Neuropsychopharmacology (2016) 41, 3042–3050
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Elevated Dopamine $D_{2/3}$ Receptor Availability in Obese Individuals: A PET Imaging Study with [^{11}C](+)PHNO

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Most prior work with positron emission tomography (PET) dopamine subtype 2/3 receptor ($D_{2/3}$R) non-selective antagonist tracers suggests that obese (OB) individuals exhibit lower $D_{2/3}$Rs when compared with normal weight (NW) individuals. A D_3-preferring $D_{2/3}$R agonist tracer, [^{11}C](+)PHNO, has demonstrated that body mass index (BMI) was positively associated with $D_{2/3}$R availability within striatal reward regions. To date, OB individuals have not been studied with [^{11}C](+)PHNO. We assessed $D_{2/3}$R availability in striatal and extrastriatal reward regions in 14 OB and 14 age- and gender-matched NW individuals with [^{11}C](+)PHNO PET utilizing a high-resolution research tomograph. Additionally, in regions where group $D_{2/3}$R differences were observed, secondary analyses of 42 individuals that constituted an overweight cohort was done to study the linear association between BMI and $D_{2/3}$R availability in those respective regions. A group-by-brain region interaction effect ($F_{7,\ 182} = 2.08$, $p = 0.047$) was observed. *Post hoc* analyses revealed that OB individuals exhibited higher tracer binding in D_3-rich regions: the substantia nigra/ventral tegmental area (SN/VTA) (+20%; $p = 0.02$), ventral striatum (VST) (+14%; $p < 0.01$), and pallidum (+11%; $p = 0.02$). BMI was also positively associated with $D_{2/3}$R availability in the SN/VTA ($r = 0.34$, $p = 0.03$), VST ($r = 0.36$, $p = 0.02$), and pallidum ($r = 0.30$, $p = 0.05$) across all subjects. These data suggest that individuals who are obese have higher $D_{2/3}$R availability in brain reward regions densely populated with D_3Rs, potentially identifying a novel pharmacologic target for the treatment of obesity.

Neuropsychopharmacology (2016) **41,** 3042–3050; doi:10.1038/npp.2016.115; published online 27 July 2016

INTRODUCTION

It has been estimated that over one-third of Americans are obese (OB) (Flegal *et al*, 2012) and thus at increased risk for developing obesity-related comorbidities such as coronary artery disease, hypertension, functional limitations, and type-2 diabetes (Field *et al*, 2001; Lakdawalla *et al*, 2004; Mokdad *et al*, 2003; Must *et al*, 1999; Sturm *et al*, 2004). Considerable preclinical and human research examining obesity has focused on dopamine 2/3 receptors ($D_{2/3}$Rs) due to their expression within brain regions associated with reward and motivation such as the striatum and the substantia nigra/ventral tegmental area (SN/VTA). In rodents, D_2R density was lower in the dorsal striatum of chronic high-fat-induced OB mice as compared with OB-resistant and low-fat-fed controls (Huang *et al*, 2006), implicating lower D_2R availability similar to rodent stimulant administration paradigms (Culver *et al*, 2008; Dalley *et al*, 2007; Ujike *et al*, 1989; Yi and Johnson, 1990). Further work has investigated a selective D_3-antagonist medication in an operant food self-administration paradigm and showed that a D_3-antagonist attenuated lever presses and food intake in both lean and OB rats, providing preliminary evidence of D_3R-related motivation to consume food (Thanos *et al*, 2008). This notion is consistent with clinical data implicating the D_3R in populations abusing substances that influence nigrostriatal reward pathway function (Boileau *et al*, 2012; Erritzoe *et al*, 2014; Matuskey *et al*, 2014; Payer *et al*, 2014).

In humans, prior work utilizing positron emission tomography (PET) and $D_{2/3}$R antagonist tracers has not yielded particularly well-defined results in obesity (for a comprehensive review of the PET dopaminergic and obesity/BMI literature, please see the work of Val-Laillet *et al* (2015). Studies employing [^{11}C]raclopride, a $D_{2/3}$R non-selective tracer, have demonstrated lower striatal $D_{2/3}$R availability in both severely obese (body mass index (BMI) $> 39.9\ kg/m^2$) (Wang *et al*, 2001) and OB (BMI 30.0–39.9 kg/m^2) individuals (Haltia *et al*, 2007) when compared with normal-weight (NW) (BMI 18.5–24.9 kg/m^2) individuals. Furthermore, within both severely obese (Wang *et al*, 2001)

