Heightened Dopaminergic Response to Amphetamine at the D₃ Dopamine Receptor in Methamphetamine Users

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Neuroimaging studies in stimulant use (eg, cocaine, methamphetamine) disorders show that diminished dopamine release by dopamine-elevating drugs is a potential marker of relapse and suggest that increasing dopamine at the $D_{2/3}$ receptors may be therapeutically beneficial. In contrast, recent investigations indicate heightened D_3 receptor levels in stimulant users prompting the view that D_3 antagonism may help prevent relapse. Here we tested whether a 'blunted' response to amphetamine in methamphetamine (MA) users extends to D_3-rich brain areas. Fourteen MA users and 15 healthy controls completed two positron emission tomographic scans with a D_3-preferring probe [^{11}C]-(+)-PHNO at baseline and after amphetamine (0.4 mg/kg). Relative to healthy controls, MA users had greater decreases in [^{11}C]-(+)-PHNO binding (increased dopamine release) after amphetamine in D_3-rich substantia nigra (36 vs 20%, $p = 0.03$) and globus pallidus (30 vs 17%, $p = 0.06$), which correlated with self-reported 'drug wanting'. We did not observe a 'blunted' dopamine response to amphetamine in D_2-rich striatum; however, drug use severity was negatively associated with amphetamine-induced striatal changes in [^{11}C]-(+)-PHNO binding. Our study provides evidence that dopamine transmission in extrastriatal 'D_3-areas' is not blunted but rather increased in MA users. Together with our previous finding of elevated D_3 receptor level in MA users, the current observation suggests that greater dopaminergic transmission at the D_3 dopamine receptor may contribute to motivation to use drugs and argues in favor of D_3 antagonism as a possible therapeutic tool to reduce craving and relapse in MA addiction.

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INTRODUCTION

Methamphetamine (MA) is a widely abused psychostimulant that has been associated with neuroadaptive changes and/or neurotoxic damage to monoaminergic neurons (in particular, dopaminergic) in the mammalian brain. Human postmortem brain studies and preclinical data show decreased levels of dopaminergic markers, including dopamine (DA) itself, tyrosine hydroxylase, and dopamine transporter (DAT), in the brains exposed to MA (see Kish, 2014 for a review).

Brain imaging of dopaminergic markers in living MA (MA and dexamphetamine) users have for the most part been in line with preclinical and postmortem data by showing reduced DAT binding (see Kish, 2014 for a review),

D_2 receptors (see Boileau et al, 2012; Trifilieff and Martinez, 2014; Volkow et al, 2015 for reviews and references), and reduced amphetamine (with [^{123}I]IBZM in light recreational users of dexamphetamine; Schrantee et al, 2015) and methylphenidate (with [^{11}C]raclopride positron emission tomography (PET)) induced DA release (Wang et al, 2012). The results of the Wang et al, 2012 PET study, although limited to the left putamen, interestingly predicted clinical outcome (relapse rate), as has been shown in cocaine addiction (Martinez et al, 2011). Recently, we reported elevated [^{11}C]-(+)-dihydrotetrabenazine binding, a vesicular monoamine transporter (VMAT2) probe sensitive to changes in vesicular DA, in the striatum of chronic MA users (Boileau et al, 2015a), suggesting low stored DA, consistent with postmortem observations (Wilson et al, 1996a). Together, clinical and preclinical studies suggest that chronic MA use in humans might be associated with a hypodopaminergic state, which could in theory be remedied by DA substitution medication (although no evidence yet exists from clinical trials).

In contrast, a recent body of work suggests that, unlike other measured dopaminergic markers, the D_3 receptor

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might be upregulated in animal models (Le Foll et al, 2005; Neisewander et al, 2004) and in postmortem (Mash, 1997; Staley and Mash, 1996) and living brain of humans with stimulant (Boileau et al, 2012; Matuskey et al, 2014; Payer et al, 2014) and perhaps alcohol (Erritzoe et al, 2014) addiction (see (Boileau et al, 2015b) for a review). General interest in D_3 receptor has developed in large part because of preferential expression of D_3 in limbic brain areas associated with reward/motivation (eg, ventral striatum) and because animal models suggested that D_3-selective antagonists decrease drug-seeking behavior. Together these studies raised the possibility that increased transmission at D_3 receptors could underlie, in part, motivation to self-administer drugs and, by extension, some aspects of psychostimulant addiction and that D_3 antagonists may have therapeutic values (Heidbreder and Newman, 2010; Le Foll et al, 2014). Nevertheless, the D_3 antagonism strategy has not been tested in large-scale clinical trials in humans (although several experimental studies showed promise; Le Foll et al, 2014). It is still unclear whether heightened D_3 levels observed in PET studies of stimulant addiction (Boileau et al, 2012; Matuskey et al, 2014; Payer et al, 2014) and postmortem brain of cocaine overdose fatalities (Mash, 1997; Staley and Mash, 1996) reflect a compensatory response to low DA levels although findings in preclinical reports (Levesque et al, 1995) and studies in Parkinson's disease (Boileau et al, 2009) of downregulated D_3 receptors upon DA loss argue against this possibility.

