Published in final edited form as:

Biol Psychiatry. 2018 April 15; 83(8): 707–714. doi:10.1016/j.biopsych.2017.11.032.

Amphetamine Induced Striatal Dopamine Release Measured with an Agonist Radiotracer in Schizophrenia

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Abstract

Background—Receptor imaging studies have reported increased amphetamine-induced dopamine release in subjects with schizophrenia relative to healthy controls. A limitation of these studies, performed with D_{2/3} antagonist radiotracers, is the failure to provide information about D_{2/3} receptors configured in a state of high affinity for the agonists (i.e., D_{2/3} receptors coupled to G-proteins, D_{2/3} HIGH). The endogenous agonist dopamine binds with preference to D_{2/3} HIGH relative to D_{2/3} LOW receptors making it critical to understand the status of D_{2/3} HIGH receptors in schizophrenia.

Methods—D_{2/3} agonist PET radiotracer [¹¹C]NPA binding potential (BP_{ND}) was measured in 14 off medication subjects with schizophrenia (SCH) and 14 matched healthy controls (HC) at baseline and after the administration of 0.5 mg kg⁻¹ oral d-amphetamine. The amphetamine-induced change in BP_{ND} (ΔBP_{ND}) was calculated as the difference between BP_{ND} in the post-amphetamine condition and BP_{ND} in the baseline condition, and expressed as a percentage of BP_{ND} at baseline.

Results—A trend-level increase was observed comparing baseline [¹¹C]NPA BP_{ND} (RM ANOVA F=3.34, df=1,26, p=0.08) between SCH and HC. Amphetamine administration significantly decreased BP_{ND} in all striatal regions across all subjects in both groups. No differences were observed in [¹¹C]NPA BP_{ND} (RM ANOVA F=1.9, df=1,26, p=0.18) between HC and SCH. Amphetamine significantly increased positive symptoms in SCH subjects (19.5 ± 5.3 vs. 23.7 ± 4.1, paired T-test p < 0.0001) however no correlations were noted with [¹¹C]NPA BP_{ND} or ΔBP_{ND}.

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DISCLOSURES

The authors report no biomedical financial interests or potential conflicts of interest.

Conclusions—This study provides in vivo indication of a role for postsynaptic factors in amphetamine-induced psychosis in schizophrenia.

Keywords

[¹¹C]N-propyl-norapomorphine (NPA); schizophrenia; dopamine; amphetamine; Positron emission tomography (PET); high-affinity

INTRODUCTION

Relative to healthy controls, subjects with schizophrenia show significant increases in the displacement of dopamine (DA) D_{2/3} receptor radiotracers in the striatum in response to psychostimulants during positron emission tomography (PET) or single photon emission computed tomography (SPECT) studies (1-4). These studies are often cited as evidence that, in schizophrenia, striatal DA neurons are overly reactive to stimulants. However, whether the increased radiotracer displacement is due to an increased D_{2/3} receptor affinity for DA or an increased presynaptic release of DA remains unknown. The interpretation of increased presynaptic release of DA following stimulant exposure is supported by recent meta-analyses of [¹⁸F]-6-L-fluorodopa imaging studies which found increases in the DA synthesis capacity in schizophrenia (5, 6). Although linking increased DA synthesis capacity to increased DA transmission should be done with caution as aromatic amino acid decarboxylase is not the rate limiting step in DA synthesis and alterations in factors such as intracellular storage and transport may also impact DA release (7).

Postsynaptic factors may provide an alternative explanation for the phenomenon of increased stimulant-induced radiotracer displacement in schizophrenia. Increased affinity at the D_{2/3} receptors for DA in subjects with schizophrenia relative to controls would be associated with increased receptor occupancy by DA following a stimulant challenge despite normal, or even reduced, presynaptic DA release. Examining this issue is important to understanding DA abnormalities in schizophrenia and requires use of an agonist radiotracer, as available imaging data do not distinguish between pre- and post-synaptic factors.

