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Varenicline-Induced Elevation of Dopamine in Smokers: A Preliminary [^{11}C]-(+)-PHNO PET Study

Patricia Di Ciano[1], Mihail Guranda[1], Dina Lagzdins[1], Rachel F Tyndale[2,3,4,5], Islam Gamaleddin[1,6], Peter Selby[4,7,8,9,10], Isabelle Boileau[5,9,11] and Bernard Le Foll*[,1,3,4,5,7,9,10]

[1]Translational Addiction Research Laboratory, CAMH, Toronto, ON, Canada; [2]Pharmacogenetics Laboratory, CAMH, Toronto, ON, Canada; [3]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada; [4]Department of Psychiatry, University of Toronto, Toronto, ON, Canada; [5]Campbell Family Mental Health Research Institute, CAMH, Toronto, ON, Canada; [6]Institute of Environmental Studies and Research, Ain Shams University, Cairo, Egypt; [7]Ambulatory Care and Structured Treatment Program, CAMH, Toronto, ON, Canada; [8]Dalla Lana School of Public Health, Toronto, ON, Canada; [9]Institute of Medical Sciences, University of Toronto, Toronto, ON, Canada; [10]Department of Family and Community Medicine, University of Toronto, Toronto, ON, Canada; [11]Addiction Imaging Group, Research Imaging Centre, CAMH, Toronto, ON, Canada

Varenicline, a nicotinic partial agonist, is the most effective treatment for tobacco use disorder. However, its mechanism of action is still unclear and may involve stimulating dopaminergic transmission. Here we used PET imaging with [^{11}C]-(+)-PHNO to explore for the first time the impact of varenicline on dopamine transmission in the D2-rich striatum and D3-rich extra-striatal regions and its relationship with craving, withdrawal and smoking. Eleven treatment-seeking smokers underwent two PET scans with [^{11}C]-(+)-PHNO, each following 12-h overnight smoking abstinence both prior to receiving varenicline and following 10–11 days of varenicline treatment (ie, at steady-state drug levels). Subjective measures of craving and urges to smoke were also assessed on the days of the PET scans. Varenicline treatment significantly reduced [^{11}C]-(+)-PHNO binding in the dorsal caudate ($p = 0.008$) and reduced some craving measures. These findings provide the first evidence that varenicline is able to increase DA levels in the human brain, a factor that may contribute to its therapeutic efficacy.

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INTRODUCTION

Smoking is a public health concern, yet pharmacological treatment strategies remain only partly effective. Current first-line medications include nicotine replacement therapy (NRT) and prescription medications such as bupropion and varenicline, with moderate effect sizes (Cahill *et al*, 2012). NRT reduces acute nicotine withdrawal while bupropion and varenicline appear to decrease the urge to smoke by reducing withdrawal symptoms and blunting the rewarding effects of smoking (Brandon *et al*, 2011; Gonzales *et al*, 2006; Jorenby *et al*, 2006; Patterson *et al*, 2009). Exploring the neurobiological underpinning of these treatments is warranted as they are poorly understood in humans.

Dopamine (DA) is a neurotransmitter believed to be important in the final common path in drug dependence, including nicotine (Pich *et al*, 1997). DA is believed to mediate both the rewarding (Brody *et al*, 2004; Corrigall *et al*, 1992; Le Foll *et al*, 2014) and withdrawal-associated (Rada *et al*, 2001; Rahman *et al*, 2004) effects of nicotine. That is, increased DA is associated with ratings of positive subjective measures (Montgomery *et al*, 2007), and decreased DA is believed to mediate the negative state during withdrawal (Hildebrand *et al*, 1998; Le Foll *et al*, 2014). As a partial agonist of the $\alpha4\beta2^*$ acetylcholine nicotinic receptor (Coe *et al*, 2005), it is possible that varenicline-induced elevations in DA levels may contribute to its therapeutic effects (Coe *et al*, 2005). However, the first study to evaluate the impact of subchronic administration of varenicline in animals that were not dependent on nicotine found no effect of varenicline on basal DA levels but an ability of varenicline to decrease nicotine-induced DA release in animals (Ericson *et al*, 2009). More recently, it has been reported that varenicline increased DA firing rates in nicotine-dependent animals that were in acute withdrawal (Perez *et al*, 2015), an effect consistent with its partial agonist profile. To date, the impact of varenicline on DA transmission in the human brain has not been reported.

