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Occupancy of Dopamine D_3 and D_2 Receptors by Buspirone: A [^{11}C]-(+)-PHNO PET Study in Humans

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There is considerable interest in blocking the dopamine D_3 receptor (DRD_3) *versus* the D_2 receptor (DRD_2) to treat drug addiction. However, there are currently no selective DRD_3 antagonists available in the clinic. The anxiolytic drug buspirone has been proposed as a potential strategy as findings suggest that this drug has high *in vitro* affinity for DRD_3, binds to DRD_3 in brain of living non-human primate, and also disrupts psychostimulant self-administration in preclinical models. No study has explored the occupancy of DRD_3 by buspirone in humans. Here, we used positron emission tomography (PET) and the D_3-preferring probe, [^{11}C]-(+)-PHNO, to test the hypothesis that buspirone will occupy (decreases [^{11}C]-(+)-PHNO binding) the DRD_3 more readily than the DRD_2. Eight healthy participants underwent [^{11}C]-(+)-PHNO scans after single oral dose administration of placebo and 30, 60, and 120 mg of buspirone in a single-blind within-subjects design. [^{11}C]-(+)-PHNO binding in DRD_2- and DRD_3-rich areas was decreased by the highest (60–120 mg), but not the lowest (30 mg), doses of buspirone. The maximal occupancy obtained was ~25% in both areas. Plasma levels of prolactin (a DRD_2 marker) correlated with percentage occupancy after orally administered buspirone. Self-reported dizziness and drowsiness increased after buspirone but that did not correlate with receptor occupancy in any region. Overall, the modest occupancy of DRD_2 and DRD_3 even at high acute doses of buspirone, yielding high levels of metabolites, suggests that buspirone may not be a good drug to preferentially block DRD_3 in humans.
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INTRODUCTION

Several lines of evidence suggest that dopamine (DA) D_3 receptors (DRD_3) may be promising targets for drug addiction treatment (Heidbreder *et al*, 2005; Le Foll *et al*, 2005). *One*, the anatomy of the DRD_3, associated with the mesolimbic DA system, suggests that it is well positioned to influence drug-seeking and relapse mechanisms (Murray *et al*, 1994). *Two*, preclinical and post-mortem human brain studies suggest elevated DRD_3 levels after chronic exposure to drugs of abuse (Staley and Mash, 1996). *Three*, positron emission tomography (PET) data from our group and others have echoed preclinical findings in showing that *in vivo* DRD_3 levels are also higher in individuals who abuse stimulants and correlates with addiction-relevant behavior and traits (Boileau *et al*, 2012; Matuskey *et al*, 2014; Payer *et al*, 2013). *Finally*, in animal models, DRD_3-selective antagonists have been shown to decrease seeking of, and relapse to, a variety of drugs of abuse and it has been hypothesized that the DRD_3 modulates the motivation to seek drugs and notably contributes to the relapse phenomenon (Heidbreder *et al*, 2005; Le Foll *et al*, 2005).

Unfortunately, there are no DRD_3-selective antagonists currently available in the clinic. It has been recently reported that buspirone, a medication used for generalized anxiety disorder (Apter and Allen, 1999), has DRD_3 antagonist properties, raising the possibility that it may be the only DRD_3 antagonist currently available. The *in vitro* data indicate a twofold affinity and an 11-fold functional selectivity for DRD_3 over $5HT_{1A}$, and 70-fold affinity over DRD_2 (Kula *et al*, 1994), with metabolites of buspirone also

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binding with higher affinity to DRD_3 relative to DRD_2 (Bergman *et al*, 2013). In addition, buspirone is able to disrupt cocaine self-administration in non-human primates (Mello *et al*, 2013). For all these reasons, buspirone is a potential promising tool to study the impact of DRD_3 blockade in humans. In support of this, a recent study in non-human primates has suggested that buspirone occupies the DRD_3 at therapeutic doses (Kim *et al*, 2014).

Here we used the DRD_3-preferring radiotracer [^{11}C]-(+)-PHNO to investigate whether oral buspirone (0, 30, 60, and 120 mg) occupies DRD_3 *vs* DRD_2 in healthy subjects. Note that [^{11}C]-(+)-PHNO binding can be interpreted in a region-dependent manner, with binding in dorsal striatum reflecting DRD_2 receptor availability, and binding in hypothalamus and substantia nigra (SN), ventral pallidum (VP), globus pallidus (GP), and ventral limbic striatum (LST) reflecting 100%, 75%, 65%, and 26% D_3 availability, respectively (Tziortzi *et al*, 2011). Our main working hypothesis was that orally administered buspirone would lead to a significant dose-dependent occupancy of DRD_3; that is, selective decrease in [^{11}C]-(+)-PHNO BP_{ND} in DRD_3-rich areas *vs* DRD_2 areas.

MATERIALS AND METHODS

Subject

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board and complied with the 1975 Helsinki Declaration (5th revision, 2000). Subjects (adults, male or female ($n=8$), >19 years old) were recruited from the community and provided written informed consent and participated in a comprehensive screening interview. All met the following criteria: (1) no past/present significantmedical condition including neurological illnesses or head trauma; (2) normal physical exam (12-lead electrocardiogram, normal routine blood tests); (3) no past/present Axis I psychiatric diagnoses as per Mini-International Neuropsychiatric Interview version 5.0.0; (4) no condition that precludes use of buspirone; (5) no MR scanning contraindications; (6) no claustrophobia; (7) no current pregnancy/breastfeeding; (8) no current use or use during the previous month of medication that may affect the CNS, including monoamine oxidase inhibitor (MAOI) or positive during drug screening for drugs of abuse; and (9) no exposure to radiation in the past 12 months exceding limit for subjects participating in research with PET.

Procedure

We used a within-subjects, fixed-order drug schedule design to characterize the dose occupancy of buspirone at the DRD_3 DA receptor. Subjects were blind to dosing regimen. Each completed four PET scans, on separate days, at least 2 days apart following the oral administration of placebo (0 mg), and 60, 30, and 120 mg of buspirone (in identical-looking capsules). Injection of the PET tracer [^{11}C]-(+)-PHNO was timed 60 min after dosing, corresponding to the expected plasma peak of buspirone (Mahmood and Sahajwalla, 1999; Meltzer *et al*, 1983).

PET Image Acquisition

The radiosynthesis of [^{11}C]-(+)-PHNO has been described in detail elsewhere (Wilson *et al*, 2005).

PET studies were performed using a high-resolution head-dedicated PET camera system, CPS-HRRT (Siemens Medical Imaging). The in-plane resolution of the scanner is ~2.8 mm full-width at half-maximum (FWHM). Transmission scans were acquired using a ^{137}Cs ($T_{\frac{1}{2}}=30.2$ years, $E=662$ keV) single-photon point source. The raw data were reconstructed by filtered-back projection. A custom-fitted thermoplastic mask (Tru-Scan Imaging) was made for each subject to reduce movement during the acquisition. A total of $\sim 370 \pm 40$ MBq ($\sim 10 \pm 1$ mCi) of [^{11}C]-(+)-PHNO was injected as a bolus into an antecubital vein. Scanning time was 90 min in list mode, and then 30 frames were defined: 1–15 of 1-min duration and 16–30 of 5-min duration.