At present, limited information is available on 'synaptic DA' status in MA users, with data employing only D_2-preferring probes that have limited ability to measure a signal in D_3-rich brain areas. This literature deficiency is relevant given the potential importance of D_3-rich areas in drug addiction and the possibility that DA level status and stimulated release might be different in D_3 vs D_2 brain areas (Drevets et al, 2001; Wilson et al, 1996a). The current study therefore employed [¹¹C]-(+)-propyl-hexahydro-naphtho-oxazin ([¹¹C]-(+)-PHNO), a D_3-preferring radiotracer having ~ 20-fold selectivity for D_3 over D_2 and allowing estimation of both D_3 and D_2 receptor signal in a region-dependent manner, with binding in dorsal striatum (high D_2/low D_3 expression) reflecting primarily D_2 receptor availability, and binding in hypothalamus and substantia nigra (SN) reflecting predominantly D_3 availability. The ventral pallidum (VP) and globus pallidus (GP) are areas of mixed D_2/D_3 binding where the D_3 fraction was estimated to represent 75 and 65%, respectively (Tziortzi et al, 2011). Furthermore, because of its presumed greater sensitivity than the more commonly used [¹¹C]raclopride, [¹¹C]-(+)-PHNO offers the advantage of detecting smaller changes in synaptic DA fluctuations and therefore may allow a better discrimination of between-group effects (Shotbolt et al, 2012a).

The specific aim of our study was to compare amphetamine-induced changes in [¹¹C]-(+)-PHNO binding between MA-abusing and healthy control subjects. We hypothesized that amphetamine would induce smaller changes in [¹¹C]-(+)-PHNO binding in D_3 as well as D_2 receptor-rich areas in MA users vs healthy controls.

MATERIALS AND METHODS

Subjects

Sixteen healthy controls and 16 chronic MA users signed consent and were enrolled to participate in an open-label amphetamine challenge PET study approved by the Centre for Addiction and Mental Health (CAMH) Research Ethics Board.

All participants underwent a comprehensive medical and psychiatric screening interview. MA users and controls were included if they were between the ages of 19 and 45 years and were free of significant medical conditions (as per medical history and standard laboratory tests) and current or previous DSM-IV Axis I disorders (excluding stimulant abuse/dependence in the MA group and nicotine dependence in both the groups). Study inclusion criteria for the MA group included: (1) self-reported use of MA as the primary drug of abuse; (2) meeting DSM-IV criteria for MA abuse or dependence; (3) testing positive for MA in scalp hair; and (4) no current (12 months) self-reported abuse of or dependence on drugs other than MA (except nicotine).

Image Acquisition and Amphetamine Challenge

On the day of the scan, all subjects were required to test negative on a urine drug screen (9-Drug Test Panel, BTNX, Markham, ON) and were asked to not smoke cigarettes or eat for at least 3 h prior to their appointment.

The scan session was comprised of two [¹¹C]-(+)-PHNO scans performed (at least) 5 h apart. One scan occurred during resting baseline and the second scan was scheduled 2 h after the administration of an oral dose of dextroamphetamine (0.4 mg/kg). Scan sessions included periodic assessments of mood and visual analog scales assessing measuring 'drug-liking', 'drug-wanting', 'energetic', 'mind-racing', 'rush', 'high', 'euphoria', 'anxious', and 'excited'. Heart rate and blood pressure (HR/BP) were monitored at 15- min intervals, and blood was drawn to measure plasma amphetamine levels.

[¹¹C]-(+)-PHNO synthesis and image acquisition protocols on the CPS-HRRT neuro-PET camera system (Siemens Medical Imaging, Knoxville, TN) are described in detail elsewhere (Boileau et al, 2012). Scans were initiated following bolus injection of [¹¹C]-(+)-PHNO (scan parameters are reported in Table 1). Raw data were reconstructed by filtered-back projection. Standard spin echo proton-density weight magnetic resonance images were obtained (Signa 1.5T MRI scanner, General Electric Medical Systems, Milwaukee, WI) for region of interest (ROI) delineation.

ROI delineation and time activity curve analyses were performed using the in-house image analysis software for automated quantification of PET data (ROMI) (details in Rusjan et al, 2006). Bilateral subcompartments of the striatum, including sensorimotor striatum (SMST), associative striatum (AST), and limbic striatum (LST), were automatically segmented as described in Martinez et al (2003). The (whole) GP and midbrain SN were automatically segmented using the atlas of Kabani et al (1998). The automatically selected VP covered approximately five coronal slices starting at the interhemispheric anterior commissural connection and was defined laterally and medially as described in Tziortzi et al (2011). Cerebellar cortex (excluding vermis) served as