D_{2/3} receptors are guanine nucleotide protein (G-protein)-coupled receptors and, as described by the ternary complex model, can be configured in interconvertible states of high (G-protein coupled) or low (uncoupled) affinity for agonists (8, 9). This means that the affinity for a specific ligand of a population of D_{2/3} receptors will be dependent not only on the affinity of the high and low states but also on the proportion of receptors in each configuration. Antagonists, such as the radiotracers [¹¹C]raclopride and [¹²³I]IBZM bind equally to the D_{2/3} HIGH and D_{2/3} LOW configurations. In contrast the binding of agonists including DA is solely dependent on the D_{2/3} HIGH state. Therefore when using an antagonist radiotracer to measure the release of DA in response to a stimulant an elevation in the ratio of D_{2/3} HIGH relative to D_{2/3} LOW would result in increased radiotracer displacement by DA. When compared to a control population without such an elevation in the D_{2/3} HIGH/D_{2/3} LOW ratio this would appear as an increase in DA release. We measured striatal D_{2/3} agonist binding in subjects with schizophrenia and matched controls at baseline and after the oral administration of amphetamine with the D_{2/3} agonist PET radiotracer [¹¹C]NPA to

investigate the hypothesis that an increase in the proportion of high-affinity sites contributes to the elevated displacement of antagonist radiotracers after amphetamine in schizophrenia.

METHODS AND MATERIALS

Human Subjects

Sixteen subjects with schizophrenia (SCH) were recruited and enrolled in this study between 02/04/2010 – 01/26/2015. Two of the subjects were unable to complete the PET scanning procedure, leaving a final cohort of fourteen (24.5 ± 4.6 years, range 18 to 32, 10M/4F; with these and subsequent values given as mean $\pm$ SD). Fourteen healthy volunteers (HC, 25.6 ± 4.1 years, range 21 to 32, 10M/4F) were recruited contemporaneously. The University of Pittsburgh IRB approved the study and all subjects provided written informed consent. Capacity to provide consent was evaluated by a psychiatrist not associated with the study for SCH. Study criteria for SCH were 1) diagnosis of schizophrenia or schizoaffective disorder according to the Diagnostic and Statistical Manual (DSM-IV); 2) no other DSM-IV axis I diagnosis; 3) no lifetime history of alcohol or substance abuse or dependence (except nicotine); 4) absence of psychotropic medication for at least 21 days (six weeks for fluoxetine, 6 months for depot antipsychotic) before the study; 5) no concomitant or past severe medical conditions; 6) not pregnant; 7) no current suicidal or homicidal ideation; 8) ability to provide informed consent. Criteria for HC included no past or present neurological or psychiatric illnesses including substance abuse/dependence. For all subjects the absence of pregnancy, medical and neurological disorders was assessed by history, ROS, physical/neurological examination, routine blood tests (including pregnancy), urine toxicology and EKG.

Clinical Assessment

Diagnosis was assessed using the Structured Clinical Interview for DSM-IV (SCID) (10). Absence of psychiatric history/symptoms in HC was determined after administration of the SCID nonpatient version (SCID-NP). The severity of symptoms in SCH subjects was assessed immediately prior to the amphetamine administration and immediately prior to the PET scan (approximately 180 min following the amphetamine administration) using the Positive and Negative Syndrome Scale (PANSS, 11).

PET Protocol and Image Analysis

All subjects were studied twice with [^{11}C]NPA, first a baseline PET scan was performed then, on a separate day, a second PET scan was performed 180 mins after administration of oral amphetamine (0.5 mg kg^{-1}). Amphetamine plasma levels were calculated as the average of three samples obtained at time 0 min, 30 min and 60 min relative to the PET scan, time points at which plasma levels have been shown to be stable (12, 13). The interval between the scans was 5.0 ± 4.6 days for SCH and 10.4 ± 6.4 days for HC ($p = 0.02$).

MRI acquisition, PET Procedures and Image Analysis—Magnetic resonance imaging (MRI) scan was obtained for determination of regions of interest (ROI) as previously described (14, 15). [^{11}C]NPA was synthesized using the methodology reported previously (16) with the PET imaging sessions conducted consistent with previous studies

for this radiotracer (14, 15, 17). The primary ROI, the striatum, was divided into five anatomical ROIs and three functional subdivisions using published criteria (18). The three functional subdivisions of the striatum: limbic striatum (LST) which includes the ventral striatum (VST), associative striatum (AST) which includes the precommisural dorsal caudate (preDCA), precommisural dorsal putamen (preDPU) and postcommisural caudate (postCA) and sensorimotor striatum (SMST) which includes the postcommisural putamen (postPU). The cerebellum (CER) was used as a reference region (15).

