.^b Healthy control nonsmokers.

TABLE 6

Effect size for amphetamine-induced dopamine release (0.5 mg · kg⁻¹ oral)

Radioligand	Region of Interest			
	VST	CAD	PUT	STR
[¹¹ C]Raclopride (this study)	2.2	2.0	3.1	2.8
[¹¹ C]NPA (this study)	2.3	2.6	4.5	4.6
[¹¹ C]PHNO (Willeit et al., 2008)	1.9	1.9	2.3	—

able in the ventral striatum ($-25 \pm 13\%$), caudate ($-13 \pm 7\%$), and putamen ($-21 \pm 9\%$) after the administration of 0.5 mg · kg⁻¹ oral amphetamine. Although the mean displacement of both these D₂ agonist radioligands is of similar magnitude (see [¹¹C]NPA displacement in Tables 2 and 3), the effect size for [¹¹C]PHNO displacement is much lower than the effect size for [¹¹C]NPA displacement (Table 6). This observation is in line with the human test–retest studies that showed a higher variability for [¹¹C]PHNO BP_{ND} (VST, $19 \pm 19\%$; CAD, $9 \pm 9\%$; and PUT, $10 \pm 8\%$; Willeit et al., 2008) relative to [¹¹C]NPA BP_{ND} (VST, $5 \pm 5\%$; CAD, $5 \pm 4\%$; PUT, $6 \pm 4\%$; Narendran et al., 2009a). Nevertheless, these data showing lower effect sizes to measure DA release for [¹¹C]PHNO relative to [¹¹C]NPA are contradictory to that observed previously in anesthetized cats (Ginovart et al., 2006) and nonhuman primates (Narendran et al., 2004, 2006). It is likely that some of the advantages of [¹¹C]PHNO over [¹¹C]NPA (such as enhanced preference to bind to D₃ receptors) that led to superior measurement of dopamine transmission in animal studies is partially offset by the relatively poor reproducibility in humans. In the absence of direct comparison studies in humans, the data seem to suggest that [¹¹C]NPA is superior to [¹¹C]PHNO for measuring dopamine release in the striatal subdivisions, despite the fact that the signal-to-noise ratio of [¹¹C]PHNO in the striatum is 2.5-fold higher than that of [¹¹C]NPA (Ginovart et al., 2006; Narendran et al., 2006).

In summary, we conducted the first comparison study of a dopamine D₂ agonist and antagonist to assess amphetamine-induced dopamine release in healthy human subjects. The results of the study failed to unequivocally demonstrate the superiority of the D₂ agonists over D₂ antagonist radioligands as preferred tools to measure dopamine release because of the nonsignificance of the finding with the BP_P outcome measure. Nevertheless, these data add to the growing literature that suggests D₂ agonist radiotracers are more vulnerable to endogenous competition by dopamine than antagonist radiotracers.

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