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ORIGINAL ARTICLE

In vivo evidence for greater amphetamine-induced dopamine release in pathological gambling: a positron emission tomography study with [^{11}C]-(+)-PHNO

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Drug addiction has been associated with deficits in mesostriatal dopamine (DA) function, but whether this state extends to behavioral addictions such as pathological gambling (PG) is unclear. Here we used positron emission tomography and the D_3 receptor-preferring radioligand [^{11}C]-(+)-PHNO during a dual-scan protocol to investigate DA release in response to oral amphetamine in pathological gamblers ($n=12$) and healthy controls ($n=11$). In contrast with human neuroimaging findings in drug addiction, we report the first evidence that PG is associated with greater DA release in dorsal striatum (54–63% greater [^{11}C]-(+)-PHNO displacement) than controls. Importantly, dopaminergic response to amphetamine in gamblers was positively predicted by D_3 receptor levels (measured in substantia nigra), and related to gambling severity, allowing for construction of a mechanistic model that could help explain DA contributions to PG. Our results are consistent with a hyperdopaminergic state in PG, and support the hypothesis that dopaminergic sensitization involving D_3-related mechanisms might contribute to the pathophysiology of behavioral addictions.

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INTRODUCTION

Pathological gambling (PG) is a psychiatric disorder that afflicts 0.2–5.3% of the population worldwide.[1] Long characterized as a behavioral addiction, PG has now been formally re-classified with substance dependence in the fifth edition of the psychiatric Diagnostic and Statistical Manual, reflecting extensive overlap in symptom profiles and shared risk.[2–4] Despite their phenomenological similarities, common neurobiological underpinnings are only beginning to be explored.

Positron emission tomography (PET) and single-photon emission computerized tomography studies with dopamine (DA) $D_{2/3}$ receptor ligands (such as [^{11}C]raclopride, [^{18}F]Fallypride and [^{123}]IBZM) in substance-dependent humans have consistently shown that substance use disorders including cocaine,[5,6] methamphetamine,[7] heroin[8] and alcohol[9] dependence are associated with a hypodopaminergic state indexed experimentally by a blunted DA response to an amphetamine or methylphenidate challenge. The significance of this deficit in DA transmission and whether it is the result of drug use or a predating vulnerability has not been fully elucidated. However, low levels of DA in substance use disorders have been linked to core clinical features and maladaptive behaviors, which are also shared by behavioral addictions and, as such, could also be a feature of PG. For example, a blunted dopaminergic response to amphetamine has been associated with compulsive use (choice to self-administer cocaine in a laboratory model[6]), relapse (in a treatment setting[7,10]) and to craving.[5] Whether disrupted DA transmission can explain these key features of PG, including compulsive pursuit of reward, is unknown.

In sharp contrast with the human *in vivo* neuroimaging literature, decades of preclinical data in animal models of addiction have shown that compulsive drug (or reward) seeking is associated with greater DA release to amphetamine. Specifically, in rodents, repeated intermittent exposure to drugs of abuse (or stress) is associated with a greater locomotor response and DA release upon re-exposure to drug. This phenomenon, known as *sensitization,* is a critical form of neuroplasticity believed to enhance the salience of a reward and thereby promote seeking,[11] and which has been associated with an upregulation of DA D_3 receptors.[12] With regards to the last point, a growing body of work, which includes our own finding of increased D_3 receptor levels in substantia nigra (SN) of stimulant users,[13,14] suggests that D_3 upregulation could be a feature of addiction and/or related to the addiction phenotype. In the current study, we sought to investigate whether level of this receptor was related to the status of DA transmission.

One of the reasons for the discrepancy between human and preclinical literature (reviewed elsewhere[15,16]) could be that chronic exposure to drugs in substance-addicted individuals (vs intermittent exposure in rodent sensitization literature) results in an enduring depletion of DA, caused by presynaptic DA cell injury (for example, see refs 17–19). Indirect support for this possibility

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comes from a PET study showing that, in contrast to drug-addicted individuals, healthy volunteers exposed to an intermittent low-dose amphetamine regimen, exhibit significantly greater amphetamine-induced striatal DA release (interpreted as sensitization) associated with greater psychomotor effects of the drug, which was predicted by trait impulsiveness.[20] Notably, the 'sensitizing' regimen in that study was not associated with a change in DA $D_{2/3}$ receptor levels, in contrast with the consistent finding of low striatal $D_{2/3}$ receptor levels in addiction.

Investigations of DA mechanisms in PG have shown ventral striatal DA release to large rewards during gambling tasks, which related to symptom severity, self-reported 'high' and self-reported 'excitement'.[21–24] Similarly, patients with Parkinson's disease (PD) with medication-induced PG displayed significantly greater DA release during a gambling-like task than PD patients without PG.[25] Of note, we and others have recently reported that in PG, $D_{2/3}$ receptor levels are also within normal range in all functional regions of the striatum and midbrain.[26,27] Together, these results indirectly support the possibility that PG without co-morbid drug abuse may not be associated with a 'blunted' DA transmission but may instead be associated with an increased DA response, which could be linked to compulsive pursuit of reward.

The present study tested the hypothesis in stimulant-naive men diagnosed with PG and healthy non-PG controls that PG, a behavioral addiction, is associated with greater DA release to amphetamine. DA release was indexed by displacement of the D_3 preferring radioligand, [^{11}C]-(+)-propyl-hexahydro-naphtho-oxazin ([^{11}C]-(+)-PHNO[28]) following d-amphetamine (0.4 mg kg^{-1}, p.o.).

