Dopamine D3 receptor alterations in cocaine-dependent humans imaged with [11C]PHNO

David Matuskey a,b,∗, Jean-Dominique Gallezot b, Brian Pittman a, Wendol Williams a,b, Jane Wanyiri a, Edward Gaiser a, Dianne E. Lee a, Jonas Hannestad a, Keunpoong Lim b, Minq-Qiang Zheng b, Shu-fei Lin b, David Labaree b, Marc N. Potenza a, Richard E. Carson b, Robert T. Malison a, Yu-Shin Ding c

a Department of Psychiatry, Yale University, New Haven, CT, USA
b Department of Diagnostic Radiology, Yale University, New Haven, CT, USA
c Department of Radiology and Psychiatry, New York University School of Medicine, New York, NY, USA

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A B S T R A C T

Background: Evidence from animal models and postmortem human studies points to the importance of the dopamine D3 receptor (D3R) in cocaine dependence (CD). The objective of this pilot study was to use the D3R-preferring radioligand [11C]PHNO to compare receptor availability in groups with and without CD.

Methods: Ten medically healthy, non-treatment seeking CD subjects (mean age 41 ± 8) in early abstinence were compared to 10 healthy control (HC) subjects (mean age 41 ± 6) with no history of cocaine or illicit substance abuse. Binding potential (BPND), a measure of available receptors, was determined with parametric images, computed using the simplified reference tissue model (SRTM2) with the cerebellum as the reference region.

Results: BPND in CD subjects was higher in D3R-rich areas including the substantia nigra ((SN) 29%; P < 0.03), hypothalamus (28%; P = 0.02) and amygdala (35%; P < 0.03). No between-group differences were observed in the striatum or pallidum. BPND values in the SN (r = +0.83; P = 0.008) and pallidum (r = +0.67; P = 0.03) correlated with years of cocaine use.

Conclusions: Between-group differences suggest an important role for dopaminergic transmission in the SN, hypothalamus and amygdala in CD. Such findings also highlight the potential relevance of D3R as a medication development target in CD.

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1. Introduction

Research suggests that chronic cocaine use exerts long-lasting effects on dopaminergic systems, and these changes have been implicated in addictive processes (Koob and Volkow, 2010; Newman et al., 2012). While all dopamine receptor subtypes (Missale et al., 1998) are likely affected, the dopamine D3 receptor (D3R) may be particularly relevant to addictions like cocaine dependence (CD).

Animal and postmortem human studies support a role for the D3R in CD. Anatomically, D3R is densely present in the mesolimbic system where reward-related learning induced by cocaine occurs (Blaylock and Nader, 2012; Stanwood et al., 2000; Xi and Gardner, 2007). Specifically, D3R mRNA and protein in these areas show increased expression after exposure to stimulants and other drugs of abuse (Caine and Koob, 1993; Heidbreder and Newman, 2010; Neisewander et al., 2004; Staley and Mash, 1996; Xi and Gardner, 2007). Although some apparently inconsistent findings exist (Caine et al., 2012), D3R antagonists and partial agonists inhibit the actions of cocaine in preclinical models (Heidbreder et al., 2005; Le Foll et al., 2005; Newman et al., 2012; Xi and Gardner, 2007). Given these findings and the ineffectiveness of current pharmacologic treatments for CD, the D3R has become a target in medication development for CD.

The development of a D3R-preferring positron emission tomography (PET) ligand, [11C]PHNO, has allowed in vivo neuroimaging investigations in schizophrenia, Parkinson’s disease and tobacco smoking (Boileau et al., 2009; Graff-Guerrero et al., 2009; Mizrahi et al., 2011; Mugnaini et al., 2012). Directly relevant to CD,
Table 1
Subject characteristics and radioligand information in cocaine-dependent (CD) and healthy control (HC) participants. Mean values (and standard deviation) are shown.

