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Heightened D_3 Dopamine Receptor Levels in Cocaine Dependence and Contributions to the Addiction Behavioral Phenotype: A Positron Emission Tomography Study with [^{11}C]-(+)-PHNO

Doris E Payer*[,1,2,10], Arian Behzadi[1,2,10], Stephen J Kish[1,2,3,4,5], Sylvain Houle[2,3,4,5,6,7,8,9], Alan A Wilson[2,5,6], Pablo M Rusjan[2,5], Junchao Tong[1,2,3,5,6], Peter Selby[5,7,8], Tony P George[6,7], Tina McCluskey[1,2,3] and Isabelle Boileau*[,1,2,3,5,6,7]

[1]Addiction Imaging Research Group, Centre for Addiction and Mental Health, Toronto, ON, Canada; [2]Research Imaging Centre and Vivian M Rakoff PET Imaging Centre, Centre for Addiction and Mental Health, Toronto, ON, Canada; [3]Human Brain Laboratory, Centre for Addiction and Mental Health, Toronto, ON, Canada; [4]Department of Pharmacology, University of Toronto, Toronto, ON, Canada; [5]Campbell Family Mental Health Research Institute, Toronto, ON, Canada; [6]Division of Addictions, Centre for Addiction and Mental Health, Toronto, ON, Canada; [7]Division of Schizophrenia, Centre for Addiction and Mental Health, Toronto, ON, Canada; [8]Division of Brain and Therapeutics, Department of Psychiatry, University of Toronto, Toronto, ON, Canada; [9]Department of Family and Community Medicine, University of Toronto, Toronto, ON, Canada

The dopamine system is a primary treatment target for cocaine dependence (CD), but research on dopaminergic abnormalities (eg, D_2 receptor system deficiencies) has so far failed to translate into effective treatment strategies. The D_3 receptor system has recently attracted considerable clinical interest, and D_3 antagonism is now under investigation as a novel avenue for addiction treatment. The objective here was to evaluate the status and behavioral relevance of the D_3 receptor system in CD, using the positron emission tomography (PET) radiotracer [^{11}C]-(+)-PHNO. Fifteen CD subjects (many actively using, but all abstinent 7–240 days on scan day) and fifteen matched healthy control (HC) subjects completed two PET scans: one with [^{11}C]-(+)-PHNO to assess D_3 receptor binding (BP_{ND}; calculated regionally using the simplified reference tissue model), and for comparison, a second scan with [^{11}C]raclopride to assess $D_{2/3}$ binding. CD subjects also completed a behavioral battery to characterize the addiction behavioral phenotype. CD subjects showed higher [^{11}C]-(+)-PHNO BP_{ND} than HC in the substantia nigra, which correlated with behavioral impulsiveness and risky decision making. In contrast, [^{11}C]raclopride BP_{ND} was lower across the striatum in CD, consistent with previous literature in $\geqslant$2 week abstinence. The data suggest that in contrast to a D_2 deficiency, CD individuals may have heightened D_3 receptor levels, which could contribute to addiction-relevant traits. D_3 upregulation is emerging as a biomarker in preclinical models of addiction, and human PET studies of this receptor system can help guide novel pharmacological strategies for treatment.
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INTRODUCTION

Cocaine is among the most commonly abused illicit substances worldwide, with cocaine dependence (CD) contributing significantly to societal burdens such as health care, criminal justice, and public safety costs and lost productivity (Degenhardt and Hall, 2012). Despite relatively high treatment-seeking rates and ongoing efforts to improve therapeutic approaches, however, clinical success rates remain low, and no pharmacotherapies have been approved to date (Haile *et al*, 2012). A better understanding of neurobiological mechanisms contributing to CD could advance treatment strategies and improve clinical outcomes.

Among the main targets of investigation is the brain dopamine (DA) system, which not only is directly modulated by the acute action of cocaine, but also has a pivotal role in the addiction and withdrawal phenotypes (Melis *et al*, 2005). Decades of preclinical research, now supported by human neuroimaging findings, suggest that DA transmission at $D_{2/3}$ receptors is diminished in addicted

*Correspondence: Dr I Boileau, Addiction Imaging Research Group, Centre for Addiction and Mental Health (CAMH), 250 College Street, Toronto, ON M5T 1R8, Canada, Tel: +416 535 8501 x34918, Fax: +416 979 6871, E-mail: isabelle.boileau@camh.ca or Dr DE Payer, Centre for Addiction and Mental Health (CAMH), 250 College Street, Toronto, ON, M5T 1R8, Canada, Tel: +416 535 8501 x36280, Fax: +416 979 6871, E-mail: doris.payer@camh.ca
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[10]These authors contributed equally to this work.
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individuals abstinent $\geqslant$2 weeks (Melis *et al*, 2005), with higher relapse risk in those with the lowest transmission (Martinez *et al*, 2011; Wang *et al*, 2012). This evidence has prompted the view that low D_2 is a useful biomarker for relapse risk, and that increasing DA transmission to ameliorate a hypo-active DA system may improve treatment success; however, this approach has yet to prove clinically efficacious.