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. Volunteers (13 non treatment seeking males with PG and 12 healthy control (HC) males matched for age, education and cigarette smoking status) were recruited by advertisements and gave written informed consent. Inclusion/exclusion criteria have been described in a previous report.[27] Briefly, PG subjects were required to meet Diagnostic and Statistical Manual of Mental Disorder (DSM IV) criteria for PG and demonstrate current clinically relevant levels of PG severity as per a DSM IV based questionnaire (DSM IV Q)[29] and the South Oaks Gambling Scale (SOGS).[30] Conversely, HC subjects were required to score 0 on the DSM IV Q and SOGS. In addition, all subjects met the following criteria: (1) no medications or significant medical conditions; (2) no past or present Axis I psychiatric diagnoses other than PG; (3) no clinically relevant depressive symptoms; and (4) negative urine drug screens (9 Drug Test Panel, BTNX Inc., Markham, ON, Canada) and blood alcohol concentration (J4X ALERT, Alcohol Countermeasures Inc., Mississauga, ON, Canada) on all study days. Current drug abuse/dependence was exclusionary for all subjects. Nicotine use of <20 cigarettes per day with a Fagerstrom Test of Nicotine Dependence[31] score <5; alcohol use of <12 alcoholic drinks per week and marijuana use of $\leqslant 1$ joint per month with tetrahydrocannabinol free urine screens and Drug Abuse Screening Test[32] scores <4 were allowed.

Study procedure

After initial screening, eligible subjects completed self report measures of trait characteristics, including the Gambler's Beliefs Questionnaire[33] and the Eysenck Impulsiveness Scale[34] based on previous use of this measure to characterize pathological gamblers.[35–37] Subjects also took part in a 15 min gambling session (see Supplementary Information; details and results have been published previously),[27] during which they reported on subjective experience via visual analog scales.

On a separate day, subjects, not blind to the procedure, completed two identical PET scans in fixed order (to avoid a conditioned response to the scanning environment)[38] with the radiotracer [^{11}C] (+) PHNO: once at baseline (no placebo capsule was administered), >5 h later, 2 h after receiving oral amphetamine (0.4 mg kg^{-1} Dexedrine® p.o.), a time at which behavioral effects of amphetamine are peaking and blood levels are on the ascending phase (peak blood levels are typically achieved 3–4 h.[39] This interval has been shown by our group to lead to robust changes in [^{11}C] (+) PHNO binding[40] (unpublished observations). Scan sessions included periodic assessments of mood (Profile of Mood States;[41]), drug effects (Addiction Research Center Inventory,[42]; Drug Effects Questionnaire) and visual analog scale ratings of energy and desire to gamble. Heart rate and blood pressure were monitored at 15 min intervals, and blood was drawn to measure plasma cortisol and amphetamine levels.

PET imaging

[^{11}C] (+) PHNO synthesis and image acquisition protocols on the CPS HRRT neuro PET camera system (Siemens Medical Imaging, Knoxville, TN, USA) are described in detail elsewhere.[13] Scans were initiated following bolus injection of [^{11}C] (+) PHNO (mean dose baseline: 9.20 ± 1.07 mCi, amphetamine: 8.82 ± 1.36 mCi; specific activity baseline: 1082 ± 239 mCi μmole^{-1}, amphetamine: 1041 ± 315 mCi μmole^{-1}; mean mass baseline: 2.16 ± 0.33 μg, amphetamine: 2.20 ± 0.40 μg; 90 min acquisition each). Raw data were reconstructed by filtered back projection. Spin echo proton density weight magnetic resonance images (Signa 1.5 T MRI scanner, General Electric Medical Systems, Milwaukee, WI, USA) were obtained for region of interest (ROI) delineation.

Data analysis

Physiological and Subjective Measures. Blood samples were analyzed for levels of plasma amphetamine and cortisol by the CAMH Clinical Laboratory. Statistical analysis of physiological and subjective measures was performed using repeated measures analysis of variances and *t* tests. Post amphetamine maximum or minimum values were used for measures with repeated time points.

PET Region of Interest Analysis. ROI delineation and time activity curve analyses were performed using ROMI (details in Rusjan *et al.*[43]). Functional sub compartments of the striatum[44] including the associative striatum (AST), limbic striatum (LST) and sensorimotor striatum (SMST) were chosen as ROIs. Delineation for the globus pallidus (GP; whole) and SN (an area where 100% of [^{11}C] (+) PHNO binding is associated with D_3 receptor levels)[45] is described elsewhere.[13]

[^{11}C] (+) PHNO specific binding (BP_{ND})[46] was estimated in each ROI using the simplified reference tissue method (SRTM),[47] with cerebellar cortex (excluding vermis and lobules IX and X) as reference region. Parameter estimation was performed using PMOD (Version 2.8.5; PMOD Technologies Ltd, Zurich, Switzerland). Percent change in BP_{ND} after amphetamine challenge was calculated. Group comparisons of these values were conducted using standard repeated measures analysis of variances and Kruskal Wallis *H* tests (for non parametric data). When appropriate, Least Significant Difference *t* tests, Bonferroni corrected, were applied.

Relationships between percent changes in BP_{ND} and changes in subjective/physiological measures were calculated using linear regression (with baseline scores as covariates). We also investigated the relationship between the magnitude of change in [^{11}C] (+) PHNO BP_{ND} and D_3 receptor levels (BP_{ND} in SN during baseline scan[45]) based on previous work indicating a link between D_3 upregulation, addiction[13] and dopaminergic sensitization.[12] Relationships to trait measures and behavior during the gambling episode were assessed using Pearson correlation.