Outcome measures—Baseline $D_{2/3}$ receptor availability for [^{11}C]NPA was estimated using the PET outcome measure BP_{ND} , i.e., binding potential relative to non-displaceable uptake (19). Arterial line placement could not be obtained in seven studies which precluded determination of the input function; two SCH in the baseline condition and two HC and three SCH in the post amphetamine condition. To utilize all brain data collected the simplified reference tissue method (SRTM, 20) was implemented for all subjects in all conditions, as previously reported (14).

The amphetamine-induced change in BP_{ND} was calculated as the difference between BP_{ND} measured in the post-amphetamine condition ($\text{BP}_{\text{ND AMPH}}$) and BP_{ND} measured in the baseline condition ($\text{BP}_{\text{ND BASE}}$) expressed as a percentage of $\text{BP}_{\text{ND BASE}}$:

$$\Delta\text{BP}_{\text{ND}} = 100 * (\text{BP}_{\text{ND AMPH}} - \text{BP}_{\text{ND BASE}}) / \text{BP}_{\text{ND BASE}}$$

Statistical analysis

Group demographic and scan parameter comparisons were performed with unpaired t tests. Group differences in [^{11}C]NPA BP_{ND} and BP_{ND} were analyzed with a repeated measures analysis of variance (RM ANOVA) with the regions of interest as the repeated measure and diagnostic group as the cofactor. Mauchly's test of sphericity was used and a Greenhouse-Geisser correction employed where appropriate. This primary analysis was followed by contrasts in the individual striatal subdivisions with two tailed unpaired t tests. Relationships between continuous variables were analyzed with the Pearson product-moment correlation coefficient. A probability value of 0.05 was selected as significance level for all analyses with Bonferroni correction to 0.01 for the correlations to account for the multiple regions examined ($n = 6$ regions, the five anatomical subdivisions and the striatum as a whole).

RESULTS

Scan parameters

Demographic data and PET scan parameters are listed in Table 1. The groups did not differ significantly in age, gender or smoking status. African Americans made up a larger proportion of the SCH compared with the HC group. Two SCH subjects had never received treatment with antipsychotic medications, the remainder were medication free for at least 21 days prior to the scan. The mean injected dose, specific activity at time of injection, injected mass and cerebellum distribution volume (V_{ND}) did not differ between baseline and post amphetamine conditions for either the HC or SCH subjects. In addition, no significant between-group differences were observed in either condition.

Baseline [^{11}C]NPA BP_{ND} and the effect of amphetamine on [^{11}C]NPA BP_{ND}

Baseline [^{11}C]NPA BP_{ND}—A trend-level difference was observed when comparing baseline [^{11}C]NPA BP_{ND} (RM ANOVA $F=3.34$, $df=1,26$, $p=0.08$) between HC and SCH subjects (Table 2, Figure 1). When comparing on a region-by-region basis SCH subjects showed trend-level higher [^{11}C]NPA BP_{ND} in the VST ($p = 0.08$), pre-DCA ($p = 0.07$) and post-PU ($p = 0.08$), see Table 2 and Figure 1.

Amphetamine Effect on [^{11}C]NPA BP_{ND}—HC Subjects: Amphetamine administration significantly decreased BP_{ND} in all striatal regions (Table 2). Examination of BP_{ND} across the component ROIs revealed a significant regional effect (RM ANOVA $F=272$, $df = 2.6,67$, $p<0.0001$), a significant region-by-condition interaction (RM ANOVA $F=6.9$, $df=2.6,67$, $p=0.001$) and a significant difference across conditions (RM ANOVA $F=14.9$, $df=1,26$, $p=0.001$). No relationship was observed between the change in [^{11}C]NPA BP_{ND} and amphetamine plasma levels.

