

## ORIGINAL ARTICLE

# Striatal dopamine release in schizophrenia comorbid with substance dependence

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Dopamine (DA) has a role in the pathophysiology of schizophrenia and addiction. Imaging studies have indicated that striatal DA release is increased in schizophrenia, predominantly in the precommissural caudate (preDCA), and blunted in addiction, mostly in the ventral striatum (VST). Therefore, we aimed to measure striatal DA release in patients with comorbid schizophrenia and substance dependence. We used [<sup>11</sup>C]raclopride positron emission tomography and an amphetamine challenge to measure baseline DA D<sub>2</sub>-receptor availability (BP<sub>ND</sub>) and its percent change post-amphetamine ( $\Delta$ BP<sub>ND</sub>, to index amphetamine-induced DA release) in striatal subregions in 11 unmedicated, drug-free patients with both schizophrenia and substance dependence, and 15 healthy controls. There were no significant group differences in baseline BP<sub>ND</sub>. Linear mixed modeling using  $\Delta$ BP<sub>ND</sub> as the dependent variable and striatal region of interest as a repeated measure indicated a significant main effect of diagnosis,  $F(1, 24) = 8.38$ ,  $P = 0.008$ , with significantly smaller  $\Delta$ BP<sub>ND</sub> in patients in all striatal subregions (all  $P \leq 0.04$ ) except VST. Among patients, change in positive symptoms after amphetamine was significantly associated with  $\Delta$ BP<sub>ND</sub> in the preDCA ( $r_s = 0.69$ ,  $P = 0.03$ ) and VST ( $r_s = 0.64$ ,  $P = 0.05$ ). In conclusion, patients with comorbid schizophrenia and substance dependence showed significant blunting of striatal DA release, in contrast to what has been found in schizophrenia without substance dependence. Despite this blunting, DA release was associated with the transient amphetamine-induced positive-symptom change, as observed in schizophrenia. This is the first description of a group of patients with schizophrenia who display low presynaptic DA release, yet show a psychotic reaction to increases in D<sub>2</sub> stimulation, suggesting abnormal postsynaptic D<sub>2</sub> function.

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## INTRODUCTION

Dopamine (DA) has a role in the pathophysiology of schizophrenia and addiction. Imaging studies using positron emission tomography (PET) or single-photon emission computed tomography (SPECT) with D<sub>2/3</sub> radiotracers and the amphetamine-challenge paradigm measure the change in D<sub>2/3</sub> radiotracer binding related to changes in synaptic DA concentration induced by amphetamine, thus providing an indirect measure of stimulated DA release. These studies have shown that, compared with healthy controls, DA release is higher in the striatum in patients with schizophrenia<sup>1–4</sup> and is blunted in those with addiction.<sup>5–7</sup> With better topographical characterization from use of higher-resolution scanners and better data analysis methods allowing reliable measurements within the striatal subdivisions,<sup>8</sup> more recent studies have suggested that in schizophrenia, DA transmission is increased in the precommissural caudate (preDCA) of the associative striatum (AST) in particular,<sup>9</sup> whereas in addiction, the ventral striatum (VST) appears to be especially affected.<sup>6</sup> In summary, DA transmission is altered in opposite directions in schizophrenia and addiction, apparently within discrete striatal subdivisions.

Epidemiological studies have shown that patients with schizophrenia are at a greater risk for developing substance-use

disorders than the general population. The Epidemiological Catchment Area Study reported a 4.6-fold increase in the prevalence of any substance abuse in patients with schizophrenia, compared with the general population.<sup>10</sup> Alcohol is most commonly used, with a lifetime prevalence of abuse or dependence of 33.7% compared with 27.5% for all other drugs. The CATIE trial reported substance use in 60% of patients with schizophrenia,<sup>11,12</sup> and higher rates of homelessness, depression and severity of psychosis among substance-using patients. In the 544 patients with a substance-use disorder, 87% used alcohol, 44% marijuana and 36% cocaine. A study examining the temporal relationship between use and symptoms in patients recorded in real time with electronic ambulatory monitoring found that sad mood and psychotic symptoms were associated with later substance use, and that substance use was associated with increased risk of subsequent anxiety and psychotic-symptom onset; these results suggest that while the intent may be to self-medicate, the consequences are that substances may exacerbate symptoms.<sup>13</sup> Comorbidity occurs early, as substance-use disorders are prevalent in first-episode psychosis patients (up to 50%).<sup>14–16</sup> These patients are more likely to show non-adherence to treatment, poor remission rates<sup>17</sup> and higher frequency of suicidal behavior.<sup>18</sup>

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In summary, patients with schizophrenia are often addicted to substances, from early on, and the effects of both factors, the psychotic illness and addiction, are intertwined throughout the stages of the disorders and difficult to disentangle. Few studies have attempted to address how the neurobiology of each disorder may be changed in cases where they co-exist in the same patients. Structural imaging studies have reported inconsistent findings, with some indicating smaller brain volumes in this population when compared with schizophrenia without comorbid substance-use disorders,<sup>19–21</sup> and others finding no change.<sup>22</sup> No studies have assessed parameters of neurotransmission in such patients. Therefore, we undertook a study to assess striatal DA dysregulation in patients with both disorders, that is, schizophrenia and substance dependence. On the basis of available findings, our working hypothesis was that the striatal DA alterations that have been associated with each disorder, that is, higher release in the preDCA with schizophrenia and lower release in the VST with addiction, coexist in these comorbid patients. Such a set of alterations could set up a vicious cycle of using drugs to self-medicate due to low ventrostriatal DA transmission, which in turn may further dysregulate DA functioning in the AST, causing or worsening psychosis. We also hypothesized that DA release in the VST would be negatively related to measures of dependence severity, whereas DA release in the preDCA would be positively associated with severity of psychosis.