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Received 28 March 2016; revised 27 May 2016; accepted 27 June 2016; accepted article preview online 4 July 2016

and OB (Haltia *et al*, 2007) individuals, a negative linear association between $D_{2/3}R$ availability and BMI was observed. This relationship did not extend to non-OB individuals, where no associations between striatal $D_{2/3}R$ availability and BMI have been found with [^{11}C]raclopride (Caravaggio *et al*, 2013). Work done with the D_2-high affinity tracer, [^{11}C]*N*-methyl-benperidol (NMB), failed to show any striatal $D_{2/3}R$ availability alterations between NW and severely obese individuals (Eisenstein *et al*, 2013). Lastly, work has also been done with [^{11}C]FLB457 and [^{18}F]fallypride, $D_{2/3}R$ tracers capable of extrastriatal reward region receptor quantification. In NW individuals, a positive linear association between amygdalar $D_{2/3}R$ availability and BMI was observed with [^{11}C]FLB457 (Yasuno *et al*, 2001), whereas work done with [^{18}F]fallypride has suggested a negative linear association in the left amygdala and the left caudate in a wide-ranged BMI (range 19–35 kg/m^2, mean 24.8 kg/m^2) cohort (Kessler *et al*, 2014). Other regions investigated with [^{18}F]fallypride have shown slightly higher $D_{2/3}R$ availability (not statistically significant) in the SN/VTA of OB as compared with NW females (Savage *et al*, 2014) and a positive linear association between $D_{2/3}R$ availability and BMI in the caudate and putamen of a large cohort of individuals with a wide BMI range (range 18–45 kg/m^2) (Guo *et al*, 2014).

The D_3-preferring $D_{2/3}R$ agonist PET tracer, [^{11}C](+) PHNO, has been found to be especially reliable (due to an excellent specific-to-nonspecific binding ratio) at quantifying extrastriatal midbrain reward regions particularly essential for the synthesis and production of dopamine such as the SN/VTA, where D_3Rs are predominant (Graff-Guerrero *et al*, 2008; Narendran *et al*, 2006; Searle *et al*, 2010; Tziortzi *et al*, 2011). In addition, [^{11}C](+)PHNO has also been shown to bind to high-affinity, presumably 'active', G-protein coupled forms of the D_2R within the striatum (Shotbolt *et al*, 2012; Willeit *et al*, 2006). Prior work done with [^{11}C](+) PHNO has indicated that BMI correlated positively with $D_{2/3}R$ availability in the ventral striatum (VST) (Caravaggio *et al*, 2013), a region of mixed D_2Rs and D_3Rs (Tziortzi *et al*, 2011), in a cohort with a relatively narrow non-OB BMI range (range 18.6–27.8 kg/m^2, mean 23.4 kg/m^2). Further work in a wide-BMI-ranged (range 21.5–36.5 kg/m^2, mean 27.9 kg/m^2) small cohort showed a positive association between $D_{2/3}R$ availability and BMI in the right dorsal caudate (Cosgrove *et al*, 2015).

The current study investigated, for the first time, differences in $D_{2/3}R$ availability between 14 OB and 14 age- and gender-matched NW individuals utilizing [^{11}C](+) PHNO PET. Additionally, in regions where $D_{2/3}R$ differences were observed, a cohort of 14 overweight (OW) individuals was added to investigate linear associations in a sample representative of the United States population based on BMI (ie, ~33% NW, ~33% OW, and ~33% OB) (Flegal *et al*, 2012). Based on prior work (Caravaggio *et al*, 2013) and tracer-binding characteristics (Shotbolt *et al*, 2012; Tziortzi *et al*, 2011), we expected to observe higher [^{11}C](+)PHNO tracer binding in important brain reward areas in OB compared with NW individuals in regions populated with D_3Rs (ie, the SN/VTA) and in mixed striatal regions where high-affinity 'active' forms of the D_2R may exist (ie, VST) as well as positive linear relationships between tracer binding and BMI in those respective regions after the OW cohort was added. Overall, this work aims to extend the obesity literature by providing useful and informative data contributing to mechanistic understanding of the disease and identify potential novel pharmacologic targets for treatment.

Table 1 Demographic, Injection, and Radioactivity Data for Normal Weight (NW) and Obese (OB) Participants

	NW ($n=14$)	OB ($n=14$)	p-value
Gender	10 M, 4 F	10 M, 4 F	–
Age (years)	34.9 (10.2)	37.0 (10.1)	0.59
BMI (kg/m^2)	**22.3 (1.8)**	**35.3 (4.5)**	**<0.01**
Specific activity (MBq/nmol)	52.1 (26.3)	53.8 (25.8)	0.87
Radioactive dose (MBq)	358.3 (181.3)	450.9 (125.6)	0.14
Cerebellar mean concentration (nM)	0.0747 (0.0169)	0.0672 (0.0224)	0.34
Injected mass (μg/kg)	0.0267 (0.0055)	0.0230 (0.0073)	0.15

Bold values indicate statistical significance.