Neuroreceptor imaging techniques with PET provide a method to investigate changes in DA in the human brain *in vivo* (Laruelle, 2000; Laruelle *et al*, 2002; Martinez and Narendran, 2010). The traditional radioligand for dopamine $D_{2/3}$ receptors ($DRD_{2/3}$) is [^{11}C]-raclopride that has relatively low sensitivity to detect changes in DA (Martinez and

*Correspondence: Dr B Le Foll, Translational Addiction Research Laboratory, Centre for Addiction and Mental Health, 33 Russell Street, Toronto, ON M5S 2S1, Canada, Tel: +416 535 8501, x34772, Fax: +416 595 6922, E-mail: Bernard.lefoll@camh.ca
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Narendran, 2010). However, [^{11}C]-(+)-PHNO (Wilson *et al*, 2005) is a $DRD_{2/3}$ agonist that is also the most sensitive PET tracer to detect relatively small fluctuations in DA (Gallezot *et al*, 2012; Ginovart *et al*, 2006, 2007; Narendran *et al*, 2006; Willeit *et al*, 2006). We have recently shown that [^{11}C]-(+)-PHNO has an enhanced ability to detect elevations in DA induced by smoking (Le Foll *et al*, 2014), with a preferential effect in the limbic striatum. In addition, [^{11}C]-(+)-PHNO allows for the measurement of occupancy of DA receptors in D2- and D3-rich areas. [^{11}C]-(+)-PHNO binding in the substantia nigra (SN) and ventral pallidum (VP) is believed to represent 100 and 75% binding to DRD_3, respectively, while [^{11}C]-(+)-PHNO binding in the dorsal caudate and dorsal putamen is accounted for by DRD_2, and the VST is about 50% of each (Ginovart *et al*, 2007; Rabiner *et al*, 2009; Tziortzi *et al*, 2011).

The purpose of the present study was to evaluate whether treatment with varenicline affects DA transmission in the D2-rich striatum and D3-rich extra-striatal regions and whether this change was associated with changes in subjective measures. Participants underwent two PET scans with [^{11}C]-(+)-PHNO after overnight abstinence from tobacco. The first PET scan was done before varenicline treatment and the second PET scan was done after 10–11 days of varenicline (at steady state). Subjective questionnaires that measured craving and reward were administered, and objective measures (ie, varenicline and plasma cotinine) were collected.

MATERIALS AND METHODS

Participants

All procedures were approved by the Centre for Addiction and Mental Health (CAMH) Research Ethics Board and complied with the Helsinki Declaration of 1975 (as revised in 1983). The study was approved by the Institutional Research Ethics Board. Thirteen participants (adult males or females 21–45 years) were recruited from the community and provided written informed consent prior to participating in any study procedures. All met the following inclusion criteria: (1) Nicotine dependent as assessed by smoking at least 10 cigarettes a day, a baseline score of $\geqslant 4$ on the Fagerstrom Test of Nicotine Dependence and expired carbon monoxide (CO) levels of at least 10 p.p.m.; (2) motivated to quit within the next 30 days; and (3) treatment-seekers who were willing to use varenicline as a quit aid. Exclusion criteria were: (1) Previous use of medication for smoking cessation within the past month; (2) Abnormal physical examination, 12-lead or routine routine blood tests or a condition that may impede memory and attention; (3) Past/present axis I psychiatric diagnoses as per MINI-International Neuropsychiatric Interview version 5.0 and the Hamilton Depression Rating Scale; (4) Magnetic resonance (MR) scanning contraindication; (5) Claustrophobia; (6) Current pregnancy/breastfeeding; (7) Current use or use during the previous month of medication that may affect the central nervous system or positive during drug screening for drugs of abuse or abuse of alcohol or drugs of abuse within the past 3 months; (8) Exposure to radiation in the past 12 months exceeding limits for participants in research with PET; and (9) Allergy to varenicline.