## METHODS

### Human subjects

This study was approved by the Institutional Review Board of New York State Psychiatric Institute (NYSPI) of Columbia University Medical Center (CUMC). All participants provided written informed consent after the procedures were fully explained to them, and all patient participants were independently assessed for capacity to provide consent by a psychiatrist who was not a member of the research team. Dual-diagnosis (DD) patients were recruited through advertisements, clinician referral, the inpatient and outpatient programs at NYSPI and the emergency department of CUMC. Healthy controls were recruited through advertisements. Medical screening procedures included a physical examination and history, blood and urine tests, an electrocardiogram and a structural magnetic resonance imaging scan of the brain. Participants were free of significant medical and neurological illnesses and were not pregnant or nursing.

Inclusion criteria for DD patients were: (1) lifetime DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th ed.) diagnosis of schizophrenia, schizoaffective or schizophreniform disorder, with the requirement of follow-up assessments of patients with schizophreniform disorder to confirm the diagnosis of schizophrenia; (2) lifetime DSM-IV diagnosis of alcohol, cannabis, and/or cocaine dependence; (3) no history of violent behavior; (4) no antipsychotic treatment for 3 weeks before PET scan participation; and (5) a negative urine drug screen prior to PET. Nicotine dependence was permitted for DD patients. Inclusion criteria for healthy controls were: (1) absence of any current or past DSM-IV Axis-I diagnosis, including substance abuse (past but not current nicotine dependence was permitted); and (2) no family history (first-degree) of psychotic illness. Current smokers were excluded from the control group with the aim of having a 'clean' comparison group with regard to any kind of substance dependence. Diagnostic status was determined with the Psychiatric Research Interview for Substance and Mental Disorders for DSM-IV<sup>23</sup> for DD patients, and with either the Diagnostic Interview for Genetic Studies<sup>24</sup> or an abbreviated version of the Structured Clinical Interview for DSM-IV Axis I disorders<sup>25</sup> for healthy controls. Participant and parental socioeconomic status were calculated according to Hollingshead.<sup>26</sup> Additionally, within 1 week before participating in PET, DD patients were assessed with the Positive and Negative Syndrome Scale (PANSS).<sup>27</sup> Clinical assessments were administered by trained interviewers.

DD patients with current drug use upon study enrollment were offered inpatient admission to assist in fulfilling the abstinence requirements for

PET participation. Patients who were psychiatrically stable were permitted to undergo detoxification as outpatients, underwent weekly urine toxicology tests (at minimum), and were scheduled for PET once they tested negative. A urine toxicology test was repeated on PET days to confirm abstinence.

### PET acquisition

All participants underwent two PET scans with an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN, USA) on the same day: at baseline and after receiving amphetamine. [<sup>11</sup>C]raclopride was administered as bolus plus constant infusion for 80 min, as previously described.<sup>28</sup> Emission scan data were collected in 3-dimensional mode 40–80 min after [<sup>11</sup>C]raclopride injection as eight frames of 5 min each. The second [<sup>11</sup>C]raclopride injection was initiated 2 min after i.v. administration of 0.3 mg/kg of dextro-amphetamine. Venous blood samples were collected 40 min after amphetamine administration to measure plasma amphetamine levels.

### Image analysis

All participants received a high-resolution structural magnetic resonance imaging scan for coregistration. Image analysis was performed using MEDx (Medical Numerics, MD, USA). The striatum was divided into five anatomical regions of interest (ROIs): VST, preDCA, precommissural dorsal putamen (preDPU), postcommissural caudate (postCA) and postcommissural putamen (postPU).<sup>29</sup> Based on the input each of these regions receives, they have been classified into three functional subdivisions: the limbic striatum (comprising the VST), the AST (comprising the preDCA, preDPU and postCA), and the sensorimotor striatum (comprising the postPU). ROIs were manually drawn on each subject's magnetic resonance image and transferred to co-registered PET data. Cerebellum (CER) was used as a reference region to estimate the concentration of free and non-specifically bound [<sup>11</sup>C]raclopride. Our analyses included the five striatal subregions and a composite 'whole striatum' region.