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>HC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>10</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>Age, in years (mean (S.D.))</td>
<td>41 (8)</td>
<td>41 (6)</td>
<td>0.98</td>
</tr>
<tr>
<td>Gender</td>
<td>8 males; 2 females</td>
<td>8 males; 2 females</td>
<td>–</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td>7 AA; 2 EA; 1 Hispanic</td>
<td>3 AA; 7 EA</td>
<td>–</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30 (7)</td>
<td>29 (6)</td>
<td>0.84</td>
</tr>
<tr>
<td>Injected mass (µg/kg)</td>
<td>0.028 (0.003)</td>
<td>0.026 (0.007)</td>
<td>0.30</td>
</tr>
<tr>
<td>Radioactive dose (MBq)</td>
<td>307 (121)</td>
<td>376 (131)</td>
<td>0.24</td>
</tr>
<tr>
<td>Specific activity (MBq/nmol)</td>
<td>32 (15)</td>
<td>46 (24)</td>
<td>0.15</td>
</tr>
</tbody>
</table>


The study was performed under protocols approved by the Yale Human Investigation, Yale University Radiation Safety, Yale-New Haven Hospital (YNHH) Radiation Safety, and Yale MRI Safety Committees. Subjects were recruited from New Haven and surrounding areas by advertisement, word of mouth and referrals. Written informed consent was obtained from all participants after a full explanation of study procedures.

2.2. Radiochemistry
Carbon 11-labeled (+)-4-propyl-9-hydroxynaphthoxazine [11C(+)PHNO] is a D3/D2 receptor agonist radiotracer that has D3/D2 preferring properties. [11C(+)PHNO] was prepared as reported before by N-acetylation of the despropyl precursor with [11C]propionyl chloride followed by reduction of the resulting amide with lithium aluminum hydride and purification by reverse-phase high performance liquid chromatography (HPLC; Gallezot et al., 2012). The requisite radioisotope [11C]CO2 was produced with the PETtrace cyclotron (GE Medical Systems, Milwaukee, WI) and purified via the PETtrace Standard Chemistry System. The fraction containing the product was formulated into 9% ethanolic saline by solid-phase extraction, followed by filtration through 0.22-µm Millipore membrane, with a mean single intravenous injection of 341 ± 128 MBq and a mean specific activity of 39 ± 20 MBq/nmol.

2.3. Scanning and imaging procedures
All scans used a high-resolution research tomograph (HRRT) (Siemens/CTI, Knoxville, TN, USA), which acquired 207 slices (1.2 mm slice separation) with a reconstructed image resolution of ~3 mm. A transmission scan with a 137Cs point source was obtained before the emission scan. The PET scans were acquired for 120 min at rest.

Structural magnetic resonance images were performed on a Siemens 3-T Trio system (Siemens Medical Solutions, Malvern, PA) with a circularly polarized head coil for each subject to exclude individuals with anatomical abnormalities and for coregistration. The dimension and voxel size of MR images were 256 mm × 256 mm × 176 mm and 0.98 mm × 0.98 mm × 1.0 mm, respectively.

Dynamic PET scan data were reconstructed with all corrections (attenuation; normalization; scatter; randoms; deadtime and motion), using the MOLAR algorithm (Carson et al., 2003) with the following frame timing: 6 × 30 s; 3 × 1 min; 2 × 2 min; 22 × 5 min. Motion correction was based on an optical detector (Vicra, ND Systems, Waterloo, Ontario, Canada).

A summed image (0–10 min after injection) was created from the motion-corrected PET data and registered to the subject’s MR image, which in turn was nonlinearly registered to a MR template (Montreal Neurological Institute space). All transformations were performed with Bioimagessuite (version 2.5; http://www.bioimagessuite.com). PET data were used to produce a time–activity curve for the cerebellum, which has minimal D3/D2 binding and was used as the reference as in previous studies (Boilleau et al., 2012; Ginovar et al., 2007; Murabito et al., 2011; Searle et al., 2010). Parametric images of the binding potential (BPND), which is linearly proportional to the density of available D3/D2 receptors, were computed using a simplified reference tissue model (2-parameter version: SRTM2). This method has been previously validated (Wu and Carson, 2002) and was used to...
optimize the statistical quality of the SRTM used in prior studies by reducing noise of the functional images.