At the same time, a novel line of research focused on the D_3 DA receptor—a member of the D_2-like family with unique features relevant to addiction (Sokoloff *et al*, 2001)—points to D_3 as a potential new treatment target. The anatomical distribution of this receptor overlaps with key addiction neurocircuitry, including midbrain projections to limbic forebrain regions (amygdala, bed nucleus of stria terminalis, nucleus accumbens shell) mediating motivation, inhibitory control, emotion, and learning (Sokoloff *et al*, 2001), which can modulate activity in cortical loops involved in motivation, salience attribution, conditioned responses, and compulsive behavior (Cole *et al*, 2012). Moreover, preclinical studies demonstrate D_3 upregulation following dopaminergic drug regimens, with corresponding behavioral sensitization, increased motivation, and drug seeking (LeFoll *et al*, 2005; Sokoloff *et al*, 2001). In line with these data, *post-mortem* human studies have reported elevated D_3 levels in striatum of cocaine overdose fatalities (Segal *et al*, 1997; Staley and Mash, 1996), and neuroimaging findings from our laboratory point to similar effects *in vivo*, showing D_3 elevation in D_3-rich regions (substantia nigra (SN), globus pallidus (GP), ventral pallidum (VP)) in methamphetamine dependence (MD), which (in SN) relates to drug wanting (Boileau *et al*, 2012b). D_3 contributions may even generalize to behavioral addictions, as we have also reported a relationship between SN D_3 and symptom severity in pathological gambling (Boileau *et al*, 2012a).

Importantly, D_3 receptor antagonists have shown promise in reversing acquisition and expression of drug-seeking and cue-induced relapse in animals (Heidbreder and Newman, 2010; Heidbreder *et al*, 2005), and clinical trials of nicotine dependence and food reward are translating this idea to humans (Mugnaini *et al*, 2013; Nathan *et al*, 2012). Taken together, the evidence suggests that activity at the D_3 receptor is pathologically increased in addiction (in contrast to D_2 downregulation), and, by extension, that a therapeutic strategy aimed at normalizing low transmission at D_2 may undermine clinical outcome by exacerbating already-exaggerated D_3 processes.

Positron emission tomography (PET) neuroimaging provides a non-invasive method to investigate DA receptors in human brain *in vivo*, but the evidence regarding D_3 is sparse, owing to a lack (until recently) of suitable radioligands. Most DA PET studies have used [^{11}C]raclopride to measure $D_{2/3}$ receptors in striatum, but this ligand binds non-selectively to D_2 and D_3, so that binding primarily reflects D_2, given the relative predominance of this receptor over D_3. [^{11}C]-(+)-propyl-hexahydro-naphtho-oxazin ([^{11}C]-(+)-PHNO; Wilson *et al*, 2005)), on the other hand, is a recently developed $D_{2/3}$ radioligand with preferential affinity for D_3 *in vivo*, allowing for measurement of this receptor in D_3-rich areas (whereas in D_3-devoid regions, signal reflects D_2; Tziortzi *et al*, 2011). Using [^{11}C]-(+)-PHNO and [^{11}C]raclopride in the same individuals, therefore, allows for a more detailed understanding of dopaminergic targets for pharmacological strategies.

In a previous study using [^{11}C]-(+)-PHNO, we reported higher D_3 receptor availability in the SN and marginally lower $D_{2/3}$ in the striatum of methamphetamine-dependent polydrug-abusing subjects compared with controls (Boileau *et al*, 2012b). The present study, conducted in an independent sample of minimally comorbid cocaine-dependent subjects (many actively using, but all abstinent with negative urine screens on scan day), tested the hypothesis that [^{11}C]-(+)-PHNO binding to D_3 would be elevated in CD and related to the addiction behavioral phenotype, whereas [^{11}C]raclopride binding was expected to be low.

