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In Vivo Imaging of Cerebral Dopamine D3 Receptors in Alcoholism

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Animal studies support the role of the dopamine D3 receptor (DRD3) in alcohol reinforcement or liking. Sustained voluntary alcohol drinking in rats has been associated with an upregulation of striatal DRD3 gene expression and selective blockade of DRD3 reduces ethanol preference, consumption, and cue-induced reinstatement. *In vivo* measurement of DRD3 in the living human brain has not been possible until recently owing to a lack of suitable tools. In this study, DRD3 status was assessed for the first time in human alcohol addiction. Brain DRD3 availability was compared between 16 male abstinent alcohol-dependent patients and 13 healthy non-dependent age-matched males using the DRD3-preferring agonist positron emission tomography (PET) radioligand [^{11}C]PHNO with and without blockade with a selective DRD3 antagonist (GSK598809 60 mg p.o.). In striatal regions of interest, where the [^{11}C]PHNO PET signal represents primarily DRD2 binding, no differences were seen in [^{11}C]PHNO binding between the groups at baseline. However, baseline [^{11}C]PHNO binding was higher in alcohol-dependent patients in hypothalamus (V_T: 16.5 ± 4 *vs* 13.7 ± 2.9, $p = 0.040$), a region in which the [^{11}C]PHNO signal almost entirely reflects DRD3 availability. The reductions in regional receptor binding (V_T) following a single oral dose of GSK598809 (60 mg) were consistent with those observed in previous studies across all regions. There were no differences in regional changes in V_T following DRD3 blockade between the two groups, indicating that the regional fractions of DRD3 are similar in the two groups, and the increased [^{11}C]PHNO binding in the hypothalamus in alcohol-dependent patients is explained by elevated DRD3 in this group. Although we found no difference between alcohol-dependent patients and controls in striatal DRD3 levels, increased DRD3 binding in the hypothalamus of alcohol-dependent patients was observed. This may be relevant to the development of future therapeutic strategies to treat alcohol abuse. *Neuropsychopharmacology* (2014) **39,** 1703–1712; doi:10.1038/npp.2014.18; published online 19 February 2014

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INTRODUCTION

Alcohol abuse contributes to an estimated 3.8% of global deaths and 4.6% of global disability-adjusted life-years, and alcoholism is particularly associated with the greatest impact (Rehm *et al*, 2009). Treatment for alcoholism can be highly effective, and although psychosocial approaches dominate the treatment field, pharmacotherapy can have an important adjunctive role if appropriately integrated (Lingford-Hughes *et al*, 2012). A wealth of evidence links dysfunction of the dopamine system with alcohol liking, self-administration, and reinforcement in animal models and also in humans under laboratory settings. Despite this, drugs modulating dopaminergic neurotransmission have limited clinical efficacy, which may be due to lack of selectivity of these medications for the dopamine D3 receptor (DRD3) (Lingford-Hughes *et al*, 2012; Volkow *et al*, 2012). The absolute density of DRD3 is high in the 'limbic' dopaminergic system, and the DRD3 is hypothesized to be particularly relevant for the treatment of addictive disorders (Heidbreder *et al*, 2005; Landwehrmeyer *et al*, 1993; Murray *et al*, 1994; Sokoloff *et al*, 1990; Suzuki *et al*, 1998).

Evidence from animal studies supports a role for the DRD3 in alcohol reinforcement or liking. Selective DRD3 antagonism has been shown to reduce ethanol preference and consumption in alcohol-preferring and non-preferring rats (Thanos *et al*, 2005) and to reduce the number of reinforcements and amount of alcohol consumed in mice (Andreoli *et al*, 2003). Furthermore, either antagonism or partial agonism of DRD3 has been shown to reduce the alcohol deprivation effect and cue-induced reinstatement (Heidbreder *et al*, 2007; Vengeliene *et al*, 2006). The DRD3 also has an important role in mediating the effects of stress leading to drug-seeking behavior (Heidbreder *et al*, 2005). It has also been reported that a year of voluntary alcohol drinking in alcohol preferring,

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in high alcohol drinking, and in Wistar rats was associated with an upregulation of DRD3 gene expression in the striatum (Vengeliene *et al*, 2006). Interestingly, in humans a single dose of a selective DRD3 antagonist was recently shown to partially alleviate cigarette craving in short-term abstinent smokers (Mugnaini *et al*, 2013).

Neuroimaging of the dopaminergic system with positron emission tomography (PET) and single-photon emission computed tomography (SPECT) has been instrumental in characterizing this system in addiction in man (Martinez *et al*, 2007). Over the past two decades, the antagonist PET and SPECT radioligands [^{11}C]raclopride, [^{11}C]fallypride, [^{123}I]epidepride, and [^{123}I]IBZM have been used to image the D2 receptor family. These *in vivo* imaging studies have revealed decreased D2 receptor availability in alcohol-dependent patients (ADP) when compared with controls and in stimulant abuse. However, quantification of the D2 receptor family has limitations, because as antagonists these ligands bind with equal affinity to both the high ($DRD2_{HIGH}$) and the low ($DRD2_{LOW}$) affinity states of the DRD2. The $DRD2_{HIGH}$ primarily mediate the effects of dopamine (George *et al*, 1985; Leff, 1995; Liu *et al*, 2000). In addition, the PET/SPECT signal from these radioligands consists of a mix of DRD2 and DRD3 binding (Halldin *et al*, 1995; Mukherjee *et al*, 1999; Narendran *et al*, 2006; Strange, 2001; Videbaek *et al*, 2000). Lastly, the receptor quantification has mostly been limited to the striatum owing to the low level of extra-striatal DRD2/3 receptors and low affinity of the radiotracers.