Procedure

PET/MRI scans. After enrolment in the study, participants had two PET scans and a 30-min MRI on a 3 Tesla GE MRI scanner (Discovery MR750, GE, Milwaukee, USA) for region of interest (ROI) delineation. On the day of the first PET scan, participants were scanned after overnight (12 h) abstinence from tobacco and given varenicline to take home, with the instructions to start taking the medication the next day (see dosing paradigm below). Seven days later, participants returned to collect refills of blister packs of varenicline and the second PET day was scheduled 10–11 days after the first PET day, after reaching maintenance doses of varenicline. Participants were asked to refrain from smoking for 12 h prior to each PET visit, and smoking abstinence was confirmed by breath CO levels below 10 p.p.m. Alcohol abstinence was also confirmed by a breath alcohol measure. At the start of each PET scan, a sample of blood was drawn to measure plasma levels of varenicline, cotinine, and nicotine. Participants completing the second PET scan were followed every 2 weeks for a total duration of 12 weeks for treatment of tobacco dependence. During follow-up visits, subjects received behavioral support adapted from the manual from the Mayo Clinic Guide (Smoke Free and Livin'it), were given blister packs of medication, and provided breath CO and completed questionnaires (see below). A final visit was scheduled 3 months after varenicline was discontinued.

Subjective measures. During each PET scan visit, participants completed the Minnesota Nicotine Withdrawal Scale (MNWS; assesses urge to smoke, depressed mood, irritability, anxiety, difficulty concentrating, restlessness, increased appetite, and sleep) and the Tobacco Craving Questionnaire (TCQ; relief from withdrawal (factor 1), anticipation of positive outcomes (factor 2), control over tobacco use (factor 3) and intention to smoke for positive outcomes (factor 4) both before and after each PET scan). The patient health questionnaire (PHQ-9; to assess depressive symptoms) was also administered on each PET scan day. All these questionnaires were also given on day 7 and on the follow-up visits with the exception of the TCQ. Participants kept a daily log of the number of cigarettes smoked. At each visit, participants were asked to indicate how many cigarettes they had smoked on each day for the 7 days prior to the visit, and an expired breath CO reading was taken. Participants were also asked how much alcohol and caffeine they had during the past 7 days.

Drug administration. Varenicline was administered as prescribed in clinical practice. For the first 3 days, participants took 0.5 mg orally once a day in the morning and then twice a day on days 4–7. After that, 1 mg varenicline was taken orally twice a day. The target quit date was set at the second PET scan visit (days 10–11 of taking varenicline). Concomitant medications and adverse events were assessed at each visit.

Table 1 Demographic Variables

Sex	
Male	7
Female	4
Race	
Black	2
Caucasian	6
Mixed	1
Asian	1
Unknown	1
	Mean ± SEM
Age	37 ± 1.85
Cigarettes	104 ± 11.36
FTND	5.68+0.55
Pack-years	16.86+2.15
CO level	17.18+2.34
Years smoking	22.64+1.99
Motivation to quit	9.36+0.31

Abbreviations: Cigarettes, number of cigarettes smoked per week; CO, exhaled carbon monoxide, parts per million (p.p.m.); FTND, Fagerstrom Test of Nicotine Dependence; motivation to quit, on a scale of 0–10 with 10 being the most motivated; pack-years, years × packs/day. Values are presented as *n* or mean ± SEM.

PET Image Acquisition

The radiosynthesis of [^{11}C]-(+)-PHNO has been described in detail elsewhere (Wilson *et al*, 2005). PET scans were performed using a Siemens-Biograph HiRez XVI (Siemens Molecular Imaging, Knoxville, TN, USA) PET/CT camera system, which measures radioactivity in 81 brain sections with a reconstructed pixel size of $1.07 \times 1.07 \times 2.00\,mm^3$ each with an in-plane resolution of 5 mm full-width at half maximum. A transmission scan was acquired and the emission scan, acquired in 32-bit list mode, began after bolus injection of [^{11}C]-(þ)-PHNO (duration of the bolus injection approximately 2 min). Emission data were reconstructed by 2D filtered back projection to yield dynamic images with 15 1-min frames and 15 5-min frames. The emission scan lasted for 90 min. The raw data were reconstructed by filtered-back projection. A custom-fitted thermoplastic mask (Tru-Scan Imaging, USA) was made for each subject to reduce movement during the acquisition. A total of ~ 370 ± 40 MBq (approximately 10 ± 1 mCi) of [^{11}C]-(+)-PHNO was injected as a bolus into an antecubital vein.