MATERIALS AND METHODS

Research participants were recruited from the greater New Haven area by advertisement, word of mouth, and referral. Once determined initially eligible through telephone questionnaire, participants reported to the Yale PET Center or the Clinical Neuroscience Research Unit of the Connecticut Mental Health Center where they were consented and screened by members of the research team. As part of the screening process, study participants underwent comprehensive medical and psychiatric histories, physical examination, neurological and mental status exam, routine laboratory studies, electrocardiogram, and a semistructured (Sheehan *et al*, 1998) or a structured clinical interview (American Psychiatric Association. and American Psychiatric Association. Task Force on DSM-IV, 2000) to confirm study eligibility. Study participants were excluded based on evidence of a current or previous major psychiatric (eg, schizophrenia or bipolar disorder) or eating disorder (eg, binge eating disorder), current or history of serious medical or neurological illness (eg, past traumatic brain injury resulting in loss of consciousness), current pregnancy (as documented by urine human chorionic gonadotropin testing at screening and on the day of PET imaging), current or history of a substance abuse disorder, evidence of drug use on day of screening and day of imaging (as documented by urine toxicology studies), taking weight loss medications or medications known to influence the dopamine system (eg, methylphenidate or amphetamine), breastfeeding, or contraindications to magnetic resonance imaging.

Fourteen otherwise healthy OB (BMI > 29.9 kg/m^2) individuals were compared with 14 age-and gender-matched healthy NW (BMI 18.5–24.9 kg/m^2) individuals. As part of the secondary analyses, 14 otherwise healthy OW (BMI 25–29.9 kg/m^2) individuals were added to the analysis to examine the linear association between BMI and $D_{2/3}R$ availability in a large, broad-BMI-ranged cohort (see Tables 1 and 2 for means and standard deviations of demographics, injection parameters, and radioactivity data for all individuals studied). All participants with the exception of one

Table 2 Demographic, Injection, and Radioactivity Data for Overweight (OW) and Combined Cohort (All)

	Gender	Age (years)	BMI (kg/m^2)	Specific activity (MBq/nmol)	Radioactive dose (MBq)	Cerebellar mean concentration (nM)	Injected mass (μg/kg)
OW ($n=14$)	13 M, 1 F	36.7 (11.5)	27.2 (1.3)	54.7 (33.5)	441.9 (197.1)	0.0797 (0.0280)	0.0283 (0.0117)
All ($N=42$)	33 M, 9 F	36.2 (10.5)	28.3 (6.1)	53.5 (28.4)	417.0 (174.1)	0.0738 (0.0235)	0.0260 (0.0087)

OW participant were non-nicotine/tobacco users and some have been reported on elsewhere (Matuskey *et al*, 2015; Matuskey *et al*, 2016). Once research participants were eligible for the study, they were asked to abstain from all food and liquids (except for water) the night prior to presenting to the Yale PET Center for imaging until the scanning procedures were completed.

[^{11}C](+)PHNO was prepared as previously reported (Gallezot *et al*, 2012). All PET scans were performed with the high-resolution research tomograph (Siemens/CTI, Knoxville, TN, USA), which acquired 207 slices separated by 1.2 mm with a reconstructed image resolution of ~3 mm. Scans were acquired over 120 min at rest. Prior to [^{11}C](+)PHNO emission imaging, a transmission scan was obtained for attenuation correction.

Motion correction was based on an optical detector (Vicra; NDI Systems, Waterloo, Ontario, Canada). Dynamic PET imaging data were reconstructed with corrections for attenuation, normalization, scatter, randoms, deadtime, and motion employing the MOLAR algorithm (Carson *et al*, 2003) with a frame timing of 6×30 s; 3×1 min; 2×2 min; and 22×5 min.

PET data were used to construct a time–activity curve for the cerebellum, a region of minimal $D_{2/3}R$ binding, which was used as the reference region as in previous studies (Boileau *et al*, 2012; Ginovart *et al*, 2007; Matuskey *et al*, 2014, 2015, 2016; Mizrahi *et al*, 2011; Payer *et al*, 2014; Searle *et al*, 2010). A summed image (0–10 min after injection) was created from the motion-corrected PET data and registered to the participant's MR image, which was nonlinearly registered to an MR template in Montreal Neurological Institute (MNI) space. All transformations were performed with Bioimagesuite (version 2.5; http://www.bioimagesuite.com). Parametric images of non-displaceable tracer binding potentials (BP_{ND}), which are linearly proportional to the density of available $D_{2/3}$Rs, were computed using a simplified reference tissue model (2-parameter version: SRTM2). This method has been previously validated for [^{11}C](+)PHNO (Gallezot *et al*, 2014b; Wu and Carson, 2002) and utilized to optimize the statistical quality of the SRTM applied in prior studies by reducing noise of the functional images (Matuskey *et al*, 2014, 2015, 2016). Unbound concentration in tissue was estimated as the radioligand mass concentration in the cerebellum at 60–90 min post injection.

Structural magnetic resonance imaging (MRI) was performed on a 3-Tesla Trio system (Siemens Medical Solutions, Malvern, Pennsylvania) with a circularly polarized head coil for reasons of excluding individuals with anatomical abnormalities and anatomically coregistering with PET images. The dimension and voxel size of MR images were $256\times256\times176$ voxels and $0.98\times0.98\times1.0$ mm^3, respectively.