Recently, the development of the agonist radioligand [^{11}C]PHNO permits imaging of the $DRD2_{HIGH}$ in both striatal and some extrastriatal regions (Ginovart *et al*, 2007b; Searle *et al*, 2013; Tziortzi *et al*, 2011). In addition, DRD3 levels can be assessed as [^{11}C]PHNO has approximately 20-fold selectivity for DRD3 over DRD2 (Graff-Guerrero *et al*, 2010; Narendran *et al*, 2006; Rabiner *et al*, 2009; Searle *et al*, 2010; Searle *et al*, 2013). In combination with a selective DRD3 antagonist (eg, GSK598809 or GSK618334), [^{11}C]PHNO PET has determined the relative contributions of DRD2 and DRD3 to the [^{11}C]PHNO PET signal in healthy volunteers (Rabiner *et al*, 2009; Searle *et al*, 2013; Tziortzi *et al*, 2011). In the substantia nigra–ventral tegmentum (SN-VTA) and hypothalamus, around 90% of [^{11}C]PHNO signal reflected DRD3. In the ventral pallidum and globus pallidus, this fraction was 71 and 66%,, respectively, whereas it was 39% in the ventral striatum, 14% in dorsal putamen, and 21% in dorsal caudate (Searle *et al*, 2013).

In four recent imaging studies, the agonist DRD2/3 radioligands [^{11}C]PHNO (Boileau *et al*, 2012; Matuskey *et al*, 2011; Payer *et al*, 2013) and [^{11}C]NPA (Narendran *et al*, 2011) were used to compare DRD2/3 receptor availability between individuals with stimulant dependence and matched healthy controls. No significant difference between the groups in striatal DRD2/3 availability was found in contrast to evidence from the previous antagonist studies. As both [^{11}C]PHNO and [^{11}C]NPA have higher affinity for DRD3 relative to DRD2 compared with the antagonist ligands (Narendran *et al*, 2006), the authors speculated that the lack of difference could be a consequence of decreased $DRD2_{HIGH}$ combined with increased DRD3 binding in stimulant dependence compared with healthy controls (Boileau *et al*, 2012; Narendran *et al*, 2011).

We therefore acquired [^{11}C]PHNO PET images before and after a dose of a DRD3 antagonist, GSK598809, to determine for the first time whether any differences between abstinent ADP and non-dependent healthy individuals in [^{11}C]PHNO signal are due to a difference in $DRD2_{HIGH}$ or DRD3 availability. Based on the pre-clinical evidence that DRD3 antagonism reduced alcohol liking or reinforcement, we hypothesized that DRD3 availability would be higher and $DRD2_{HIGH}$ lower in abstinent ADP compared with non-dependent healthy individuals. Specifically, we expected to observe higher total [^{11}C]PHNO binding in DRD3 signal-rich regions such as SN-VTA, as reported in the three recent [^{11}C]PHNO studies in stimulant users (Boileau *et al*, 2012; Matuskey *et al*, 2011; Payer *et al*, 2013), and hypothalamus and lower total [^{11}C]PHNO binding in caudate and putamen where signal mostly represents DRD2 binding. In regions where the [^{11}C]PHNO signal represents both DRD2 and DRD3, such as thalamus, globus pallidum, and ventral striatum, we expected to detect a more pronounced decrease in binding after DRD3 blockade in patients with alcohol dependence, reflecting a higher fraction of DRD3 in these individuals.

METHODS

Participants

Abstinent ADP were recruited from outpatient programs in Central and North West London NHS Foundation Trust and associated local addiction services. Healthy volunteers were recruited from newspaper advertisements. All ADP met DSM-IV criteria for alcohol dependence and had been abstinent from alcohol after undergoing detoxification with benzodiazepines for a minimum of 4 weeks at the time of scanning. Control participants (CTR) had no history of alcohol abuse or dependence and drank a maximum of 25 units of alcohol per week (200 g/week). Additional inclusion criteria for all participants were: (1) male; (2) age 24–60 years; (3) no previous or current Axis I disorders although a history of major depression was allowed for ADP; (4) no current or past history of abuse of or dependence on drugs other than nicotine, with a negative urine toxicology (a history of use of but not dependence on cannabis was allowed but not within the past 4 weeks before scanning); and (5) no significant medical condition or current use of medications. Participants were instructed to refrain from consuming caffeine- or xanthine-containing products on the day of the scans and underwent urine and breathalyzer tests to rule out recent drug and alcohol use. Participants were not allowed to smoke for 1 h before each brain (MRI and PET) scan.

Semi-structured interviews were administered to obtain lifetime history of substance use, including approximate quantification of lifetime alcohol and substance use (interviews adapted from Skinner and Sheu, 1982 and Copenhagen Stimulant Screening Questionnaire). Degree of alcohol dependence was assessed with Severity of Alcohol Dependence Questionnaire (SADQ) (Stockwell *et al*, 1994), alcohol craving with Alcohol Urge Questionnaire (Bohn *et al*, 1995), and nicotine dependency with the Fagerstrom Test for Nicotine Dependence (Heatherton *et al*, 1991). Symptoms of depression and anxiety were characterized using Beck Depression Inventory (BDI; Beck, 1961), Spielberger Trait

Anxiety Scale, and Spielberger State Anxiety Scale (SSAI; Spielberger *et al*, 1983). Premorbid IQ was assessed with the National Adult Reading Test (NART-R) (Blair and Spreen, 1989).

The study was sponsored by the Imperial College London, London, UK and approved by local ethics committee and all participants provided informed, written consent. Brain scans were carried out at GSK Clinical Imaging Centre (now Imanova), Hammersmith Hospital, London, UK.

Imaging

All participants underwent two [^{11}C]PHNO PET scans before and 3 h after administration of 60 mg of GSK598809. For details regarding preparation of [^{11}C]PHNO, see Plisson *et al* (2012) and for acquisition of PET images, collection of arterial blood samples, derivation of input function, and motion correction, see Searle *et al* (2013). Venous blood was sampled for measurements of GSK598809 concentration at the beginning of the second PET scan.

To aid the definition of the regions of interest (ROIs), high resolution structural MRIs and two components of the FLAWS sequence (Tanner *et al*, 2012) were acquired on 3-Tesla MR scanner (Magnetom Trio Syngo MR B13 Siemens 3T; Siemens AG, Medical Solutions) to provide a T1-weighted contrast and WM nulled image in a single scan. T1-weighted 3D MPRAGE volumes were acquired using the ADNI-GO recommended parameters: 256×192 mm field of view, 1 mm^3 isotropic resolution. The inversion times for the T1-weighted and the WM nulled images were 409 and 1100 ms, respectively.