Equilibrium analysis was used to derive the outcome measure BP<sub>ND</sub> (binding potential relative to the nondisplaceable compartment in the brain):

$$BP_{ND} = \frac{\text{Activity(ROI)} - \text{Activity(CER)}}{\text{Activity(CER)}}$$

BP<sub>ND</sub> reflects DA D<sub>2/3</sub> receptor availability, as measured by [<sup>11</sup>C]raclopride. The primary outcome measure for this study was the percent change in BP<sub>ND</sub> from baseline to post-amphetamine scan ( $\Delta BP_{ND}$ ); this measure reflects the relative reduction in D<sub>2/3</sub> receptor availability for [<sup>11</sup>C]raclopride binding after amphetamine-induced DA release, and thus is used to index amphetamine-induced DA release.

### Cardiovascular and behavioral responses to amphetamine

Blood pressure and heart rate of participants were measured at baseline and at regular intervals after amphetamine administration (approximately every 2.5 min for the first 20 min, every 5 min from 20–40 min and every 10 min from 40–80 min). Subjective responses to amphetamine were measured using a simplified version of the Amphetamine Interview Rating Scale (AIRS);<sup>30,31</sup> Supplementary Table S1. The area under the curve (AUC) was calculated, relative to baseline, for each cardiovascular measure and AIRS item. The PANSS was used to evaluate behavioral responses to amphetamine. Change scores from pre- to post-amphetamine were calculated for the PANSS (Positive-symptom and Negative-symptom subscales); Supplementary Table S2.

### Statistical analysis

Group comparisons on demographic features were performed with independent-samples *t*-tests or  $\chi^2$ -tests. Within-subject comparisons on scan parameters across scan conditions were performed with paired *t*-tests, and between-group comparisons on scan parameters, striatal volumes and AUC values (cardiovascular and AIRS) were performed with independent-samples *t*-tests. In the case of non-normally distributed data, comparisons were repeated with non-parametric tests (Wilcoxon for paired tests, Mann–Whitney *U*-test for independent-samples tests); non-parametric test results are included here only if they differed from

parametric test results. Group comparisons on our primary PET outcome measures, baseline BP<sub>ND</sub> and  $\Delta$ BP<sub>ND</sub>, were performed using linear mixed modeling for which diagnosis (DD versus control) and region of interest (ROI; five striatal subregions) were treated as fixed effects, and ROI was treated as a repeated-measures variable; between-group planned comparisons were also conducted for each striatal subregion. Cohen's *d* was used as an index of effect size; the s.d. pooled across the two groups was used for this calculation. Correlational analyses were performed using Spearman's rank correlations, because of non-normal distributions (skew and/or kurtosis) observed for several variables. For exploratory analyses, we used Bonferroni correction for comparisons across six ROIs (five striatal subregions and whole striatum), which resulted in an adjusted *P* value of 0.008. All statistical tests were two-tailed.

## RESULTS

### Demographic and clinical characteristics

Twenty-one patients were enrolled to participate in this study; however, because of technical difficulties or participant withdrawal, only 13 of these 21 patients completed the scanning procedures. Data from two of these patients were excluded from analyses (one because of excessive movement in the scanner, and the other because additional diagnostic information on follow-up suggested that her initial presentation of schizoaffective disorder

was due to Lyme disease), resulting in a final patient group of 11. Fifteen healthy adults matched to the patient group for age, sex, ethnicity and parental socioeconomic status were included as a comparison group (Table 1). DD patients had significantly lower participant socioeconomic status compared with controls,  $t(17.91) = -2.97$ ,  $P = 0.008$ ; Table 1.

Six of the eleven DD patients met the criteria for schizophrenia, four for schizoaffective disorder and one for schizophreniform disorder (with a later confirmed diagnosis of schizophrenia). Their mean age of psychosis onset was  $18.09 \pm 4.16$ , and duration of psychotic illness averaged to  $11.0 \pm 8.73$  years (Table 1). Two patients were antipsychotic-naïve. DD patients met the criteria for current or past cannabis dependence ( $n = 10$ ) or abuse ( $n = 1$ ), alcohol dependence ( $n = 8$ ), and/or cocaine dependence ( $n = 3$ ); Supplementary Table S3. Eight DD patients reported current regular tobacco use (all controls were nonsmokers). Patients began using drugs on a regular basis at a mean age of  $16.45 \pm 2.46$ , which was, on average,  $12.90 \pm 7.68$  years before study participation.

### Striatal subregion volumes

There were no significant group differences in any of the ROI volumes. Across the total sample, age was negatively associated with volumes of the preDCA ( $r_s = -0.49$ ,  $P = 0.011$ ) and whole