Regions of interest (ROIs) included the amygdala, caudate, hypothalamus, pallidum, putamen, SN/VTA, thalamus, and VST. ROIs were based on the automated anatomical labeling template delineated in MNI space (Tzourio-Mazoyer *et al*, 2002), with the exception of the hypothalamus, SN/VTA, and VST. More specifically, the hypothalamus was manually delineated on the MRI template image, the template SN/VTA ROI was manually delineated using [^{11}C](+)PHNO BP_{ND} images from a previous study (Gallezot *et al*, 2014b), and individual, hand-drawn VST delineations were performed in MNI space on each subject's MRI based on the guidelines described by Mawlawi *et al* (2001). ROIs were applied to the parametric BP_{ND} images to extract individual values.

In addition to ROI analyses, a voxel-wise analysis was performed on parametric BP_{ND} images with SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). Similar to the ROI methods, summed PET images were registered to participants' MR images before optimized unified segmentation (Ashburner and Friston, 2005) was used to determine nonlinear registrations into MNI standard space. Transformations were applied to parametric BP_{ND} images, resliced into $2\times2\times2$ mm isotropic voxels and smoothed using a 2-mm FWHM Gaussian kernel. Group differences were assessed using a two-sample *t*-test at a voxel-level threshold of $p<0.001$ with a cluster extent threshold (k) > 10 voxels.

Data were summarized descriptively and assessed for normality prior to analyses employing normality probability plots and Kolmogorov test statistics. Linear mixed models were used to examine the independent and joint effects of obesity (between-group factor) and ROIs (within-group) on BP_{ND} values. Between-group differences within each region were estimated to explain significant interactions. Within-group associations were accounted for by fitting three variance—covariance structures to the data (unstructured, compound symmetry, and heterogeneous compound symmetry) with an unstructured form fitting the data best according to the Bayesian Information Criterion. Secondary correlation analyses were not adjusted for multiple tests given the targeted nature of the analyses (ie, based on the between-group findings). All analyses were conducted using SAS version 9.3 (Cary, NC) or SPSS version 22 (Armonk, NY) and considered significant at the two-tailed $\alpha=0.05$ threshold.

RESULTS

We observed a main effect of obesity ($F_{1,26}=6.11$, $p=0.020$) demonstrating that OB and NW individuals differed in overall $D_{2/3}R$ availability as well as a group-by-brain region interaction effect ($F_{7,182}=2.08$, $p=0.047$) between OB and NW individuals. *Post hoc* analyses revealed that OB participants exhibited higher $D_{2/3}R$ availability compared

Table 3 Regional [^{11}C](+)PHNO BP_{ND} (with SD) and Relative Differences Between Normal Weight (NW) and Obese (OB) Participants

	Amygdala	Caudate	Hypothalamus	Pallidum	Putamen	SN/VTA	Thalamus	VST
NW ($n=14$)	0.27 (0.08)	1.88 (0.32)	1.24 (0.42)	**3.37 (0.39)**	2.52 (0.35)	**1.85 (0.36)**	0.35 (0.09)	**4.12 (0.52)**
OB ($n=14$)	0.30 (0.07)	1.98 (0.42)	1.27 (0.24)	**3.73 (0.47)**	2.73 (0.41)	**2.21 (0.42)**	0.37 (0.07)	**4.73 (0.58)**
ΔOB %	+13	+5	+2	**+11**	+8	**+20**	+6	**+14**
p-Value	0.22	0.47	0.81	**0.02**	0.14	**0.02**	0.54	**<0.01**

Bold values indicate statistical significance.

Table 4 Details of BMI-Related Clusters Identified by Whole-Brain Voxel-Wise Analysis: Spatial Extent (*k*) in Voxels, MNI Coordinates (*x*, *y*, *z*) and *t*-score (*t*) of Peak Value

Region	*k*	*x*	*y*	*z*	*t*
L/R midbrain (SN/VTA)	74	6	−22	−14	5.42
R pallidum	61	14	−6	−6	6.22
L pallidum	46	−8	−8	−6	5.22
L VST	13	−10	6	−10	4.67
R VST	11	8	6	−8	5.34

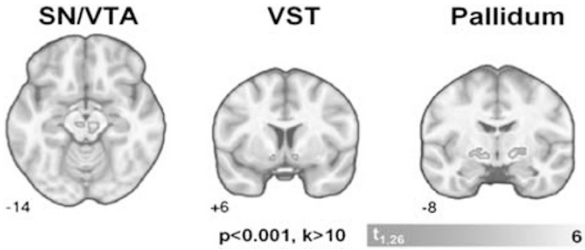

Figure 1 Voxel-wise analyses of obese relative to normal weight [^{11}C](+)PHNO BP_{ND} in the substantia nigra/ventral tegmental area (SN/VTA), the ventral striatum (VST), and the pallidum. Whole-brain results displayed at uncorrected $p<0.001$ and $k>10$; coronal coordinates in MNI space.