MATERIALS AND METHODS

Subjects

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board and complied with ethical standards of the Helsinki Declaration (1975; updated 1989). Fifteen CD volunteers were recruited from a community program ('Getting Started,' an ambulatory twice-weekly psychoeducation and support program aimed at engaging clients and increasing readiness for intensive treatment, but itself providing no therapeutic intervention). For comparison, 15 healthy control (HC) volunteers were recruited from the community via flyers and advertisements. After complete description of the study, all volunteers gave written informed consent.

Subjects in the CD group were required to (1) self-report cocaine as the primary currently abused drug; (2) meet DSM-IV criteria for CD (as per SCID); and (3) provide a scalp hair sample and/or urine screen positive for cocaine/metabolites. HC subjects were required to have no history of cocaine use, and no more than five lifetime occasions of recreational drug use (except cannabis). All subjects were required to be 18–55 years old and meet the following criteria: (1) no current Axis I disorder (as per SCID), except CD or substance-induced mood disorder in CD; (2) no lifetime history of alcohol or substance dependence (except caffeine or nicotine, and cocaine in CD; cannabis abuse was not exclusionary); (3) no medical conditions likely to affect the brain; (4) no current use of antidepressant or psychotropic medications; and (5) no PET or magnetic resonance image (MRI) contraindications (eg, radiation exposure exceeding guidelines, metal implants, pregnancy/lactation).

Study Procedure and Outcome Measures

Screening session. Screening for inclusion/exclusion consisted of the SCID for DSM-IV, detailed medical history, and questionnaires assessing alcohol and drug use patterns and depressive symptomatology (Beck Depression Inventory (Beck and Steer, 1984), Snaith–Hamilton Pleasure Scale (Snaith *et al*, 1995)). For CD subjects, we additionally assessed drug use history, severity, and toxicology (via scalp hair (US Drug Testing Laboratory) to assess drug use in recent months, and a comprehensive urine screen (9-Drug

Test Panel, BTNX; broad spectrum, GC–MS) to test for prescription and illicit drugs.

Behavioral battery. Subjects underwent a brief battery of tests aimed at characterizing the CD behavioral phenotype. Tests included: the Halsted-Reitan Trail-Making Task (Reitan, 1955) to assess attention and set-shifting, Continuous Performance (Conners, 1994) and Go/No-Go tasks to assess attention and inhibitory control, the Balloon Analogue Risk Task (Lejuez *et al*, 2002) and Game of Dice Task (Brand *et al*, 2005) to assess risky decision making, the Kirby Delay Discounting Task (Kirby, 2000) to assess temporal discounting of reward, the Wisconsin Card Sorting Test to assess perseverative responding, the Barratt Impulsiveness Scale (Patton *et al*, 1995) and a Finger Tapping task to assess psychomotor function.

Neuroimaging session. Neuroimaging consisted of two PET scans, [^{11}C]-(+)-PHNO and [^{11}C]raclopride, conducted on the same day ([^{11}C]raclopride first), along with a MRI to help delineate regions of interest (ROI) in PET analyses. CD subjects were not admitted to an inpatient unit for withdrawal management, but were asked to discontinue all drug use (except cigarette smoking) at least 10 days before the PET session. Compliance was assessed with a urine drug screen on scan day, requiring a negative result for cocaine. To avoid nicotine withdrawal on PET day, cigarette smokers were advised to smoke to satiation before each scan (up until 1 h before each injection). At the beginning of the PET day, CD subjects completed assessments of cocaine craving and withdrawal (Cocaine Selective Severity Assessment (Kampman *et al*, 1998), Cocaine Urge Questionnaire (adapted from Bohn *et al* (1995), Desire for Cocaine Scale (adapted from James *et al* (2004)).

[^{11}C]-(+)-PHNO and [^{11}C]raclopride synthesis and image acquisition protocols are described in detail elsewhere (Graff-Guerrero *et al*, 2008). PET scanning was performed on a high-resolution PET camera system (CPS-HRRT, Siemens Medical Imaging, Knoxville, TN), using a custom fitted thermoplastic mask to reduce head movement (TruScan Imaging, Annapolis, USA). Following a 15-min transmission scan, a bolus injection of [^{11}C]-(+)-PHNO or [^{11}C]raclopride was given into an antecubital vein ([^{11}C]-(+)-PHNO mean dose = 9.04 mCi, specific activity = 1345.73 mCi/μmole, mass = 2.21 μg; [^{11}C]raclopride mean dose = 9.2 mCi, specific activity = 1862.31 mCi/μmole, mass = 1.9 μg; no group differences). [^{11}C]raclopride data were acquired for 60 min, and [^{11}C]-(+)-PHNO data for 90 min, as ~80 min of data acquisition have been shown to yield stable binding estimates (Ginovart *et al*, 2007). The MRI (Signa 1.5T, General Electric Medical Systems, Milwaukee, WI) consisted of a standard proton density sequence acquired over the whole brain.