Blood samples for plasma level of buspirone and metabolites were taken before and 60 and 180 min after dosing. Prolactin levels were measured before and 60, 90, and 180 min following dosing (to investigate DRD_2 antagonist effect). Subjective assessments of drug effects were conducted at each PET visit with Visual Analogue Scale (VAS) (0, 60, and 180 min after dosing).

MRI Image Acquisition

Subjects underwent standard proton density-weighted brain magnetic resonance imaging (MRI) on a Discovery MR750 3T MRI scanner (General Electric, 3T MR750) (slice thickness 2 mm; interleaved; slice number, 84; repetition time, 6000 ms; echo time, 8 ms; number of excitations, 2; acquisition matrix, 256×192; FOV, 22×16.5 cm) to aid region-of-interest (ROI) delineation of the PET images.

Plasma Levels of Buspirone

Plasma levels of buspirone and two major metabolites, 5-hydroxybuspirone and 6-hydroxybuspirone, were measured in plasma by LC/MS/MS (see Supplementary Material) and were used in the one-site binding model described below.

PET Image Analysis

ROI-based analysis. ROI delineation and time activity curve analyses were performed using ROMI (details in Rusjan *et al*, 2006). Functional subcompartments of the striatum (Martinez *et al*, 2003) including the associative striatum (AST), limbic striatum (LST), and sensorimotor striatum (SMST) were chosen as ROIs. Delineation for the GP (whole), VP, and SN is described elsewhere (Boileau *et al*, 2012).

The [^{11}C]-(+)-PHNO-specific binding (BP_{ND}) was estimated in each ROI using the simplified reference tissue method (SRTM; Lammertsma and Hume, 1996), with cerebellar cortex as reference region. The cerebellar cortex template excludes the vermis as well as lobules IX and X (uvula and nodulus) and a tissue-classification process (described in Rusjan, *et al.* 2006) removes all voxels with the cerebellar ROI that contain white matter (that is, voxels with a probability of gray matter <99% are excluded). Parameter estimation was performed using PMOD (version

2.8.5; PMOD Technologies, Zurich, Switzerland). Because of the low signal present in hypothalamus and the difficulty in delineating this structure, we did not attempt to quantify the [^{11}C]-(+)-PHNO BP_{ND} in this brain area.

Receptor occupancy, defined as the percentage reduction in [^{11}C]-(+)-PHNO BP_{ND} from 0 mg (placebo) scan to the buspirone-exposed state, was calculated for each subject using the following Equation (1):

$$\%Occupancy = \frac{BP_{ND}baseline - BP_{ND}buspirone}{BP_{ND}baseline} \times 100 \qquad (1)$$

Comparisons between [^{11}C]-(+)-PHNO BP_{ND} in ROIs as well as regional receptor occupancies were conducted by using repeated-measures ANOVAs (SPSS 20.0, SPSS). Sphericity was assessed with the Mauchly test and, when indicated, correction was made with Greenhouse–Geisser adjustments. When appropriate, least significant difference *t*-tests, Bonferroni corrected, were applied to determine the significance of regional differences in BP_{ND} between conditions (doses).

As we are interested in the DRD_3 *vs* DRD_2 selectivity of buspirone, we estimated the DRD_3 fraction (fD_3) in areas of moderate to high DRD_3 signal using previously established regional fractions (Tziortzi *et al*, 2011). This was done by multiplying [^{11}C]-(+)-PHNO BP_{ND} in each ROI by their estimated regional fraction (as per Girgis *et al*, 2011). The average [^{11}C]-(+)-PHNO BP_{ND} within the DRD_3 'compartment' (that is, fD_3) as well as occupancy values within the fD_3 were calculated (as described above) and compared with the D_2 'compartment' (that is, [^{11}C]-(+)-PHNO BP_{ND} in dorsal striatum where 100% of the signal is associated with D_2; fD_2) by using a repeated-measures ANOVA (as described above). Relationship between [^{11}C]-(+)-PHNO BP_{ND}, occupancies and continuous variables including plasma buspirone (and metabolites), prolactin, and self-reported drug effects were investigated using correlation analysis (Pearson's product moment correlation).

Voxel-Wise Analysis

Voxel-wise parameter estimation of [^{11}C]-(+)-PHNO BP_{ND} was implemented using receptor parametric mapping (RPM) (Gunn *et al*, 1997). The BP_{ND} map images were spatially normalized into the Montreal Neurological Institute (MNI) brain space by nearest neighbor interpolation and with a voxel size fixed in $2 \times 2 \times 2$ mm using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK). The normalized images were smoothed with a Gaussian filter in each coordinate direction with a kernel of 3 mm and an average [^{11}C]-(+)-PHNO BP_{ND} was created at every dose for visualization purposes. Normalized [^{11}C]-(+)-PHNO BP_{ND} images were also entered into a GLM and statistically investigated in SPM 8. To minimize multiple comparisons and given our *a priori* regional hypotheses, voxels investigated for significant effects were limited to voxels with a BP_{ND} value >0.1.

One-Site Modeling

Regional brain [^{11}C]-(+)-PHNO occupancy and buspirone/metabolites dose–response relationship was analyzed by a one-site binding model. Plasma levels of buspirone and its major metabolites (presumably binding to DRD_3/DRD_2) 5-hydroxybuspirone and 6-hydroxybuspirone (average between 60 and 180 min after dose; corresponding to the start and end of the PET acquisition time and the peak in buspirone plasma levels) were entered into the analyses (Equation 2):

$$\%Occupancy = \frac{(A1 - A2) \times [Buspirone \text{ or } \mathbf{5} \text{ and } 6' - hydroxybuspirone]^h}{ED_{50}{}^h + [Buspirone \text{ or } \mathbf{5} \text{ and } 6' - hydroxybuspirone]^h} + A2 \qquad (2)$$

where ED_{50} represents the plasma level of buspirone or it metabolites that results in 50% receptor occupancy, $A1$ is the saturated occupancy, $A2$ is the baseline occupancy, and h is the Hill constant. The model constraints were $ED_{50} > 0$, $h > 0$, $A1 \leq 100$, and $|A2| \leq 10$ allowing variance of binding at baseline given the test–retest variability, with $A1$ and $A2$ shared between buspirone and metabolites in each area being analyzed. Despite the presence of at least two brain binding sites for buspirone and metabolites in some brain areas, the simplified one-site model is useful to estimate the efficacy (maximal %Occupancy) and apparent efficiency (ED_{50}) of blocking of tracer binding among the brain regions given variable dose adsorption among subjects (see Supplementary Figures 3 and 5). The one-site nonlinear least square fitting and comparison (Akaike information criterion (AIC)) with simpler one-site hyperbolic or two-sites functions (Graff-Guerrero *et al*, 2010) were performed in GraphPad Prism software (Version 4, GraphPad Software).