Table 1 Subject Demographic Information

	Control subjects (*n* = 15), mean ± SD	Methamphetamine users (*n* = 14), mean ± SD	Group difference *p*-value
Age (years)	28.53 ± 5.18	27.57 ± 5.96	0.64
Gender	13 (M)	10 (M)	0.29
Ethnicity	13 (W)	12 (W)	0.17
Weight (kg)	74.45 ± 17.53	78.86 ± 14.49	0.69
Years of education	16.73 ± 2.71	12.57 ± 2.68	*<0.01*
Premorbid IQ (NART)	117.43 ± 5.77	115.1 ± 4.50	0.27
Beck Depression Inventory	1.93 ± 2.19	5.36 ± 6.45	0.06
Nicotine smokers	4	7	0.18
Cigarettes/day	2.93 ± 2.25	7.43 ± 1.51	*0.03*
Cannabis (⩾1 week last month)	3	8	*0.05*
Alcohol misuse[a]	0	1	0.48
[^{11}C]-(+)-PHNO dose (mCi)			
Baseline scan	8.3 ± 1.6	8.1 ± 1.4	0.69
Amphetamine scan	8.3 ± 1.4	8.1 ± 1.9	0.78
[^{11}C]-(+)-PHNO mass (μg)			
Baseline scan	2.3 ± 0.4	2.4 ± 0.1	0.27
Amphetamine scan	2.2 ± 0.4	2.3 ± 0.3	0.43
[^{11}C]-(+)-PHNO Spec. Act.			
Baseline scan	1764 ± 3363	819 ± 151	0.30
Amphetamine scan	989 ± 335	897 ± 276	0.49

Abbreviations: NART, National Adult Reading Test; Spec. Act., [^{11}C]-(+)-PHNO specific activity at the time of the injection.
[a]The National Institute on Alcohol Abuse and Alcoholism defines Alcohol Misuse (ie, more than moderate alcohol use) as a pattern of drinking that exceeds drinking one drink per day in women and two drinks a day in men or drinking more than five alcoholic drinks on a single occasion for men and three drinks on a single occasion for women (in the past 30 days). *p*-values in italics represent significance at *p* < 0.05.

reference region. [^{11}C]-(+)-PHNO time activity curves were obtained from dynamic data, and specific binding (BP$_{ND}$) was estimated in each ROI using the simplified reference tissue method (SRTM) (Lammertsma and Hume, 1996). Parameter estimation was performed with PMOD (Version 2.8.5; PMOD Technologies, Zurich, Switzerland).

Estimation of Amphetamine Effect on [^{11}C]-(+)-PHNO and Statistical Analysis

Amphetamine-induced changes in [^{11}C]-(+)-PHNO BP$_{ND}$ were calculated in each ROI as the difference between [^{11}C]-(+)-PHNO BP$_{ND}$ measured in the baseline condition and that measured in the amphetamine condition, expressed as a percentage of baseline as described in the equation below.

$$\Delta BP_{ND} = \frac{BP_{ND}\text{baseline} - BP_{ND}\text{amphetamine}}{BP_{ND}\text{baseline}} \times 100 \quad (1)$$

Group comparisons of [^{11}C]-(+)-PHNO BP$_{ND}$ and of Δ[^{11}C]-(+)-PHNO BP$_{ND}$ across ROIs were conducted using standard repeated-measures ANOVAs or ANCOVAs. When indicated, sphericity corrections were made with Greenhouse–Geisser adjustments. Least Significant Difference *t*-tests, Bonferroni corrected for planned comparisons, were applied to determine the significance of regional differences in BP$_{ND}$ between groups. Relationships between continuous

variables were analyzed with Pearson product moment correlation coefficient and Spearman's Rank test for categorical data.

RESULTS

Participant Demographic and Drug Use History

Two MA users were excluded: one for claustrophobia and the other for providing a urine sample positive for MA at the time of the scan. The data from one control was lost to [^{11}C]-(+)-PHNO-induced nausea. The final sample size was 14 MA users and 15 controls. Part of the baseline data from some controls (12) and MA users (10) has been reported previously (Boileau *et al*, 2012). Groups were matched with respect to age, gender, and ethnicity. MA users self-reported marginally greater depressive symptoms (Beck Depression Inventory) without being clinically depressed. The MA user group also had more moderate cannabis smokers and reported smoking more nicotine cigarettes daily but did not report drinking more alcohol (Table 1).

MA users had been using MA for an average of ~ 5 years. The typical dose of MA per occasion at the time of the scan was ~ 0.3 g. Forty percent of the sample smoked crystal MA *vs* 60% who preferred intranasal or oral administration. Ten of the 14 users had been abstinent for > 10 days at the time

of the scan (10–90 days), whereas 4 reported using MA ~ 7 days before the scan. Hair analysis not only confirmed use of MA in all MA users (and none in controls) but also revealed the presence of other drugs in the hair of the MA users; particularly cocaine metabolites (Table 2).

PET [^{11}C]-(+)-PHNO BP$_{ND}$

Seven subjects had scans performed on separate days (during the same week). The order of administration was reversed (ie: amphetamine session first) for one control and one MA user who received scans on separate days.