**Table 1.** Sample characteristics and scan parameters

| Sample Characteristic  | Controls ( <i>n</i> = 15) |  | DD Patients ( <i>n</i> = 11)                            | <i>P</i> <sup>a</sup> |
|--|---------------------------|--|---|-----------------------|
| Age (years)  | $30.69 \pm 7.57$          |  | $29.54 \pm 8.62$  | 0.72                  |
| Sex ( <i>n</i> )   | 4 female, 11 male         |  | 1 female, 10 male                                       | 0.26                  |
| Ethnicity ( <i>n</i> )   | 3C, 4AA, 3H, 2As, 3 mixed |  | 1C, 4AA, 4H, 1As, 1 mixed                               | 0.45 <sup>b</sup>     |
| Parental SES <sup>c</sup>  | $40.87 \pm 10.87$         |  | $36.36 \pm 13.74$ <sup>d</sup>                          | 0.41                  |
| Participant SES <sup>c</sup>   | $32.07 \pm 16.56$         |  | $18.45 \pm 5.47$  | 0.01                  |
| Psychosis age of onset   | —                         |  | $18.09 \pm 4.16$  | —                     |
| Duration of psychotic illness (years)                                      | —                         |  | $11.00 \pm 8.73$  | —                     |
| Weeks since antipsychotic medication exposure (median, range) <sup>e</sup> | —                         |  | 10, 1.71 <sup>f</sup> –313                              | —                     |
| Drugs of dependence or abuse ( <i>n</i> <sup>g</sup> )                     | —                         |  | 11 cannabis, 8 alcohol, 3 cocaine, 1 other <sup>h</sup> | —                     |
| Age of onset of regular drug use   | —                         |  | $16.45 \pm 2.46$  | —                     |
| Duration since regular use initiated (years)                               | —                         |  | $12.90 \pm 7.68$  | —                     |
| Weeks since last drug use (median, range) <sup>i,j</sup>                   | —                         |  | 4.93, 1.71–15   | —                     |

| Scan Parameter <sup>k</sup>            | Controls ( <i>n</i> = 15) |                  | DD Patients ( <i>n</i> = 11) |                  |
|--|---------------------------|------------------|------------------------------|------------------|
|  | Baseline                  | Post-amph        | Baseline                     | Post-amph        |
| Injected dose (mCi)                    | $8.93 \pm 2.29$           | $8.48 \pm 2.42$  | $7.59 \pm 1.35$              | $8.14 \pm 1.38$  |
| Injected mass (μg)                     | $4.30 \pm 1.64$           | $3.70 \pm 1.40$  | $4.21 \pm 1.77$              | $3.63 \pm 1.53$  |
| Specific activity (Ci/mmol)            | $1287 \pm 621$            | $1416 \pm 755$   | $1159 \pm 600$               | $1430 \pm 759$   |
| Amphetamine level <sup>l</sup> (ng/ml) | —                         | $42.53 \pm 7.28$ | —                            | $38.45 \pm 9.04$ |

Abbreviations: AA, African American; As, Asian; C, Caucasian; DD, dual-diagnosis; H, Hispanic; post-amph, post-amphetamine; SES, socioeconomic status.

Note: Mean  $\pm$  s.d. unless otherwise indicated.

<sup>a</sup>Independent-samples *t*-tests for continuous variables;  $\chi^2$ -tests for dichotomous variables.

<sup>b</sup>Groups were compared on a dichotomized ethnicity variable (Caucasian vs ethnic minority).

<sup>c</sup>Socioeconomic status was calculated according to Hollingshead.<sup>26</sup>

<sup>d</sup>Parental SES was missing for four patients.

<sup>e</sup>*n* = 9 (two patients were naïve to antipsychotics).

<sup>f</sup>Twelve days for one patient, and a minimum of 3 weeks for all others.

<sup>g</sup>Overlapping; see Supplementary Table S3 for drugs of dependence and/or abuse by subject.

<sup>h</sup>One subject also met criteria for amphetamine, sedative, opioid and hallucinogenic dependence.

<sup>i</sup>*n* = 10; for one patient, duration of drug-free period unclear because of discrepant results between the urine toxicology test (dipstick) conducted on scan day and the quantitative laboratory results of the same urine sample. Results were essentially unchanged when excluding this patient from analyses.

<sup>j</sup>Three patients met criteria for current drug dependence; for one of these, duration of drug-free period was unclear (see above footnote); for the other two patients, days since last use were 12 (cannabis and alcohol) and 17 (cannabis), respectively. Of the remaining patients, five were in early remission and three in sustained remission (see Supplementary Table S3 for details).

<sup>k</sup>No significant within- or between-group differences.

<sup>l</sup>Forty minutes after amphetamine administration; missing for one control.

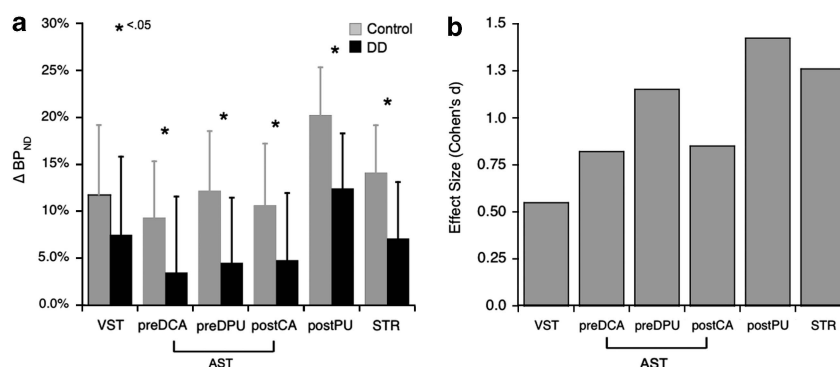
**Table 2.** [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  and  $\Delta\text{BP}_{\text{ND}}$  by diagnostic group