ROIs and Derivation of [^{11}C]PHNO PET Outcome Measures

Ten ROIs were analyzed: A [^{11}C]PHNO-binding dopaminergic midbrain region (consisting of substantia nigra–ventral tegmentum (SN-VTA)), ventral pallidum (VP), globus pallidum internal (GPi), globus pallidum external (GPe), ventral striatum (VST), thalamus (THA), caudate (CD), putamen (PU), amygdala (AMY), and hypothalamus (HYP). The ROIs, except SN-VTA, were defined manually on each subject's MRI according to Tziortzi *et al* (2011) and amygdala according to Colasanti *et al* (2012). The delineation of the GPi and GPe was performed at first on the transverse slices using the white matter nulled image. The delineation started at the most dorsal slices where only the GPe is visible. Moving ventral, the thin white matter lamina, which separates the GPi and GPe, comes into view. The lamina is used to define the lateral and medial boundaries of the GPi and GPe, respectively. After the delineation of the two structures on the transverse plane, the operator switches to the coronal plane to refine the definition of the structures. This is particularly important for the delineation of the medial area of the GPi. SN-VTA was defined on each subject's baseline PET integral image, as the contrast between SN and surrounding tissue was insufficient for accurate delineation on the T1 MR images (Tziortzi *et al*, 2011). The gray matter (GM) of the dorsal cerebellum (CER), used as a reference region, was defined via nonlinear registration (using SPM5b; Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm) of a template MRI (ICBM 52, http://www.bic.mni.mcgill.ca/ServicesAtlases/ICBM152NLin2009) and corresponding brain atlas (Tziortzi *et al*, 2011). The cerebellar ROI did not include the vermis in order to minimize the presence of DRD3. Each ROI was then applied to the dynamic PET data to derive regional time–activity curves (TACs). The T1-weighted images were segmented into GM, white matter (WM), and cerebrospinal fluid (CSF) images that represent the probability of any given voxel containing GM, WM, or CSF. Each subject's GM image was used to mask the cortical regions and the cerebellum, and the masked ROIs were subsequently used to estimate the cortical GM volumes. For the manually defined subcortical ROIs, the entire ROI was used to estimate the GM volumes.

[^{11}C]PHNO rate constants (K_1–k_4) were estimated using a two tissue compartment (2TC) plasma input model as described previously (Searle *et al*, 2013). Subsequently, the total volume of distribution (V_T), defined as the equilibrium partition coefficient between tissue and plasma, was derived for each ROI;

$$V_T = (K_1/k_2)(1 + (k_3/k_4)) \quad (1)$$

Subsequently, BP_P and BP_{ND} in the target regions were calculated:

$$BP_P = V_T - V_{ND} \quad (2)$$

$$BP_{ND} = (V_T - V_{ND})/V_{ND} \quad (3)$$

where V_{ND} is the nondisplaceable volume of distribution and BP_P and BP_{ND} are the binding potentials derived as the ratio at equilibrium of specifically bound radioligand to that of total parent radioligand in plasma and to that of nondisplaceable radioligand in tissue, respectively.

In addition, the Simplified Reference Tissue Model (SRTM) (Gunn *et al*, 1997; Lammertsma and Hume, 1996), using cerebellum as the reference region, was applied to derive BP_{ND}. In order to distinguish the two BP_{ND} outcome measures from SRTM and 2TC kinetic analysis, they will be referred to as $BP_{ND(SRTM)}$ and $BP_{ND(2TC)}$, respectively.

For V_T, BP_P, and BP_{ND} values, a DRD3/DRD2 binding ratio measure was created by dividing SN-VTA with caudate–putamen (average binding in these two regions) binding (Boileau *et al*, 2012). By re-scanning with [^{11}C]PHNO after administration of a selective DRD3 antagonist, the degree of blockade of the [^{11}C]PHNO signal reflects a decrease in DRD3 binding. This blockade, referred to as 'Δ DRD3 (%)', also served as outcome measure and for V_T and BP_{ND} was calculated as follows:

$$\Delta\,\mathrm{DRD3}\,V_T(\%) = \left(V_{T(\mathrm{pre-block})} - V_{T(\mathrm{post-block})}/V_{T(\mathrm{pre-block})}\right) * 100 \quad (4)$$

$$\Delta\,\mathrm{DRD3}BP_{ND}(\%) = \left(BP_{ND(\mathrm{pre-block})} - BP_{ND(\mathrm{post-block})}/BP_{ND(\mathrm{pre-block})}\right) * 100 \quad (5)$$

Plasma Measurements of GSK598809

High-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was used to quantify plasma levels of GSK598809 sampled 3 h post administration (beginning of the second PET scan). Two hundred