with NW individuals in the SN/VTA (+20%; $F_{1,182}=5.13$, $p=0.025$), the VST (+14%; $F_{,182}=8.14$, $p=0.005$), and the pallidum (+11%; $F_{1,182}=5.30$, $p=0.022$). Group-by-brain interaction results persisted after independently adjusting for potential confounding effects of age ($F_{7,182}=2.06$, $p=0.050$), gender ($F_{7,182}=2.08$, $p=0.048$), specific activity at time of injection ($F_{7,182}=2.08$, $p=0.048$), and injected mass ($F_{7,182}=2.07$, $p=0.048$). *Post hoc* results were unaffected by these confounding variables similar to the unadjusted models. Further, there were no differences observed between groups in mean cerebellar mass concentration of unbound [^{11}C](+)PHNO, thus providing additional evidence that these results were not driven by injected mass. Of the three regional findings, the VST was the only brain region that survived correction for multiple comparisons utilizing the Bonferroni method (adjusted α threshold $=0.05/8=0.006$). Mean BP_{ND} values for both OB and NW individuals along with relative group differences are shown in Table 3. Voxel-wise analyses confirmed significant ROI findings of higher $D_{2/3}R$ availability in OB as compared with NW individuals in the SN/VTA, VST, and pallidum (Table 4 and Figure 1).

The secondary analyses employing a larger cohort to include OW individuals in the above-mentioned significant regions indicated that BMI was positively associated with $D_{2/3}R$ availability in the SN/VTA ($r=0.34$, $p=0.029$; Figure 2a), the VST ($r=0.36$, $p=0.018$; Figure 2b), and the pallidum ($r=0.30$, $p=0.051$; Figure 2c) across all subjects. Although these regions were targeted based on our between-group comparisons, none of these associations survived correction for multiple comparisons using Bonferroni correction (adjusted α threshold $=0.05/3=0.017$). That stated, the associations persisted after independently adjusting for age (SN/VTA: $r=0.32$, $p=0.043$; VST: $r=0.37$, $p=0.018$; pallidum: $r=0.31$, $p=0.045$), gender (SN/VTA: $r=0.34$, $p=0.029$; VST: $r=0.37$, $p=0.017$; pallidum: $r=0.30$, $p=0.060$), specific activity at time of injection (SN/VTA: $r=0.34$, $p=0.031$; VST: $r=0.36$, $p=0.020$; pallidum: $r=0.31$, $p=0.049$), injected mass (SN/VTA: $r=0.32$, $p=0.043$; VST: $r=0.39$, $p=0.011$; pallidum: $r=0.29$, $p=0.071$), and mean cerebellar mass concentration of unbound [^{11}C](+)PHNO (SN/VTA: $r=0.32$, $p=0.041$; VST: $r=0.39$, $p=0.013$; pallidum: $r=0.27$, $p=0.087$).

DISCUSSION

To the best of our knowledge, the current study is the first to utilize the D_3-preferring $D_{2/3}R$ agonist tracer [^{11}C](+)PHNO to assess both striatal and extrastriatal $D_{2/3}R$ availability differences between otherwise healthy OB and age- and gender-matched NW individuals. Specifically, this work demonstrated that OB individuals exhibited higher $D_{2/3}Rs$ in both extrastriatal (ie, SN/VTA and pallidum) and striatal (ie, VST) regions associated with reward and motivation with the latter finding being the first published difference in any addiction-like condition with [^{11}C](+)PHNO in the striatum. These results were unaffected by adjustment for potential confounding factors such as age (Ishibashi *et al*, 2009; Kim *et al*, 2011; Matuskey *et al*, 2016; Nakajima *et al*, 2015; Volkow *et al*, 2000) or tracer injection parameters, and were confirmed with a whole-brain voxel-wise analysis. Moreover, after adding a cohort of OW individuals, we observed a positive linear association between BMI and $D_{2/3}R$ availability in the aforementioned regions that also persisted after adjusting for age, gender, and tracer injection parameters.

Prior work with [^{11}C](+)PHNO has demonstrated in non-OB individuals a positive linear association between BMI and tracer binding in the VST (Caravaggio *et al*, 2013). Our work extends those findings into an OB population when BMI categorical cohorts were combined. Other work with [^{11}C](+)PHNO has also yielded a positive linear association