Plasma Levels of Varenicline, Nicotine and Cotinine

Consistent with its elimination half-life of approximately 24 h, steady-state conditions for varenicline are reached within 4 days of repeat dosing (Faessel *et al*, 2010). Plasma levels of nicotine, cotinine and varenicline, were measured by LC/MS/MS using previously established methods (St Helen *et al*, 2012; Tanner *et al*, 2015) for nicotine, cotinine and 3-hydroxycotinine modified to additionally detect varenicline. Varenicline measured by this method provided identical levels as observed using other methods.

PET Image Analysis

ROI delineation and time activity curve analyses were performed using ROMI (details in (Rusjan *et al*, 2006). ROI included the dorsal caudate (DC), dorsal putamen (DP), ventral striatum (VST) as well as globus pallidus (GP; whole), VP, and SN. Delineation is described elsewhere (Boileau *et al*, 2012). [^{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 (excluding vermis) as reference region. Parameter estimation was performed using PMOD (Version 2.8.5; PMOD Technologies, Zurich, Switzerland). Percentage of occupancy was calculated as [^{11}C]-(+)-PHNO binding after varenicline/([^{11}C]-(+)-PHNO binding at baseline − 1).

Voxel-wise parameter estimation of [^{11}C]-(+)-PHNO binding was generated using the basis function implementation of SRTM (Lammertsma and Hume, 1996), with the tissue time activity curve of cerebellar cortex as the reference region. Normalized BP_{ND} maps (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK) were statistically investigated to assess significant contrasts between conditions at every voxel using paired sample *t*-test analysis. The threshold for significant clusters was set to a family-wise error corrected $p = 0.05$.

Comparisons between [^{11}C]-(+)-PHNO BP_{ND} in ROIs were conducted by using repeated-measures Day (two levels; baseline, post-varenicline) × Region (six levels; ROIs: SN, GP, VP, VST, DC, DP) ANOVAs (SPSS 20.0, SPSS, USA). Sphericity was assessed with Mauchly test, and corrections were made when indicated. Bonferonni-corrected paired *t*-tests were conducted between the baseline scan and scan under varenicline for each ROI.

Subjective questionnaire data obtained prior to and following each PET scan were averaged for each PET scan day. Questionnaires were analyzed with Day (two levels; baseline PET scan days *vs* PET scan after 11 days of treatment with varenicline) × Measure (six levels; TCQ1, TCQ2, TCQ3, TCQ4, MNWS, and PHQ) ANOVAs. All objective measures (number of cigarettes smoked and plasma cotinine) were analyzed with ANOVAs on the effect of Day (two levels). Correlations between questionnaire measures and BP_{ND} were conducted for any ROIs that had any significant changes in BP_{ND}.

RESULTS

Thirteen participants were recruited for this study. One participant withdrew after the first scan. For another participant, there was a movement artifact in the PET scan that prevented proper determination of [^{11}C]-(+)-PHNO BP_{ND}, and data were not included. A total of 11 subjects were included in the final analysis.

All participants tested negative for drugs of abuse at the time of scanning. Varenicline was detected in all participants on the day of second PET scan. Demographic variables are provided in Table 1. A mixed ANOVA of Measure

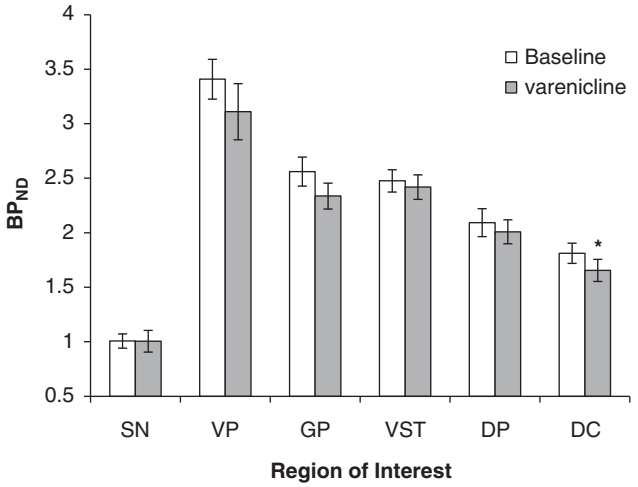

Figure 1 Mean ± SEM [^{11}C]-(+)-PHNO BP$_{ND}$ before varenicline (open bars) and after 10–11 days of varenicline (filled bars) presented for individual participants for ROIs (DC, dorsal caudate; DP, dorsal putamen: GP, globus pallidus; SN, substantia nigra; VP, ventral pallidum; VST, ventral striatum). [^{11}C]-(+)-PHNO BP$_{ND}$ was significantly decreased in the DC after treatment with varenicline (*$p < 0.05$, paired *t*-tests).