PET Voxel wise Analysis. Voxel wise parameter estimation of [^{11}C] (+) PHNO BP_{ND} was implemented using RPM.[48] Normalized BP_{ND} maps were investigated to assess significant contrasts between conditions using paired *t* tests under SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). An implicit mask was applied removing voxels with BP_{ND} values <0.1. The threshold for significant clusters was set to a family wise error corrected $P<0.05$.

RESULTS

Subject characteristics and traits

Of the 13 PG and 12 HC subjects, one per group was lost during scanning, resulting in a final sample of 12 PG and 11 HC. Subject characteristics are summarized in Table 1 (see also previous publication[27]).

Physiological and subjective effects of amphetamine

Detailed physiological and subjective data are presented in Table 2. Overall amphetamine increased physiological outcome

Table 1. Subject demographic and trait characteristics

	HC (n 11) mean ± s.d.	*PG (n 12) mean ± s.d.*	*Group difference P value*
Age (years)	34.5 ± 11.5	32.7 ± 8.9	0.68
Education (years)	15.9 ± 0.5	15.4 ± 1.3	0.26
WAIS verbal score (out of 70)	59.7 ± 10.2	54.8 ± 8.7	0.22
BMI	26.6 ± 3.0	27.0 ± 3.9	0.79
Gambling characteristics			
SOGS score	0.0 ± 0.0	12.0 (3.5)	**<0.01**[a]
DSM IV PG score	0.0 ± 0.0	13.1 (5.3)	**<0.01**[a]
Money spent gambling ($CAD per week)	1.09 ± 1.76	207.92 ± 248.09	**<0.01**
Monthly income spent gambling (%)	0.2 ± 0.4	30.0 ± 21.1	**<0.01**
Trait characteristics			
EIS impulsiveness	3.2 ± 1.7	8.1 ± 4.9	**<0.01**
GBQ luck/ perseverance	76.6 ± 9.8	46.5 ± 14.2	**<0.01**
GBQ illusion of control	39.6 ± 13.6	20.8 ± 5.2	**<0.01**
Drug use characteristics			
Alcoholic drinks per week	1.6 ± 1.5	2.7 ± 3.5	0.37
ADS	1.0 ± 1.4	2.6 ± 2.4	0.07
Number of smokers (out of total)	3 (11)	3 (12)	0.64[b]
Cigarettes per day (in smokers)	4.7 ± 1.2	8.7 ± 5.0	0.25
FTND (in smokers)	1.3 ± 0.6	4.0 ± 0.0	**<0.01**
Cannabis users (last 12 months)	3 (11)	2 (12)	0.46
DAST	0.4 ± 0.7	0.1 ± 0.3	0.20
Depressive symptoms			
BDI (short form)	0.8 ± 1.5	5.8 ± 4.9	**<0.01**
Hamilton depression scale (HAM D)	0.7 ± 1.4	6.5 ± 5.3	**<0.01**

Abbreviations: ADS, Alcohol Dependence Scale; BDI, Beck depression inventory; BMI, body mass index; DAST, Drug Abuse Screening Test; DSMIV PG, Diagnostic and Statistical Manual of Mental Disorders Pathological gambling criteria; EIS, Eysenck Impulsiveness Scale; FTND, Fagerstrom test for nicotine dependence; GBQ, Gamblers' Beliefs Questionnaire (lower scores indicate more severe cognitive distortions); HC, healthy control; PG, pathological gambling; SOGS, South Oaks Gambling Scale; WAIS, Wechsler Adult Intelligence Scale verbal IQ. [a]Results of a one sample *t* test assessing difference from zero. [b]Results of a Chi square test. *P* values shown in bold denote a statistically significant difference between the groups.

(heart rate and blood pressure), plasma levels of amphetamine and cortisol, self-reported effects of amphetamine (Addiction Research Center Inventory, Drug Effects Questionnaire) and desire to gamble (visual analog scale) but this did not differ between groups. Desire to gamble was greater in PG vs HC, and increased with amphetamine (vs baseline), but there was no interaction; confidence in ability to refrain from gambling showed the opposite pattern (PG<HC and amphetamine<baseline). Cortisol levels were greater in HC relative to PG but there was no interaction.

PET measures

There was no significant effect of group or condition on [^{11}C]-(+)-PHNO mass injected, dose and specific activity (all $P>0.1$). Between-group differences in regional [^{11}C]-(+)-PHNO binding at baseline are detailed in another report.[27] Following amphetamine, [^{11}C]-(+)-PHNO binding was decreased, indicative of DA release, in all ROIs in both groups (ROI × condition $F(4, 84)=5.1$; $P=0.001$), ranging from a maximum of 36 ± 22% in SN to a minimum of 16 ± 7% in AST; percent change in [^{11}C]-(+)-PHNO BP_{ND} in other ROIs was 24 ± 11% in LST, 23 ± 11% in SMST and 18 ± 14% in GP (all $P<0.001$; Figure 1).

Comparing percent change in [^{11}C]-(+)-PHNO BP_{ND} between groups (Figures 1 and 2), we found greater percent change after amphetamine in PG than HC in striatal ROIs (ROI × group $F(3,19)=4.12$, $P=0.02$), in line with our hypothesis. This effect corresponded to a 63% ($P=0.01$) and 54% ($P=0.05$) difference in [^{11}C]-(+)-PHNO BP_{ND} change in PG relative to HC in AST and SMST, respectively, and marginally greater [^{11}C]-(+)-PHNO BP_{ND} change (63%, $P=0.07$) in GP. In LST and SN, [^{11}C]-(+)-PHNO BP_{ND} change was, respectively, 7% lower and 29% greater in PG than HC, but these effects did not reach statistical significance ($P>0.1$). Controlling for baseline BP_{ND} showed that baseline values were significant covariates in SMST ($P=0.03$) and GP ($P=0.03$), but did not change the group differences in these regions (AST $P=0.01$, SMST $P=0.03$, GP $P=0.03$). In addition, controlling for potential differences in depressive symptomatology (BDI, HAM-D scores) and alcohol use did not change the significant ROI × group interaction (all $P<0.05$).