VP values in one control could not be estimated owing to poor segmentation of the VP. A repeated-measure ANOVA without the VP was conducted; group differences in the VP were assessed using a separate ANOVA (on [^{11}C]-(+)-PHNO BP$_{ND}$) and a univariate test (on percentage difference in [^{11}C]-(+)-PHNO BP$_{ND}$). Regional [^{11}C]-(+)-PHNO BP$_{ND}$ in the amphetamine and baseline conditions together with amphetamine-induced Δ[^{11}C]-(+)-PHNO BP$_{ND}$ (%) are reported in Table 3.

The repeated-measure ANOVA (condition (amphetamine vs baseline) × ROI (SN, GP, AST, LST, SMST) × group (controls vs MA users)) investigating differences in [^{11}C]-(+)-PHNO BP$_{ND}$ indicated a main effect of condition (F(1, 27) = 180.73, $p < 0.001$), suggesting that amphetamine led to a significant decrease in [^{11}C]-(+)-PHNO BP$_{ND}$ across ROIs. This effect corresponded to an overall decrease in [^{11}C]-(+)-PHNO BP$_{ND}$ of 23% ($p < 0.0001$). The ANOVA also yielded a significant group × ROI × condition interaction (F(4, 108) = 4.444, $p = 0.002$) driven by greater baseline [^{11}C]-(+)-PHNO BP$_{ND}$ values in the SN and GP of MA users relative to controls (SN: +59%, $p = 0.001$; GP: +14%, $p = 0.02$). This finding at baseline in an overlapping group of individuals was presented in our previous publication (Boileau et al, 2012).

Taking into account cigarettes per day and cannabis use as covariates (as both variables were significantly different between groups and could impact [^{11}C]-(+)-PHNO BP$_{ND}$) did not change this effect (main effect of condition: F(1, 25) = 28.598, $p < 0.001$; three-way interaction: F(4, 100) = 3.592, $p = 0.009$, pairwise comparison SN: 65%, $p = 0.002$; GP: 13% $p = 0.067$).

The repeated-measure ANOVA in the VP revealed a main effect of condition (F(1, 26) = 85.932, $p < 0.001$) and a marginal effect of group ([^{11}C]-(+)-PHNO BP$_{ND}$ MA users > [^{11}C]-(+)-PHNO BP$_{ND}$ controls) (F(1, 26) = 3.786, $p = 0.06$) but no group × condition interaction. Taking into account cigarettes per day and recent cannabis use did not change the effect of condition but the marginal effect of group disappeared ($p = 0.22$).

Testing whether amphetamine-induced Δ[^{11}C]-(+)-PHNO BP$_{ND}$ (%) were different across groups (repeated-measures ANOVA: ROI (SN, GP, AST, LST, SMST) × group (controls vs MA users)) revealed a significant interaction (F(4, 108) = 3.12, $p = 0.02$) which indicated that, relative to controls, MA users had significant, in the SN, and marginally significant, in the GP, greater decreases in [^{11}C]-(+)-PHNO BP$_{ND}$ after amphetamine (Figure 1) (SN: controls, 20 ± 21%; MA users, 36 ± 17%; $p = 0.03$; GP: controls, 17 ± 13%; MA users, 30 ± 21%; $p = 0.06$). In MA users (but not in controls), greater amphetamine-induced displacement (ΔBP$_{ND}$, %)

Table 2 Drug Use Characteristics and Co-Used Substances

	Methamphetamine users ($n = 14$)
Years of MA use	5 ± 3 (2–11)
Frequency of use (days a week)	2 ± 1 (1–5)
Estimated typical dose (mg)	310 ± 174 (100–500)
[a]Binges in the last 30 days (n)	5 ± 3 (0–10)
Route of administration	6 (40%) smoked; 8 (60%) oral/ intranasal
Days since last MA use	20 ± 21 (6–90)
Severity of Dependence Scale[b]	4 ± 2 (2–8)
MA/amphetamine in hair (n, %)	14, 100%
Cocaine/cocaine metabolites in hair (n, %)	10, 71%
MDMA/MDA/MDEA in hair (n, %)	8, 57%
Morphine/codeine in hair (n, %)	6, 42%

Abbreviations: MA, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxyethylamphetamine; MDMA, 3,4-methylenedioxymethamphetamine.
[a]Period of 2–3 days of use.
[b]Gossop et al (1995).