RESULTS

Eight subjects (4 male/4 female, all Caucasians) were recruited for this study. Two subjects were nauseous after [^{11}C]-(+)-PHNO; this led to scan interruption and study termination. These two subjects provided partial data: one completed the baseline scan and the other completed the baseline scan as well as the 60 and 30 mg scans. The average age of the sample was 35.4 ± 13.6 (23–56 years old), their body mass index was within normal range (23.4 ± 3.7 kg/m^2), all tested negative for drugs of abuse, and none were nicotine smokers. The dose per kg of buspirone corresponded to 0.44 ± 0.11 mg/kg for the 30 mg scan, 0.87 ± 0.22 mg/kg for the 60 mg scan, and 1.74 ± 0.44 mg/kg for the 120 mg scan. There were no differences in scan parameters across doses (mass injected (μg) 2.0 ± 0.4; amount injected (mCi) 9.1 ± 1.0; specific activity (mCi/μmol) 1146.0 ± 316.3).

ROI Analysis

The [^{11}C]-(+)-PHNO binding was moderately blocked by buspirone in a dose-dependent manner ($F(5, 25) = 6.420$; $P = 0.005$). Pairwise comparison revealed that [^{11}C]-(+)-PHNO BP_{ND} was reduced from placebo after 120 and 60 mg of oral buspirone but not after 30 mg (−5%, $P = 0.50$; Table 1) (effect size in D_3-rich SN and dorsal striatum: Cohen's *d*: 1.2 and 2.4, respectively). This effect corresponded to a mean decrease in [^{11}C]-(+)-PHNO BP_{ND} (all ROIs included) of −22% ($P = 0.012$) and −15% ($P = 0.007$) after 120 and 60 mg of buspirone respectively relative to 0 mg. The [^{11}C]-(+)-PHNO BP_{ND} at the 30 mg dose was also higher than at the 120 mg dose (−18%, $P = 0.027$). The ROI × dose interaction was not significant ($F(15, 75) = 1.524$;

Table 1 Percentage Receptor Occupancy by Buspirone in ROIs

	[^{11}C]-(+)-PHNO BP_{ND} (mean ± SD)					Buspirone occupancy (%)			
	0 mg	30 mg	60 mg	120 mg	$P^{\S}$	30 mg	60 mg	120 mg	$P^{\S}$
LST	2.8 ± 0.5	2.5 ± 0.5	**2.2 ± 0.4**	**2.1 ± 0.3***	<0.01	9 ± 17	19 ± 10	23 ± 14*	<0.005
AST	2.4 ± 0.3	2.1 ± 0.3	**1.9 ± 0.3**	**1.8 ± 0.1**	<0.005	11 ± 15	20 ± 5	25 ± 10*	<0.05
SMST	2.4 ± 0.4	2.2 ± 0.2	**2.1 ± 0.4**	**1.9 ± 0.2***	<0.01	10 ± 15	16 ± 6	23 ± 14*	<0.01
SN	1.0 ± 0.2	1.0 ± 0.4	0.9 ± 0.4	0.9 ± 0.4*	0.32	3 ± 23	16 ± 26	19 ± 26*	<0.05
GP	2.9 ± 0.2	2.7 ± 0.4	**2.6 ± 0.2**	**2.2 ± 0.6**	<0.05	5 ± 17	10 ± 5	23 ± 21	NS
VP	3.7 ± 0.5	3.5 ± 0.7	3.0 ± 0.7	**2.7 ± 0.6**	<0.05	5 ± 23	17 ± 24	24 ± 20	NS
fD_3	1.6 ± 0.1	1.5 ± 0.3	**1.4 ± 0.2**	**1.2 ± 0.3**	<0.05	6 ± 17	16 ± 14	23 ± 18	NS
fD_2	2.4 ± 0.3	2.2 ± 0.3	**2.0 ± 0.3**	**1.8 ± 0.2***	<0.005	*14 ± 14*	18 ± 6	24 ± 11	NS

Abbreviations: AST, associative striatum; GP, globus pallidus; LST, limbic striatum; SMST, sensorimotor striatum; SN, substantia nigra; VP, ventral pallidum.
fD_3: DRD_3 fraction calculated in areas of moderate to high DRD_3 signal using previously established regional fractions (Tziortzi *et al*, 2011). This was done by multiplying [^{11}C]-(+)-PHNO BP_{ND} in each ROI by their estimated regional fraction (as per Girgis *et al*, 2011). fD_2: DRD_2 fraction corresponding to [^{11}C]-(+)-PHNO BP_{ND} in dorsal striatum.
Bold numbers indicate significance from 0 mg; italicized numbers indicate significance from fD_3.
$^{\S}P$-value for difference between 0 mg and value in bold.
*Significantly different from 30 mg.

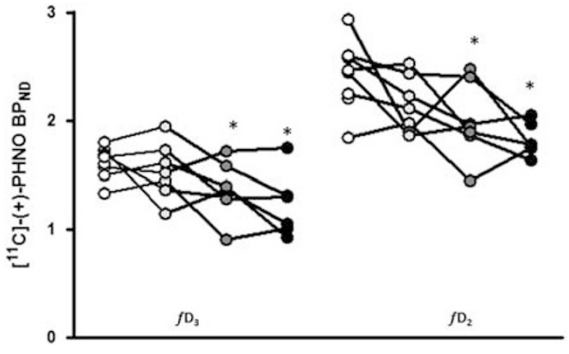

Figure 1 [^{11}C]-(+)-PHNO BP_{ND} associated with fD_3 and fD_2.Shaded circles represent dose: from lightest to darkest, respectively 0, 30, 60, and 120 mg. *Significantly different from 0 mg. fD_3 : DRD_3 fraction calculated in areas of moderate to high DRD_3 signal using previously established regional fractions (Tziortzi *et al*, 2011). This was done by multiplying [^{11}C]-(+)-PHNO BP_{ND} in each ROI by their estimated regional fraction (as per Girgis *et al*, 2011). fD_2:DRD_2 fraction corresponding to [^{11}C]-(+)-PHNO BP_{ND} in dorsal striatum.

$P = 0.118$), suggesting that buspirone administration did not differentially affect [^{11}C]-(+)-PHNO BP_{ND} across ROIs (Table 1) (effect size for the interaction: Cohen's *d*: 1.3). An ANOVA investigating [^{11}C]-(+)-PHNO BP_{ND} associated with the fD_3 and fD_2 yielded the same results: that is, an effect of dose ($F(3, 15) = 6.981$; $P = 0.004$), suggesting that 120 and 60 mg of orally administered buspirone reduced [^{11}C]-(+)-PHNO BP_{ND} by 25% and 16%, respectively ($P < 0.05$). Pairwise comparisons investigating differences in occupancy associated with the fD_3 and fD_2 suggested that at the lowest dose of buspirone, occupancy was greater in fD_2 *vs* fD_3 ($P = 0.015$; Table 1 and Figure 1).