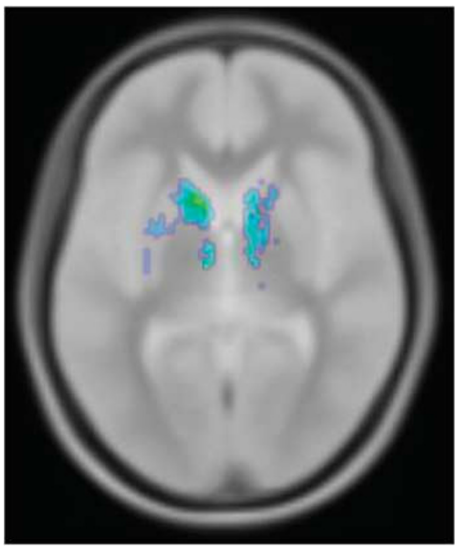

Figure 2 Voxel-wise comparison between baseline and varenicline illustrating lower [^{11}C]-(+)-PHNO BP$_{ND}$ in the DC ($t_{max} = 5.92$; $p = 0.05$). The image is not corrected for multiple comparisons.

(mass injected, amount injected, specific activity) × Day (PET baseline, PET after varenicline) revealed no effects, suggesting that there were no differences in scan parameters across scans or between groups (mass injected (μg) 2.09 ± 0.09; amount injected (mCi) 9.45 ± 0.25; specific activity (mCi/μmol) 1695.59 ± 99.81).

Changes in Binding Potential

A two-way repeated-measures Region (six levels) × Day (two levels) revealed no interaction and no effect of Day. *t*-Tests corrected for multiple comparisons (Bonferroni) indicated that BP$_{ND}$ differed between baseline and while taking varenicline only in the DC (Figure 1; $p = 0.008$; Bonferroni uncorrected to 0.0083; Cohen's *D*: VST: 0.168; VP: 0.41; SN: 0; GP: 0.53; DC: 0.49; DP: 0.20).

Voxel-Wise Analyses

In the voxel-wise statistical analyses (SPM8), we identified small clusters of lower [^{11}C]-(+)-PHNO BP$_{ND}$ in the varenicline condition as compared with baseline in areas corresponding to the ventral lateral nucleus of the thalamus and the head/body of the caudate nucleus (on left voxel 48 with peak threshold of 4.15 and on the right a cluster of 57 voxels with a peak threshold of 4.01; Figure 2). No other clusters of significantly lower [^{11}C]-(+)-PHNO BP$_{ND}$ was significant after correction for multiple comparisons.

Objective and Subjective Measures

On the first PET session, prior to taking varencline, the number of cigarettes smoked in the 7 days prior was 98 ± 12. On the day of the second PET scan, after taking varenicline for 11 days, the number of cigarettes smoked in the 7 days prior was 93 ± 8. A one-way ANOVA revealed no significant effect of Day for the objective measures of number of cigarettes smoked or cotinine levels (day 1: 210 = 42; day 11: 149 ± 29). A two-way repeated-measures Day (two levels) × Measure (six levels; TCQ1, TCQ2, TCQ3, TCQ4, MNWS, PHQ) ANOVA revealed a significant interaction ($F(5, 45) = 5.040$, $p = 0.001$). Main effects of Day were found for TCQ2 ($F(1, 9) = 10.229$, $p < 0.011$) and TCQ4 ($F(1, 10) = 3.494$, $p = 0.091$). For the MNWS and PHQ, main effects of Day approached significance (MNWS: $F(1, 10) = 3.564$, $p = 0.088$; PHQ: $F(1, 10) = 3.494$, $p = 0.091$). Bonferroni-corrected *t*-tests (corrected $p = 0.0083$) revealed differences between the 2 days only for TCQ factor 4 ($p = 0.006$), suggesting that the intention and planning to smoke for positive outcomes was reduced after varenicline. The effect of Day for TCQ factor 2 was not statistically significant after correction for multiple comparisons ($p = 0.011$, see Figure 3). One participant was missing subjective data from day 1 and was not included in this analysis.