Regions of interest (ROI) included the amygdala, caudate, hypothalamus, pallidum, putamen, SN, thalamus and ventral striatum and were based on the Anatomical Automatic Labeling (AAL) template delineated on MR (Tzourio-Mazoyer et al., 2002) with the exception of hand-drawn ventral striatum and SN templates. The ventral striatum template was based on guidelines from Mawlawi et al. (2001). The SN was manually delineated on $BP_{ND}$ images in template space as previously described (Lee et al., 2012).

2.4. Statistical analysis

All outcomes were summarized descriptively and assessed for normality prior to analysis using normal probability plots and Kolmogorov test statistics. All outcomes were approximately normal. Linear mixed models were used to examine the independent and joint effects of group (between-subjects factor) and region of interest (within-subjects) on $BP_{ND}$ values. Between-group contrasts within each region were estimated to explain significant interactions. Within-subject correlations were accounted for by fitting three variance–covariance structures to the data (unstructured, compound symmetry, and heterogeneous compound symmetry) with an unstructured form fitting the data best according to the Bayesian Information Criterion (BIC). Gender, age, BMI, and injection dose were considered as covariates in the above models but were not significant and dropped for parsimony. Among CD subjects, potential associations between background variables (e.g., years of use, age) and $BP_{ND}$ levels within each region were evaluated using correlation analysis. Correlations were not adjusted for multiple tests given the exploratory nature of this analysis. All analyses were conducted using SAS, version 9.1 (Cary, NC).

3. Results

The main effect of diagnostic group on $BP_{ND}$ levels was not significant ($F_{1,18} = 1.34, P = 0.26$). However, a significant diagnostic-group-by-region interaction effect ($F_{7,18} = 3.53, P = 0.01$) was observed. Table 3 shows mean $BP_{ND}$ values for all ROIs. Fig. 1 shows individual subject $BP_{ND}$ values within each group and region. Higher $BP_{ND}$ values in CD (versus HC) individuals were seen in the amygdala ($F_{1,18} = 5.82, P = 0.03$; +35%), hypothalamus ($F_{1,18} = 6.22, P = 0.02$; +28%) and SN ($F_{1,18} = 5.96, P = 0.03$; +29%). Findings persisted when covarying for age, gender, body mass index (BMI) and PET injection parameters.

Within CD individuals, positive associations were observed between years of cocaine use and $[^{11}C](+)^{3}PHNO$ $BP_{ND}$ in the SN ($r = +0.83; P = 0.008$) and pallidum ($r = +0.67; P = 0.03$). Weekly cocaine use (in dollars spent) correlated with the amygdala ($r = +0.66; P = 0.04$). No other factors examined (age, gender, BMI, radioligand parameters, alcohol use, nicotine use, or days since last cocaine use) correlated with regional brain $BP_{ND}$ availability within this cohort.

4. Discussion

In this study, our major findings were higher $[^{11}C](+)^{3}PHNO$ $BP_{ND}$ values in the SN, hypothalamus and amygdala in CD as compared with HC individuals. Among CD subjects, years of cocaine use were correlated with $BP_{ND}$ values in the SN and pallidum. Despite differences in abstinence periods (on average 7 days for the current study versus 19 days from the polysubstance methamphetamine and 50 days for the previous CD study), overall the recent findings complement those from previous studies showing higher $[^{11}C](+)^{3}PHNO$ binding in the SN (Boileau et al., 2012; Payer et al., 2014). The results in the hypothalamus and amygdala have not been reported before, and as such represent novel findings. Implications are discussed in further detail below.