We performed partial volume effect correction (as described in Rousset *et al*, 1998) and this did not change the results (Supplementary Figure 1). A main effect of dose was observed ($F(3, 15) = 4.791$; $P = 0.016$); however, the effect was not region dependant ($F(12, 60) = 0.564$; $P = 0.862$). Note that we did not correct for partial volume effect in the VP as our template for partial volume effect does not include this region.

One case had lower [^{11}C]-(+)-PHNO BP_{ND} during placebo *vs* 60 and 120 mg doses that could not be explained by motion artifact, other drug on board (a clean toxicology screen was provided). Removing this case from the analysis (outlier test on raw data does not suggest that he is an outlier, but outlier test on occupancy value does) revealed a significant ROI × dose interaction ($F(15, 60) = 3.707$; $P < 0.001$). Follow-up pairwise comparison suggested that relative to placebo the 60 mg dose significantly decreased [^{11}C]-(+)-PHNO BP_{ND} in all ROIs (SN: $P = 0.05$, AST: $P = 0.002$, LST: $P = 0.006$, SMST: $P = 0.009$, GP: $P = 0.007$) except VP ($P = 0.08$), and that the 120 mg dose reduced [^{11}C]-(+)-PHNO BP_{ND} in all ROIs (AST: $P = 0.014$, LST: $P = 0.015$, SMST: $P = 0.04$, GP: $P = 0.009$, VP: $P = 0.002$) except SN ($P = 0.09$), suggesting overall a more variable occupancy in D_3-rich SN and VP. The ANOVA investigating [^{11}C]-(+)-PHNO BP_{ND} associated with the fD_3 and fD_2 without the 'outlier' yielded the same results: that is, no interaction ($F(3, 12) = 1.981$; $P = 0.225$).

Effect on Prolactin

An ANOVA investigating whether buspirone (dose) affected prolactin levels yielded a significant dose × time interaction ($F(9, 45) = 4.479$; $P < 0.001$), suggesting that orally administered buspirone increased prolactin levels after 60 mg ($P = 0.02$) and 120 mg ($P = 0.02$) of buspirone. Peak effects occurred 60 min after buspirone. There were no significant differences in baseline, predrug prolactin level between doses (all $P > 0.05$). See Supplementary Figure 2A. Plasma levels of

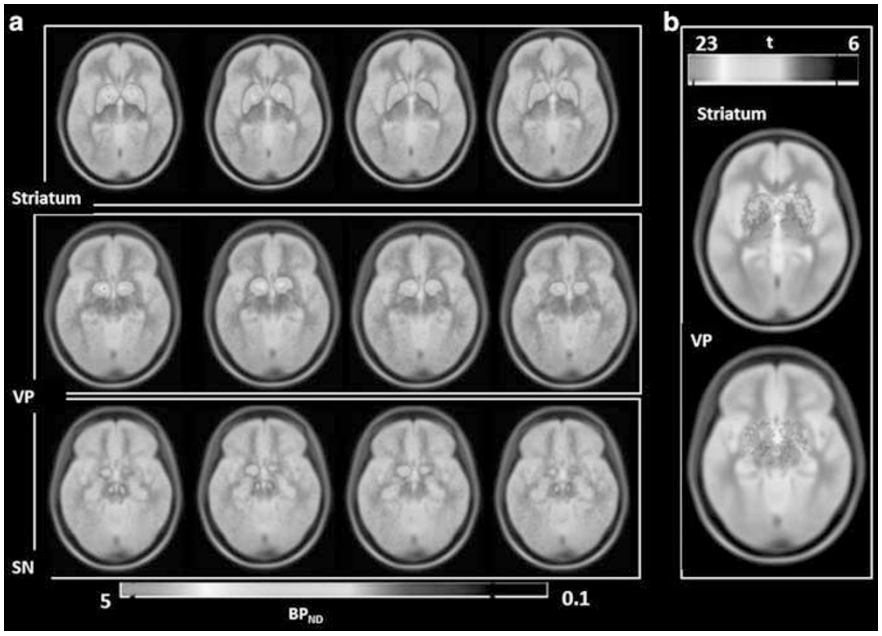

Figure 2 (a) Average [^{11}C]-(+)-PHNO BP_{ND} maps after 0, 30, 60, and 120 mg of orally administered buspirone, overlaid on top of PD MRI template in MNI space. (b) *T*-statistical map of difference between [^{11}C]-(+)-PHNO BP_{ND} at baseline and at dose 120 mg. *P* (FEW-corrected) = 0.008; KE = 249, peak *T* = 23.83 in (*x*: 18, *y*: 8, *z*: 10). SN: substantia nigra, VP: ventral pallidum.

prolactin correlated with occupancy after orally administered buspirone (120 mg in fD_2: −0.87, $P < 0.05$; see Figure 2b).

Effect on Plasma Levels of Buspirone and Metabolites

Orally administered buspirone increased plasma levels of buspirone after 60 and 120 mg, at 60 and 180 min of dosing ($F(6, 30) = 8.906$; $P = 0.01$ and $P < 0.05$), whereas the 30 mg dose only increased buspirone plasma levels 180 min after dosing ($P < 0.05$). The ANOVA for 6-OH and 5-OH buspirone yielded the same finding (6-OH: dose × time interaction $F(6, 30) = 5.893$; $P < 0.001$; 5-OH dose × time interaction $F(6, 30) = 5.009$ $P = 0.001$), suggesting again that oral administration significantly increase metabolites after 60 and 120 mg, 60 and 180 min after dosing ($P < 0.05$), whereas the 30 mg dose only increased metabolites 180 min after dosing ($P < 0.05$). Supplementary Figure 3.

Effect on Subjective Measures

Buspirone significantly increased self-reported 'Dizziness' ($F(3, 18) = 14.798$; $P < 0.001$) and 'Drowsiness' ($F(3, 21) = 4.591$; $P = 0.013$). Pairwise comparisons revealed that the effect on 'Dizziness' was present at all doses, whereas 'Drowsiness' was only reported after the highest doses (60 and 120 mg; $P < 0.05$; Supplementary Figure 4). Self-reported behaviors 'Dizziness' and 'Drowsiness' did not correlate with percentage of occupancy in any region.

Voxel-Wise Analysis

Results of our voxel-wise analysis is consistent with our ROI analyses in showing an effect of buspirone on [^{11}C]-(+)-PHNO BP_{ND} at higher doses. Figure 2a illustrates average [^{11}C]-(+)-PHNO BP_{ND} maps after orally administered buspirone showing a progressive decreases of binding at higher doses. The voxel-wise *t*-statistical comparison is concordant with this in showing a large ($k = 249$) cluster of significant difference in dorsal and ventral striatum expanding to the ventral pallidum (Figure 2b). Peak ($T = 23.83$) occurred in the dorsal striatum in (18, 8, 10) and survived correction for multiple comparisons (P(FEW-corrected) = 0.008).