SCH Subjects: Amphetamine administration significantly decreased BP_{ND} in all striatal regions (Table 2). Examination of BP_{ND} across the component ROIs revealed a significant regional effect (RM ANOVA $F=209$, $df = 2.6,68$, $p<0.0001$), a significant region-by-condition interaction (RM ANOVA $F=9.1$, $df=2.6,68$, $p<0.0001$) and a significant difference across conditions (RM ANOVA $F=23$, $df=1,26$, $p<0.0001$). No relationship was observed between the change in [^{11}C]NPA BP_{ND} and amphetamine plasma levels.

HC and SCH Comparison: No differences were observed in [^{11}C]NPA BP_{ND} (RM ANOVA $F=1.9$, $df=1,26$, $p=0.18$) between HC and SCH subjects (Table 2, Figure 2). Examining this measure on a region-by-region basis revealed a trend-level *lower* displacement in the pre-DCA for SCH subjects compared with HC (SCH -11.2 ± 7.2 vs HC -19.1 ± 13.0 , $p = 0.06$).

Relationship Between [^{11}C]NPA BP_{ND}, BP_{ND}, and Clinical Measures

Baseline [^{11}C]NPA BP_{ND} was negatively correlated with age in the HC group in the AST ($r = -0.60$, $p = 0.02$), SMST ($r = -0.62$, $p = 0.02$) and as well as the striatum as a whole ($r = -0.62$, $p = 0.02$). The same was not found for SCH subjects, where no significant relationship between age and baseline [^{11}C]NPA BP_{ND} was noted. However, age was negatively correlated with the *change* in radiotracer displacement in the AST ($r = -0.54$, $p = 0.04$) and striatum as a whole ($r = -0.56$, $p = 0.04$) in the SCH subjects. Notably, none of these findings survived correction for multiple comparison but are included due to the known relationship between D_{2/3} receptor density and age (21).

In the SCH subjects baseline [^{11}C]NPA BP_{ND} binding in the AST ($r = -0.56$, $p = 0.04$) and the striatum as a whole ($r = -0.58$, $p = 0.03$) was negatively correlated with time off medications, suggestive of an effect of medications on D_{2/3} HIGH receptor density, however this did not survive correction for multiple comparisons, but is reported due to the impact of medications on D_{2/3} receptor density (22). While amphetamine administration significantly increased positive symptoms on the PANSS (19.5 ± 5.3 vs. 23.7 ± 4.1 , paired T-test $p <$

0.0001) no correlations were noted for [^{11}C]NPA BP_{ND} or BP_{ND} with PANSS total score/subscores or with the change in PANSS total score/subscores after amphetamine.

DISCUSSION

This is the first study utilizing an agonist radiotracer to measure amphetamine-induced DA release in subjects with schizophrenia. Unlike previous reports (1-4) we did not find elevated radiotracer displacement in response to the stimulant challenge. Rather we observed a similar magnitude of change in each group with numerically lower change in SCH subjects which reached trend-level significance in the dorsal caudate. This is the first study of subjects with schizophrenia in which normal-to-low DA release was measured in response to a stimulant challenge. In addition, this is the first study in which the amphetamine associated increase in psychotic symptoms was not correlated with degree of change in radiotracer binding in subjects with schizophrenia. Both of these findings were surprising given the fact that a greater decrease in radiotracer binding after amphetamine has been replicated multiple times in this disorder with each study demonstrating the increase in psychosis correlated with the change in radiotracer binding. The previous studies in this area demonstrated an effect sizes of 1.49 (1), 0.95 (2), 1.06 (3), and 3.8 (4). The current study was appropriately powered to detect a difference between SCH subjects and HC subjects anticipating an effect size of similar magnitude and direction.