| ROI    | Controls ( $n = 15$ ) |                 |                               | DD patients ( $n = 11$ ) |                 |                               | Group comparisons <sup>a</sup> |                                 |
|--------|-----------------------|-----------------|-------------------------------|--------------------------|-----------------|-------------------------------|--------------------------------|---------------------------------|
|        | Baseline              | Post-amph       | $\Delta\text{BP}_{\text{ND}}$ | Baseline                 | Post-amph       | $\Delta\text{BP}_{\text{ND}}$ | $P \text{ BP}_{\text{ND}}$     | $P \Delta\text{BP}_{\text{ND}}$ |
| VST    | $2.52 \pm 0.30$       | $2.22 \pm 0.31$ | $-12 \pm 7\%$                 | $2.48 \pm 0.36$          | $2.28 \pm 0.27$ | $-7 \pm 8\%$                  | 0.76                           | 0.18                            |
| AST    | $2.90 \pm 0.28$       | $2.59 \pm 0.30$ | $-11 \pm 6\%$                 | $2.77 \pm 0.35$          | $2.65 \pm 0.33$ | $-4 \pm 7\%$                  | 0.29                           | 0.01                            |
| preDCA | $2.77 \pm 0.31$       | $2.51 \pm 0.32$ | $-9 \pm 6\%$                  | $2.69 \pm 0.35$          | $2.59 \pm 0.33$ | $-3 \pm 8\%$                  | 0.57                           | 0.04                            |
| preDPU | $3.24 \pm 0.31$       | $2.84 \pm 0.35$ | $-12 \pm 6\%$                 | $3.06 \pm 0.37$          | $2.91 \pm 0.35$ | $-5 \pm 7\%$                  | 0.19                           | 0.01                            |
| postCA | $2.19 \pm 0.27$       | $1.95 \pm 0.19$ | $-11 \pm 7\%$                 | $2.07 \pm 0.37$          | $1.97 \pm 0.37$ | $-5 \pm 7\%$                  | 0.35                           | 0.04                            |
| postPU | $3.45 \pm 0.35$       | $2.75 \pm 0.32$ | $-20 \pm 5\%$                 | $3.20 \pm 0.43$          | $2.80 \pm 0.42$ | $-12 \pm 6\%$                 | 0.12                           | <0.01                           |
| STR    | $3.02 \pm 0.30$       | $2.60 \pm 0.29$ | $-14 \pm 5\%$                 | $2.87 \pm 0.37$          | $2.66 \pm 0.34$ | $-7 \pm 6\%$                  | 0.25                           | <0.01                           |

Abbreviations: AST, associative striatum; DD, dual-diagnosis; post-amph, post-amphetamine; postCA, postcommissural caudate; postPU, postcommissural putamen; preDCA, precommissural dorsal caudate; preDPU, precommissural dorsal putamen; STR, whole striatum; VST, ventral striatum.

Note: Mean  $\pm$  s.d. (observed).

<sup>a</sup>Group comparisons of estimated marginal means derived from linear mixed modeling (see text) with the exception of AST and STR; these composite regions were compared with independent-samples *t*-tests.



**Figure 1.** (a)  $\Delta\text{BP}_{\text{ND}}$  for dual-diagnosis (DD;  $n = 11$ ) and control ( $n = 15$ ) groups.  $*P < 0.05$  per between-group planned comparisons of the estimated marginal means derived from linear mixed modeling (see text) with the exception of STR, which was assessed with an independent-samples *t*-test. (b) Effect sizes (Cohen's *d*) for group differences between DD ( $n = 11$ ) and controls ( $n = 15$ ) in  $\Delta\text{BP}_{\text{ND}}$ . See Table 2 for abbreviations.

striatum ( $r_s = -0.47$ ,  $P = 0.016$ ); however, these associations were not significant after Bonferroni correction.

#### Scan parameters

There were no significant differences between conditions (baseline and post-amphetamine) or between groups in injected dose, injected mass or specific activity of [ $^{11}\text{C}$ ]raclopride (Table 1). Likewise, the groups did not differ significantly in their plasma amphetamine levels.

#### Group comparisons on baseline $\text{BP}_{\text{ND}}$ and $\Delta\text{BP}_{\text{ND}}$

**Baseline  $D_{2/3}$  receptor availability.** Linear mixed modeling using baseline  $\text{BP}_{\text{ND}}$  as the dependent variable and striatal ROI (the five subregions) as a repeated measure showed no significant effect of diagnosis ( $F(1,24) = 1.12$ ,  $P = 0.301$ ; Table 2). There was a significant main effect of ROI ( $F(4,24) = 317.69$ ,  $P < 0.001$ ), and the diagnosis  $\times$  ROI interaction was also significant ( $F(4,24) = 2.78$ ,  $P = 0.050$ ). However, as indicated by planned pairwise comparisons of the estimated marginal means derived from the linear mixed model, there were no significant group differences in baseline  $\text{BP}_{\text{ND}}$  in any of the striatal subregions (Table 2).