Model Fit

The plots of %Occupancy *versus* plasma levels of buspirone and metabolites (Figure 3 and Supplementary Figure 5) suggest that maximal blocking of [^{11}C]-(+)-PHNO binding was reached at lower plasma levels of buspirone and metabolite in fD_3 and in D_3-rich brain areas (SN, VP, GP, and LST) as compared with that in fD_2 and D_2-rich areas (AST, SMST, or whole dorsal striatum). Curve fitting with the one-site model (Equation 2, see Table 2 and Supplementary Table 1, $R^2 = 0.19$–0.71) confirmed that fD_3 and the individual D_3-rich areas have lower ED_{50} values of buspirone (3.1–5.2 ng/ml, $P = 0.003$), 5-OH (2.1–2.7 ng/ml, $P = 0.003$), and 6-OH (42–55 ng/ml, $P = 0.005$) with larger Hill constants (2–44) than fD_2 and D_2 areas (buspirone, 6.1–13 ng/ml; 5-OH, 3.1–5.5 ng/ml; 6-OH, 62–129 ng/ml; Hill, 1.1–4.4). This analysis also showed a maximal occupancy of 30–36% in fD_3 and D_3 areas *versus* 42–51% in fD_2 and D_2 areas, with the latter having larger uncertainty (see 95% CI in Table 2 and Supplementary Table 1) as the fitted curves were not plateaued in the concentration range, suggesting that higher doses of buspirone could have bigger occupancy in the D_2 areas but not for D_3. Indeed, compared with a simple one-site hyperbolic function with Hill constant fixed to 1, Equation 2 was the preferred model for data in fD_3

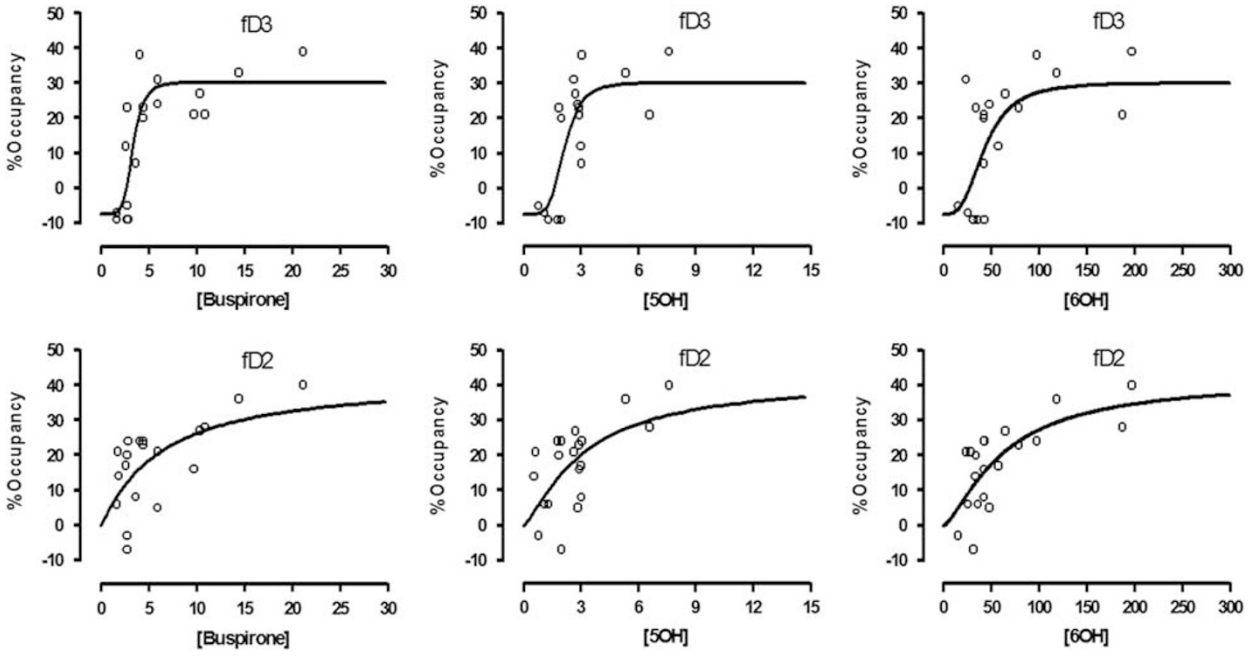

Figure 3 Plots of [^{11}C]-(+)-PHNO occupancy against plasma concentration of Buspirone and 5OH and 6OH buspirone in fD_2 and fD_3. Solid lines represent model fits (via Equation 2 above) to the measured data. fD_3 : DRD_3 fraction calculated in areas of moderate to high DRD_3 signal using previously established regional fractions (Tziortzi *et al*, 2011). This was done by multiplying [^{11}C]-(+)-PHNO BP_{ND} in each ROI by their estimated regional fraction (as per Girgis *et al*, 2011). fD_2:DRD_2 fraction corresponding to [^{11}C]-(+)-PHNO BP_{ND} in dorsal striatum.

Table 2 ED_{50} for Buspirone and Metabolites Calculated Using a One-Site Binding Model

One-site model with Hill

	Buspirone			5-OH Buspirone			6-OH Buspirone			All
	ED_{50} (95% CI)	**Hill (95% CI)**	**R^2**	**ED_{50} (95% CI)**	**Hill (95% CI)**	**R^2**	**ED_{50} (95% CI)**	**Hill (95% CI)**	**R^2**	**A1 (95% CI)**
fD_2	6.1 (0–20)	1.1 (0–2.7)	0.42	3.1 (0–7.7)	1.3 (0–3.6)	0.35	62 (0–295)	1.4 (0–3.4)	0.53	42 (0–50)
fD_3	3.4 (0–4.4)	5.9 (2.6–9.3)	0.71	2.1 (0–2.7)	5.0 (2.1–7.8)	0.65	43 (0–88)	3.0 (0.7–5.2)	0.44	30 (23–37)

ED_{50} in ng/ml. ED_{50} represents the plasma level of buspirone or it metabolites that results in 50% receptor occupancy, A1 is the estimated saturated occupancy shared between buspirone and metabolites, and Hill is the Hill constant. fD_3: DRD_3 fraction calculated in areas of moderate to high DRD_3 signal using previously established regional fractions (Tziortzi *et al*, 2011). This was done by multiplying [^{11}C]-(+)-PHNO BP_{ND} in each ROI by their estimated regional fraction (as per Girgis *et al*, 2011). fD_2: DRD_2 fraction corresponding to [^{11}C]-(+)-PHNO BP_{ND} in dorsal striatum.