Voxel-wise analysis confirmed ROI findings, showing that amphetamine significantly decreased [^{11}C]-(+)-PHNO BP_{ND} in striatum, and produced greater clusters of significance in PG than HC (Figure 2).

Relationships among measures

Percent change in [^{11}C]-(+)-PHNO BP_{ND} vs Reinforcement. Across PG, amphetamine-induced changes in [^{11}C]-(+)-PHNO BP_{ND} in dorsal striatum correlated with the average bet per spin and the number of spins during the slot machine game (bet per spin: AST $r(12)=0.83$, $P<0.01$; SMST $r(12)=0.60$, $P=0.04$; number of spins: anterior dorsal putamen: $r(12)=0.59$, $P=0.04$), indicating that PG with lower bet size and faster rate of play had greater striatal displacement after amphetamine. Percent change in [^{11}C]-(+)-PHNO BP_{ND} in striatum did not correlate with the subjective experience of the slot machine game (composite score, see Boileau *et al.*[27]), or self-reported 'desire to gamble'/'confidence in ability to refrain from gambling' following the gambling episode. However, percent change in [^{11}C]-(+)-PHNO BP_{ND} in LST showed a significant relationship with lower 'confidence in ability to refrain from gambling' measured after amphetamine ($F(2,11)=10.04$, $P<0.01$; $\beta=0.548$, $P=0.01$). Amphetamine-induced percent change in [^{11}C]-(+)-PHNO BP_{ND} did not significantly correlate with physiological measures or behavioral effects of amphetamine.

Percent change in [^{11}C]-(+)-PHNO BP_{ND} vs Trait Measures. Across PG, amphetamine-induced percent change in [^{11}C]-(+)-PHNO BP_{ND} in LST correlated at a trend level with SOGS scores ($r(12)=0.56$, $P=0.056$), suggesting that PG subjects with greater LST DA release tended to have greater gambling severity (Figure 3). Behavioral/trait predictors of gambling severity included self-reported impulsiveness (Eysenck Impulsiveness Scale correlation with SOGS: $r(12)=0.72$, $P<0.01$; with DSM-IV-Q: $r(12)=0.75$, $P<0.01$), and greater gambling-related cognitive distortions (Gambler's Beliefs Questionnaire luck/perseverance) correlation with SOGS (lower scores denote greater distortions): $r(12)=0.58$, $P=0.04$; with DSM-IV-Q: $r(12)=0.59$, $P=0.04$).

Relationship to Baseline Binding: Toward a model of dopaminergic mechanisms in PG. As D_3 receptor levels have been related to sensitization,[12] we tested for a relationship between baseline [^{11}C]-(+)-PHNO binding in SN and amphetamine-induced change in [^{11}C]-(+)-PHNO BP_{ND}. We found that percent change in LST

Table 2. Amphetamine induced changes in physiological and subjective measures

Measure	*PG (n 12) mean (s.d.)*		*HC (n 11) mean (s.d.)*		*Effect of group*	*Effect of Amph*	*Interaction*
	BL	*Amph*	*BL*	*Amph*			
Physiological							
Heart rate (BPM)	73.9 (8.3)	86.7 (9.6)	69.4 (9.3)	88.3 (8.5)	$F(1,18)<1$	**$F(1,18)$ 77.9****	$F(1,18)$ 2.8
Systolic BP (mm Hg)	120.3 (12.2)	146.6 (9.8)	125.2 (8.7)	151.3 (14.9)	$F(1,18)$ 1.1	**$F(1,18)$ 95.7****	$F(1,18)<1$
Diastolic BP (mm Hg)	70.5 (10.5)	116.7 (7.3)	67.6 (8.2)	122.2 (15.3)	$F(1,18)<1$	**$F(1,18)$ 567.2****	$F(1,18)$ 4.0
Plasma cortisol (nmol l ^{1})	240.7 (75.1)	454.0 (74.8)	339.4 (133.5)	521.6 (129.1)	**$F(1,20)$ 5.4***	**$F(1,20)$ 48.6****	$F(1,20)<1$
Plasma Amph (ng ml ^{1})	0.0 (0.0)	57.4 (15.1)	0.8 (1.5)	63.9 (16.9)	$F(1,20)$ 1.1	**$F(1,20)$ 310.9****	$F(1,20)<1$
VAS							
Desire to gamble	3.3 (3.0)	5.3 (3.2)	0.5 (1.0)	1.6 (2.4)	**$F(1,22)$ 13.5****	**$F(1,22)$ 8.90****	$F(1,22)<1$
Ability to refrain from gambling	7.1 (2.5)	5.7 (3.0)	9.8 (0.6)	9.4 (1.3)	**$F(1,22)$ 19.5****	**$F(1,22)$ 5.05***	$F(1,22)$ 1.5
ARCI							
Amph	4.4 (2.5)	7.7 (2.5)	3.0 (1.6)	7.0 (2.5)	$F(1,22)<1$	**$F(1,22)$ 71.9****	$F(1,22)$ 1.6
Euphoria	2.3 (2.1)	4.7 (2.7)	1.8 (2.0)	3.8 (2.2)	$F(1,22)<1$	**$F(1,22)$ 26.7****	$F(1,22)<1$
DEQ							
Drug liking		5.8 (4.4)		4.0 (5.0)	$t(22)<1$	NA	NA
Good effects		6.1 (3.2)		7.5 (2.4)	$t(22)$ 1.2	NA	NA
Bad effects		2.79 (3.0)		3.7 (3.2)	$t(22)<1$	NA	NA
Desire to take again		5.08 (3.4)		3.5 (4.1)	$t(22)<1$	NA	NA

Abbreviations: Amph, oral amphetamine (0.4 mg kg ^{1}) 2 h before [^{11}C] (+) PHNO injection; ARCI, Addiction Research Center Inventory; BL, baseline; BP, blood pressure; DEQ, Drug Effects Questionnaire; HC, healthy control; NA, not available; PG, pathological gambling; VAS, Visual Analog Scale. *$P<0.05$; **$P<0.01$. *F* values shown in bold denote statistically significant effects.