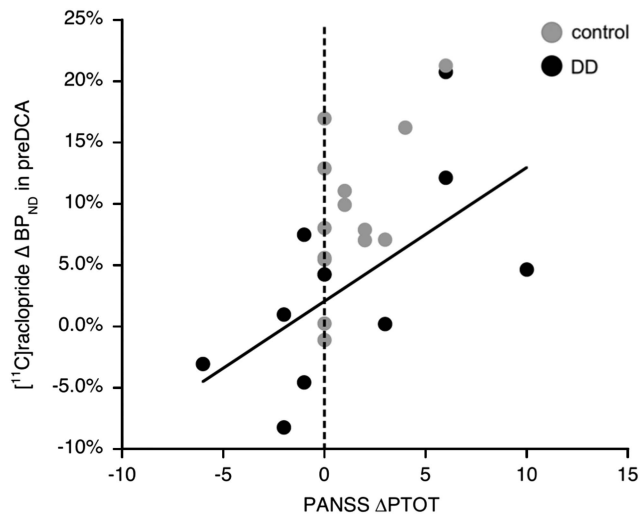
**Amphetamine-induced reduction in  $D_{2/3}$  receptor availability.** Linear mixed modeling using  $\Delta\text{BP}_{\text{ND}}$  as the dependent variable and striatal ROI as a repeated measure indicated significant main effects of diagnosis ( $F(1,24) = 8.38$ ,  $P = 0.008$ ; Table 2 and Figure 1a) and

ROI ( $F(4,24) = 46.51$ ,  $P < 0.001$ ); however, the diagnosis  $\times$  ROI interaction was not significant ( $F(4,24) = 0.67$ ,  $P = 0.618$ ). Between-group planned comparisons of the estimated marginal means indicated that DD patients had significantly smaller amphetamine-induced  $\Delta\text{BP}_{\text{ND}}$  in all striatal subregions except for VST (Table 2 and Figure 1a). The effect sizes (Cohen's *d*) for group differences in  $\Delta\text{BP}_{\text{ND}}$  were large for putamen (preDPU = 1.15, postPU = 1.42) and caudate (preDCA = 0.82, postCA = 0.85), and moderate for the VST (0.55); Figure 1b.

#### Cardiovascular and behavioral responses to amphetamine

The groups did not differ significantly in their mean change (AUC) in systolic or diastolic blood pressure or heart rate following amphetamine administration. As compared with controls, DD patients reported experiencing a greater increase in happiness ( $t(22) = 2.68$ ,  $P = 0.014$ ) and energy ( $t(22) = 2.03$ ,  $P = 0.054$ ; Mann–Whitney *U*-test,  $P = 0.032$ ) after amphetamine administration (mean AUC of self-report AIRS ratings;  $n = 9$  for patients because of missing data); these differences were not considered significant after Bonferroni correction. Groups did not differ significantly in their amphetamine-related restlessness or anxiety ratings (Supplementary Table S1). Among controls but not patients, changes in ratings of energy were positively associated with  $\Delta\text{BP}_{\text{ND}}$  in the preDPU ( $r_s = 0.72$ ,  $P = 0.002$ ), preDCA ( $r_s = 0.76$ ,  $P = 0.001$ ), VST ( $r_s = 0.69$ ,  $P = 0.005$ ), and whole striatum ( $r_s = 0.74$ ,  $P = 0.002$ ).





**Figure 2.** Precommissural dorsal caudate (PreDCA)  $\Delta BP_{ND}$  and Positive and Negative Syndrome Scale (PANSS) Positive-Symptom change scores ( $\Delta PTOT$ ; that is, amphetamine-induced change in PANSS positive symptoms) among dual-diagnosis (DD;  $n = 10$ ) and control ( $n = 14$ ) participants.  $\Delta PTOT$  could not be calculated for one DD patient and one control because of missing data. For DD:  $r_s = 0.69$ ,  $P = 0.029$ . The solid line indicates the least-squares fit for DD patients, and the dashed line marks the 'zero' value for PANSS  $\Delta PTOT$ , that is, no change in PANSS Positive-Symptom scores from baseline to the post-amphetamine assessment.

Supplementary Table S2 provides the pre-amphetamine and amphetamine-related change scores for the PANSS (Positive-symptom and Negative-symptom subscales) by group. There was a large degree of inter-subject variability in positive-symptom change scores ( $\Delta PTOT$ ) among patients, in contrast to controls (DD range =  $-6.0$  to  $10.0$ ; control range =  $0.0$  to  $6.0$ ; Figure 2). For controls, the mean increase in positive symptoms after amphetamine ( $1.36 \pm 1.87$ ,  $n = 14$ ; Supplementary Table S2) was primarily driven by the excitement item of the Positive-Symptom subscale. As predicted, among DD patients,  $\Delta PTOT$  ( $n = 10$ ; Figure 2) was significantly associated with  $\Delta BP_{ND}$  in the preDCA ( $r_s = 0.69$ ,  $P = 0.029$ ; Figure 2). This relationship was also observed in the VST ( $r_s = 0.64$ ,  $P = 0.048$ ), although it was not considered significant after multiple-comparisons correction.  $\Delta PTOT$  was not significantly related to  $\Delta BP_{ND}$  at the level of the whole striatum ( $r_s = 0.36$ ,  $P = 0.306$ ).