($\Delta AIC = -12$) and D_3 areas ($\Delta AIC = -9.9, -8.1$, and -1.9 for SN, VP, and GP, respectively), whereas the simpler model was preferred for data in fD_2 ($\Delta AIC = +9.1$) and D_2 areas ($\Delta AIC = +5.8$, +6.8, and +5.7 for SMST, AST, and LST, respectively). With the simpler model, the estimated maximal occupancy for fD_2 and D_2 areas increased to 55–90% and ED_{50} increased to 10–26, 5–12, and 102–265 ng/ml for buspirone, 5-OH, and 6-OH, respectively, reflecting the limited buspirone concentration range for D_2 areas. A two-site model could not converge for data in fD_3 and D_3 areas and did not improve the fitting for data in fD_2 and D_2 areas, namely, the second site was not identifiable (data not shown).

DISCUSSION

Our data indicate for the first time that acute doses of buspirone can occupy DRD_2 and DRD_3 in human subjects. However, this occupancy was in the same range for the DRD_2 *vs* DRD_3 and the maximum occupancy achieved during acute single-dose regimen was modest with the doses of buspirone tested.

One possible explanation for our finding of a modest occupancy may be that the plasma concentrations of the parent drug and/or metabolites reached by the dose of buspirone were not high enough. The average dose per kg body weight of the subjects was 0.87 ± 0.22 mg/kg for the 60 mg scan (the highest dose approved for clinical use) and 1.74 ± 0.44 mg/kg for the 120 mg scan, yielding plasma concentrations of buspirone (>8 ng/ml after 120 mg and >4 ng/ml after 60 mg) slightly higher than what is reported in the literature during daily 60 mg exposure of buspirone (2.7 ng/ml) (Dockens *et al*, 2006). A recent[^{11}C]-(+)-PHNO study by Kim *et al* (2014) in female baboons indicated that 3 mg/kg of buspirone p.o. (but not 1 mg/kg) led to a significant occupancy in DRD_3-rich areas (up to 74 % in midbrain). We cannot directly compare these data with ours,

because plasma levels of buspirone were not reported and we cannot say whether higher dosage of buspirone (for example, 3 mg/kg) would have increased occupancy. However, our data would argue against the possibility that higher occupancy of DRD_3 sites or preferential DRD_3 over DRD_2 occupancy would be achieved by increasing the dose of buspirone in humans (see Figure 3). It is possible that the differences when compared with the study of Kim *et al* (2014) may be because of interspecies differences. Although a repeated dosing regimen study is required to rule out that chronic exposure would have an accumulative effect increasing the DRD_3 occupancy, our results showing a lack of selectivity for DRD_3 over DRD_2, similar to antipsychotic drugs, might induce a decreased occupancy of DRD_3 after chronic exposure (Graff-Guerrero *et al*, 2009; McCormick *et al*, 2010).

Another issue is whether buspirone's major metabolites had differential effects on [^{11}C]-(+)-PHNO binding. In this regard, Bergman *et al* (2013) have shown that some of buspirone metabolites have high affinities for DRD_3 *vs* DRD_2. (ki were: 98, 261, and 795 nM for buspirone, 5-OH buspirone and 6-OH buspirone, respectively). In line with this, Kim *et al* (2014) have reported in baboons that there is significantly higher occupancy of DRD_2 following intramuscular administration and that 6'-hydroxybuspirone affected [^{11}C]-(+)-PHNO in midbrain, suggesting a possible important role of metabolites in the occupancy of DRD_3. Curve fitting of our data suggests faster occupancy of DRD_3 *vs* DRD_2 sites that may be translated in a higher DRD_3 affinity and is consistent with *in vitro* findings. Based on plasma levels of metabolites achieved in the current study (in literature range: 37 ng/ml; Dockens *et al*, 2006) our conditions allowed for (some) metabolism of buspirone and accumulation of high dosages of metabolites (see Supplementary Figure 3).

Another issue that could have affected our measure is the possible elevation of DA induced by DRD_2 antagonism. It could also be speculated that because of the higher affinity of endogenous DA for the DRD_3 (*vs* DRD_2), increases in DA would have interfered with buspirone in fD_3 more so than in fD_2. However, several studies reported no significant effects of buspirone on DA release using microdialysis in striatal areas in rats (Kaariainen *et al*, 2008; Liu *et al*, 2004). In addition, the elevation of DA induced by acute antipsychotics is in the range of 100% (Ichikawa and Meltzer, 1991), and therefore it is unlikely that potential elevation of DA induced by buspirone would lead to significant occupancy of receptors as assessed by PET (Martinez and Narendran, 2010).

Our findings that higher doses of buspirone were associated with self-reported drowsiness and dizziness are in line with clinical observation in patients receiving this drug and were within tolerable limits. Subjects who reported negative side effects from buspirone reported maximal effects during the ascending limb of the plasma buspirone curve (that is, at 60 min) and were back to normal at the end of the session (~200 min after buspirone). We found that plasma prolactin levels were increased after buspirone and correlated with DRD_2 occupancy: this is in line with the known effects of DRD_2 antagonism on prolactin release from the anterior pituitary gland and suggests that even low brain occupancy of DRD_2 stimulates prolactin increases. Note that because of the low signal, we did not measure [^{11}C]-(+)-PHNO binding in hypothalamus (arcuate nucleus) where DA DRD_2 antagonism would have, via hypothalamic hormones, stimulated prolactin release.

It may be assumed that one reason for a lack of difference in occupancy of the DRD_2 and DRD_3 by buspirone may be that it is not feasible to image both the DRD_2 and the DRD_3 using a single PET tracer. However, converging evidence suggests that it is possible to differentially measure these receptors that are differentially expressed in the brain. A number of occupancy studies (using the DRD_3 selective/preferential drugs ABT-925 and GSK598809) have suggested that the DRD_2 and DRD_3 fraction of the [^{11}C]-(+)-PHNO signal can be measured simultaneously (Graff-Guerrero *et al*, 2009, 2010; Searle *et al*, 2010). Preclinical studies performed with [^{3}H]-(+)-PHNO and use of DRD_3 knockout mice and of DRD_3-selective antagonist also clearly demonstrated that [^{11}C]-(+)-PHNO is suitable to visualize DRD_3 sites (Rabiner *et al*, 2009). In contrast to the high occupancy that was reported with a highly selective DRD_3 ligand such as GSK598809, here we have obtained modest occupancy of DRD_3 sites using buspirone. It is unlikely that this is because of the inability of [^{11}C]-(+)-PHNO to measure occupancy at the DRD_3.