The fact that we did not detect increased radiotracer displacement raises questions about the nature of the disturbance in DA transmission in schizophrenia. The possibility exists that some variable in our subject sample accounted for this finding. Laruelle et al reported that subjects in remission, as opposed to exacerbation, exhibited amphetamine-induced radiotracer displacement similar to controls (2). Remission is difficult to quantify; all subjects were experiencing positive symptoms at the time of the baseline scan similar in magnitude (PANSS Positive 19.5 ± 5.3) to the patients experiencing exacerbation in the Laruelle et al study (PANSS Positive 18.9 ± 6.5). The amphetamine-induced increase in positive symptoms was higher in the subjects in our study (4.2 ± 2.2) than reported for the exacerbation subjects (3.8 ± 6.0) in Laruelle et al. Using published remission criteria (23) one subject in our study met remission, with that subject exhibiting a change in radiotracer binding after amphetamine ($20.7\% \pm 4.4\%$) higher than the SCH group average, suggesting that the phase of illness was not a factor in our findings. The fact that we do not have follow-up data on the subjects, particularly in the area of antipsychotic treatment response, is a limitation. DA imaging studies have shown a correlation between reduced presynaptic DA synthesis (24) or reduced baseline DA levels (25) and treatment resistance or poor response to antipsychotic medications, respectively. Although no study has explored the relationship between amphetamine-induced DA release and treatment responsiveness, it is possible that our sample was inadvertently enriched for treatment non-responders which could have contributed to our findings.

Notably a similar finding to ours was seen in subjects with schizophrenia with comorbid substance dependence using an antagonist radiotracer (26). Thomson et al reported a blunting of DA release in the striatum in schizophrenia. In the current study we excluded a history of substance dependence/abuse however 6 SCH subjects had used cannabis in the six

months prior to the study, 4 had lifetime use, and 4 denied history of use. While the impact of cannabis use on DA release remains unclear - work from the same group indicates no impact of mild-to-moderate use on amphetamine induced DA release (27, 28) - we found no differences in either the baseline [^{11}C]NPA BP_{ND} binding or BP_{ND} when looking at those with cannabis use separately (data not shown). Despite noting blunted release of DA, Thomson et al report a significant correlation between change in positive symptoms and change in radiotracer binding in the subjects with schizophrenia and comorbid substance dependence/abuse, similar to the previous studies in this area. Although we did not observe a difference in DA release we did observe an increase in psychotic symptoms post amphetamine in the context of normal-to-low DA release. Taken together this may indicate that elevated DA release is not necessary for psychosis but rather postsynaptic factors may be primarily responsible for the development of positive symptoms in response to amphetamine.

It has been well documented that patients with schizophrenia are ‘supersensitive’ to the effects of psychostimulants, an effect mediated via DA (for review see 29). Studies have demonstrated that ~ 50% – 75% of patients with schizophrenia will develop new or worsening psychosis when exposed to a psychostimulant as opposed to 10% – 25% of controls (30, 31). The DA hypothesis of schizophrenia postulates that this effect is due to an excess of presynaptic DA release resulting in increased D_{2/3} stimulation (32). However, alternative mechanisms for increased transmission through D_{2/3} receptors in schizophrenia include increased receptor density or alterations in the affinity of D_{2/3} receptors for DA or a combination of factors (33). Post mortem studies report consistent elevation of D_{2/3} receptors in schizophrenia relative to controls whereas in vivo imaging studies have reported more mixed results, with some studies showing elevation of D_{2/3} receptors and others normal or even reduced density, a discrepancy which is likely secondary to the confounds of previous medication treatment in the post mortem studies (for review see 34). In the current study we don’t see definitive evidence for elevated D_{2/3} HIGH however we note a trend-level increase in D_{2/3} HIGH receptor density in the subjects with schizophrenia; ~50 subjects per group would be necessary to detect a significant difference at the observed effect size. The influence of prior medication treatment on this finding is difficult to estimate. Given the fact that we note a moderate negative correlation between time off medications and [^{11}C]NPA binding it is possible treatment with antipsychotic medications selectively increases D_{2/3} HIGH receptors. Post mortem studies which have examined the binding affinity of D_{2/3} receptors for agonists have not found elevated binding in schizophrenia (35), although the degree to which G protein coupling and cycling is preserved in post mortem tissue remains unclear. In our study, the SCH subjects were off medications for an average of ~40 weeks with a range of 7–113 weeks, suggesting a fairly long lasting effect of medications. The fact that minimal information on total exposure to antipsychotic medications is available for the previously treated group limits our ability to explore the relationship between time on and type of medications and represents a limitation of this study. Future studies with agonist radiotracers which include a larger proportion of medication naïve subjects are needed to understand this issue better.