#### Correlations with baseline symptoms and other clinical features

Among the DD patients, there was a significant negative association between the age of psychosis onset and  $\Delta BP_{ND}$  in the preDPU ( $r_s = -0.77$ ,  $P = 0.005$ ), indicating that DD patients who developed psychotic symptoms at an earlier age had greater  $\Delta BP_{ND}$  in the preDPU (or conversely that those with a later age of onset had lower preDPU  $\Delta BP_{ND}$ ). There were no other significant associations between psychosis age of onset, duration of psychotic illness, or weeks since antipsychotic medication exposure and baseline  $BP_{ND}$  or  $\Delta BP_{ND}$  across the striatal subregions. There were also no significant associations between age of onset of regular drug use, years since regular drug use was initiated, or weeks since last drug use and  $BP_{ND}$  or  $\Delta BP_{ND}$  across the striatal subregions. Baseline symptom severity as measured by the PANSS (using the standard 7-day assessment time frame) was not significantly associated with baseline  $BP_{ND}$  or  $\Delta BP_{ND}$  in any striatal subregion.

## DISCUSSION

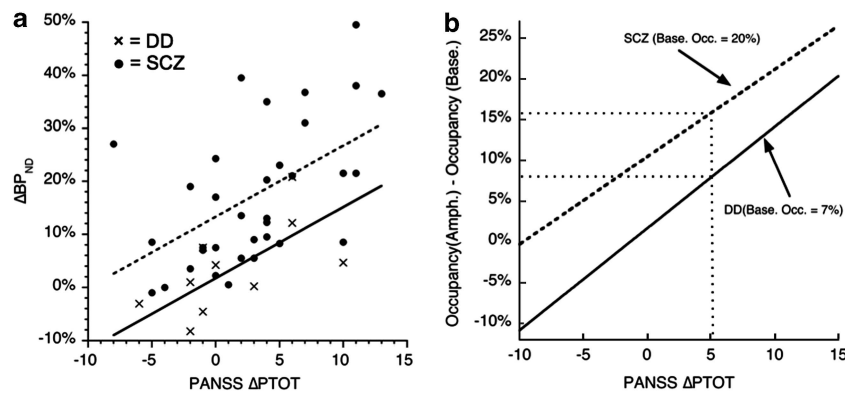
This is the first description of a group of patients with schizophrenia who display low presynaptic DA release, yet show a psychotic reaction to increases in  $D_2$  stimulation, suggesting abnormal postsynaptic  $D_2$  function. In this small group of patients with comorbid schizophrenia and substance dependence, we observed a generalized blunting of DA release in all striatal subregions, to varying degrees, with putamen most affected and VST least affected. Despite the low range of DA release displayed by DD patients compared with controls and with previously published reports in schizophrenia, DA release was significantly associated with the amphetamine-induced change in positive symptoms, as previously observed in schizophrenia.<sup>4</sup> Amphetamine-induced changes in positive symptoms were most strongly associated with  $\Delta BP_{ND}$  in the preDCA and VST, adding to the data linking psychosis more specifically with the AST, as well as the limbic striatum, and confirming a prominent role for these two areas in the neurobiology of psychosis. Quantitatively, the relationship between  $\Delta BP_{ND}$  and change in positive symptoms following amphetamine ( $\Delta PTOT$ ) observed in this study is strikingly similar to that observed in patients with schizophrenia.<sup>4</sup> When data from the two studies are pooled, analysis of model order suggests that linear regression with a shared slope is more parsimonious than fitting the two studies separately with two different slopes,  $F(1,40) = 1.15$ ,  $P = 0.71$  ( $\Delta BP_{ND} = 1.34 \times \Delta PTOT + \text{intercept}$ , where the fitted intercept is 13.13 for schizophrenia and 1.73 for DD; Figure 3). That is, a unit difference in  $\Delta PTOT$  results from 1.34 units of difference in  $\Delta BP_{ND}$  in both studies.