Correlations

Correlations (Figure 4) between changes in BP$_{ND}$ in the DC and subjective measures (PHQ, TCQ1, TCQ2, TCQ3, TCQ4, MNWS) revealed that the correlation between changes in BP$_{ND}$ in the DC and changes in TCQ factor 2 (anticipation of positive outcomes) approached, but did not reach, significance ($r^2 = -0.505$, $p = 0.136$). One participant was missing subjective data from day 1 and was not included in the analyses. A significant correlation between change in plasma cotinine and change in BP$_{ND}$ in the DC was found ($r^2 = -0.742$, $p = 0.009$)

DISCUSSION

The purpose of the present study was to investigate the effects of varenicline treatment on [^{11}C]-(+)-PHNO BP$_{ND}$ in

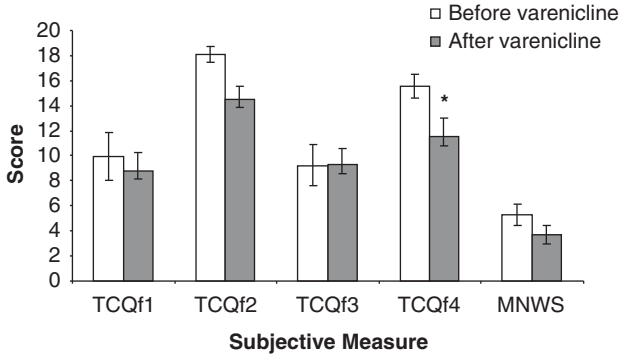

Figure 3 Mean ± SEM scores on the Tobacco Craving Questionnaire (TCQ) factors 1–4 and the Minnesota Nicotine Withdrawal Scale (MNWS) presented before (open squares) and after (closed squares) treatment with varenicline. *Significant effects of treatment with varenicline were found in the scores on the TCQ factor 4 (intention and planning to smoke for positive outcomes; *t*-test, $p = 0.006$). TCQ factor 2 (anticipation of positive outcomes) was significant but did not survive corrections for multiple comparisons (paired *t*-test: $p = 0.011$; Bonferroni $p = 0.0083$).

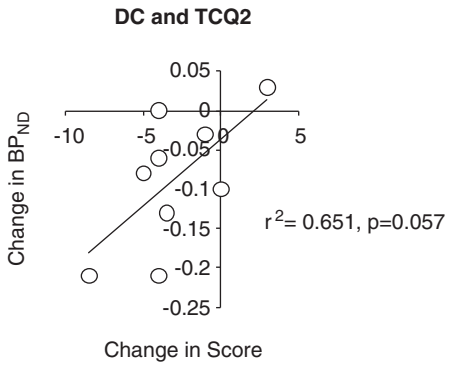

Figure 4 Correlations changes in TCQ factor 2 and changes in BP_{ND} in the DC approached significance. Improvements in the TCQ score were correlated with decreased BP_{ND} after varenicline (ie, increased DA).

ROIs in treatment-seeking smokers. PET scans were performed before and after 10–11 days of varenicline treatment (at steady state). Subjective mood and craving questionnaires as well objective measures of plasma levels of varenicline and cotinine were also taken. After treatment with varenicline, [^{11}C]-(+)-PHNO BP_{ND} was significantly decreased in the DC, as assessed with voxel-wise and ROI analyses. Subjective measures of TCQ factor 4 (intention to smoke for positive outcomes) were also decreased, while TCQ factor 2 (anticipation of positive outcomes from smoking) failed to reach significance after correction for multiple comparisons.

This study revealed that [^{11}C]-(+)-PHNO BP_{ND} was lower in DC after treatment with varenicline, as compared with baseline in treatment-seeking smokers. Changes in BP_{ND} could be obtained not only by changes in DA but possibly also by changes in DA receptor expression. However, the latter is unlikely here as preclinical studies suggest that if varenicline has any effects on DA receptor expression it would be an increase in receptor number (Crunelle *et al*, 2012), which would then be producing an increase in [^{11}C]-(+)-PHNO binding. Therefore, it is more likely that the lowest [^{11}C]-(+)-PHNO BP_{ND} reflects an elevation of DA in DC. This effect was consistently found with both our ROI analysis and with the voxel-wise analysis approach. The voxel-wise approach is aimed at detecting differences in neuroreceptor ligand binding at the voxel level, with no *a priori* anatomical hypothesis, and enables circumvention of some limitations of ROI placement, as well as investigation of regions not included in our ROI template. It should be noted that, in this study, participants were abstinent from tobacco use, in order to avoid the confound of tobacco-induced elevations in DA (Le Foll *et al*, 2014).