Nevertheless, our findings should be interpreted in light of some limitations including small sample size as well as caveats of the [^{11}C]-(+)-PHNO PET approach, namely scanning at nontracer doses, and specific binding in the reference tissue (for a detailed discussion of these issues, refer to Shotbolt *et al*, 2012). Importantly, it is not likely that specific binding in the cerebellum contributed to the finding as AUC cerebellum was not different between conditions and mass was not different between scans. Furthermore, the difference in measurement sensitivity between D_3-rich areas *vs* D_2 areas (that is, D_3-rich areas are more difficult to delineate, prone to greater partial volume effect, and in some cases may be affected by white matter content (globus pallidus)) may have limited our ability of finding a region-dependant effect. This study has some implications for the mechanism of action of buspirone. Although initially developed to be an antipsychotic drug, the initial clinical trial with buspirone showed a lack of antipsychotic activity. Our results indicating low occupancy of DRD_2 is in line with this, as it has been shown that higher degree of DRD_2 occupancy is required for antipsychotic activity. It has been previously reported that buspirone has a low occupancy ($<26\%$) of the $5HT_{1A}$ in clinical doses (Rabiner *et al*, 2000). We cannot exclude that the therapeutic efficacy is therefore related to a combined moderate occupancy of the HT_{1A}, DRD_2, and DRD_3. In addition, it has some affinity toward the DRD_4 (Bergman *et al*, 2013) that can also contribute to its effects on animal models of substance use disorders (Di Ciano *et al*, 2014). It is not currently feasible to determine the occupancy of DRD_4 in humans because of the lack of a proper PET tracer.

There has been considerable interest in using buspirone for drug addiction treatment. In animal models of drug dependence, it was recently reported by Mello *et al* (2013) and Bergman *et al* (2013) that buspirone can significantly decrease cocaine self-administration in non-human primates. It is notable that, in the former study, buspirone

was administered chronically, and that it has been previously demonstrated that i.v. or i.m. injections of higher, but not lower, doses of buspirone decreased cocaine seeking (Gold and Balster, 1992). It is possible that higher doses of buspirone are needed to disrupt the primary reinforcing properties of drug, because acute and moderate i.p. doses of buspirone decreased drug seeking as measured in the reinstatement model (Shelton *et al*, 2013). As mentioned above, it is difficult to compare human doses with animal doses, but it is possible that the dose of buspirone needed to treat addictions is too high to be considered free from adverse events.

In human clinical studies, promising effects of buspirone have been reported for treatment of substance dependence, including tobacco (Cinciripini *et al*, 1995; Hilleman *et al*, 1992, 1994), marijuana (McRae *et al*, 2006), and opiates (McRae *et al*, 2004), but not alcohol (Malec *et al*, 1996), although it was effective in reducing alcohol withdrawal (Dougherty and Gates, 1990). However, recently the clinical results of a trial evaluating buspirone for cocaine dependence have been reported and no significant effects were observed (Winhusen *et al*, 2014). If anything, there was higher rate of relapse to cocaine use in the female participants in the trial. This possible worsening effect has been reported in different trials performed with dopamine antagonists while being tested for substance use disorder treatment and may reflect the impact of DADRD_2 blockers (as SUD has been already associated with lower DRD_2 function, Volkow *et al*, 1993).

CONCLUSION

Our study provides the first evidence of $DRD_{2/3}$ occupancy by buspirone in humans. However, contrary to our expectation, its occupancy at the DRD_3 was modest despite plasma levels (of drugs and metabolites) higher than that obtained at the usual therapeutic regimen. Our data do not suggest that increasing the dose would lead to higher occupancy of DRD_3 without concurrent occupancy of DRD_2; chronic studies would be needed to conclude with certainty on this. Buspirone may not be a good candidate to test specifically the role of DRD_3 in drug addiction. It is not excluded that the therapeutic effects of buspirone could be mediated by a combination of its effects at HT_{1A}, DRD_2, and DRD_3.

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REFERENCES

Apter JT, Allen LA (1999). Buspirone: future directions. *J Clin Psychopharmacol* **19**: 86–93.

Bergman J, Roof RA, Furman CA, Conroy JL, Mello NK, Sibley DR *et al* (2013). Modification of cocaine self-administration by buspirone (buspar(R)): potential involvement of D3 and D4 dopamine receptors. *Int J Neuropsychopharmacol* **16**: 445–458.

Boileau I, Payer D, Houle S, Behzadi A, Rusjan PM, Tong J *et al* (2012). Higher binding of the dopamine D3 receptor-preferring ligand [11C]-(+)-propyl-hexahydro-naphtho-oxazin in methamphetamine polydrug users: a positron emission tomography study. *J Neurosci* **32**: 1353–1359.

Cinciripini PM, Lapitsky L, Seay S, Wallfisch A, Meyer W Jr, van Vunakis H (1995). A placebo-controlled evaluation of the effects of buspirone on smoking cessation: differences between high- and low-anxiety smokers. *J Clin Psychopharmacol* **15**: 182–191.

Di Ciano P, Grandy DK, Le Foll B (2014). Dopamine D4 receptors in psychostimulant addiction. *Adv Pharmacol* **69**: 301–321.

Dockens RC, Salazar DE, Fulmor IE, Wehling M, Arnold ME, Croop R (2006). Pharmacokinetics of a newly identified active metabolite of buspirone after administration of buspirone over its therapeutic dose range. *J Clin Pharmacol* **46**: 1308–1312.

Dougherty RJ, Gates RR (1990). The role of buspirone in the management of alcohol withdrawal: a preliminary investigation. *J Subst Abuse Treat* **7**: 189–192.

Girgis RR, Xu X, Miyake N, Easwaramoorthy B, Gunn RN, Rabiner EA *et al* (2011). In vivo binding of antipsychotics to D(3) and D(2) receptors: a PET study in baboons with [(11)C]-(+)-PHNO. *Neuropsychopharmacology* **36**: 887–895.

Gold LH, Balster RL (1992). Effects of buspirone and gepirone on i.v. cocaine self-administration in rhesus monkeys. *Psychopharmacology (Berl)* **108**: 289–294.

Graff-Guerrero A, Mamo D, Shammi CM, Mizrahi R, Marcon H, Barsoum P *et al* (2009). The effect of antipsychotics on the high-affinity state of D2 and D3 receptors: a positron emission tomography study With [11C]-(+)-PHNO. *Arch Gen Psychiatry* **66**: 606–615.

Graff-Guerrero A, Redden L, Abi-Saab W, Katz DA, Houle S, Barsoum P *et al* (2010). Blockade of [11C](+)-PHNO binding in human subjects by the dopamine D3 receptor antagonist ABT-925. *Int J Neuropsychopharmacol* **13**: 273–287.

Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997). Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage* **6**: 279–287.

Heidbreder CA, Gardner EL, Xi ZX, Thanos PK, Mugnaini M, Hagan JJ *et al* (2005). The role of central dopamine D3 receptors in drug addiction: a review of pharmacological evidence. *Brain Res Brain Res Rev* **49**: 77–105.

Hilleman DE, Mohiuddin SM, Del Core MG, Sketch MH Sr. (1992). Effect of buspirone on withdrawal symptoms associated with smoking cessation. *Arch Intern Med* **152**: 350–352.

Hilleman DE, Mohiuddin SM, Delcore MG (1994). Comparison of fixed-dose transdermal nicotine, tapered-dose transdermal nicotine, and buspirone in smoking cessation. *J Clin Pharmacol* **34**: 222–224.

Ichikawa J, Meltzer HY (1991). Differential effects of repeated treatment with haloperidol and clozapine on dopamine release

and metabolism in the striatum and the nucleus accumbens. *J Pharmacol Exp Ther* **256**: 348–357.