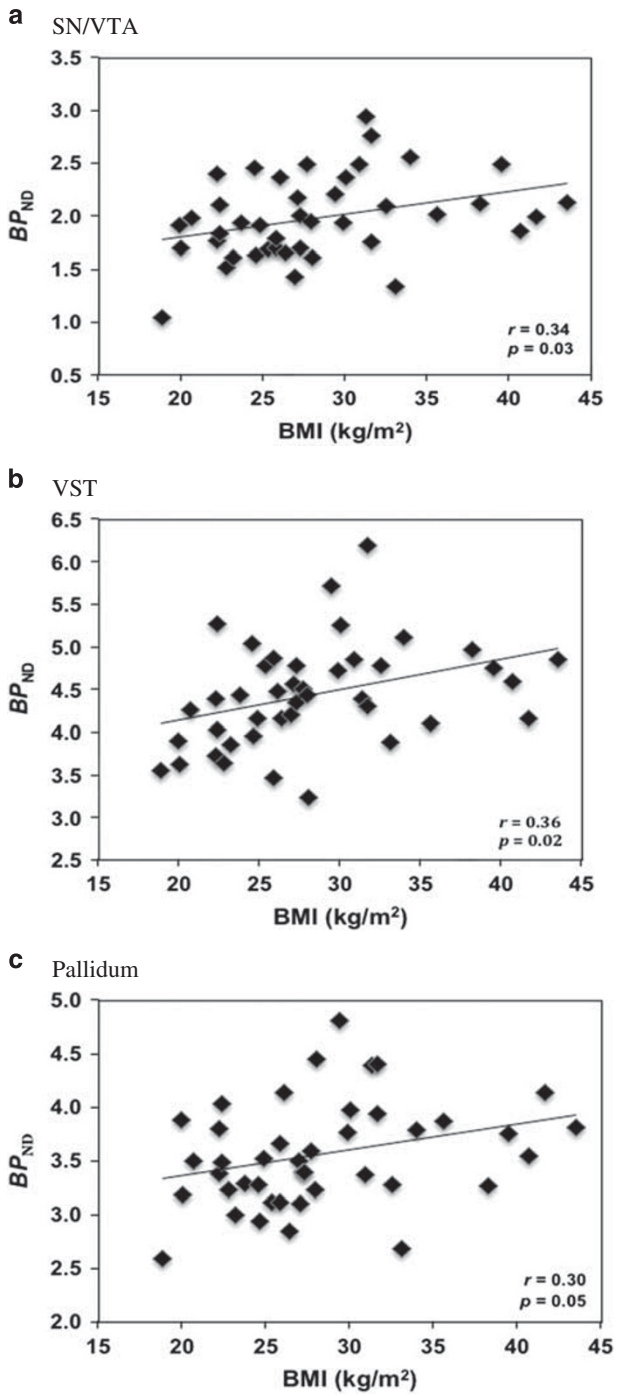

Figure 2 Unadjusted correlations between body mass index (BMI) and [^{11}C](+)PHNO BP_{ND} in the substantia nigra/ventral tegmental area (SN/VTA) (a), in the ventral striatum (VST) (b), and in the pallidum (c).

between BMI and right dorsal caudate tracer binding in a wide-BMI-ranged cohort of 12 individuals (Cosgrove *et al*, 2015). The small cohort studied, as well as the unilateral nature of the finding in the dorsal caudate, warrants caution however. Additionally, the current work, one that includes the largest cohort to date examining $D_{2/3}R$ alterations imaged with [^{11}C](+)PHNO as related to BMI, did not observe any receptor differences between OB and NW individuals in the dorsal striatum.

Much prior PET and obesity research has utilized $D_{2/3}R$ antagonist tracers. Like our current work, studies that used [^{18}F]fallypride have demonstrated positive associations between BMI and striatal $D_{2/3}R$ availability (although in the dorsal and not the VST) in a large, wide-BMI-ranged cohort (Guo *et al*, 2014) as well as non-statistically significant higher tracer binding in the SN/VTA of OB as compared with NW females (Savage *et al*, 2014). That stated, our work is not consistent with studies that used other $D_{2/3}R$ antagonist tracers. For instance, work with [^{11}C]raclopride has demonstrated reductions in striatal $D_{2/3}R$ availability in both severely obese (Wang *et al*, 2001) and OB (Haltia *et al*, 2007) individuals when compared with NW controls. Complicating the picture further, [^{11}C]raclopride findings are not consistent with studies that utilized [^{11}C]FLB457, which demonstrated BMI was positively associated with amygdalar $D_{2/3}R$ availability (Yasuno *et al*, 2001) or [^{11}C]NMB, where no association between BMI and striatal $D_{2/3}R$ availability was found (Eisenstein *et al*, 2013). Such lack of agreement across the obesity and PET dopamine literature is complex and several factors could be responsible including differences in tracers and research participants. To that end, the radiotracer employed, [^{11}C](+)PHNO, is responsible for at least part of these observed differences and worth further commentary as it has unique properties.