was found to correlate with higher levels of baseline binding in the SN ($r = 0.64$, $p = 0.015$) and AST ($r = 0.69$, $p = 0.007$). An ANCOVA controlling for cigarettes per day and cannabis use again yielded a significant group × ROI interaction (F(4, 100) = 3.59, $p = 0.009$). Pairwise contrasts revealed that MA use was associated with significantly greater decreases in [^{11}C]-(+)-PHNO BP$_{ND}$ in the SN ($p = 0.003$); effects were marginal in the GP ($p = 0.07$). We did not observe group difference in response to amphetamine in the D$_2$-rich dorsal striatum or VP ($p > 0.05$). There were no differences in regional volume or cerebellar time activity curves (area under the curve) between groups ($p > 0.05$; time activity curves for groups and conditions are represented in Supplementary Figure S2). Taking into account interscan interval did not affect any of the results. In line with previous reports (Shotbolt et al, 2012b), amphetamine-induced percent change in [^{11}C]-(+)-PHNO BP$_{ND}$ were smaller for studies completed on separate days but this effect was not significant (eg, dorsal striatum same day = − 18%, different day = − 16%, SN same day = − 27%, different day = − 19%; $p > 0.05$). Similarly percent change in [^{11}C]-(+)-PHNO BP$_{ND}$ in the two cases (one MA user and one HC) who completed the amphetamine scan first were smaller relative to the cases who had baseline scans first (respectively, − 12 vs − 17% in the dorsal striatum and − 13 vs − 29% in the SN).

A voxel-wise approach (SPM8) echoed results of our ROI analysis identifying no differences in amphetamine-induced changes in the striatum of MA users vs HC. Instead we found that changes in [^{11}C]-(+)-PHNO BP$_{ND}$ after amphetamine led to smaller, less significant clusters of significant voxel in HC relative to MA users in an area corresponding to the midbrain (SN/VTA) (see Supplementary Figure S1).

We tested for relationships between amphetamine-induced changes in [^{11}C]-(+)-PHNO BP$_{ND}$ (Δ[^{11}C]-(+)-PHNO BP$_{ND}$) in the MA users with drug use pattern and self-report effects of amphetamine. In the MA group, smaller

Table 3 Mean Regional [¹¹C]-(+)-PHNO BP$_{ND}$ in Methamphetamine (MA) Users and Healthy Controls at Baseline, After Amphetamine and Relative Changes in [¹¹C]-(+)-PHNO BP$_{ND}$ (Δ[¹¹C]-(+)-PHNO BP$_{ND}$) After Oral Amphetamine (0.3 mg/kg Given 2 h Prior to Tracer Injection)

	Healthy control subjects ($n = 15$)			Methamphetamine users ($n = 14$)		
	Baseline (mean ± SD)	Amphetamine (mean ± SD)	ΔBP$_{ND}$ (%) (mean ± SD)	Baseline (mean ± SD)	Amphetamine (mean ± SD)	ΔBP$_{ND}$ (%) (mean ± SD)
AST	2.36 ± 0.28	2.01 ± 0.31	14 ± 8	2.47 ± 0.72	2.07 ± 0.45	14 ± 10
LST	2.98 ± 0.46	2.23 ± 0.12	24 ± 17	3.00 ± 0.65	2.41 ± 0.12	23 ± 17
SMST	2.61 ± 0.37	1.99 ± 0.44	23 ± 8	2.57 ± 0.58	2.04 ± 0.48	20 ± 11
GP	2.94 ± 0.46	2.41 ± 0.46	17 ± 13	3.36 ± 0.49[a]	2.33 ± 0.67	30 ± 21[b]
VP	4.19 ± 0.61	2.89 ± 0.66	30 ± 19	4.60 ± 0.89	3.45 ± 0.79	25 ± 9
SN	1.15 ± 0.28	0.91 ± 0.29	20 ± 21	1.82 ± 0.66[a]	1.09 ± 0.32	36 ± 17[c]

Abbreviations: AST, associative striatum; GP, globus pallidus; LST, limbic striatum; SMST, sensorimotor striatum; SN, substantia nigra; VP, ventral pallidum.
[a]Indicating a significant difference in baseline [¹¹C]-(+)-PHNO BP$_{ND}$ ($p < 0.05$) between methamphetamine users and healthy controls.
[b]Indicating a non-significant trend in ΔBP$_{ND}$ ($p = 0.06$) between methamphetamine users and healthy controls.
[c]Indicating a significant difference in ΔBP$_{ND}$ ($p < 0.05$) between methamphetamine users and healthy controls.
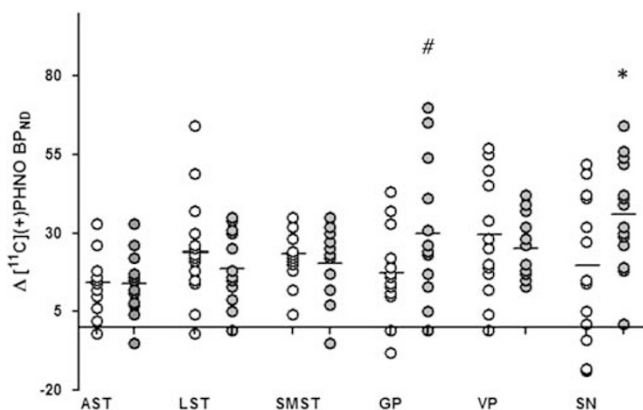