Reported intention to smoke for the positive effects of tobacco (TCQ factor 4) were decreased. This decrease in the positive effects of smoking is consistent with both preclinical (George *et al*, 2011; Le Foll *et al*, 2012) and clinical (Gonzales *et al*, 2006; Jorenby *et al*, 2006) studies that have demonstrated that varenicline affects the rewarding properties of smoking. Given that positive reactions to smoking have been shown to predict relapse (Strong *et al*, 2011), decreases in measures of positive reinforcement may provide an explanation as to the efficacy of varenicline (Gonzales *et al*, 2006; Jorenby *et al*, 2006). In addition, varenicline also decreased the negative reinforcement of withdrawal (Gonzales *et al*, 2006; Jorenby *et al*, 2006). However, changes in brain DA and subjective appraisals of smoking occurred in the absence of any changes in plasma cotinine or the number of cigarettes smoked per day from baseline. Thus changes in subjective values or BP_{ND} do not reflect alterations in smoking habits *per se*. This is compelling as it suggests that varenicline may act to alter the brain and subjective response prior to quitting, thus enabling the mechanism by which smokers may subsequently quit. It may also explain the delayed quitting observed up to 4 weeks after taking varenicline (Agboola *et al*, 2010, 2015; Kasza *et al*, 2013). It has been shown that treatment with the nicotine patch prior to the quit date can improve smoking cessation (Rose *et al*, 2009); in smokers who did not decrease smoking prior to their quit date while undergoing nicotine replacement therapy, they could be 'rescued' by bupropion augmentation of the patch or with varenicline treatment alone (Rose and Behm, 2013). Future studies can address how brain response and subjective measures can predict the success of smoking cessation interventions.

Varenicline and tobacco smoke affect DA differently. Varenicline appears to produce elevations in DA in a D_2-rich areas (DC) but not in D_3-rich areas (SN, VP). In contrast, tobacco produced elevations in both limbic striatum and in D_3-rich area (VP), but not in the DC, as assessed with [^{11}C]-(+)-PHNO (Le Foll *et al*, 2014). These differential effects may be due to the different pharmacological agonist properties (full agonist for nicotine *vs* partial agonist for varenicline) or the involvement of different subtypes of nicotinic acetylcholine receptors (Coe *et al*, 2005). Another possibility is that varenicline predominantly stimulated nicotinic acetylcholine receptors located in DA neurons that preferentially project to DC. Further studies should explore for such effects. Regardless of the receptor target (D_2 or D_3), [^{11}C]-(+)-PHNO has been shown to have a greater sensitivity in detecting smaller changes in synaptic DA levels as compared with [^{11}C]-raclopride (Ginovart *et al*, 2007; Narendran *et al*, 2006; Willeit *et al*, 2006). This is supported by the direct comparison of the dose–effect of amphetamine

(0.1, 0.5, and 2 mg/kg; i.v.) on binding of [^{11}C]-(+)-PHNO and [^{11}C]-raclopride in cats (Ginovart *et al*, 2006) and humans (Shotbolt *et al*, 2012).

To our knowledge, no study has investigated the effects of bupropion or NRT using [^{11}C]-(+)-PHNO. Previous studies measuring the impact of bupropion on DA transmission using [^{11}C]-raclopride found no effect on ventral caudate/nucleus accumbens binding induced by bupropion (Brody *et al*, 2010). Limited work has been carried out assessing the impact of NRT on dopamine transmission with PET. Nicotine gum (Takahashi *et al*, 2008), but not nicotine spray (Montgomery *et al*, 2007), was effective in decreasing [^{11}C]-raclopride in striatal area. As compared with the traditional radiotracer [^{11}C]-raclopride, [^{11}C]-(+)-PHNO allows for greater sensitivity in the measurement of change in DA levels (Gallezot *et al*, 2012; Ginovart *et al*, 2006, 2007; Narendran *et al*, 2006; Willeit *et al*, 2006). It is possible that our ability to detect significant elevations in DA induced by varenicline was due to the use of [^{11}C]-(+)-PHNO *vs* [^{11}C]-raclopride. As response to treatment for varenicline and for NRT has been related to nicotine metabolic rate (Lerman *et al*, 2015), further studies exploring the impact of nicotine metabolic rate on [^{11}C]-(+)-PHNO would be informative.