As previously mentioned, [^{11}C](+)PHNO is a $D_{2/3}R$ agonist tracer that has demonstrated higher binding in D_3R-predominant extrastriatal regions compared with D_2R-predominant dorsal striatal regions (Graff-Guerrero *et al*, 2008; Narendran *et al*, 2006; Searle *et al*, 2010; Tziortzi *et al*, 2011). Such a preference might explain why the current study demonstrated higher tracer binding in regions densely populated with D_3Rs (ie, the SN/VTA and the pallidum) and in the mixed $D_{2/3}R$ area of the VST when compared with studies that utilized $D_{2/3}R$ antagonist tracers that predominantly estimate D_2Rs. These distinctions suggest that the D_3R subtype and the D_2R subtype may be regulated differently with respect to obesity, a finding also observed in the stimulant-use-disorders literature (Boileau *et al*, 2012; Lee *et al*, 2009; Martinez *et al*, 2004; Matuskey *et al*, 2014; Payer *et al*, 2014; Volkow *et al*, 1990, 2001). Further, the D_3R has been implicated to be positively associated with behavioral impulsiveness and risky decision making, thus our findings of higher D_3R availability in OB may be explained by underlying trait impulsivity (Payer *et al*, 2014). As such, these data suggest, albeit speculatively, that the D_3R may be a novel pharmacologic target for the treatment of obesity. In fact, a prior study has demonstrated preliminary evidence that a D_3R antagonist, when compared with placebo, may attenuate motivational attractiveness to palatable food cues in otherwise healthy OW and OB individuals (Mogg *et al*, 2012). However, a follow-up study done by the same group using the same D_3R antagonist compound failed to show any evidence of changes in neural activation in response to food images on fMRI (Dodds *et al*, 2012), thus demonstrating the need for clarity in further studies involving D_3R antagonist compounds in obesity.

In addition to providing an estimate of D_3R sites, [^{11}C](+)PHNO, as an agonist tracer, could bind to high-affinity G-protein-coupled forms of the D_2R within the striatum that are presumed to be 'active' (Shotbolt *et al*, 2012; Willeit *et al*, 2006). Although debate still exists whether this can be

reliably measured *in vivo* (Seeman, 2012; Skinbjerg *et al*, 2012), in theory, only agonist tracers like [^{11}C](+)PHNO would prefer these sites as $D_{2/3}R$ antagonist tracers typically bind with equal preference to both high- and low-affinity forms of the $D_{2/3}R$. Therefore, it is possible that these differential binding characteristics could also help explain our VST findings such that the regulation of 'active' high-affinity forms *vs* 'inactive' low-affinity forms of the $D_{2/3}R$ may be altered in obesity. Our finding in the VST is also consistent with the previous [^{11}C](+)PHNO work in non-OB individuals (Caravaggio *et al*, 2013) and preclinical research that posits the nucleus accumbens, a central component of the VST, plays a key role in the formation/development of the OB phenotype (Davis *et al*, 2008; Geiger *et al*, 2009; Hryhorczuk *et al*, 2016; Rada *et al*, 2010; Valenza *et al*, 2015). This regional specificity, along with tracer properties, may help explain why differences were not found in the dorsal striatum. Evidence does exist that obesity may be mediated by the dorsal striatum through work with preclinical research (Huang *et al*, 2006), functional connectivity (Contreras-Rodriguez *et al*, 2015), and other PET studies done in humans utilizing antagonist tracers (Haltia *et al*, 2007; Wang *et al*, 2001). However, our lack of findings here are not surprising considering [^{11}C](+)PHNO has only recently been found to be significantly different in the dorsal striatum of humans with any addiction-like phenotype (Worhunsky *et al*, 2016), suggesting decreased sensitivity in this region.

Another potential explanation for dissimilarities between this study and other studies that employed antagonist tracers may be due to endogenous dopamine competition. Without utilizing a pharmacologic intervention such as the tyrosine-hydroxylase inhibitor α-methly-para-tyrosine (AMPT) to deplete tonic dopamine (Abi-Dargham *et al*, 2000; Caravaggio *et al*, 2014; Laruelle *et al*, 1997; Martinez *et al*, 2009), our outcome measure, BP_{ND}, reflects tracer bound to receptors not currently occupied by the endogenous neurotransmitter (ie, dopamine) and therefore measures receptor availability (Innis *et al*, 2007). [^{11}C](+)PHNO has been shown to be substantially more sensitive to endogenous dopamine competition (ie, increased tracer displacement resulting in lower BP_{ND}) compared with the antagonist tracers used in prior studies (Cropley *et al*, 2008; Gallezot *et al*, 2014a; Ginovart *et al*, 2006; Moerlein *et al*, 1997; Shotbolt *et al*, 2012; Willeit *et al*, 2008); thus, our receptor availability findings could also be due to OB individuals exhibiting lower levels of tonic dopamine. In fact, this concept is consistent with prior reports that have shown diet-induced OB and OB-prone rats exhibited lower levels of tonic dopamine or less dopaminergic-mediated behaviors (ie, conditioned place preference or amphetamine-induced locomotor activity) compared with standard chow fed or OB-resistant controls (Geiger *et al*, 2009; Hryhorczuk *et al*, 2016; Rada *et al*, 2010).