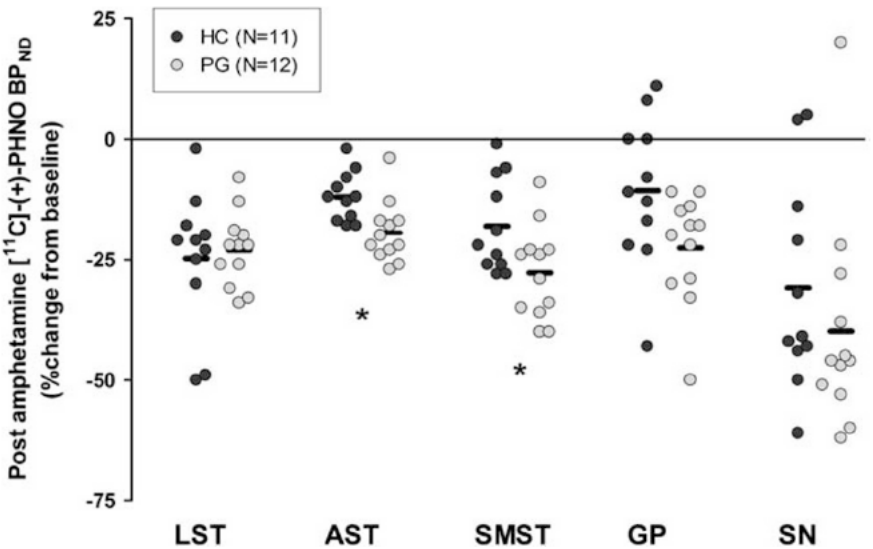

Figure 1. Scattergram showing lower [^{11}C]-(+)-PHNO BP_{ND} after oral amphetamine in striatum and midbrain in pathological gamblers (PGs) and healthy controls (HCs). Displacement of [^{11}C]-(+)-PHNO, indicating dopamine release, was observed in all regions of interest, and percent change in binding potential (BP_{ND}) was significantly more negative in PG (light circles) than HC (dark circles) in associative striatum (AST) and sensorimotor striatum (SMST); *$P<0.05$. The same pattern was observed in globus pallidus (GP) and substantia nigra (SN), but the difference did not reach statistical significance. LST, limbic striatum.

correlated with SN binding ($r(12) = 0.64$, $P<0.05$), which, in turn, predicted gambling severity and impulsiveness (see Boileau *et al.*[27]). This result indicated that higher D_3 receptor availability in SN was associated with greater DA release in LST, and predicted severity of the addiction (see Figure 3).

DISCUSSION

To our knowledge, this study provides the first neuroimaging evidence of an elevated DA response to a stimulant challenge in otherwise healthy, stimulant-naive PG, suggesting greater DA release (vs a 'blunted' response[5,6]) in putatively addicted individuals. These results indicate that despite their overlapping clinical features, dopaminergic mechanisms may contribute differentially to behavioral vs substance addictions, or that greater dopaminergic transmission is differentially discernible in behavioral addictions free of potential substance-specific confounds. In addition, the data support preclinical and recent human data pointing to a putative role for the D_3 receptor in the addiction phenotype.[13,14,49]