### Table 3

<table>
<thead>
<tr>
<th>ROI</th>
<th>$BP_{ND}$ mean (S.D.)</th>
<th>CD</th>
<th>HC</th>
<th>ΔCD (%)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>0.28 (0.11)</td>
<td>0.28 (0.28)</td>
<td>+5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>1.81 (0.53)</td>
<td>1.86 (0.45)</td>
<td>-2</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>1.52 (0.33)</td>
<td>1.19 (0.25)</td>
<td>+28</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Pallidum</td>
<td>3.53 (0.39)</td>
<td>3.56 (0.55)</td>
<td>-1</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Putamen</td>
<td>2.45 (0.33)</td>
<td>2.54 (0.39)</td>
<td>-4</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>2.05 (0.50)</td>
<td>1.59 (0.34)</td>
<td>+29</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.49 (0.07)</td>
<td>0.38 (0.09)</td>
<td>+6</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Ventral striatum</td>
<td>3.74 (0.89)</td>
<td>3.57 (0.63)</td>
<td>+5</td>
<td>0.63</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Individual subject $BP_{ND}$ values are shown for each region (N = 10 for each group; CD in blue and HC in red). Short bold lines denote group mean values (per Table 3). Asterisks denote statistical significance ($P = 0.03$ for both the amygdala and SN and $P = 0.02$ for the hypothalamus). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
Previous work with $^{11}$C]raclopride and a D$_3$R antagonist have indicated that the $^{11}$C]raclopride signal can be considered to be relatively specific for D$_3$R or D$_2$R depending upon brain region (Graff-Guerrero et al., 2008; Searle et al., 2010; Tziortzi et al., 2011), with some studies attributing 100% of the SN and hypothalamus to D$_3$R (Gallezot et al., 2012; Searle et al., 2010; Tziortzi et al., 2011). Therefore, these two regions are arguably a reasonable representation of a “pure” D$_3$R signal with $^{11}$C]raclopride and these elevated $B_{PD}$ values could represent a global D$_3$R up-regulation in CD. Further, the correlation between $B_{PD}$ values and years of cocaine use suggests that chronic cocaine use may lead to D$_3$R up-regulation, although longitudinal studies are needed to test this hypothesis directly. While up-regulation of the D$_3$R seems the most plausible explanation, the increase in $B_{PD}$ values may alternatively result from decreased endogenous dopamine (leading to higher ligand binding) in CD participants. Any possible differences in endogenous dopamine levels between the groups could be especially sensitive to $^{11}$C]raclopride as the binding values of D$_3$R have higher affinities for dopamine than do other dopamine receptors, making D$_3$R-preferring ligands particularly sensitive to endogenous dopamine levels (Schotte et al., 1996; Sokoloff et al., 1992).

While the SN and hypothalamus have been previously studied with $^{11}$C]raclopride and found to be rich in D$_3$R, the amygdala has not and our findings here should be viewed cautiously due to relatively low $B_{PD}$ values in this region (which adds more overall variability to these results). With that caveat stated, if confirmed, these preliminary results could be a potentially important finding as the amygdala contributes to learned associations between rewarding properties of drugs and cues (Koob, 2003; Koob and Volkow, 2010). In chronic cocaine users, decreased amygdalar volume and changes in functional connectivity involving the amygdala have been described (Gu et al., 2010; Makris et al., 2004) In rodents, D$_3$R antagonists injected into the amygdala have decreased cocaine self-administration under second-order schedules of reinforcement (Di Ciano, 2008), anxiety-like behaviors (Diaz et al., 2011) and more recently cocaine seeking (Xi et al., 2013). In addition, the amygdala has been implicated in cocaine-induced behavioral inflexibility (Stalnaker et al., 2007), cocaine memory reconsolidation (Wells et al., 2012) and stress-induced relapse (Smith and Aston-Jones, 2008). Despite not knowing the attributable D$_3$R percentage in the amygdala of humans, these findings suggest that the D$_3$R receptors represent an attractive pharmacologic target in this region.