Table 1 Demographics, Personality, and Symptom Measures

Demographics, personality, and symptoms	Alcohol-dependent patients ($n=16$)			Healthy controls ($n=13$)			ADPs *vs* HCs
	Mean (median)	SD	Range	Mean (median)	SD	Range	*p*-value (two-tailed)
Age	42.4 (45.5)	9.4	24–55	41.5 (39.0)	10.3	27–60	0.822
NART	18.6	10.6	7–37	17.2 (18)	7.3	3–30	0.679
Body weight (kg)	78.5 (75.4)	13.2	56.6–109.0	78.0 (81.6)	8.9	60.5–90.5	0.91
Ethnicity	13 Caucasians 2 Indian 1 Black	NA	NA	11 Caucasians 1 Indian 1 Black	NA	NA	NA
Beck' Depression Inventory score	11.6 (10)	7.7	2–26	1.1 (1)	1.3	0–4	<0.001
Spielberger State Anxiety score	38.6 (38)	6.8	28–53	28.8 (30)	5	20–36	<0.001
Barret's Impusivity Scale score	72.3 (71.5)	10.8	57–98	61.9 (62)	11.1	45–81	0.025
Smoking status	11 smokers 3 ex-smokers 2 non-smokers	NA	NA	10 smokers 2 ex-smokers 1 non-smoker	NA	NA	NA
Cigarettes per day among smokers	17 (20)	7	4–25	10.9 (12.5)	4.5	1.5–15	0.027
Cigarette package years	28.1 (22.7)	29	7.8–123.0	15.0 (15.1)	11.7	1.4–38.3	0.139
Smoking dependence (Fagerstrom score)	5.9 (6.0)	1.6	3–9	3.3 (3.0)	1.5	1–5	0.001
Alcohol dependence severity (SADQ score)	45.7 (50)	14.7	14–60	2.2 (1)	2.6	0–7	<0.001
Alcohol units per week (up to last detox for ADPs)	348 (338)	131	112–560	9.8 (10.4)	7.6	0–23	<0.001
Years of alcohol abuse	26.4 (30.5)	9.5	9–38	NA	NA	NA	NA
Accumulated lifetime alcohol intake (1000 units)	181 (185)	92	35–351	NA	NA	NA	NA
Days since last alcohol	414 (355)	254	39–893	Min 24 h	NA	NA	NA

microliters acetonitrile, containing haloperidol-d4 as internal standard, was added to 50 µl of standard. QC or sample was put in a 2-ml polypropylene tube and mixed for 1 min. Following centrifugation at 12000 rpm to compact the protein pellet, 100 µl of the supernatant was then mixed with 100 µl 0.1% formic acid and 25 µl injected onto the HPLC/MS/MS. Column was a Grace Altima $150 \times 2.1\,mm^2$ 5-µm C18 held at 60 °C. Mobile phase was 55% acetonitrile/45% 0.1% formic acid, pumped at 400 µl/min. Detection was by positive ion-spray and MRM masses: GSK598809 $482.0 \rightarrow 286.0$, Haloperidol-d4 $380.1 \rightarrow 169.1$.

Statistics

Group differences in PET outcome measures, in socio-demographic data, tobacco and alcohol use, and in scanning-related variables were tested using the Student's unpaired two-tailed *t*-test. To explore whether PET outcome measures and other variables, such as drinking parameters, smoking parameters, and psychometric data, were related, multiple linear regression analysis with V_T or Δ DRD3 V_T (%) was included as the dependent variable. Age was also included as a covariate in these analyses as DRD2/3 levels decline with age (Antonini *et al*, 1993; Ichise *et al*, 1998; Ishibashi *et al*, 2009; Rinne *et al*, 1990; Severson *et al*, 1982; Volkow *et al*, 1998; Wong *et al*, 1997). To assess whether there was a significant V_T blockade in the cerebellum, one-tailed paired *t*-test for pre- *vs* post-blockade cerebellar V_T values for each group was applied. When comparing injected masses for the four conditions (pre- and post-blockade for both the groups), one-way analysis of variance was conducted. In cases where assumptions of normally distributed data were violated, two-tailed nonparametric test (Mann–Whitney) and nonparametric signed rank test (Kruskal–Wallis) were used.

RESULTS

Participants and Demographics

Sixteen male ADP (42.4 ± 9.4 years) and 13 male CTR (41.5 ± 10.3 years) were included in the study. For one ADP an arterial line could not be inserted, and consequently only SRTM BP_{ND}, and not V_T, could be estimated. Table 1 describes demographic and clinical variables of mood, alcohol, tobacco and drug use, and personality measures. No group differences were observed for the following: age ($p=0.82$), body weight ($p=0.91$), ethnicity, premorbid IQ (NART) score ($p=0.70$), or composition of current/former/non-smokers. However, ADP smoked more cigarettes per day than CTR ($p=0.03$) and had higher Fagerstrom Nicotine Dependence Scale scores ($p<0.01$). ADP scored higher than CTR on all measures of alcohol consumption and dependence ($p<0.01$) and had been abstinent for 415 ± 254 (range: 39–893) days before scanning. Although participants with current clinical depression or anxiety were excluded, ADP reported higher scores on depressive mood and anxiety (all: $p<0.01$; Table 1).

PET Results

Regional pre- and post-blockade [^{11}C]PHNO V_T, BP_P, and BP_{ND} values are presented in Table 2. Administration of

GSK598809 resulted in a significant decrease in cerebellar V_T (8.3 ± 7.4 and 11.4 ± 9.7%, $p = 0.002$ and $p < 0.001$ for CTR and ADP, respectively). The presence of a specific binding component in the [^{11}C]PHNO signal in the cerebellum led us to use the V_T as the primary outcome measure.

Group comparison of the total [^{11}C]PHNO binding. Total (or baseline) [^{11}C]PHNO V_T was significantly higher in ADP compared with CTR in the hypothalamus (17.2% difference, $p = 0.04$, uncorrected) but not in any other region: striatum (caudate, putamen, and ventral striatum; 4.8%, $p = 0.40$; 1.9%, $p = 0.82$; and 1.5%, 0.88; respectively), amygdala (6.1%, $p = 0.12$), external pallidum (− 5.7%, $p = 0.54$), cerebellum (6.0%, $p = 0.16$), and SN-VTA region (5.1%, $p = 0.67$). In the thalamus, ventral pallidum, and internal pallidum, there were trends towards higher binding in ADP compared with CTR (9.4%, $p = 0.08$; 16.2%, $p = 0.08$; and 22.7%, $p = 0.09$; respectively). The calculated VTA-SN/CD-PU binding ratios did not differ between the ADP and CTR groups, see Table 2. Correction for age did not change any of these regional findings significantly (data not shown).

Plasma levels of GSK598809. Plasma levels of GSK598809 at the beginning of the post-GSK598809 PET scan (3 h after dosing) did not differ between the two groups (ADP: 359 ± 113 ng/ml *vs* CTR: 401 ± 144 ng/ml, $p = 0.409$).