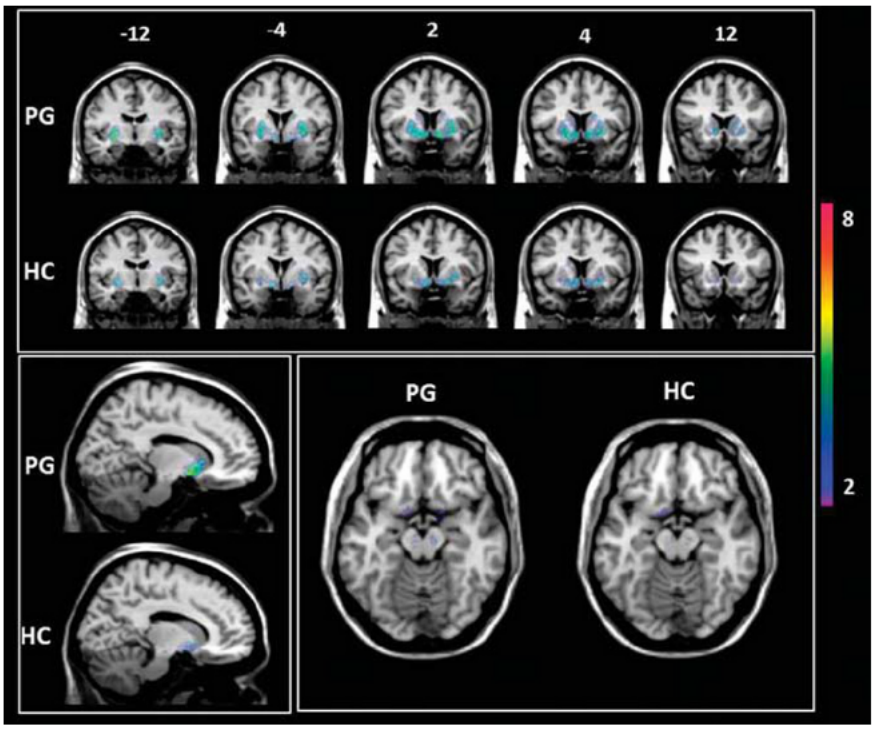

Figure 2. T-statistical maps overlaid onto a single-subject T1-weighted magnetic resonance image template showing greater amphetamine-induced dopamine release ([^{11}C]-(+)-PHNO BP_{ND} changes from baseline) in striatum and midbrain in pathological gamblers (PGs; *n* 12) than healthy controls (HCs; *n* 11; PG: *t*-max 13.4; HC: *t*-max 8.3; threshold for visualization *P* 0.01).

Our neuroimaging finding of exaggerated DA response to amphetamine in PG is in line with the 'incentive sensitization' model of addiction.[11] The model posits that compulsive 'reward seeking' in addiction involves long-lasting changes within the brain's DA circuitry normally involved in incentive motivation, rendering it hypersensitive to drug and non-chemical reward and thus driving exaggerated approach behaviors. In addition to a wealth of evidence for sensitization to drugs, preclinical work also supports the view that non-drug rewards can lead to sensitization. Specifically, increased 'incentive salience attribution' and 'compulsive pursuit' have been demonstrated for food and sexual partners.[50] Interestingly, a recent study,[51] reported that chronic exposure to unpredictable reinforcement (saccharine), a model of gambling, leads to amphetamine sensitization in rats. The authors concluded that repeated exposure to gambling-like conditions could induce neuroadaptations in DA transmission similar to those produced by amphetamine exposure, and may account for the development of gambling addiction. This is not to say that greater DA release in PG, indexed here by amphetamine-induced change in [^{11}C]-(+)-PHNO BP_{ND}, may not have existed (as a vulnerability factor) before the onset of problematic gambling, nor does it exclude the possibility that in substance use disorder, sensitization could have acted in the initial phase of the disorder and then become masked or extinguished by chronic drug use.

In contrast to the preclinical literature, neuroimaging data supporting a hyper dopaminergic state have been sparse in humans with a behavioral addiction, and inconsistent in those with a chemical addiction. With regard to behavioral addiction, previous neuroimaging studies in patients with medicated PD exhibiting a range of impulse control disorders (ICDs; including PG) have provided some evidence of DA system hypersensitivity associated with addiction-like behaviors. Specifically, these PET/[^{11}C]raclopride studies have shown that relative to their counterparts without ICD, patients with ICD have enhanced DA release in LST in response to rewarding stimuli,[52] to a gambling task[25] and to their dopaminergic medication.[53] As well, functional magnetic resonance imaging studies present seemingly parallel, although indirect findings showing increased reward-related blood–oxygen level-dependent activity in the ventral striatum in patients with ICD.[54] Although these findings suggest that the pathological pursuit of natural rewards or appetitive cues may be modulated by neuroadaptations within the DA system, it is possible that the observed effects are attributable to conditioned responses to reward-related cues (that is, gambling-associated stimuli). In contrast, our study provides direct evidence that the hyper-dopaminergic response can also be observed in response to pharmacological challenge—a stimulus with which these PG subjects had no conditioning history.

Our results are in striking contrast with the accepted finding that drug addiction is associated with a hypodopaminergic sate, characterized by blunted [^{11}C]raclopride displacement following amphetamine or methylphenidate.[55] Although [^{11}C]-(+)-PHNO has been shown to be more sensitive to changes in endogenous DA levels (in the current study and in previous published work[56]), which could have helped in detecting between-group differences, we do not believe that the discrepancy between the current finding and the reports of blunted DA release in drug addiction can be explained by choice of radioligand. In a previous study with these subjects, we report binding of both the D_3 preferring agonist [^{11}C]-(+)-PHNO and the antagonist [^{11}C]raclopride within control range, suggesting that differences in D_3 or high affinity $D_{2/3}$ receptors are unlikely to explain the present findings.[27]

One likely explanation for the discrepancy between the current finding and those reported in chemical addiction is that 'expression of sensitization' in substance-addicted individuals may be masked by an extensive depletion of DA stores during early withdrawal, as evidenced by both animal models and human brain data.[55,57] In this regard, most PET studies looking at amphetamine-triggered DA release (PET/[^{11}C]raclopride) have measured neurochemical outcomes at least 14 days after