Image Analysis and Statistical Approach

Delineation of ROIs including whole striatum, sensorimotor striatum (SMST), associative striatum (AST), limbic striatum (LST), VP, GP, SN, and cerebellar cortex (excluding vermis and lobules IX and X) is described in Martinez *et al* (2003) and Boileau *et al* (2012b). SN, VP, and GP ROIs were included because they are rich in D_3 and a high proportion of [^{11}C]-(+)-PHNO signal (100%, 75%, and 65%, respectively) reflects D_3 binding (Tziortzi *et al*, 2011). [^{11}C]-(+)-PHNO and [^{11}C]raclopride time activity curves were obtained from dynamic data using ROMI (Rusjan *et al*, 2006). Specific binding (BP_{ND}) in ROIs was estimated using the simplified reference tissue method (Lammertsma and Hume, 1996), implemented in PMOD (v 2.8.5; PMOD Technologies Ltd, Zurich, Switzerland); this method is most suitable for simplified analysis, as it minimizes BP_{ND} under-estimation and inter-individual variability (Ginovart *et al*, 2007).

Group comparisons of [^{11}C]-(+)-PHNO and [^{11}C]raclopride BP_{ND} were performed using repeated-measures ANOVAs or ANCOVAs (with ROIs as the repeated measure and CD *vs* HC as the between-groups factor), using Greenhouse-Geisser sphericity corrections when indicated. Regional group differences were examined for significance using *t*-tests, Bonferroni corrected for planned comparisons. Relationships between PET measures and continuous behavioral variables were analyzed with Pearson product moment correlations and Spearman's Rank tests for categorical data.

RESULTS

Subject Characteristics

Subject characteristics and demographic information are described in Table 1. CD and HC groups were matched for age, sex, ethnicity, body mass, and cigarette and alcohol use, but CD subjects had lower education levels and consumed more cannabis. CD also reported more depressive symptomatology and anhedonia than HC, and two CD subjects met criteria for a current substance-induced depressive episode at study entry. Control analyses for these factors (see below) confirmed that they did not influence the results.

Cocaine use characteristics in the CD group are described in Table 2. During the screening/intake session, 10 out of 15 CD provided a cocaine-positive urine test, confirming recent cocaine use, and hair analysis confirmed cocaine use in the remaining five subjects (and all other subjects with scalp hair, 12 out of 15). In a minority of the sample, toxicology screening (performed during the screening/intake session) also revealed (limited) use of other drugs: opiate metabolites in hair (3 out of 15) and urine (1 out of 15), cannabis in urine (4 out of 15), and benzodiazepine in hair (1 out of 15). Although subjects were asked to remain at least 10 days abstinent between screening and PET visits, two subjects only achieved 7 days abstinence between visits; however, their urine screens still met the requirement of testing negative for cocaine on scan day.

PET Results

All subjects completed both PET scans, but one CD [^{11}C]raclopride scan was lost due to radiochemistry problems. [^{11}C]raclopride and [^{11}C]-(+)-PHNO BP_{ND} correlated (controlling for group) in SMST (where both tracers are thought to predominantly measure D_2; $r(26) = 0.45$,

Table 1 Participant Characteristics

	Cocaine dependent ($N=15$)		Healthy control ($N=15$)		Group comparison (P)
Sex (male/female)	13/2		13/2		1.00[a]
Ethnicity (Caucasian/Asian/Hispanic/African)	12/0/1/2		10/3/1/1		0.32[a]
Cigarette smokers	6		5		0.50[a]
	Mean	*SD*	*Mean*	*SD*	
Years of age	41.8	8.7	42.4	11.2	0.87
Years of education	12.9	2.9	15.7	2.7	**0.01**
Body mass index (BMI)	25.3	2.5	25.6	2.6	0.75
Fagerstrom test for nicotine Dependence (smokers only)	5.3	2.7	6.2	2.1	0.57
Cannabis use occasions (last 30 days)	7.3	9.0	0	0	**<0.01**
Alcoholic drinks (last 30 days)	5.1	6.1	10.5	15.2	0.22
Beck depression inventory	15.0	11.1	2.2	2.5	**<0.01**
Snaith-Hamilton pleasure scale	10.8	2.9	13.8	1.3	**<0.01**

[a]Chi-square test.
Bold values indicate statistically significant ($p<0.05$).