It is noteworthy to consider the absence of between-group differences in $^{11}$C]raclopride binding potential values in D$_3$R-rich regions like the caudate and putamen. These findings contrast with those using the D$_2$/D$_3$ antagonist ligand $^{11}$C]raclopride to examine CD (Martinez et al., 2004, 2011; Volkow et al., 1997, 2006; Wong et al., 2006). Several explanations exist. First, low endogenous dopamine levels in CD could differentially increase available $^{11}$C]raclopride binding, muting more robust differences seen in D$_2$R-rich areas with antagonist tracers. This possibility was given more credence recently as $^{11}$C]raclopride was found to be more sensitive than $^{11}$C]raclopride in detecting the fluctuations of extracellular dopamine in humans (Shotbolt et al., 2012), complementing earlier work on anesthetized animals in the striatum (Giovanardi et al., 2006). Second, given the high affinity of dopamine for D$_3$R (Schotte et al., 1996; Sokoloff et al., 1992), another plausible explanation could relate to striatal D$_3$R. Although previous $^{11}$C]raclopride studies have suggested relatively negligible D$_3$R binding in the caudate and putamen (Gallezot et al., 2012; Searle et al., 2010; Tziortzi et al., 2011), repeated exposure to l-DOPA in rats can increase striatal D$_3$R (Bordet et al., 1997). Thus, potential D$_3$R losses could be concealed by up-regulation of the D$_3$R (Boileau et al., 2012). Third, the agonist profile of $^{11}$C]raclopride has increased affinity to a proposed high-affinity state (D$^{high}_{3}$) that reflects receptors that are coupled to G-proteins (as opposed to a low-affinity state where receptors are uncoupled from G-proteins, D$^{low}_{3}$; Graff-Guerrero et al., 2009; Seeman, 2012). This differential binding could have also blunted differences, but this possibility has been called into question recently with evidence that agonists (including $^{11}$C]raclopride) are not selective for dopamine D$^{high}_{3}$ receptors, but also bind to the D$^{low}_{3}$ state of the dopamine receptors (Seeman, 2012). Evidence for this phenomena explaining our results seems inconclusive, with a current review describing the controversy of whether D$^{high}_{3}$ can be measured in vivo (Skinnberg et al., 2012).

The current study was limited by a small sample size that may have precluded the identification of some between-group differences in $^{11}$C]raclopride binding, particularly in other regions demonstrating moderate levels of D$_3$R expression (e.g., the thalamus, ventral striatum and pallidum). In addition, despite not finding any correlations with the use of other substances (e.g., alcohol and nicotine) in the CD group, we cannot exclude the possibility that differences in non-cocaine substance use between the CD and HC groups and/or ethnicity may have partially confounded the results. That being said, we believe such effects are explanatory for the SN given previously positive findings in matched cohorts (Payer et al., 2014). Despite such limitations and some unresolved questions, this study lends support to the continued development of D$_3$R-related treatments for CD. The first report of a D$_3$R antagonist in the clinical treatment of substance abuse was recently reported to reduce self-reported craving in cigarette smoking (Mugnaini et al., 2012), and it possible that D$_3$R antagonism might normalize a hypofunctional dopamine system in CD (Heidbreder et al., 2005; Xi and Gardner, 2007). Given these prospects, further studies can focus on reproducing these findings with a larger sample size to improve generalizability to CD clinical populations, investigating how long-lasting these differences exist in abstinence, and

![Graph of A) Substantia Nigra and B) Pallidum](image)
potentially linking clinically relevant constructs like craving, impulsivity, drug self-administration and deficits in executive functions (Ersche et al., 2012) with D3R availability.

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Conflict of interest

The authors report no conflicts of interest related to the current study. Dr. Potenza has served as a consultant or advisor to Boehringer Ingelheim, Somaxon, gambling businesses and organizations, law offices, the federal defender’s office in issues regarding impulse control disorders. He has received research support from the National Institutes of Health, Veteran’s Administration, Mohegan Sun Casino, the National Center for Responsible Gaming, Psyadon, Forest Laboratories, Ortho-McNeil, Oyo-Control/Biotei, and GlaxoSmithKline.

Authors’ contributions

DM, REC, RTM and YSD wrote the protocols and designed the study in this manuscript. JDG, DEL, KL, MQZ, SFL, DL preprocessed data and DM and JDG prepared data for final analyses. JW and JH provided clinical expertise. EG completed the background literature search. BP completed the statistical analyses. DM wrote the first draft of the manuscript. All authors have approved the final manuscript.

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