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Title Page

A comparative evaluation of the dopamine $D_{2/3}$ agonist radiotracer [^{11}C]NPA and antagonist [^{11}C]raclopride to measure amphetamine-induced dopamine release in the human striatum

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Running title: Amphetamine-induced displacement of [^{11}C]NPA

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List of non standard abbreviations

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PET - positron emission tomography

MRI - magnetic resonance imaging

RAC - raclopride

NPA - (-)-N-propyl-nor-apomorphine

MNPA - 2-methoxy-N-propyl-nor-apomorphine

PHNO - (+)-4-propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9 –ol

D_{2high} - G-protein coupled D_2 receptors

D_{2low} - G-protein uncoupled D_2 receptors

VST - ventral striatum

CAD - caudate

PUT - putamen

STR - whole striatum

RM ANOVA - repeated measures analysis of variance

% R_{high} -fraction of D_2 receptors configured in a state of high affinity for the agonist dopamine

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)

Abstract

(-)-N-Propyl-norapomorphine is a full dopamine $D_{2/3}$ receptor agonist, and [^{11}C]NPA is a suitable radiotracer to image $D_{2/3}$ receptors configured in a state of high affinity for agonists with Positron Emission Tomography. In this study, the vulnerability of the in vivo binding of [^{11}C]NPA to acute fluctuation in synaptic dopamine was assessed with PET in healthy humans, and compared to that of the reference $D_{2/3}$ receptor antagonist radiotracer [^{11}C]raclopride. Ten subjects (8 Females/2 Males) were studied on two separate days, a minimum of one week apart, both with [^{11}C]raclopride and [^{11}C]NPA at baseline and following 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, BP_{ND} in the ventral striatum (VST), caudate (CAD), putamen (PUT). [^{11}C]raclopride BP_{ND} was significantly reduced by 9.7 ± 4.4 %, 8.4 ± 4.2 % and 14.7 ± 4.8% following amphetamine administration in the VST, CAD and PUT. [^{11}C]NPA BP_{ND} was also reduced significantly, by 16.0 ± 7.0%, 16.1 ± 6.1% and 21.9 ± 4.9% following the same dose of amphetamine in the VST, CAD and PUT. Although these results suggest that [^{11}C]NPA is more vulnerable to endogenous competition by dopamine compared to [^{11}C]raclopride by a factor of 1.49 to 1.90, the same data for a related outcome measure binding potential, BP_P, 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.

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INTRODUCTION

PET studies comparing $D_{2/3}$ (hereafter referred to as D_2) agonist and antagonist radiotracers with respect to their vulnerability to endogenous competition by dopamine suggests that the agonist radiotracers such as [^{11}C]NPA (Narendran et al., 2004), [^{11}C]-MNPA (Seneca et al., 2006) and [^{11}C]PHNO (Ginovart et al., 2006) are more displaceable than the antagonist radiotracer [^{11}C]raclopride following an acute amphetamine challenge. This increased vulnerability to endogenous competition by dopamine for D_2 agonist radiotracers has been attributed to the fact that agonists but not antagonists distinguish between G-protein coupled and uncoupled high and low affinity D_2 receptor states in vivo (Zahniser and Molinoff, 1978; George et al., 1985). As the endogenous agonist dopamine competes only at D_{2high} receptors, which are the same sites that the agonist radiotracers bind with preference, a relatively larger fraction of the agonist radiotracers' in vivo binding is vulnerable to endogenous competition by dopamine. In contrast, a smaller fraction of the antagonist radiotracers' in vivo binding is vulnerable to endogenous competition by dopamine because it binds to both high and low affinity states with equal affinity.

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 than that observed for the antagonist radiotracer [^{11}C]raclopride under un-anesthetized 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]MNPA 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 non tracer 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 following 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 un-anesthetized 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 following 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%) following the administration of 0.5 mg kg^{-1} oral amphetamine. As 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 dataset whether or not D_2 agonist radiotracers are superior tools to measure amphetamine-induced dopamine release in humans. In order 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.