$p=0.02$), but not AST or LST (both $p>0.25$). Neither ROI volumes nor standard uptake values for cerebellum differed significantly between groups (all $p>0.19$).

A repeated-measures ANOVA investigating regional differences in [^{11}C]-(+)-PHNO BP_{ND} between groups yielded a significant Group × ROI interaction ($F(2.7,70.8)=3.63$, $p=0.02$). Pairwise contrasts revealed that CD subjects had higher [^{11}C]-(+)-PHNO BP_{ND} in the SN than HC (+24%, $p=0.06$, Cohen's $d=0.71$; Figure 1a). Group differences were not statistically significant in other D_3-rich regions (VP: +5%; GP: −9%) or the striatum (−4 to 5%).

A second ANOVA investigating [^{11}C]raclopride BP_{ND} found lower binding in CD than HC (Figure 1b); this effect was not region dependent, but occurred in the striatum as a whole (−11%; $F(1,27)=4.32$, $p<0.05$). Group differences were not statistically significant in individual striatal subdivisions (AST: −11%, $p=0.20$, Cohen's $d=0.49$; LST: −10%, $p=0.14$, Cohen's $d=0.57$; SMST: −11%, $p=0.06$; Cohen's $d=0.72$).

Several follow-up tests were performed to rule out potentially confounding factors. In all ROIs, PET measures (BP_{ND}) did not differ between CD subjects who did and did not ($n=5$) provide a cocaine-positive urine at study entry (indicating recent use *vs* abstinence; all $p>0.25$), between those who did ($n=4$) and did not currently abuse cannabis (all $p>0.25$), or between those who did ($n=2$) and did not experience current drug-induced depressive symptoms (all $p>0.1$). Further, removing abstinent subjects and subjects co-morbid for cannabis/depression from analyses did not affect the ROI × Group effect in [^{11}C]-(+)-PHNO BP_{ND} (recency: $F(2.6,54.6)=3.50$, $p=0.03$; cannabis: $F(2.7,58.7)=4.06$, $p=0.01$; depression: $F(2.9,69.4)=2.56$, $p=0.06$), or the group difference in [^{11}C]raclopride BP_{ND} (recency: $F(1,23)=5.50$, $p=0.03$; cannabis: $F(1,22)=3.02$, $p=0.10$; depression: $F(1,23)=5.50$, $p=0.03$). PET findings did not correlate with addiction severity measures, and testing whether time from last use influenced binding showed no correlation between days abstinent and [^{11}C]raclopride ($0.06<r<0.30$, all $p>0.29$) or [^{11}C]-(+)-PHNO ($-0.32<r<0.07$, all $p>0.24$) BP_{ND} in any of the ROIs.

Table 2 Cocaine Use Patterns and Severity in CD Sample ($N=15$)

	Mean	SD	Range
Years of cocaine use	19.5	7.4	6–32
Days since last use	50.1	64.4	7–240
Average days per week used	4.7	2.2	1–7
Average amount per week (g)	11.7	10.3	1–35
Number of days used in last 30	10.3	10.8	0–28
Estimated dose per occasion (g)	2.4	1.8	0.5–7
Route of administration (smoke/nasal/i.v.)	14/5/4		
Number of attempts to quit	7.9	13.0	0–50
Readiness to change scale[a] ($_{max}$ score 8)	6.4	1.7	3–8

[a]$N=13$.

Behavioral Battery and Relationships with PET Measures

As the behavioral battery was aimed at characterizing the CD phenotype, all CD subjects but not all HC subjects, completed the battery, so that group comparisons are not presented here (means are available in Supplementary Table 1). Across CD subjects, assessing correlations between [^{11}C]-(+)-PHNO BP_{ND} in SN (index of brain D_3 levels) and task performance identified a relationship with number of risky choices on the Game of Dice Task ($r=0.51$, $p=0.05$), and with commission errors on the Continuous Performance Task ($r=0.52$, $p=0.05$; Figure 2).