Effect of GSK598809 on [^{11}C]PHNO binding. Reductions of [^{11}C]PHNO V_T after GSK598809 blockade were most pronounced in the SN-VTA, hypothalamus, and ventral pallidum (approximately 40%), whereas V_T changed to a lesser extent in the caudate, putamen, and cerebellum (10–14%), see Table 2 (lower section). There was no significant difference between groups in the degree of post-block V_T decrease in any region. Adjusting the change in V_T for plasma levels of GSK598809 measured at the start of the post-GSK598809 PET scan did not change this result.

Injected Mass of PHNO and Plasma-Free Fraction

There was no difference in average PHNO mass injected across the four conditions (CTR baseline: 1.5 ± 0.3 μg, CTR post GSK598809: 1.4 ± 0.3 μg, ADP baseline: 1.4 ± 0.3 μg, ADP post GSK598809 : 1.3 ± 0.3 μg, ANOVA: F[3,57], $p = 0.577$). Also, no differences were found after correcting for injected mass per kg body weight (ANOVA: F[3,57], $p = 0.340$). There were no significant relationships between injected mass per kg body weight and regional V_T values (p-values between 0.286 and 0.938, data not shown). The plasma-free fraction, f_p, did not differ between the groups (CTR: 0.33 ± 0.05, ADP: 0.34 ± 0.05, $p = 0.589$).

Effect of Age

There was a significant negative association between age and baseline V_T in SN-VTA ($R = 0.26$, F[1,26] = 9.3, $p = 0.005$) and in putamen ($R = 0.18$, F[1,26] = 5.6, $p = 0.025$) when ADP and CTR were combined. In SN-VTA, this effect was mainly driven by the CTR group (ADP: $R = 0.26$, F[1,13] = 4.47, $p = 0.054$; CTR: $R = 0.38$, F[1,11] = 6.8, $p = 0.025$) and in putamen mainly by the ADP group (ADP: $R = 0.38$, F[1,13] = 8.0, $p = 0.014$; CTR: $R = 0.014$, F[1,11] = 0.16, $p = 0.697$). In the hypothalamus, there was a trend to a negative association with age in the full sample ($R = 0.13$, F[1,26] = 4.0], $p = 0.057$), an effect driven by the ADP subgroup (ADP: $R = 0.57$, F[1,13] = 17.1, $p = 0.001$; CTR: $R = 0.008$, F[1,11] = 0.1], $p = 0.773$). No significant relationships between V_T and age were detected in other regions.

In the ADP group ($R = 0.33$, F[1,12] = 6.0, $p < 0.031$) but not in the full sample or in the CTR group, the degree of DRD3 blockade was negatively associated with age in both thalamus and putamen. No other significant relationships between degree of DRD3 blockade and age were detected in any other regions.

GM Volumes

There was no significant overall effect of group (Wilks' λ 0.51, F[1,29] = 1.61, $p = 0.186$) or age (Wilks' λ 0.62, F[1,29] = 1.05, $p = 0.448$) when entering all regional GM volumes into a multivariate linear regression analysis. However, *post-hoc* analysis and after correcting for age revealed that only the cerebellum was significantly smaller (8.5%, F[1,29] = 4.64, $p = 0.041$) in ADP compared with CTR. Correction for GM volumes did not change the group results (only data without correction for volume is presented in Table 2) from the group comparison of V_T values in any of the regions.

Relationships between V_T Data and Clinical Measures

Alcohol consumption. Neither SADQ score nor length of abstinence were significantly related to V_T or to the degree of DRD3 blockade in any of the regions among ADP. The lifetime alcohol intake was positively associated with V_T in the caudate but not in VST, putamen, or SN-VTA. In the thalamus, V_T as well as the degree of DRD3 blockade was positively associated with lifetime alcohol intake.

Smoking. The full sample was divided into three groups; current smokers ($n = 20$), former smokers ($n = 5$), and never-smokers ($n = 3$). There were no significant differences in V_T in any ROI between these groups. Similarly, there were no significant group difference in regional V_T when dividing the entire group of currently smoking ADP and CTR into two groups based on low and high scores on the Fagerstrom Test for Nicotine Dependence (low dependence: 0–4 *vs* medium-to-high dependence: 5–10). Additionally, in a regression analysis the score was not associated with V_T in any region (data not shown).

Barrett's Impulsiveness Scale (BIS). The ADP scored significantly higher than CTR (BIS: 72 ± 11 *vs* 62 ± 11, respectively, $p = 0.025$). The BIS score was not significantly correlated with V_T or DRD3 blockade in any region.

Symptom scores. There were no significant relationships between depression (BDI) score and V_T in any region. In contrast, there was a significant positive correlation between SSAI score and V_T in the thalamus, amygdala, and hypothalamus in the full sample. The degree of DRD3

Table 2 Regional 11C-PHNO Binding Data; Baseline (upper section) and after Blockade with the DRD3 Antagonist, GSK598809 (lower section)