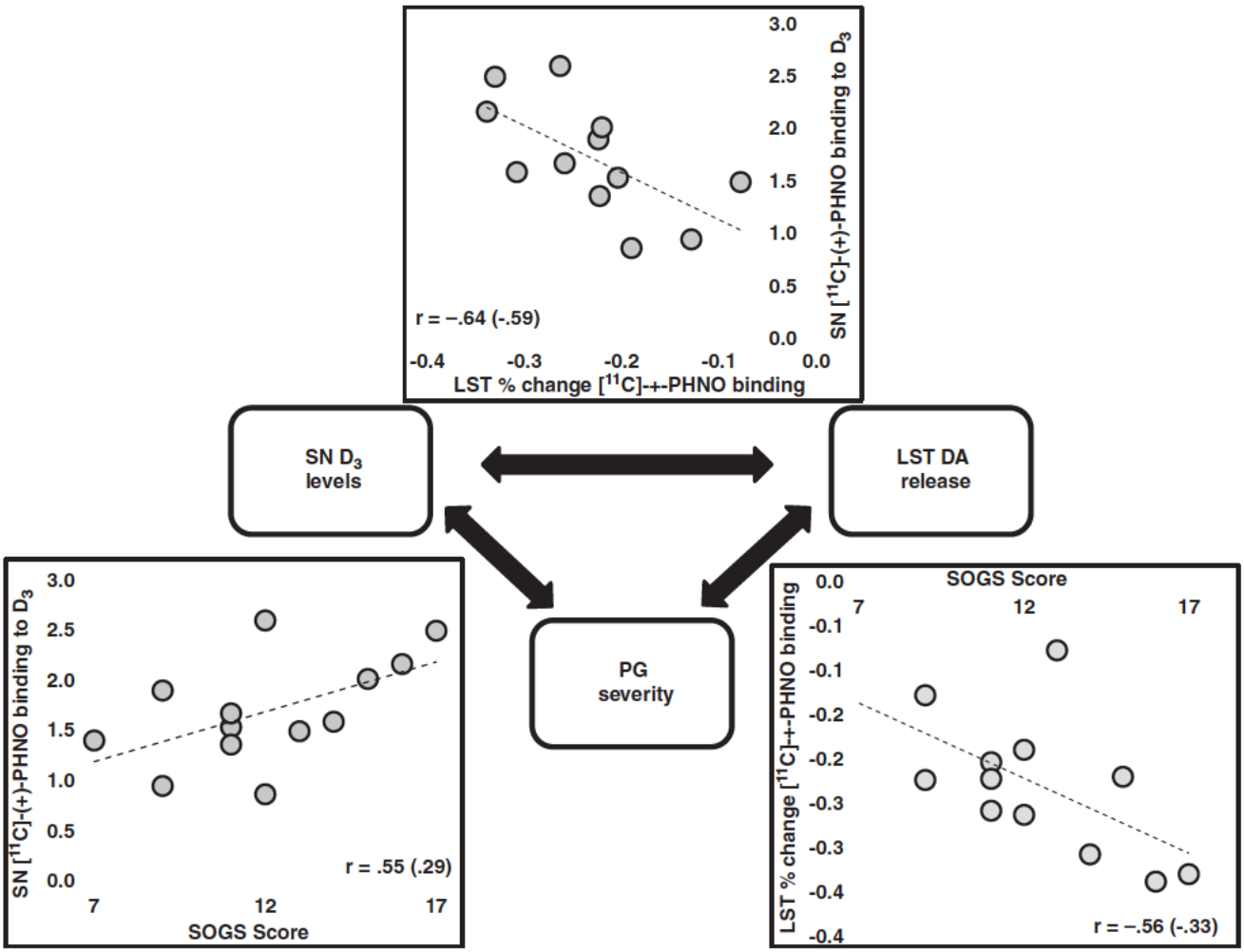

Figure 3. Model representing the interrelationships between D_3 dopamine (DA) receptor levels, ventral striatal dopamine release ([^{11}C]-(+)-PHNO BP_{ND} % change from baseline) and gambling severity (South Oaks Gambling Screen (SOGS)) that may characterize pathological gambling. DA release in limbic striatum (LST) correlates with (previously reported[27]) D_3 receptor levels in substantia nigra (SN), and both measures predict pathological gambling (PG) severity, implicating these neurobiological measures in gambling outcomes. Numbers in parentheses next to Pearson *r*'s represent the partial correlations between the two associated variables, when variance contributed by the third variable was partialled out.

exposure to drug.[15] This drug-free interval is perhaps sufficient to avoid residual effects of drugs, but it may not be long enough to allow recovery, if any, of DA stores. Although there is no consensus on whether chronic exposure to drugs in humans is associated with neurotoxicity, the possibility that damage to DA neurons can explain failure to find a hyperdopaminergic response (similar to that observed in animals) cannot be entirely ruled out. The literature in animals suggests that this may be the case, as neurotoxic regimens of high-dose amphetamine are not associated with sensitization.[17]

With respect to the regional distribution of our findings, previous imaging studies reported a greater dopaminergic response in the ventral LST, in agreement with microdialysis studies of the nucleus accumbens.[11] However, 'dopaminergic sensitization' affecting dorsal striatum has also been reported in human[20,58] and animals studies,[11] and in the current study, we find that the greater dopaminergic response in PG vs HC is expressed in the dorsal striatum, an area which interestingly has been linked with sensitization in healthy humans[20] and in patients with schizophrenia.[59] This area is traditionally believed to be involved in 'habit formation' as addiction takes hold,[60] but has also more recently been implicated in incentive salience processes. Here we find that in PG, greater DA release in dorsal striatum is related to faster rate of play and smaller bet size on the slot machine, a strategy that maximizes number of spins. Speed of play has previously been found to contribute to the perceived reinforcing effects of slot machines in PG,[61] and might be interpreted as hyperlocomotion, which accompanies amphetamine sensitization in rats. With regard to the ventral striatum, it is interesting to note that several individual differences were found in this region (correlations), despite a lack of group differences in DA release. For example, we found that DA release related to gambling severity (SOGS scores) in PG subjects, suggesting that although greater DA was only evident in dorsal regions, DA release in LST may still contribute to progressive severity of the disorder; this suggests that ventral striatal findings may capture individual differences in reward-based decision making, rather than an overall index of habitual/compulsive responding (see, for example, Everitt and Robbins[62]). The lack of overall group difference in LST (a smaller, noisier striatal ROI prone to partial volume effect) does not appear to be a statistical artifact, given that effect size for the group difference in this region was small, Cohen's $d = 0.14$.