Kaariainen TM, Lehtonen M, Forsberg MM, Savolainen J, Kaenmaki M, Mannisto PT (2008). Comparison of the effects of deramciclane, ritanserin and buspirone on extracellular dopamine and its metabolites in striatum and nucleus accumbens of freely moving rats. *Basic Clin Pharmacol Toxicol* **102**: 50–58.

Kim SW, Fowler JS, Skolnick P, Muench L, Kang Y, Shea C *et al* (2014). Therapeutic doses of buspirone block D3 receptors in the living primate brain. *Int J Neuropsychopharmacol* **17**: 1257–1267.

Kula NS, Baldessarini RJ, Kebabian JW, Neumeyer JL (1994). S-(+)-aporphines are not selective for human D3 dopamine receptors. *Cell Mol Neurobiol* **14**: 185–191.

Lammertsma AA, Hume SP (1996). Simplified reference tissue model for PET receptor studies. *Neuroimage* **4**: 153–158.

Le Foll B, Goldberg SR, Sokoloff P (2005). The dopamine D3 receptor and drug dependence: effects on reward or beyond? *Neuropharmacology* **49**: 525–541.

Liu YP, Wilkinson LS, Robbins TW (2004). Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex. *Psychopharmacology (Berl)* **173**: 175–185.

Mahmood I, Sahajwalla C (1999). Clinical pharmacokinetics and pharmacodynamics of buspirone, an anxiolytic drug. *Clin Pharmacokinet* **36**: 277–287.

Malec TS, Malec EA, Dongier M (1996). Efficacy of buspirone in alcohol dependence: a review. *Alcohol Clin Exp Res* **20**: 853–858.

Martinez D, Narendran R (2010). Imaging neurotransmitter release by drugs of abuse. *Curr Top Behav Neurosci* **3**: 219–245.

Martinez D, Slifstein M, Broft A, Mawlawi O, Hwang DR, Huang Y *et al* (2003). Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. *J Cereb Blood Flow Metab* **23**: 285–300.

Matuskey D, Gallezot JD, Pittman B, Williams W, Wanyiri J, Gaiser E *et al* (2014). Dopamine D(3) receptor alterations in cocaine-dependent humans imaged with [(1)(1)C](+)PHNO. *Drug Alcohol Depend* **139**: 100–105.

McCormick PN, Kapur S, Graff-Guerrero A, Raymond R, Nobrega JN, Wilson AA (2010). The antipsychotics olanzapine, risperidone, clozapine, and haloperidol are D2-selective ex vivo but not in vitro. *Neuropsychopharmacology* **35**: 1826–1835.

McRae AL, Brady KT, Carter RE (2006). Buspirone for treatment of marijuana dependence: a pilot study. *Am J Addict* **15**: 404.

McRae AL, Sonne SC, Brady KT, Durkalski V, Palesch Y (2004). A randomized, placebo-controlled trial of buspirone for the treatment of anxiety in opioid-dependent individuals. *Am J Addict* **13**: 53–63.

Mello NK, Fivel PA, Kohut SJ, Bergman J (2013). Effects of chronic buspirone treatment on cocaine self-administration. *Neuropsychopharmacology* **38**: 455–467.

Meltzer HY, Flemming R, Robertson A (1983). The effect of buspirone on prolactin and growth hormone secretion in man. *Arch Gen Psychiatry* **40**: 1099–1102.

Murray AM, Ryoo HL, Gurevich E, Joyce JN (1994). Localization of dopamine D_3 receptors to mesolimbic and D_2 receptors to mesostriatal regions of human forebrain. *Proc Natl Acad Sci USA* **91**: 11271–11275.

Payer DE, Behzadi A, Kish SJ, Houle S, Wilson AA, Rusjan PM *et al* (2013). Heightened D3 dopamine receptor levels in cocaine dependence and contributions to the addiction behavioral phenotype: a positron emission tomography study with [C]-(+)-PHNO. *Neuropsychopharmacology* **39**: 311–318.

Rabiner EA, Gunn RN, Wilkins MR, Sargent PA, Mocaer E, Sedman E *et al* (2000). Drug action at the 5-HT(1A) receptor *in vivo:* autoreceptor and postsynaptic receptor occupancy examined with PET and [carbonyl-(11)C]WAY-100635. *Nucl Med Biol* **27**: 509–513.

Rabiner EA, Slifstein M, Nobrega J, Plisson C, Huiban M, Raymond R *et al* (2009). In vivo quantification of regional dopamine-D3 receptor binding potential of (+)-PHNO: studies in non-human primates and transgenic mice. *Synapse* **63**: 782–793.

Rousset OG, Ma Y, Evans AC (1998). Correction for partial volume effects in PET: principle and validation. *J Nucl Med* **39**: 904–911.

Rusjan P, Mamo D, Ginovart N, Hussey D, Vitcu I, Yasuno F *et al* (2006). An automated method for the extraction of regional data from PET images. *Psychiatry Res* **147**: 79–89.

Searle G, Beaver JD, Comley RA, Bani M, Tziortzi A, Slifstein M *et al* (2010). Imaging dopamine D(3) receptors in the human brain with positron emission tomography, [(11)C]PHNO, and a selective D(3) receptor antagonist. *Biol Psychiatry* **68**: 392–399.

Shelton KL, Hendrick ES, Beardsley PM (2013). Efficacy of buspirone for attenuating cocaine and methamphetamine re-instatement in rats. *Drug Alcohol Depend* **129**: 210–216.

Shotbolt P, Tziortzi AC, Searle GE, Colasanti A, van der Aart J, Abanades S *et al* (2012). Within-subject comparison of [(11)C]-(+)-PHNO and [(11)C]raclopride sensitivity to acute amphetamine challenge in healthy humans. *J Cereb Blood Flow Metab* **32**: 127–136.

Staley JK, Mash DC (1996). Adaptive increase in D3 dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci* **16**: 6100–6106.

Tziortzi AC, Searle GE, Tzimopoulou S, Salinas C, Beaver JD, Jenkinson M *et al* (2011). Imaging dopamine receptors in humans with [11C]-(+)-PHNO: dissection of D3 signal and anatomy. *Neuroimage* **54**: 264–277.

Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ *et al* (1993). Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse* **14**: 169–177.

Wilson AA, McCormick P, Kapur S, Willeit M, Garcia A, Hussey D *et al* (2005). Radiosynthesis and evaluation of [11C]-(+)-4-propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9 -ol as a potential radiotracer for in vivo imaging of the dopamine D2 high-affinity state with positron emission tomography. *J Med Chem* **48**: 4153–4160.

Winhusen TM, Kropp F, Lindblad R, Douaihy A, Haynes L, Hodgkins C *et al* (2014). Multisite, randomized, double-blind, placebo-controlled pilot clinical trial to evaluate the efficacy of buspirone as a relapse-prevention treatment for cocaine dependence. *J Clin Psychiatry* **75**: 757–764.

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