Interestingly, in the exact same region we see trend-level increased baseline [^{11}C]NPA binding and a trend-level *lower* displacement, the dorsal caudate, a previous study found DA

occupies a higher proportion of $D_{2/3}$ receptors in patients with schizophrenia utilizing a DA depletion paradigm (36). The elevated $D_{2/3}$ occupancy by DA seen by Kegeles et al could be secondary to an increased proportion of receptors in the $D_{2/3}$ HIGH configuration rather than increased basal activity of the presynaptic neurons. However, it should be noted that many, although not all, studies assessing DA synthesis capacity find elevated DA synthesis in subjects with schizophrenia, pointing to a presynaptic abnormality of increased DA production (37). In addition, a study with [^{11}C]-(+)-PHNO which binds to both D_3 and $D_{2\text{ HIGH}}$ receptors, with a 25- to 48-fold higher affinity for D_3 (38), did not detect any increase in $D_{2\text{ HIGH}}$ receptor density in this region in schizophrenia (39). Although, as the authors point out, the signal in the dorsal striatum is a mixture of that from D_3 (8% - 50%) and $D_{2\text{ HIGH}}$ receptors confounding interpretations about the level of $D_{2\text{ HIGH}}$ receptors in this region. A more recent study measured the effect of a stress task compared with a sensory motor task on [^{11}C]-(+)-PHNO binding in subjects with schizophrenia and healthy controls (40). While the authors did not report whether the [^{11}C]-(+)-PHNO BP_{ND} differed significantly from healthy controls, Mizrahi et al found a greater reduction in binding of [^{11}C]-(+)-PHNO during the stress task in subjects with schizophrenia when compared with healthy controls, suggestive of increased DA release. However, a similar study by the same group found no difference in [^{11}C]-(+)-PHNO BP_{ND} comparing subjects with schizophrenia and healthy controls while performing a cognitive task (41).

It is important to highlight that additional post synaptic factors may contribute to enhancing the DA signal through $D_{2/3}$ receptors beyond increased $D_{2/3}$ HIGH receptor density, including alterations in the cellular processes which modulate the activation of G proteins. For example, Gainetdinov et al reported that knockout mice for G protein coupled receptor kinase 6, a protein implicated in the regulation of post synaptic $D_{2/3}$ receptors, displayed enhanced sensitivity to stimulants and increased affinity of $D_{2/3}$ receptors for DA in both the heterozygous and homozygous knockout animals (42).

Additional limitations of this study relate to the PET methodology. First, the baseline and post amphetamine PET scans were performed on separate days. This was done to ensure all study procedures on the post amphetamine day could be completed in a timely manner. However, while we do not have data comparing between day reliability to the within day reliability of the PET scans for [^{11}C]NPA, it is likely that the inter-subject variability is higher when scans are performed across days as we have observed this with other radiotracers (unpublished data), which could have reduced our ability to detect between-group differences.

Somewhat related to the previous point, while we obtained the arterial plasma data necessary to estimate regional VT for many studies, arterial lines could not be obtained for 2 studies on the baseline day and 5 studies on the post-amphetamine day. Therefore we elected to utilize the reference tissue approach so as to include all the collected data in the analysis. Our previous work has shown that [^{11}C]NPA BP_{ND} values derived using SRTM were lower than those obtained using arterial input with a more pronounced underestimation of BP_{ND} values in regions of higher receptor density relative to that in regions of low receptor density (14). No difference was observed when comparing BP_{ND} obtained using the arterial input analysis ($n = 12$ for HC and $n = 11$ for SCH) vs. BP_{ND} obtained via SRTM (data not shown).

Moreover, the results of the study were of similar magnitude and directionality when the [^{11}C]NPA BP_{ND} was restricted to studies obtained with arterial input. However, we cannot exclude the possibility of a differential effect in the underestimation of BP_{ND} values derived using SRTM between SCH and HC groups.