Figure 1 Scattergram of relative changes in regional [¹¹C]-(+)-PHNO BP$_{ND}$ (Δ[¹¹C]-(+)-PHNO BP$_{ND}$) induced by an oral dose of amphetamine (0.4 mg/kg given 2 h before the tracer injection). Changes in [¹¹C]-(+)-PHNO BP$_{ND}$ correspond to the difference between [¹¹C]-(+)-PHNO BP$_{ND}$ during baseline and amphetamine conditions, expressed as a percentage of baseline (see equation (1)). AST, associative striatum; GP, globus pallidus; LST, limbic striatum; SMST, sensorimotor striatum; SN, substantia nigra; VP, ventral pallidum. *$p = 0.03$, #$p = 0.06$, methamphetamine users (closed circles) vs healthy controls (open circles).

decreases in [¹¹C]-(+)-PHNO binding in the striatum (implying less DA release) was associated with years of MA use (full striatum with years of use: $r = -0.60$, $p = 0.02$; Figure 2a) and frequency of use (full striatum with days a week of MA use: $r = -0.62$, $p = 0.017$). There was no relationship between Δ[¹¹C]-(+)-PHNO BP$_{ND}$ and days of abstinence. Greater decreases in amphetamine-induced [¹¹C]-(+)-PHNO binding were related to overall greater self-reported effects of amphetamine (full striatum with ARCI: $r = +0.74$, $p = 0.006$) as well as greater alertness (full striatum with VAS energetic: $r = +0.56$, $p = 0.039$, VAS mind racing: $r = +0.64$, $p = 0.015$) and greater self-reported 'drug wanting' (SN with VAS drug-wanting: $r = +0.63$, $p = 0.016$) (Figure 2b).
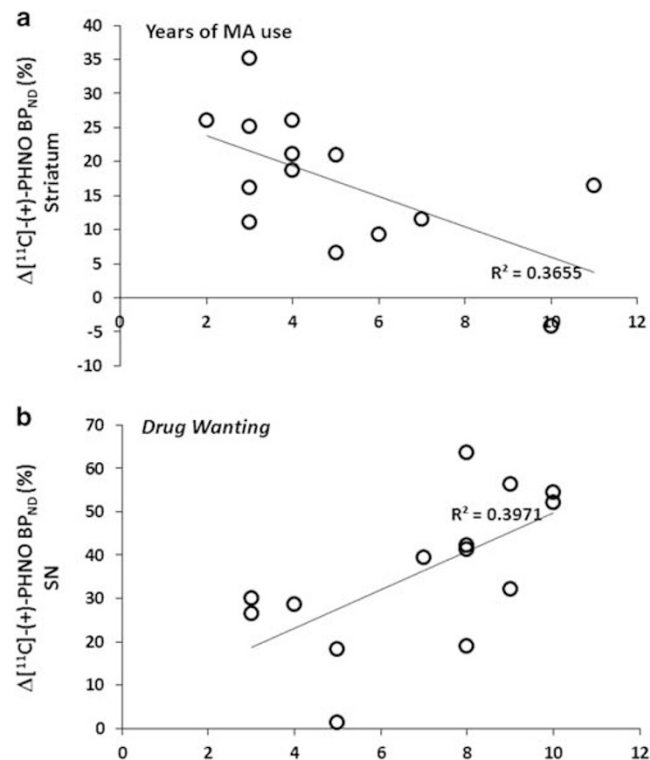

Figure 2 Correlation plot indicating a relationship between relative changes in regional [¹¹C]-(+)-PHNO BP$_{ND}$ (Δ[¹¹C]-(+)-PHNO BP$_{ND}$) induced by an oral dose of amphetamine (0.4 mg/kg given 2 h before the tracer injection) and (a) years of methamphetamine use and (b) self-reported 'drug-wanting' after a priming dose of amphetamine.

DISCUSSION

Contrary to our hypothesis and in contradistinction to some previous work showing that stimulant use disorder is associated with reduced amphetamine or methylphenidate-induced DA release (the so called 'blunted response') (Narendran and Martinez, 2008; Schrantee et al, 2015;

Trifilieff and Martinez, 2014; Volkow et al, 2015; Wang et al, 2012), here we find instead that, in a group of MA-preferring polystimulant users, DA release (as indexed by decreased amphetamine-stimulated [^{11}C]-(+)-PHNO PET binding) is within the normal range in striatum and is actually increased in the D₃-rich SN and (marginally in) GP relative to controls. Of potential clinical relevance, greater DA release in the SN was associated with higher self-reported drug-wanting, suggesting that exaggerated transmission at the D₃ receptor level could increase risk for relapse, therefore providing further evidence for testing the D₃ antagonism strategy in human stimulant users (Heidbreder and Newman, 2010; Le Foll et al, 2014).