It could equally be argued that greater neurochemical response to the amphetamine challenge is a risk phenotype, which predates exposure to appetitive stimuli (drugs or other) and, in some cases, can lead to addictive-like behaviors. In agreement with this, a number of studies in non-addicted individuals have reported a link between greater amphetamine-triggered DA release and trait impulsiveness.[63] Although this suggests that between-group differences in impulsiveness in the current study could partly explain the finding, we found that, unlike severity of the addiction (SOGS), impulsiveness (Eysenck Impulsiveness Scale) scores were not related to dopaminergic response to amphetamine in any of the striatal subregions. Furthermore, our finding of greater DA release in PG did not appear to be driven by trait impulsiveness, as controlling for impulsiveness scores yielded a similar effect.

The second critical finding in this study is the relationship between amphetamine-induced striatal DA release and D_3 receptor levels. The D_3 receptor, first described by Sokoloff[64] as having a unique mesocorticolimbic distribution, has been investigated as a drug-addiction target based on convincing evidence that D_3 antagonism decreases addiction-relevant behaviors in animals.[49] The present finding that D_3 receptor levels (indexed by SN [^{11}C]-(+)-PHNO BP_{ND}) predict LST DA response to amphetamine as well as severity of the syndrome (SOGS scores), suggests that D_3 levels may serve as an early marker of PG vulnerability. This is in line with preclinical studies[49] and with our recent work in humans linking D_3 receptor levels to features of the addiction phenotype.[13,14,27] It is also in direct agreement with some, although not all, animal studies suggesting that D_3 receptor levels are critical to the development of dopaminergic sensitization.[49] Reports linking D_3 receptor level and occurrence of dopaminergic sensitization have not measured D_3 in the SN and only report D_3 upregulation in striatum.[12,65] Note that in the current study we are limited to pre- and post-synaptic D_3 receptors measured in the SN as an index of D_3 levels[45] and thus do not know whether this finding is region specific or occurs in other regions (for example, striatum). Furthermore, this method also does not allow a distinction between hetero- and auto-receptors. Pharmacological studies conducted with D_3 ligands (most nonspecific to D_3 but some with higher potency for D_3 (SB-277011-A)), seem to contradict the view that D_3 receptors are involved in increased DA transmission by showing an inhibitory effect of D_3 receptor agonism on DA release.[66] Antisense and gene knockout studies collectively suggest that D_2 auto-receptors appear to be the primary mediators of inhibitory control of DA release, whereas D_3 receptors may contribute only indirectly (via DA transport).[67] This view appears to be supported by the finding of a relationship between lower $D_{2/3}$ receptor levels measured with [^{18}F]fallypride (presumably D_2 auto-receptors) and increased DA release.[63] Currently, the exact role and conditions in which D_3 receptors modulate DA transmission or whether this effect is through auto-receptors (whose function is still elusive[68]), or hetero-receptors, remain unknown. We therefore emphasize that whether our finding of a relationship between D_3 receptor levels, DA release, and gambling severity are causally/mechanistically linked will need to be established in future studies.

A surprising result was that of plasma cortisol levels, which (although increased by amphetamine as expected) was significantly lower in PG than HC at both baseline and post-amphetamine time points. This finding is difficult to interpret, given that the literature on cortisol in PG is inconsistent and often shows no difference from controls,[69–71] but is in line with a report of hypoactive HPA response in pathological vs recreational gamblers.[72]

Our results should be interpreted in light of the following limitations. For one, the study was carried out in a small sample composed of only men; however, this gender homogeneity combined with the absence of comorbid psychiatric conditions enabled us to characterize PG in the absence of moderating factors or potential confounds. Other caveats include the use of a ligand that is selective to D_3 receptors only in the SN, hence, limiting our D_3 finding to this region. Other generic limitations of using this ligand include scanning at non-tracer doses, the possibility of carryover effects from the first [^{11}C]-(+)-PHNO scan to the second (reducing the power to find additional effects), and specific binding in the reference tissue—for a detailed discussion of these issues refer to Shotbolt *et al*.[56] In this regard, a previous study investigating the confound of carry-over effect on [^{11}C]-(+)-PHNO BP_{ND} did not detect a difference between same-day and separate-day procedures but did note that the magnitude of displacement tended to be greater for same-day vs different-day scans (for example, in the LST 22% vs 20%; SMST; 18% vs 14%).[56] Regardless, the carry-over confound should not affect the between-group difference or within-group correlational findings in PG because all subjects were scanned twice on the same day. Further, we did not find that PG reported greater subjective effects of amphetamine than HC, although increased DA response in LST was associated with decreased confidence to refrain from gambling after amphetamine. One reason for the general dearth of effects may be that the highly restrictive environment (PET camera) together with the negative side effects of [^{11}C]-(+)-PHNO itself (queasiness, nausea felt by some, although not most, individuals) obscured group differences in the mood-related effects of amphetamine.

Despite these limitations, our data help to elucidate the role of dopaminergic mechanisms in behavioral addictions such as PG, suggesting that dopaminergic sensitization in dorsal striatum, along with D_3-linked ventral striatal mechanisms, influence gambling behavior and severity of the pathology. At the clinical level, the present findings suggest that dopaminergic sensitization may mediate enhanced response to both chemical and non-chemical rewards, contributing to the high prevalence of substance addictions in PG.[73] More fundamentally, the present data are consistent with the pivotal role of neuroplasticity in addictive pathology,[74] and corroborate early claims that many environmental stimuli apart from drugs can produce sensitization.[75] By extension, the data also support the view that sensitization of the DA system may have a role in (at least the early phases of) substance addiction in humans, whose expression may, however, be masked by a progressive DA deficiency.

CONFLICT OF INTEREST

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