METHODS

General Design

The study was approved by the Institutional Review Board of the University of Pittsburgh Medical Center. A total of 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 post-amphetamine scan with the same radiotracer. All subjects returned for a second experimental session in a minimum of one week (but no longer than three weeks) identical to the first, but with the other radiotracer (a total of 4 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]raclorpide scans during the first experimental session and the remaining five received [^{11}C]NPA scans during the first experimental session. The post-amphetamine scan occurred three hours following the administration of 0.5 mg kg-1 oral d-amphetamine.

PET Protocol

The radiolabeling of [^{11}C]NPA and [^{11}C]raclopride were performed using previously published procedures (Halldin et al., 1991; Hwang et al., 2000).

Imaging experiments were conducted on the ECAT EXACT HR+ camera consistent with previously described image acquisition protocols (Narendran et al., 2009a). Briefly, following 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 20s. Based on previous reports, the maximum injected mass for [^{11}C]raclopride and [^{11}C]NPA was restricted to 6 µg 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 minutes. The post-amphetamine scan was performed three hours after the administration of 0.5 mg kg^{-1} oral amphetamine.

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Input function measurement

Following radiotracer injection, arterial samples were collected manually approximately every 6s for the first two min and thereafter at longer intervals. A total of 35 samples were obtained per scan. Following 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, 50) seven samples were further processed using high performance liquid chromatogram 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 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 liter/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 were performed using ultrafiltration units (Gandelman et al., 1994).

In the post-amphetamine condition, amphetamine plasma levels were measured in three arterial samples obtained at time 0 min, 30 min and 60 min relative to the PET scan as previously described (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 using a 1.5 T GE Medical Systems Signa Scanner 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 the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) and SPM2 (www.fil.ion.ucl.ac.uk/spm). Frame-to-frame motion correction for head movement and MR-PET image alignment were performed using a mutual information algorithm implemented in SPM2.

Time activity curves were generated for the three anatomical subdivisions of the human striatum: ventral striatum, caudate (which included both the pre- and post-commissural caudate) and putamen (included both pre- and post-commissural putamen) using criteria previously outlined in (Martinez et al., 2003). In addition, a whole striatum region (STR) was derived as the weighted average of the ventral striatum (VST), caudate (CAD) and putamen (PUT). The cerebellum was sub sampled in fifteen consecutive coronal MRI slices caudal to the cerebellar penduncle 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 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}, mL cm^{-3}), regions of interest binding potential relative to plasma concentration ($BP_{P,}$ mL cm^{-3}),

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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 post-amphetamine condition ($BP_{ND\ AMPH}$) and BP_{ND} measured in the baseline condition on that day ($BP_{ND\ BASE}$), and expressed as a percentage of $BP_{ND\ BASE}$:

$$\Delta BP_{ND} = 100 * \frac{BP_{ND_{AMPH}} - BP_{ND_{BASE}}}{BP_{ND_{BASE}}} \qquad \text{Eq. 1}$$

ΔBP_{ND} is generally preferred to ΔBP_P (which is derived using Eq1 as well by 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 report 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 pre-amphetamine value measured the same day.

Derivation of [^{11}C]raclopride and [^{11}C]NPA V_T (distribution volume) was performed 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 [^{11}C]raclopride (Lammertsma et al., 1996). A two-tissue compartment model was used to describe both the cerebellar and striatal kinetics for [^{11}C]NPA (Narendran et al., 2009a).

Statistical analysis

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

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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 using repeated measures (RM ANOVA), with the amphetamine effect on the outcome measure as dependent variable (ΔBP_{ND} or ΔBP_P) the tracer as repeated condition, and the region cofactor. The significance levels of the tracer condition and the region*condition interaction are reported. A two-tailed $p = 0.05$ was selected as the significance level all statistical tests.

RESULTS

Demographics: 10 subjects (2 males/8 females; 1 African American /9 Caucasian) participated in the study. The mean age of the subjects was 28 ± 9 (range 19 to 50). All ten subjects who participated in the study were non-smokers.