There are several potential limitations of the current study worth discussing. First, this was a retrospective study, and we did not collect information such as percent adiposity, eating and exercising behavior questionnaires, impulsivity measures, and nutritional intake that would be potentially useful to relate to our PET measures. Future prospective studies may include collecting those measures as well as investigating exercise interventions to examine $D_{2/3}R$ transformations as BMI decreases. In fact, one study using [^{18}F]fallypride has recently demonstrated that exercise intervention for 8 weeks, compared with psychoeducation, normalized lower striatal $D_{2/3}Rs$ in humans with methamphetamine-use disorder (Robertson *et al*, 2015). Second, although research participants were gender- and age-matched in the primary between-group comparisons, the cohort for the secondary correlational analyses was heavily dominated by males (33 males *vs* 9 females); thus, further correlational analyses confirming our findings are warranted in a gender-balanced cohort. Lastly, as previously mentioned, BP_{ND} only reflects PET tracer binding to available receptors and since [^{11}C](+)PHNO is susceptible to endogenous dopamine fluctuations, it is not possible to reconcile whether our findings in OB individuals are driven by an upregulation of $D_{2/3}Rs$ or accounted for by lower levels of endogenous dopamine. Future studies employing an AMPT tonic dopamine depletion paradigm may be in order to resolve the underlying effect obesity is having on brain neurochemistry.

In summary, this study, to the best of our knowledge, is the first to examine both striatal and extrastriatal $D_{2/3}R$ availability differences between otherwise healthy OB and age-and gender-matched NW individuals utilizing the D_3-preferring $D_{2/3}R$ agonist tracer [^{11}C](+)PHNO. Our data suggest that $D_{2/3}Rs$ are higher in OB compared with NW individuals in regions densely populated with D_3Rs, thus implicating the D_3R as a potential pharmacologic target for the treatment of obesity. These data also implicate the same nigrostriatal pathway alterations in obesity that have been previously observed with [^{11}C](+)PHNO in cocaine (Matuskey *et al*, 2014; Payer *et al*, 2014), methamphetamine (Boileau *et al*, 2012), and alcohol (Erritzoe *et al*, 2014) abusers, indicating excessive food consumption might alter reward pathways in similar ways as do substances of abuse. The direct role of the D_3R in addiction is not currently clear (Boileau *et al*, 2015). A full discussion is beyond the scope of the current work because of the dynamic interactions of D_3Rs that include dopamine autoreceptor properties and heterodimers that have diverse direct and indirect effects on GABA, adenosine, and glutamate systems (Casado-Anguera *et al*, 2016; Fiorentini *et al*, 2015; Leggio *et al*, 2015; Sokoloff *et al*, 2013). That being stated, one speculative mechanism to explain these phenomena could be that initial responses to ingestion of an addictive substance (ie, drug of abuse or high-fat palatable food) causes increases of dopamine which leads to a desensitization of postsynaptic striatal D_2Rs and a compensatory presynaptic D_3R upregulation in the SN/VTA on either autoreceptors or postsynaptic GABA neurons, both of which have negative control on dopamine neurons (Sokoloff *et al*, 2013).

FUNDING AND DISCLOSURE

This work was supported by the National Institute on Drug Abuse (NIDA) (K24 DA017899, 1R03DA027456-01, K02DA031750 to RTM; K12DA00167, JH; P20 DA027844 to RTM, MNP, and REC), the National Institute of Mental Health (NIMH; T32 MH019961 to DM/RTM), NARSAD Young Investigator Award Grant (M132018 to DM), the Yale PET Center, and the Yale Center for Clinical Investigation (YCCI) Pilot Projects Utilizing Core Technologies. This work was also made possible by CTSA Grant Number UL1

TR000142 from the National Center for Advancing Translational Science (NCATS), a component of the National Institutes of Health (NIH). MNP has received financial support or compensation for the following: MNP has been consulted for and advised Lundbeck, Ironwood, Shire, INSYS, Lakelight Therapeutics and Rivermend Health; has received research support from the National Institutes of Health, Veteran's Administration, Mohegan Sun Casino, the National Center for Responsible Gaming, and Pfizer; has participated in surveys, mailings or telephone consultations related to drug addiction, impulse control disorders or other health topics; has consulted for gambling entities, law offices and the federal public defender's office in issues related to impulse control disorders; provides clinical care in the Connecticut Department of Mental Health and Addiction Services Problem Gambling Services Program; has performed grant reviews for the National Institutes of Health and other agencies; has edited journals; has given academic lectures in grand rounds, CME events, and other clinical or scientific venues; and has written books or book chapters for publishers of mental health texts. The remaining authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank the staff of the Yale PET Center, the Clinical Neuroscience Research Unit at the Connecticut Mental Health Center, the Hospital Research Unit of the Yale Center for Clinical Investigation at Yale-New Haven Hospital, the Yale Magnetic Resonance Research Center, and especially Nina Levine, Julie Holub, Jane Wanyiri, RN, Samantha Massaro, Elisa Hidalgo, Peter T Morgan, MD, PhD, and Margaret Dion-Marovitz, RN, MSN.

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