Region	V_T Av (SD)			BP_P Av (SD)			BP_{ND} (kinetic) Av (SD)			BP_{ND} (SRTM) Av (SD)		
	CTR	ADP	*p*-value	CTR	ADP	*p*-value	CTR	ADP	*p*-value	CTR	ADP	*p*-value
VTA-SN	14.4 (3.2)	15.1 (5.8)	0.666	9.5 (3.1)	9.9 (5.6)	0.790	2.0 (0.6)	1.9 (0.9)	0.868	1.3 (0.2)	1.3 (0.4)	0.972
Hypothal	13.7 (2.9)	16.5 (4.0)	0.040*	8.8 (2.7)	11.3 (3.7)	0.050	2.3 (1.8)	2.2 (0.6)	0.762	1.4 (0.2)	1.5 (0.3)	0.135
Ventral Pall	35.1 (7.9)	41.9 (11.0)	0.075	30.3 (7.7)	36.7 (10.7)	0.080	6.2 (1.5)	7.1 (1.7)	0.194	4.3 (0.4)	4.5 (0.7)	0.252
Pall Glob Ext	29.5 (6.9)	27.9 (5.7)	0.537	24.6 (6.8)	22.8 (5.2)	0.468	5.0 (1.4)	4.4 (0.7)	0.211	3.0 (0.2)	3.0 (0.3)	0.778
Pall Glob Int	26.1 (4.4)	33.8 (14.5)	0.091	21.1 (4.2)	28.7 (14.1)	0.089	4.3 (0.9)	5.4 (2.2)	0.094	2.9 (0.4)	3.0 (0.5)	0.371
VST	23.5 (6.3)	23.9 (5.9)	0.878	18.6 (6.2)	18.7 (5.6)	0.983	3.8 (1.2)	3.6 (0.9)	0.557	2.8 (0.2)	2.8 (0.5)	0.646
Putamen	18.6 (3.1)	18.9 (5.1)	0.824	13.7 (3.0)	13.7 (4.8)	0.980	2.8 (0.7)	2.6 (0.8)	0.474	2.3 (0.1)	2.3 (0.2)	0.492
Caudate	13.9 (2.2)	14.6 (2.0)	0.399	9.0 (2.1)	9.4 (1.5)	0.553	1.9 (0.4)	1.8 (0.2)	0.851	1.5 (0.2)	1.6 (0.2)	0.331
Thalamus	7.3 (0.8)	8.1 (1.4)	0.080	2.4 (0.5)	2.9 (1.0)	0.138	0.5 (0.1)	0.6 (0.2)	0.333	0.4 (0.1)	0.5 (0.1)	0.102
Amygdala	6.9 (0.6)	7.3 (0.9)	0.124	2.0 (0.4)	2.1 (0.5)	0.446	0.4 (0.1)	0.4 (0.1)	0.981	0.3 (0.1)	0.3 (0.1)	0.828
Ratio VTA-SN/Caud-Put	0.9 (0.2)	0.9 (0.3)	0.769	0.9 (0.3)	0.8 (0.1)	0.452	0.9 (0.3)	0.8 (0.1)	0.452	0.7 (0.1)	0.7 (0.2)	0.946
Cerebellum	4.9 (0.5)	5.2 (0.6)	0.164	NA	NA	NA	NA	NA	NA	NA	NA	NA

Region	Δ V_T (%) Av (SD)			Δ BP_P (%) Av (SD)			Δ BP_{ND} (2TC) (%) Av (SD)			Δ BP_{ND} (SRTM) (%) Av (SD)		
	CTR	ADP	*p*-value	CTR	ADP	*p*-value	CTR	ADP	*p*-value	CTR	ADP	*p*-value
VTA-SN	38.6 (13.2)	41.2 (11.9)	0.602	54.8 (14.7)	57.8 (17.1)	0.632	51.0 (15.7)	51.3 (23.9)	0.962	47.3 (9.8)	45.0 (10.8)	0.552
Hypothal	40.6 (11.1)	41.1 (11.6)	0.905	57.0 (13.6)	55.0 (16.9)	0.744	52.3 (15.6)	48.3 (22.6)	0.607	48.9 (14.8)	46.5 (14.8)	0.677
Ventral Pall	36.2 (15.4)	42.0 (15.4)	0.410	40.8 (16.7)	46.6 (17.6)	0.438	35.2 (20.2)	39.1 (21.7)	0.667	32.0 (13.6)	34.0 (13.3)	0.693
Pall Glob Ext	25.5 (38.4)	27.8 (20.3)	0.871	27.4 (46.4)	31.4 (24.8)	0.819	19.2 (50.2)	22.6 (26.7)	0.856	19.7 (9.3)	18.5 (10.4)	0.746
Pall Glob Int	31.4 (21.5)	35.9 (39.1)	0.749	36.5 (26.3)	40.8 (45.7)	0.789	29.2 (28.3)	32.0 (55.5)	0.882	35.9 (12.2)	35.2 (11.7)	0.881
VST	22.6 (12.4)	18.4 (11.0)	0.384	26.1 (13.5)	20.7 (12.6)	0.315	19.5 (12.8)	10.3 (13.0)	0.087	8.4 (8.5)	5.9 (10.4)	0.493
Putamen	15.2 (12.5)	13.0 (13.0)	0.666	17.1 (15.0)	13.1 (14.3)	0.505	9.3 (15.9)	1.6 (16.0)	0.235	− 3.7 (8.7)	− 6.1 (7.8)	0.453
Caudate	11.2 (13.4)	11.8 (10.0)	0.905	11.8 (18.3)	12.3 (10.2)	0.935	3.5 (20.5)	1.3 (6.9)	0.741	− 8.2 (9.4)	− 7.7 (7.5)	0.880
Thalamus	18.4 (4.9)	22.3 (10.6)	0.229	37.4 (8.5)	40.6 (16.3)	0.530	31.1 (12.5)	32.4 (19.9)	0.835	15.1 (18.2)	15.8 (23.4)	0.923
Amygdala	10.8 (7.0)	14.4 (10.1)	0.301	13.6 (32.7)	18.4 (25.6)	0.690	3.7 (43.9)	7.2 (31.5)	0.821	− 16.6 (44.8)	− 14.6 (23.2)	0.887
Cerebellum	8.3 (7.4)	11.4 (9.7)	0.368	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 2 Regional 11C-PHNO binding data; baseline (upper section) and after blockade with the DRD3 antagonist, GSK598809 (lower section). *$P<0.05$.

blockade was also positively related to SSAI score in the thalamus ($p = 0.050$) and trend-wise positively related in the amygdala ($p = 0.058$).

DISCUSSION

We report here the first *in vivo* brain imaging study investigating DRD3 receptor levels in addiction using the DRD3-preferring agonist [^{11}C]PHNO as PET radioligand with a selective DRD3 blocker. We did not confirm our hypothesis of global increase in DRD3 receptor availability in abstinent ADP when compared with controls. In particular, we did not see any group differences in total [^{11}C]PHNO binding or in the degree of DRD3 blockade in striatal regions or in the SN-VTA region as we had hypothesized. Interestingly, we did find evidence of higher DRD3 binding in hypothalamus among abstinent ADP. A lack of difference in total [^{11}C]PHNO binding in the dorsal part of the striatum (caudate and putamen) is suggestive of unaltered $DRD2_{HIGH}$ binding in abstinent ADP.