Partial agonists are believed to be able to decrease drug-induced elevations in DA and also to elevate DA levels during withdrawal (Childress and O'Brien, 2000). Therefore, increases in DA levels in the DC in the present study may reflect a reversal of attenuated DA levels normally seen during withdrawal. It should be noted that the present study did not measure whether varenicline could decrease elevations in DA levels induced by smoking in humans. Further studies could explore this as a further test of the partial agonist properties of varenicline.

This study has several limitations. First, the study has a limited sample size. However, despite this limited sample size, we were able to detect significant changes in [^{11}C]-(+)-PHNO BP_{ND} in the DC that were still significant after corrections for multiple testing. But it is possible that with a larger sample size we would have detected an effect of varenicline in more brain areas and also had more power to detect changes in subjective measures. Indeed, previous studies have found alterations in measures of withdrawal following varenicline (Gonzales *et al*, 2006; Jorenby *et al*, 2006). Further, we were not able to analyze the data in terms of those who respond to treatment compared with those who do not respond to treatment (only three responded to treatment). Future investigations may reveal the predictive relationship between early changes in BP_{ND} and response to treatment in smokers, as already identified in subjects with cocaine use disorders undergoing behavioral treatment (Martinez *et al*, 2011).

Related to the relatively small sample size is the inability in the present study to look at individual differences. One of these is gender effects (Cosgrove *et al*, 2014). It has been shown, using [^{11}C]raclopride, that DA levels in the ventral striatum are increased in males as compared with females during cigarette smoking. In the present study, the sample consisted of seven males and four females, and thus the data may disproportionately represent one gender, with not enough power to compare the two. It is noteworthy that effects found in the present study in the VST were minimal (Cohen's *D* of 0.168), and thus it is possible that gender effects are more pronounced during smoking than withdrawal *per se*, as studied in the present study. Future studies will need to determine whether gender differences exist in the neurochemical response to treatment approaches for smoking cessation.

Further, there are some limitations owing to the PET scanning parameters. The injected mass of the radiotracer was slightly above the limit suggested by others (Gallezot *et al*, 2012), potentially leading to underestimation of DA occupancy in both conditions (Shotbolt *et al*, 2012). The fact that the mass injected was similar in the two conditions suggests that it did not interfere with the results. Although our analysis based on the brain area suggests that varenicline has an impact predominantly at the level of the DRD_2 receptor in the DC, we do not have a clear explanation as to why there would be no effect at the level of the DRD_3. In our previous studies, we have found that DRD_3 levels are increased in the brains of people with psychostimulant use disorder (Boileau *et al*, 2012; Payer *et al*, 2013).

It should be considered, given the lack of a control group, that the present results may be due, perhaps in part, to a placebo effect. The placebo effect is common in the treatment of pain, depression, and Parkinson's disease and is set up by an expectation on the part of the participant that a treatment will be successful (for reviews, see Lidstone and Stoessl, 2007; Murray and Stoessl, 2013). Particularly powerful in establishing a placebo effect are environmental considerations such as a hospital and controlled clinical trial setting, both of which were true here. With the use of [^{11}C]raclopride, it has been shown that the placebo effect can be associated with increases in DA in the dorsal and ventral striatum, the same brain regions that are involved in reward expectation (de la Fuente-Fernandez *et al*, 2001, 2002, 2006; de la Fuente-Fernandez and Stoessl, 2002; Strafella *et al*, 2001, 2003, 2006). Given the increase in DA observed in the DC in the present study, the possibility that this may be partly explained by a placebo effect cannot be ruled out. However, this suggestion is somewhat tempered by the fact the placebo effect also elevates DA in the VST, something that was not found in the present study.

CONCLUSIONS

The purpose of the present study was to determine whether steady-state levels of varenicline can increase DA levels in the brain of abstinent smokers. It was found that DA was increased in the DC after treatment with varenicline and that this increase was (nearly) correlated with decreases in ratings of the positive effects of smoking. These findings indicate for the first time that varenicline increases DA transmission in human smokers scanned under abstinence. This effect may contribute to its therapeutic efficacy, and future studies would be needed to determine this relationship, especially as varenicline negatively affects positive ratings of smoking (Agboola *et al*, 2010, 2015; Cahill *et al*, 2012).

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