Enhanced Amphetamine-Induced DA Release in D₃ Brain Areas

The main novel finding of the current study is that amphetamine-stimulated release of DA is greater, in particular in the DA cell body regions, in MA users (who also have greater D₃ receptor levels—this finding is discussed in Boileau et al, 2012) relative to HC. This finding is rather surprising in light of the 'generally accepted' 'blunted theory' of addiction—which includes the finding of smaller DA release not only in the striatum but also in the extrastriatal regions (Narendran et al, 2014) of stimulants (Martinez et al, 2011; Narendran and Martinez, 2008; Schrantee et al, 2015; Trifilieff and Martinez, 2014; Wang et al, 2012) as well as opiate and alcohol users (Trifilieff and Martinez, 2014). However, it is in line with some (but see Richtand, 2006) preclinical findings suggesting that intermittent repeated exposure to stimulants is associated with a greater dopaminergic response to drugs (ie: sensitization) (Robinson and Berridge, 2001) and that heightened D₃ receptor may be critically involved (Guillin et al, 2001). In this regard, elevation in D₃ receptor level in rodents has been shown to accompany sensitization to DA-elevating drugs after repeated stimulation of DA (D1) receptors (Guillin et al, 2001) and inversely DA depletion has been related to downregulation of the D₃ receptor (Boileau et al, 2009; Levesque et al, 1995). In line with studies linking D₃ receptor upregulation and dopaminergic sensitization, our group reported in a sample of pathological gamblers a correlation between elevated D₃ receptor level in SN and exaggerated limbic striatal dopaminergic response to an amphetamine challenge (Boileau et al, 2014). In the present study, enhanced DA response to an amphetamine challenge in MA users is in fact observed in areas with elevated baseline D₃ receptor levels and the two outcome measures are positively correlated with each other. How the D₃ might modulate DA transmission is not yet entirely clear. Pharmacological studies conducted with D₃ antagonist ligands (including the striatum SB-277011-A with 70-fold affinity for D₃ over D₂) suggest that prolonged transmission at the D₃ receptor could provoke increases in synaptic DA levels via modulation of DAT activity (Castro-Hernandez et al, 2015; Zapata et al, 2007). Specifically, some data suggest that subchronic (Joyce et al, 2004) (vs acute see (Zapata et al, 2007)) exposure to D₃ agonists decreases DAT activity and that MA-induced deficits in DAT, a rather constant finding in many human MA studies, could be in part D₃ mediated (Baladi et al, 2014).

The Lack of Blunted DA Response in the Striatum in MA Users

Previous studies investigating synaptic DA levels in stimulant users with [^{11}C]raclopride or [^{123}I]IBZM have, for the most part, shown that cocaine users and perhaps, to a lesser extent, MA users, have smaller or no changes in [^{11}C]raclopride binding after IV or oral administration of amphetamine or methylphenidate, findings which have been interpreted as a blunted DA release (Martinez et al, 2011; Narendran and Martinez, 2008; Schrantee et al, 2015; Trifilieff and Martinez, 2014; Wang et al, 2012). These data have further been strengthened by the observation in cocaine users of smaller changes in striatal [^{11}C]raclopride binding after a DA-depletion paradigm (with alpha-methyl-para-tyrosine) (Martinez et al, 2009) and with findings of reduced VMAT2 levels (interpreted as a loss of presynaptic DA terminal) in some cocaine users (Narendran et al, 2012; Wilson et al, 1996b). In the case of MA, it was assumed, based on postmortem brain data (Wilson et al, 1996a) indicating a severe loss of tissue levels of DA in MA (but not in cocaine (Wilson et al, 1996b) users), that changes in [^{11}C]raclopride binding after methylphenidate or amphetamine would likely reflect this 'blunted' response. However, more recent in vivo data employing the VMAT2 probe [^{11}C]DTBZ suggest that a MA-induced DA deficiency in the humans might only be short-lived (Boileau et al, 2015a). One study investigating this question in a group of 15 MA users finds a small effect (slightly blunted DA release employing methylphenidate-induced changes in PET [^{11}C]raclopride binding) but limited to a small area in the left putamen (Wang et al, 2012)—a 'marginal' finding not inconsistent with our observation that using a tracer highly sensitive to changes in synaptic DA levels (Shotbolt et al, 2012a) we do not find that MA users have a blunted striatal response to amphetamine.

Differences in the amount of daily MA used in the subjects of the different studies could explain inconsistent findings. In this regard, cases in the study by Wang et al (2012) used MA for 13 years on average and were using 1.2 g per day at the time of the scan. In contrast, our sample of MA users was using for an average of 5 years and were taking 0.3 g a day. In light of the relationship found between years of use and changes in [^{11}C]-(+)-PHNO binding (such that the more chronic the use the lesser the changes in [^{11}C]-(+)-PHNO binding), it could be argued that our failure to find a blunted DA signal (but instead a greater dopaminergic response to amphetamine) could be related to severity of the MA use disorder. In this regard, preclinical studies have shown that, in contrast to chronic high-dose exposure to MA, a regimen of repeated intermittent lower doses is associated with dopaminergic sensitization. Arguing against this explanation, however, is the recent report of Schrantee et al (2015) who find in their [^{123}I]IBZM study a robust blunting of DA release after amphetamine in a group of non-addicted, 'light' dexamphetamine users.