Finally, an ongoing discussion in the literature exists regarding whether the two affinity states of the D_{2/3} receptor occur in vivo and, if so, whether the high affinity state can be measured with an agonist radiotracer (for review see 43). The overall goal in developing and characterizing an agonist D_{2/3} radiotracer was to provide more physiologically relevant information about the DA system in various illnesses (44) based on the hypothesis that the DA D_{2/3} receptors can be configured in the G-protein coupled or uncoupled state (8, 9). DA and other agonists will bind with high affinity to the G-protein coupled receptor (D_{2/3} HIGH) whereas antagonists bind equally to both the D_{2/3} HIGH and D_{2/3} LOW (45). Consistent with this, previous work in non-human primates has shown that [^{11}C]NPA binds with high affinity to a fraction (70 to 80%) of the sites labeled by the antagonist [^{11}C]raclopride and is more vulnerable to endogenous competition than [^{11}C]raclopride suggesting that, in vivo, about 70 to 80% of D_{2/3} receptors are configured in the high-affinity state for agonists (46, 47). Furthermore, in healthy humans, amphetamine-induced increase in synaptic DA in the striatum resulted in a 49% to 90% larger decrease in the agonist radiotracer [^{11}C]NPA BP_{ND} compared with the antagonist [^{11}C]raclopride BP_{ND} in the same subjects (15). Similar findings have been demonstrated with the agonist D_{2/3} radiotracer, [^{11}C]-(+)-PHNO (48). Taken together these finding indicate that, compared with antagonist radiotracers, [^{11}C]NPA is a superior probe for measuring endogenous DA fluctuations and support the ability to measure D_{2/3} HIGH state in vivo (44).

While previous PET imaging studies using the amphetamine induced DA release paradigm have demonstrated that amphetamine leads to a greater stimulation of D_{2/3} receptors in schizophrenia compared with controls they were unable to provide insight into the mechanism underlying this effect - increased DA concentration versus increased affinity of D_{2/3} receptors for DA, or some combination of both factors. The current study provides the first indication that postsynaptic alterations in D_{2/3} receptor affinity may play a significant role in the pathophysiology of this illness. Future [^{11}C]NPA PET studies in schizophrenia following the depletion of synaptic DA with alpha-methyl-para-tyrosine as well as direct comparison of the impact of amphetamine on the radiotracer binding with both an agonist and antagonist radiotracer in the same subjects is necessary to further elucidate this issue.

Acknowledgments

The project described was supported by Award Number R01 MH086523A1 from the National Institute of Mental Health (NIMH).

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Figure 1.

Baseline [^{11}C]NPA BP_{ND} in healthy controls (white bars) and subjects with schizophrenia (blue bars) in the striatal subdivisions. A trend-level difference was observed between HC and SCH subjects (RM ANOVA $F=3.34$, $df=1,26$, $p=0.08$). SCH subjects showed trend-level higher [^{11}C]NPA BP_{ND} in the VST ($p = 0.08$), pre-DCA ($p = 0.07$) and post-PU ($p = 0.08$).

Figure 2.

Amphetamine induced percent change in $[^{11}\text{C}]\text{NPA BP}_{\text{ND}}$ in healthy controls (white bars) and subjects with schizophrenia (blue bars) in the striatal subdivisions. Amphetamine administration significantly decreased BP_{ND} in all striatal regions in both groups. No differences were observed in between HC and SCH subjects on the omnibus RM ANOVA test. A region-by-region comparison revealed a trend-level lower displacement in the pre-DCA for SCH subjects compared with HC (SCH -11.2 ± 7.2 vs HC -19.1 ± 13.0 , $p = 0.06$).

Figure 3. Scatterplot of the preDCA BP_{ND} (%) for HC (n = 14, left) and SCH (n = 14, right) with horizontal bars denoting the group means.