DISCUSSION

The present study supports our predictions of high D_3 (compared with low D_2) receptor availability in CD. The findings add to a small but growing human neuroimaging literature pointing to D_3 as a novel biomarker and potential treatment target, and are also in line with the extensive body of research showing downregulation of D_2. In addition to

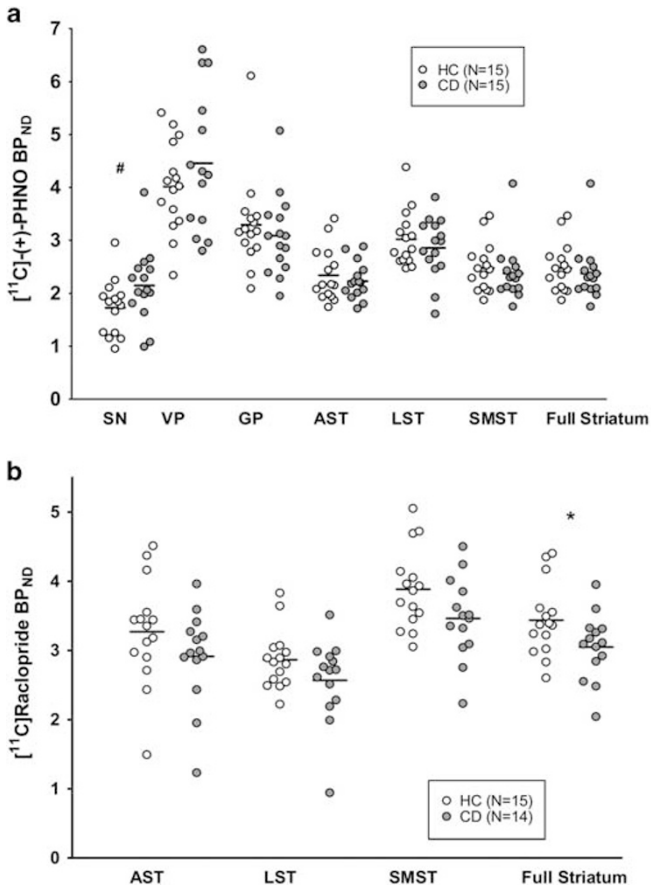

Figure 1 Individual binding potential (BP_{ND}) values across regions of interest for each PET tracer in cocaine-dependent (CD) and healthy control (HC) subjects. (a) [^{11}C]-(+)-PHNO: as predicted, CD showed higher [^{11}C]-(+)-PHNO BP_{ND} than HC in the substantia nigra (SN; $^{\#}p = 0.06$). Ventral pallidum (VP), another D_3-rich region, also showed this pattern, but this effect was not statistically significant. No group differences were found in globus pallidus (GP), striatal subregions, or whole striatum. (b) [^{11}C]Raclopride: CD showed lower [^{11}C]raclopride BP_{ND} in the striatum as a whole ($^{*}p < 0.05$), but not individual subregions. AST, associative striatum; LST, limbic striatum; SMST, sensorimotor striatum.

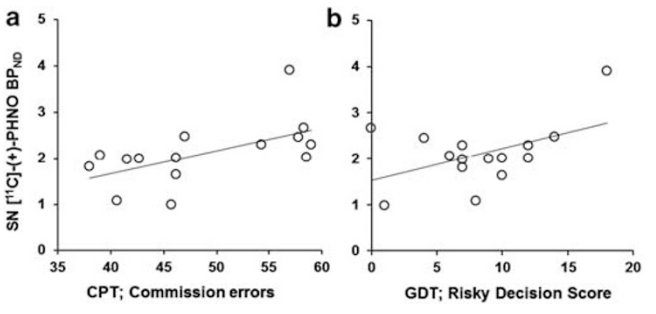

Figure 2 Correlations across cocaine-dependent (CD) subjects ($N = 15$) illustrating relationships between [^{11}C]-(+)-PHNO BP_{ND} in substantia nigra (SN; index of brain D_3 levels) and (a) commission errors during the Continuous Performance Task (CPT), indicative of behavioral impulsivity; (b) number of risky choices during the Game of Dice Task (GDT), indicative of risky decision making.

extending our understanding of neurobiological and behavioral components of addiction, our findings may have important clinical implications: pharmacological strategies aimed at normalizing D_2 deficiencies may inadvertently exacerbate an exaggerated D_3 response, and D_3 antagonism may provide a viable alternative or adjunct.