Previous PET/SPECT studies in alcoholism conducted with the DRD2/3 antagonist radiotracers [^{11}C]raclopride, [^{18}F]desmethoxyfallypride, [^{123}I]IBZM, and [^{123}I]epidepride have consistently reported lower (7–22%) DRD2/3 availability in the striatum of patients compared with controls (Martinez *et al*, 2007). Our finding of no differences in striatal [^{11}C]PHNO binding between abstinent ADP and controls with or without DRD3 blockade initially appears therefore inconsistent. However, recent studies using [^{11}C]PHNO (Boileau *et al*, 2012; Matuskey *et al*, 2011; Payer *et al*, 2013) or [^{11}C]NPA (Narendran *et al*, 2011) reported no significant differences in striatal binding in stimulant-dependent individuals compared with controls. In one, *post-hoc* analysis showed that heavy, but not moderate, methamphetamine users had slightly decreased [^{11}C]PHNO binding in dorsal striatum (Boileau *et al*, 2012). However, in the absence of a DRD2 or DRD3 antagonist, the relative contributions of lower DRD2 or $DRD2_{HIGH}$ and higher DRD3 binding could not be determined in these studies.

We were able to combine the DRD3-preferring [^{11}C]PHNO with GSK598809, a selective DRD3 antagonist, and did not detect any differences between abstinent ADP and controls in the degree of DRD3 blockade in the striatum or any region. Consistent with previous studies, the largest

reduction of the [^{11}C]PHNO V_T after DRD3 blockade was detected in the SN-VTA, hypothalamus, and ventral pallidum (~40%), reflecting levels of DRD3, whereas binding changed very little in the caudate, putamen, and cerebellum (10–14%) where DRD2 predominate. In the thalamus and VST, the blockade-induced reduction is intermediate (~20%) owing to a mixed DRD2/3 signal with [^{11}C]PHNO in these regions. The incomplete blockade of [^{11}C]PHNO signal in the DRD3-rich regions such as hypothalamus and SN-VTA is due to the moderate dose of GSK598809 used so as not to block DRD2. The 60-mg dose of GSK598809 resulted in equivalent reductions in [^{11}C]PHNO signal to those previously reported (Searle *et al*, 2013).

Importantly, similarity in the baseline V_T between groups, as seen in striatum, is open to the argument that this could be due to a change in DRD3 and a compensatory change in $DRD2_{HIGH}$. However, in that case the occupancy by GSK598809 would have been greater in the group with the higher DRD3 availability—as at the dose used GSK598809 has negligible binding to $DRD2_{HIGH}$. Therefore, our data strongly suggest that it is unlikely that higher DRD3 levels, alongside lower DRD2 levels, result in similar striatal [^{11}C]PHNO binding levels in addiction.

Could a difference in the fraction of $DRD2_{HIGH}$ explain the discrepancy between the newer PET 'agonist studies' ([^{11}C]PHNO and [^{11}C]NPA) and the previous 'antagonist studies' (eg, mostly [^{11}C]raclopride)? Although there is controversy whether $DRD2_{HIGH}$ and $DRD2_{LOW}$ are detectable *in vivo* (Seeman, 2012; Skinbjerg *et al*, 2012), a larger fraction of receptors in the high affinity state among ADP compared with controls could in theory explain the lack of difference in binding between these two groups when assessed with an agonist in contrast to the lower total DRD2/3 binding in ADP found with antagonists. Although we cannot rule out this possibility in the current data set, a recent study does not support this. A study using both the antagonist [^{11}C]raclopride and the agonist [^{11}C]NPA to measure $DRD2_{HIGH}$ and $DRD2_{LOW}$ reported no differences in $DRD2_{HIGH}$ between cocaine addicts and controls (Narendran *et al*, 2011).

Lastly, a difference in synaptic dopamine levels may contribute to DRD2/3 availability. As a DRD3-preferring DRD2/3 agonist, [^{11}C]PHNO is more sensitive to fluctuations in dopamine levels than an antagonist radioligand (Narendran *et al*, 2004; Seneca *et al*, 2006; Shotbolt *et al*, 2012b). Such differential sensitivity could also contribute to the lack of differences in total [^{11}C]PHNO signal but a lower [^{11}C]raclopride binding in addicts compared with controls if there are fewer $DRD2_{HIGH}$ combined with less synaptic dopamine in striatum among addicts compared with controls.

Although we found no differences in [^{11}C]PHNO binding in the DRD3-rich region SN-VTA, studies using a reference region approach reported an increase (Boileau *et al*, 2012; Matuskey *et al*, 2011). This may be due to us using an arterial input function for full quantification compared with their reference region approach. The latter approach relies on the assumption that the reference region has no specific binding of the PET tracer (Lammertsma and Hume, 1996). However, we found that DRD3 blockade reduces [^{11}C]PHNO V_T in the reference region, cerebellum, which suggest the presence of specific binding and therefore contravenes the reference region approach. Consequently, BP_{ND} and BP_P values should be regarded with caution. We therefore chose V_T as the main outcome measure but reported BP_{ND} values for comparison with previous reports using [^{11}C]PHNO (Table 2). In addition, due to its close relationship with the pure measure of receptor binding, BP_F, BP_P is presented. BP_F is equivalent to the B_{avail}/K_d (where B_{avail} = the total concentration of available receptors, K_d = 1/the affinity of radioligand to the receptor) and can be calculated by dividing BP_P by the plasma-free fraction, f_P. In the present data set, f_P did not differ between the groups, and thus group differences in BP_P reflect differences in B_{max}/K_d. The regional group differences in [^{11}C]PHNO binding as measured by V_T were confirmed when looking at BP_P data. Importantly, without derivation of V_T in the reference region, cerebellum, the observed significant difference in binding in hypothalamus would have been masked by the slightly higher [^{11}C]PHNO V_T in cerebellum among the ADP.