Baseline scan parameters

Injected dose and mass: The mean injected dose, mass and specific activity at the time of injection for the baseline and post-amphetamine condition for both radiotracers [^{11}C]raclopride and [^{11}C]NPA are listed in Table 1. No significant differences were observed between the baseline and post-amphetamine condition in injected radiation dose and injected mass for [^{11}C]raclopride and [^{11}C]NPA.

Plasma Analysis

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

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

Amphetamine Plasma Levels: No significant differences in the amphetamine plasma levels were observed between the post-amphetamine [^{11}C]raclopride and [^{11}C]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 mm^3, 5535 ± 514 mm^3, 8337 ± 851 mm^3. The mean volumes of the whole striatum and sub-sampled cerebellum were 15912 ± 1313 mm^3 and 20397 ± 3071 mm^3

Reference region analysis

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

Region of interest analysis: Binding Potential BP_{ND}

Table 2 lists the values of [^{11}C]raclopride and [^{11}C]NPA BP_{ND} under baseline and post-amphetamine conditions.

Under baseline conditions, [^{11}C]raclopride BP_{ND} was significantly higher than [^{11}C]NPA BP_{ND} (RM ANOVA, BP_{ND} as dependent variable; tracer factor, $p < 0.001$; tracer*region interaction, $p < 0.001$).

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

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

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

Region of Interest analysis: Binding Potential BP_P

Table 3 lists the values of [^{11}C]raclopride and [^{11}C]NPA BP_P under baseline and post-amphetamine conditions.

Under baseline conditions, [^{11}C]NPA BP_P was significantly higher than [^{11}C]raclopride BP_P (RM ANOVA, BP_P as dependent variable; tracer factor, $p < 0.001$; tracer*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 non specific binding (V_{ND}) of [^{11}C]NPA relative to [^{11}C]raclopride, since the variable BP_{ND} is derived as the ratio of BP_P to V_{ND}. Thus, a higher BP_P for [^{11}C]NPA does not indicate a higher signal to noise ratio in tissue compared to [^{11}C]raclopride.

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

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Amphetamine produced a statistically significant decrease in [^{11}C]NPA BP_P in the striatal subdivisions (RM ANOVA, Δ BP_P as dependent variable; tracer factor, p =0.049; tracer*region interaction, p = 0.011).

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

Amphetamine-induced decreases in [^{11}C]raclopride or [^{11}C]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 (p > 0.05, Pearson Correlation Coefficient). 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 [^{11}C]raclopride or [^{11}C]NPA (RM ANOVA, p > 0.05).

DISCUSSION

The data from this comparison study raises two relevant questions regarding *D_2 agonist radiotracers.*

1. Are D_2 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 [^{11}C]raclopride and [^{11}C]NPA in vivo specific binding in healthy human subjects. This was undertaken in response to recent investigations suggesting that the results of previous comparison studies showing greater vulnerability of the in vivo binding of D_2 agonists relative to D_2 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 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 D_2 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 [^{11}C]NPA BP_{ND} compared to [^{11}C]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 [^{11}C]NPA BP_P relative to [^{11}C]raclopride BP_P, however, this was not statistically significant. The discordance between significance level of the findings for BP_{ND} and BP_P was caused by the reduction in [^{11}C]raclopride V_{ND} in the cerebellum following the administration of amphetamine. This reduction in V_{ND} led to a poorly correlated ΔBP_{ND} and ΔBP_P for [^{11}C]raclopride. For example, the mean striatal displacement of [^{11}C]raclopride after amphetamine was 12% for BP_{ND} and 19% for BP_P. In contrast, the amphetamine-induced displacement of [^{11}C]NPA in the striatum was a nearly identical -20% and -22% for BP_{ND} and BP_P. 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 Δ BP_{ND} rather than Δ BP_P is used as an outcome measure. Nevertheless, this issue is rarely discussed in depth in the clinical PET literature in which the measured [^{11}C]raclopride cerebellum V_{ND} is lower more often than not following an acute amphetamine (or methylphenidate) challenge (see Table 4). Thus, a question that is critical to the interpretation of these studies is whether Δ BP_{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 non displaceable (V_{ND}) distribution volumes at equilibrium (Innis et al., 2007). Compared to 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;