The main finding of the study is that brain D_3 levels may be heightened in CD (*vs* HC), as indicated by higher [^{11}C]-(+)-PHNO binding in the SN (where 100% of signal reflects D_3; Tziortzi *et al*, 2011). The pattern is reiterated in the D_3-rich VP (where 75% of signal reflects D_3), but this difference was not statistically significant. Importantly, we also identified a relationship between SN [^{11}C]-(+)-PHNO binding and impulsivity/risky decision making, suggesting that D_3 is relevant to behavioral contributors to addiction. The finding is consistent with *post-mortem* data showing elevated D_3 levels in cocaine abuse (Segal *et al*, 1997; Staley and Mash, 1996), as well as a sizeable body of preclinical data, which, although not entirely consistent (Richtand *et al*, 2001), mainly demonstrates D_3 upregulation and related locomotor sensitization, drug cue reactivity, drug-seeking, and motivation to take drugs (LeFoll *et al*, 2005; Sokoloff *et al*, 2001). Our finding of 24% higher SN D_3 binding in CD *vs* HC is remarkably in line with a preliminary report that reported 22% greater [^{11}C]-(+)-PHNO BP_{ND} in SN (Matuskey *et al*, 2011). It also echoes our previous neuroimaging studies of MD (Boileau *et al*, 2012b), where we showed heightened SN [^{11}C]-(+)-PHNO binding that related to self-reported drug wanting, and of pathological gambling (Boileau *et al*, 2012a), where SN [^{11}C]-(+)-PHNO binding related to impulsiveness and gambling severity. It should be noted that the magnitude of SN D_3 elevation (relative to respective HC groups) differed between our CD and MD samples (Boileau *et al*, 2012b; 24% here *vs* 46% in MD), which could be explained by age differences between samples (mean 42 years here *vs* 28 years in MD), or differences between the two drugs (eg, mechanism of action, duration of effects, route of administration).

An interesting (although controversial) point is that in our previous report, we calculated *relative* D_3 levels, ie, individual D_3-to-D_2 ratios estimated as [^{11}C]-(+)-PHNO BP_{ND} in SN (100% of signal thought to reflect D_3) to [^{11}C]-(+)-PHNO BP_{ND} in dorsal striatum (devoid of D_3 so signal is thought to reflect D_2 (Tziortzi *et al*, 2011)), and showed that this measure was greater in stimulant dependent than control subjects (Boileau *et al*, 2012b). Here, too, we find that CD subjects had a 32% greater D_3-to-D_2 binding fraction than HC ($p = 0.01$), possibly providing further evidence for heightened D3 receptor expression (outside of SN). However, the biological relevance of this index is under debate, and the implications of this finding therefore highly speculative.

Together, [^{11}C]-(+)-PHNO PET studies suggest that D_3 elevation may be a consistent and predictive biomarker for addiction, with potential use for clinical innovation. Indeed, the clinical utility of targeting D_3 in addiction treatment is currently under intensive investigation: Preclinical studies with highly selective D_3 receptor antagonists have observed attenuation of drug-seeking, self-administration, and cue- and stress-induced reinstatement in animal models of addiction (Heidbreder and Newman, 2010; Heidbreder *et al*, 2005; LeFoll *et al*, 2007), and in humans, clinical trials have reported D_3 antagonist effects on food reward and nicotine craving in smokers (Mugnaini *et al*, 2013;

Nathan *et al*, 2012), supporting the potential clinical efficacy of D_3 antagonism.

Mechanisms underlying the paradoxical upregulation of D_3 (contrasting with D_2 downregulation) and its downstream effects are still unclear (see Boileau *et al* (2012b) for discussion). Briefly, upregulation is thought to result from repeated stimulation of D_1 receptors during cocaine use, leading to release of brain-derived neurotrophic factor (BDNF), which is, in turn, linked to increased D_3 expression (Guillin *et al*, 2001), and is elevated in CD (D'Sa *et al*, 2011). Although D_3 receptors are expressed on all DA neurons in the SN, their physiological role there remains under debate (Davila *et al*, 2003); moreover, D_3 receptors are both reciprocal autoreceptors and heteroreceptors (Sokoloff *et al*, 1990), and it is unknown whether the D_3 upregulation occurs on dopaminergic or other (eg, GABAergic) neurons (Bordet *et al*, 1997; Guillin *et al*, 2001). Increased transmission at D_3 appears to modify limbic outputs and functional connectivity between orbitofrontal cortex and networks involved in cognitive control and goal-directed behavior (Cole *et al*, 2012), thereby modulating motivation to use drugs. Future studies will be important in determining the effects of D_3 antagonism on these outcomes.

A secondary finding of the study is that [^{11}C]raclopride binding in striatum was lower in CD than HC subjects, suggesting low D_2 availability (although [^{11}C]raclopride cannot distinguish between D_2 and D_3). This effect was statistically significant only when considering the striatum as a whole, but the 10–11% difference we observed in individual subregions is within the range of what has been previously reported (-6–17%; Martinez *et al*, 2004, 2011; Narendran *et al*, 2011), and effect size estimates are in the medium range, suggesting that our failure to find significant group differences in individual subregions may have been due to low statistical power. The pattern of low $D_{2/3}$ signal is consistent with preclinical and other PET imaging evidence, supporting the notion that low D_2 availability is a hallmark of addiction across a range of substances, reflecting a hypodopaminergic state that can contribute to relapse to drug use (Martinez *et al*, 2011; Wang *et al*, 2012). Our finding therefore confirms that our CD sample has comparable dopaminergic characteristics to previous study samples (Martinez *et al*, 2011; Wang *et al*, 2012), suggesting that the D_3 finding reflects a previously unobserved process rather than sample differences.