Another important issue is the cold mass of PHNO. To minimize the risk of confound, we kept the injected PHNO mass low, around 1.4 μg (as also recommended by Searle *et al*, 2013). In addition, the aim of the DRD3 blockade in this study was not to evaluate a numerically accurate *in vivo* affinity for GSK598809 in this population but to block the DRD3 component of the [^{11}C]PHNO signal in order to evaluate whether any differences between the healthy volunteers and the alcoholics are due to the DRD2 or the DRD3 component of [^{11}C]PHNO. For this purpose, any carry-over of PHNO to the second PET scan added to the GSK598809 would be believed to be negligible as PHNO has a ~20-fold selectivity for DRD3 over DRD2, and at the levels of DRD3 occupancy likely induced by PHNO carry-over (<50%), the effects on DRD2 would be minor (<5%).

Our finding of similar striatal [^{11}C]PHNO binding in abstinent ADP and controls could reflect a true lack of difference in dopamine receptor binding between these groups. One important factor that could explain some of the discrepancy between the current and previous studies is the duration of abstinence before entering the study. Like in most of the antagonist studies, the methamphetamine addicts (Boileau *et al*, 2012) and cocaine addicts using [^{11}C]PHNO (Matuskey *et al*, 2011; Payer *et al*, 2013), were only about 2 weeks abstinent when scanned. Studies assessing the effect of prolonged alcohol abstinence on the regulation of DRD2/3 binding (Heinz *et al*, 2004; Hietala *et al*, 1994; Volkow *et al*, 1996; Volkow *et al*, 2002) have generally not detected any signs of recovery. On the other hand, recently (Rominger *et al*, 2012) striatal DRD2/3 binding has been reported to increase significantly by 29% between the first day and 1 year of abstinence. Furthermore, it is interesting to note that in a recent [^{11}C]raclopride study by Martinez *et al* (2011), in cocaine addiction the patients who responded to contingency management treatment and thus remained abstinent did not differ from healthy controls in DRD2/3 levels measured pre-treatment. Similar results in methamphetamine-dependent patients have been reported by Wang *et al* (2012). Therefore, if successful abstinence is associated with striatal DRD2/3 levels similar to healthy controls, this could explain our lack of difference as some of the ADP in our study had been abstinent for more than a year, with the minimum length of abstinence being 39 days. It should be noted that a recent [^{11}C]PHNO PET study of cocaine-dependent individuals with a

7–240-day abstinence range were in line with the results obtained in the studies by Boileau *et al* (2012) and Matuskey *et al* (2011), pointing to increased [^{11}C]PHNO binding in substantia nigra (Payer *et al*, 2013).

Based primarily on preclinical literature, we hypothesized that DRD3 binding would be globally elevated in ADP compared with controls; however higher levels were restricted to the hypothalamus. We believe this to be the first such description in humans as in previous studies by Boileau *et al* (2012) Matuskey *et al* (2011) did not report binding in the hypothalamus. The hypothalamus is a relatively small region but the quantification of DRD3 binding here using [^{11}C]PHNO has recently been validated (Tziortzi *et al*, 2011). Our finding of higher DRD3 binding in the hypothalamus in alcoholism adds to the growing interest in the role of hypothalamus in addiction (Aston-Jones *et al*, 2009). Increases in dopamine in the hypothalamus are associated with release of beta-endorphin in nucleus accumbens (Roth-Deri *et al*, 2003), with DRD2/3 blockade from eticlopride in the hypothalamus reducing beta-endorphin (Doron *et al*, 2006). Given the high level of DRD3 in the hypothalamus, it is likely that DRD3 contributes to this effect. Therefore, antagonism of the higher hypothalamic DRD3 levels resulting in decreased beta-endorphin release in the ventral striatum might underpin how DRD3 antagonists impact on addiction. Consistent with this hypothesis, *in vivo* human PET imaging data have shown that alcohol consumption induces endogenous opioid release in the ventral striatum (Mitchell *et al*, 2012).

Concerning the relationship with clinical variables and [^{11}C]PHNO levels, we observed a significant negative relationship between baseline [^{11}C]PHNO and age in some of the brain regions, such as SN-VTA (combined, controls), hypothalamus (ADP), and putamen (combined, ADP). This is in accordance with existing *in vitro* and *in vivo* evidence of a significant decline in DRD2/3 density with age (Antonini *et al*, 1993; Ichise *et al*, 1998; Ishibashi *et al*, 2009; Rinne *et al*, 1990; Severson *et al*, 1982; Volkow *et al*, 1998; Wong *et al*, 1997). In addition, among our ADP, DRD3 blockade in the putamen and thalamus was negatively associated with age. Overall, these results are suggestive of a decline in DRD3 binding with age, in particularly among ADP. No previous [^{11}C]PHNO study has reported a relationship between age and DRD2/3 binding (Boileau *et al*, 2009; Boileau *et al*, 2012; Ginovart *et al*, 2007a; Graff-Guerrero *et al*, 2009a; Graff-Guerrero *et al*, 2009b; Shotbolt *et al*, 2012a; Willeit *et al*, 2006), which could be due to the narrower age range in their reports and/or to the effect of alcohol.

In conclusion, our study with [^{11}C]PHNO and a DRD3 antagonist, GSK598809, allows for the first time assessment of DRD2 and DRD3 levels in abstinent ADP. Although we did not find decreased striatal DRD2/3 binding in alcoholism as reported using antagonist radiotracers, we were able to show that this was not caused by higher DRD3 levels alongside lower DRD2 as the DRD3 blockade after administration of a selective antagonist was similar between ADP and controls. We did not confirm our hypothesis of a global increase in DRD3 in abstinent alcohol dependence although increased DRD3 binding was detected in the hypothalamus, a region involved in the control of opioid neurotransmission that is a key modulator of the dopaminergic mesolimbic pathway. In the light of evidence for a role of selective DRD3 antagonism in controlling behavior relevant to addiction, this increased DRD3 level in the hypothalamus is likely to be relevant to the therapeutic strategies targeting the DRD3.

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