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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, Δ BP_{ND} is typically preferred over Δ BP_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 f_{ND} (i.e., non displaceable free fraction in the brain, 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 (see Table 1; Note [^{11}C]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 Δ BP_{ND} and Δ BP_P for [^{11}C]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 [^{11}C]raclopride-[^{11}C]NPA comparison data is whether the amphetamine-induced decrease in [^{11}C]raclopride BP_{ND} was underestimated by changes in V_{ND} and if so, by what magnitude? This issue was assessed by contrasting the Δ BP_{ND} of [^{11}C]raclopride obtained in this study with that reported for other D_2 antagonist radiotracers following 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 [^{11}C]raclopride BP_{ND} following 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 Δ BP_{ND} comparison as valid in suggesting that the agonist [^{11}C]NPA is more

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vulnerable to endogenous competition by dopamine compared to the antagonist [^{11}C]raclopride. Such an interpretation is also consistent with our previous report in non human primates in which in vivo binding of [^{11}C]NPA was more displaceable than [^{11}C]raclopride by 42% following an acute amphetamine challenge. Nevertheless, future amphetamine studies comparing D_2 agonists and antagonist radiotracers in humans are necessary to confirm our interpretation of these data.

2. How do the D_2 agonist radiotracers [^{11}C]NPA and [^{11}C]PHNO compare as tools to study amphetamine-induced DA release in humans?

In a previous human study, the D_2 agonist radiotracer [^{11}C]PHNO was displaceable in the ventral striatum (-25 ± 13%) , caudate (-13 ± 7%) and putamen (-21 ± 9%) following the administration of 0.5 mg kg^{-1} oral amphetamine. Although the mean displacement of both these D_2 agonist radioligands is of similar magnitude (see [^{11}C]NPA displacement in Table 2 and Table 3), the effect size for [^{11}C]PHNO displacement is much lower than the effect size for [^{11}C]NPA displacement (see Table 6). This observation is in line with the human test-retest studies that showed a higher variability for [^{11}C]PHNO BP_{ND} (VST 19 ± 19%; CAD 9 ± 9% and PUT 10 ± 8%, Willeit et al., 2008) relative to [^{11}C]NPA BP_{ND} (VST 5 ± 5%; CAD 5 ± 4%; PUT 6 ± 4%, Narendran et al., 2009a). Nevertheless, these data showing lower effect sizes to measure DA release for [^{11}C]PHNO relative to [^{11}C]NPA is contradictory to that previously observed in anesthetized cats (Ginovart et al., 2006) and non human primates (Narendran et al., 2004; Narendran et al., 2006). It is likely that some of the advantages of [^{11}C]PHNO over [^{11}C]NPA (such as enhanced preference to bind to D_3 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 available data seems to suggest that [^{11}C]NPA is superior than [^{11}C]PHNO to measure dopamine release in the striatal

subdivisions, despite the fact that the signal to noise ratio of [^{11}C]PHNO in the striatum is 2.5-fold higher that that of [^{11}C]NPA (Ginovart et al., 2006; Narendran et al., 2006).

In summary, we conducted the first comparison study of a dopamine D_2 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 D_2 agonists over D_2 antagonist radioligands as preferred tools to measure dopamine release due to the non significance of the finding with the BP_P outcome measure. Nevertheless, these data add to the growing literature that suggests D_2 agonist radiotracers are more vulnerable to endogenous competition by dopamine than antagonist radiotracers.