Table 1

Demographic data and scan parameters

	Healthy Controls (n=14/group)		Subjects with Schizophrenia (n=14/group)	
Age	25.6 ± 4.1		24.5 ± 4.6	
Gender	10M/4F		10M/4F	
Race	3AA/11C		10AA/4C	
Smoking Status	8 Yes, 6 No		8 Yes, 6 No	
Treatment Status	–		2 MN/12 MF ^a	
Time off medications (weeks)	–		39.6 ± 41.4	
Scan Parameter	Baseline	Post Amphetamine	Baseline	Post Amphetamine
Injected dose (mCi)	7.9 ± 0.5	8.1 ± 0.7	8.2 ± 0.5	8.1 ± 0.8
Specific activity (Ci/mmoles)	2557 ± 1103	2290 ± 659	2464 ± 904	2173 ± 697
Injected Mass (µg)	1.1 ± 0.5	1.1 ± 0.3	1.1 ± 0.4	1.2 ± 0.3
Cerebellum V _{ND} (mL cm ⁻³)	2.5 ± 0.9	2.0 ± 1.0 ^b	3.0 ± 0.8 ^c	2.7 ± 1.0 ^d
Plasma Amphetamine (ng mL ⁻¹)	0.0	82.6 ± 9.4	0.0	80.3 ± 15.5
PANSS Positive Score	–	–	19.5 ± 5.3	23.7 ± 4.1 [†]
PANSS Negative Score			17.1 ± 4.1	16.6 ± 5.5
PANSS General Score			33.5 ± 6.7	36.6 ± 7.1
PANSS Total Score			70.1 ± 13.9	77.0 ± 12.8 [‡]

No differences within subject group nor between controls and subjects with schizophrenia for both baseline and post amphetamine scan parameters with $p > 0.05$, two-tailed, unpaired t-test.

^aMN = Medication naïve, MF = Medication free.

^b_{n=12}

^c_{n=12}

^d_{n=11}.

Amphetamine induced change in PANSS paired t-test

[†]_{p < 0.0001}

[‡]_{p = 0.02}.

Table 2Amphetamine-induced change in [¹¹C]NPA binding potential (BP_{ND}, unitless)

Striatal Subdivision (Anatomic ROIs)	Healthy Controls (n =14/group)		Subjects with Schizophrenia (n =14/group)				HC v SCH Baseline BPND		HC v SCH	BPND
	Baseline BP _{ND}	Post amphetamine BP _{ND}	BP _{ND} (%)	Baseline BP _{ND}	Post amphetamine BP _{ND}	BP _{ND} (%)	p*	d	p*	d
LST (VST)	0.87 ± 0.15	0.68 ± 0.14	-21.6 ± 10.8	0.96 ± 0.09	0.78 ± 0.07	-17.6 ± 7.8	0.08	0.72	0.27	0.44
AST	0.77 ± 0.12	0.62 ± 0.13	-19.6 ± 12.3	0.84 ± 0.11	0.73 ± 0.09	-12.8 ± 7.6	0.16	0.57	0.09	0.69
(Pre-DCA)	0.74 ± 0.13	0.60 ± 0.14	-19.1 ± 13.0	0.82 ± 0.08	0.73 ± 0.09	-11.2 ± 7.2	0.07	0.75	0.06	0.78
(Post-CA)	0.51 ± 0.15	0.39 ± 0.14	-21.9 ± 20.8	0.59 ± 0.11	0.50 ± 0.12	-16.1 ± 14.2	0.10	0.68	0.40	0.34
(Pre-DPU)	1.01 ± 0.15	0.81 ± 0.13	-18.8 ± 10.4	1.06 ± 0.17	0.91 ± 0.09	-12.5 ± 11.6	0.40	0.34	0.14	0.59
SMST (Post-PU)	1.11 ± 0.12	0.82 ± 0.13	-25.7 ± 10.2	1.20 ± 0.13	0.90 ± 0.11	-24.4 ± 7.2	0.08	0.71	0.72	0.15
Striatum	0.90 ± 0.13	0.69 ± 0.13	-22.5 ± 10.6	0.97 ± 0.11	0.79 ± 0.08	-18.0 ± 6.8	0.12	0.63	0.20	0.52

Values are Mean ± SD, in HC (n = 14) and SCH (n = 14); p is the significance level for an unpaired t-test between the groups, d is the Cohen's effect size of this difference.

* RM ANOVA did not reach significance for either group comparison.