One question raised by our use of two radiotracers is why low striatal $D_{2/3}$ levels were detected with [^{11}C]raclopride but not [^{11}C]-(+)-PHNO, including in dorsal striatum, where [^{11}C]-(+)-PHNO binding is thought to predominantly reflect D_2. Although low statistical power is a possibility, there are also several biological explanations. It is possible that our failure to detect group differences with [^{11}C]-(+)-PHNO reflects an ectopic upregulation of D_3 in dorsal stratum, which has been observed in animals (Bordet *et al*, 1997) and would mask lowered D_2 receptor binding. Alternatively, as an agonist radiotracer, [^{11}C]-(+)-PHNO is more sensitive to endogenous DA levels (Shotbolt *et al*, 2012; Willeit *et al*, 2008), so that low tonic DA levels in CD subjects could have led to more available receptor sites, masking lowered D_2 levels. Last, [^{11}C]-(+)-PHNO only labels receptors in the G-protein-coupled high-affinity state (D_{2HIGH}), and as animal literature suggests that $D2_{HIGH}$ proportion is relevant to addiction (Seeman *et al*, 2007), it is possible that our failure to detect group differences with [^{11}C]-(+)-PHNO reflects a higher D_{2HIGH} fraction in CD. We tested for this possibility by comparing [^{11}C]-(+)-PHNO binding to [^{11}C]raclopride (which binds $D2_{HIGH}$ and $D2_{LOW}$ indiscriminately) in SMST (presumably devoid of D_3). This analysis is highly theoretical and additionally complicated by potential ectopic upregulation of D_3, but revealed a (statistically non-significant, $p=0.18$) 14% greater D_{2HIGH} fraction in CD than HC. This failure to find an effect is in line with a previous study using [^{11}C]NPA (Narendran *et al*, 2011), but nonetheless could have contributed to the discrepancy between the [^{11}C]raclopride and [^{11}C]-(+)-PHNO findings in the present report. However, it is not possible to distinguish between these possibilities here.

Several limitations of the study should be noted. The first concerns the study sample, which included CD subjects with a wide range of abstinence durations (which was not supervised) and in some cases co-morbid drug use or mood symptoms. Our inclusion of 10 out of 15 actively using (albeit urine-negative) CD subjects distinguishes our studies from previous reports, which imposed a monitored/supervised abstinence period of at least 14 days (eg, Martinez *et al*, 2004, 2011; Wang *et al*, 2012). We conducted explicit follow-up analyses to test for differences between actively using and abstinent subjects in our sample, and found no modulating effects of abstinence (or mood/comorbid drug use), although these had low power and require confirmation in larger samples. At the same time, we excluded other co-morbidities to strengthen our ability to attribute effects to CD, limiting generalizability to the broader CD population. Another set of limitations concerns the radiotracer [^{11}C]-(+)-PHNO, including previously noted limitations (see Rabiner and Laruelle (2010)) such as scanning at non-tracer doses (Shotbolt *et al*, 2012), using a reference tissue with known specific binding (cerebellum (Murray *et al*, 1994)—although vermis and lobules 9 and 10 were excluded), and long wash-out times in D_3-rich regions that may limit adequate quantification of receptor binding (Willeit *et al*, 2006). In addition, as SN is the only region where 100% of [^{11}C]-(+)-PHNO signal reflects D_3 binding, with all other ROIs reflecting some mixture of D_2 and D_3 (Tziortzi *et al*, 2011), interpretation of findings is anatomically limited. Finally, as in all cross-sectional studies of drug abuse, we acknowledge that group differences could have predated drug use, and that longitudinal designs must be employed to distinguish cause and effect.

Despite these limitations, the present study is consistent with preclinical and *post-mortem* data, replicates our previous finding of heightened SN D_3 levels in psychostimulant addiction, and is the first to address the combination of D_3 and D_2 receptor states in CD. D_3 upregulation is emerging as a biomarker of the addicted state, and the newly gained ability to examine pharmacological features in living humans with [^{11}C]-(+)-PHNO holds promise for the guidance of clinical investigations, and the development of novel D_3 antagonist strategies in the treatment of addictions.

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