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FOOTNOTES.

a. Unnumbered Footnote

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Table 1. Baseline scan parameters and Plasma analysis (n =10 subjects)

	[^{11}C]Raclopride		[^{11}C]NPA	
	Baseline	Post-amphetamine	Baseline	Post-amphetamine
Injected dose (mCi)	8.4 ± 0.3	7.8 ± 0.9	8.2 ± 0.3	8.4 ± 0.3
SA (Ci/mmoles)	1574 ± 476	1723 ± 770	2270 ± 491	2018 ± 520
Injected Mass (µg)	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%
Non displaceable 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 VT (mL cm^{-3})	0.41 ± 0.07	0.38 ± 0.08*	3.31 ± 0.77	3.20 ± 0.86
Plasma Amphetamine (0 min, ng mL^{-1})		65.9 ± 12.7		73.9 ± 5.4
Plasma Amphetamine (30 min, ng mL^{-1})		61.8 ± 11.2		69.7 ± 8.2
Plasma Amphetamine (60 min, ng mL^{-1})		61.1 ± 11.5		67.0 ± 8.2

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

Table 2. Effect of amphetamine on [^{11}C]raclopride and [^{11}C]NPA BP_{ND}

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

Values are mean ± SD; n =10 subjects. *P* values indicate paired t-tests; *p < 0.001

Table 3. Effect of amphetamine on [^{11}C]raclopride and [^{11}C]NPA BP_P

	[^{11}C]raclopride BP_P			[^{11}C]NPA BP_P			Delta Ratio
Region	Baseline	Post amphetamine	Difference (%)	Baseline	Post amphetamine	Difference (%)	NPA/RAC
Ventral Striatum	0.98 ± 0.16	0.83 ± 0.20	-16.1 ± 9.5*	3.42 ± 0.75	2.82 ± 0.87	-18.4 ± 12.9*	1.14
Caudate	1.01 ± 0.16	0.86 ± 0.18	-14.9 ± 9.4*	2.86 ± 0.61	2.35 ± 0.64	-18.4 ± 13.7*	1.23
Putamen	1.33 ± 0.19	1.06 ± 0.19	-20.7 ± 8.9*	4.09 ± 0.84	3.11 ± 0.84	-24.4 ± 9.4*	1.18
Striatum	1.18 ± 0.17	0.96 ± 0.18	-18.5 ± 9.0*	3.58 ± 0.76	2.80 ± 0.75	-22.1 ± 10.5*	1.19

Values are mean ± SD; n =10 subjects. *P* values indicate paired t-tests; *p < 0.001

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

Reference	N	Stimulant Challenge	Baseline V_{ND} (ml cm^{-3})	Post-challenge V_{ND} (ml cm^{-3})	Mean Difference (%)
This study	*10*	*Amphetamine 0.5 mg/kg, oral*	*0.41 ± 0.07*	*0.38 ± 0.08**	*-7%*
(Volkow et al., 1997)	23	Methylphenidate 0.5 mg/kg, intravenous	0.42 ± 0.06	0.38 ± 0.08*	-10%
(Wang et al., 1999)	14	Methylphenidate 0.5 mg/kg, intravenous	-	-	-13%
(Volkow et al., 2001)	11	Methylphenidate 0.8 mg/kg, oral	0.38 ± 0.05	0.36 ± 0.05	-5%
(Drevets et al., 2001)	6	Amphetamine 0.3 mg/kg, intravenous	0.38 ± 0.03	0.35 ±0.03	-6%
(Martinez et al., 2003)	14	Amphetamine 0.3 mg/kg, IV	0.34 ± 0.08	0.34 ± 0.08	0%
(Martinez et al., 2005)	15	Amphetamine 0.3 mg/kg, IV	0.40 ± 0.08	0.36 ± 0.08*	-10%
(Martinez et al., 2007)	24	Amphetamine 0.3 mg/kg, IV	0.39 ± 0.07	0.36 ± 0.07*	-8%

* reported as statistically significant

Table 5. Displacement of dopamine D_2 antagonist radioligands in human striatum following oral amphetamine (~0.5 mg kg^{-1})

		Region of Interest			
Healthy Controls	Radioligand	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
(Cardenas et al., 2004)	[^{11}C]raclopride	-	-	-	13.3 ± 5.0
(Busto et al., 2009) †	[^{11}C]raclopride	-	-	-	11.1 ± 3.6
(Busto et al., 2009) ††	[^{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	-

† Healthy control smokers

†† Healthy control non-smokers

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	-