Alternatively, the difference in striatal DA response between chronic MA and cocaine exposure might be at least partly explained by differential adaptations in dopaminergic system. In this regard, whereas loss of DAT has been replicated in postmortem brain studies of MA users by independent groups and in six brain-imaging studies (see Kish, 2014 for a review), the data concerning DAT loss in

cocaine users are negative (showing even elevation of DAT in early abstinence)/inconclusive and/or suggests a much less robust effect (see Narendran and Martinez, 2008 for a review). Given the role of DAT in removing DA after release, it is plausible that lower levels of DAT in MA *vs* cocaine addiction may lead to greater amphetamine-induced DA occupancies (recorded with PET) in MA users, ie, an apparent lack of 'blunting'. Indeed, a recent study suggests that in MA users lower DAT levels are associated with increased methylphenidate-induced DA signal (Volkow *et al*, 2015); heterozygote DAT KO mice $(+/-)$, unlike homozygotes $(-/-)$ (Jones *et al*, 1998), had increased amphetamine-induced DA response in the striatum compared wth the wild type $(+/+)$ (Ji and Dluzen, 2008); low dose of amphetamine as used in our human neuroimaging studies might depend more on DAT blocking (*vs* vesicular depletion) to increase extracellular DA (Siciliano *et al*, 2014).

Another possible explanation for our inability to find a 'blunted' DA response in the striatum could be the use of an agonist tracer $[^{11}C]$-(+)-PHNO (which measures the high-affinity state of the receptor; ie: $D_{2/3}$ receptors coupled to G-proteins) as compared with the antagonist tracer $[^{11}C]$ raclopride used in previous studies (which does not discern between high- and low-affinity states). In principle, a greater proportion of $D_{2/3}$ receptors in the low-affinity functional state, which can be measured with $[^{11}C]$raclopride but not $[^{11}C]$-(+)-PHNO, would lead to a smaller DA occupancy and a perceived blunted DA response as detected by $[^{11}C]$ raclopride. Although no differences in DA receptor in high- *vs* low-affinity states ($[^{11}C]NPA/[^{11}C]$raclopride) have been noted in cocaine use disorder (Narendran and Martinez, 2008), this possibility cannot be ruled out in users of MA. Given the on-going debate on the selectivity of functional D_2 receptor status by the current agonist tracers including $[^{11}C]$-(+)-PHNO and the validity of the dichotomy of 'high' *vs* 'low' D_2 status *in vivo* (Seeman, 2012; Skinbjerg *et al*, 2012; van Wieringen *et al*, 2013), the significance of possible differences in functional status of striatal D_2/D_3 receptors in MA users in the interpretation of our finding is not entirely clear.

Clinical Relevance and Limitations

Any significance of our study needs to be interpreted in light of many limitations, including the following: the study was not placebo controlled and therefore increased DA release in MA users could be the result of a conditioned DA release to drug cues; the study was, for the most part, not counterbalanced with most of the scans conducted on the same day; however, we can rule out tracer mass carryover effect given the between-subject design and > 5-h interval between scans. Most users in our studies reported being M- preferring users but also tested positive in the hair for cocaine (though not in urine); therefore, it is unclear if some past use of cocaine contributed to the finding. This study was not performed with arterial sampling, and given the finding of specific $[^{11}C]$-(+)-PHNO binding in the cerebellum, we cannot rule out the possibility that the true effect of amphetamine on $[^{11}C]$-(+)-PHNO binding may have been underestimated and that difference in $D_{2/3}$ receptor levels and therefore of specific binding in the cerebellum of MA users *vs* HC could have influenced the finding. However, analysis of

cerebellar time activity curves suggested no differences between conditions and across groups (see Supplementary Figure S1). Generic caveats related to $[^{11}C]$-(+)-PHNO imaging are given in (Boileau *et al*, 2012).

The clinical implications of our finding and function of the D_3 receptor in the SN still need to be clarified. In theory, increased neurotransmission at the D_3 receptor (in the SN and GP), which receive afferent ventral striatum projections (Haber *et al*, 2000), could modify output to the LST affecting addiction-relevant behaviors. Perhaps increased DA release in somatodendritic field could be explained by diminished D_2 autoreceptor inhibition (D_2 receptor desensitization, leading to more easily depolarized neurons) owing to chronic stimulation during binge drug use (Calipari *et al*, 2014). In the current study, we found that greater changes in $[^{11}C]$-(+)-PHNO binding (enhanced DA release) correlated with drug-wanting, suggesting that D_3 receptor activation could contribute to craving and motivation to use drugs. Our findings are in line with preclinical work suggesting that D_3 receptor antagonists block drug-seeking behaviors, self-administration, and cue- and stress- induced drug reinstatement (a model of relapse) (for a review, see Le Foll *et al*, 2014) and suggest that increased D_3 receptor activity might contribute to the development of MA addiction.

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