To explore this further, we reframed this correlation in terms of  $D_2$  receptor occupancy by DA rather than  $\Delta BP_{ND}$ , assuming that the main effect in  $\Delta BP_{ND}$  is due to competition with DA and using existing information regarding baseline receptor occupancy by DA:

$$\begin{aligned} & \text{post-amphetamine occupancy} - \text{baseline occupancy} \\ &= 1.34 \times (1 - \text{baseline occupancy}) \times \Delta PTOT + \text{intercept} \\ & \times (1 - \text{baseline occupancy}); \text{ see derivation in Supplement} \end{aligned}$$

DA depletion studies have suggested that average baseline  $D_2$  receptor occupancy by DA is  $\sim 20\%$  in schizophrenia<sup>32</sup> and  $\sim 7\%$  in substance dependence.<sup>33</sup> It is not known which of these might be more reflective of baseline occupancy in DD, but any baseline occupancy in the range of 7–20% results in the relationship between amphetamine-induced change in occupancy and  $\Delta PTOT$  being nearly parallel for both groups, with DD biased downward compared with schizophrenia (Figure 3b). The lower intercept observed for DD compared with schizophrenia suggests that, assuming similar levels of receptor availability, a smaller increase in  $D_2$  receptor occupancy by DA in DD compared with schizophrenia leads to a similar magnitude of symptom change. These results suggest that  $D_2$  receptors are more sensitive to DA in terms of psychosis exacerbation in DD compared with schizophrenia. Thus, our findings suggest that  $D_2$  function may be abnormal in this group of patients, such that small variations in agonist stimulation have amplified effects. These may be due to abnormal intracellular signaling events, or alternatively, could be an indirect effect of  $D_2$  stimulation on other non-dopaminergic components of the network that may be abnormal in schizophrenia. The exact mechanism of this  $D_2$ -mediated effect is unclear.

Prior research suggests that the blunted DA release observed among the DD patients is related to their comorbid drug use. The primary drugs of dependence included alcohol and cannabis, and the majority also reported current tobacco use. Previous studies have shown that alcohol dependence is associated with blunted DA release,<sup>6,34</sup> whereas cannabis dependence, at least of moderate severity, is not.<sup>35</sup> With regard to tobacco use, there are reports of both decreased  $D_2$  receptor availability<sup>36</sup> and



**Figure 3.** (a) The association between  $\Delta BP_{ND}$  and Positive-Symptom change scores ( $\Delta PTOT$ ; that is, amphetamine-induced change in Positive and Negative Syndrome Scale (PANSS) positive symptoms) for dual-diagnosis (DD;  $n = 10$ , data from current study;  $\Delta BP_{ND}$  for precommissural dorsal caudate (preDCA)) and schizophrenia (SCZ;  $n = 34$ , data from Laruelle *et al.*<sup>4</sup>  $\Delta BP_{ND}$  for whole striatum). The lines result from a regression with a common slope and study-specific intercepts:  $\Delta BP_{ND} = 1.34 \times \Delta PTOT + 13.13$  (SCZ, dashed) or  $\Delta BP_{ND} = 1.34 \times \Delta PTOT + 1.73$  (DD, solid). The fit demonstrates that the same change in symptoms is associated with a smaller change in tracer binding in DD compared with SCZ. (b) The same fits, transformed to the increase in  $D_2$  receptor occupancy by dopamine (DA) following amphetamine. Baseline occupancy in DD is assumed to be similar to that previously reported for substance-dependent patients.<sup>33</sup> The main qualitative result is that a given increase in symptoms (five on the Positive-Symptom subscale of the PANSS in this example) results from a smaller increase in the number of receptors being stimulated in DD than in SCZ. Abbreviations: Amph, amphetamine; Base, baseline; Occ, occupancy.

reduced amphetamine-induced DA release in the striatum of nicotine-dependent subjects.<sup>37</sup> A limitation of this study is that we cannot distinguish the respective contributions of any one of these drugs to the overall decrease in DA transmission. For example, most of the DD patients reported current regular cigarette smoking, and this tobacco use may have contributed to our results. However, given that all of the patients used more than one drug and the sample is too small to examine the effect of each drug separately, this study cannot address such questions.

There are no clear mechanisms at this point to explain the decrease in presynaptic DA either in this study or in prior studies reporting a similar decrease in substance-use disorders. One view is that the decrease may precede drug use and predispose patients to develop addictions, whereas another is that the decrease is a result of chronic substance use (for discussion, see Volkow and colleagues<sup>38–40</sup>).

In contrast to the findings in addiction,<sup>6,34,36</sup> we did not observe a downregulation of  $D_2$  receptors. This may be because of the small sample size and/or the confounding effect of previous antipsychotic exposure, which is known to induce an upregulation of  $D_2$ .

Our sample size is relatively small because of the difficulty in recruiting this group of patients. Nevertheless, our results are clear and compelling regarding the magnitude of DA blunting detected in these patients. However, the small sample size limited our ability to confidently characterize some of the clinical correlates of this blunted DA release.

In summary, we observed that patients with comorbid schizophrenia and mixed substance dependence displayed significant blunting of striatal DA release. Despite this blunting, DA release was associated with acute and transient increases in positive symptoms. Overall, these findings suggest that in substance-dependent patients with schizophrenia, hypersensitivity of  $D_2$  receptors to dopaminergic stimulation is the predominant dopaminergic alteration, as opposed to excess presynaptic release.<sup>41</sup> This hypersensitivity may be related to intrinsic factors within the  $D_2$  signaling cascade<sup>42</sup> and/or from the effects of excess  $D_2$  signaling on the rest of the circuitry. Translational studies of altered pre- and postsynaptic mechanisms are needed to clarify this issue.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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