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REGULAR RESEARCH ARTICLE

Influence of Nicotine Metabolism Ratio on [^{11}C]-(+)-PHNO PET Binding in Tobacco Smokers

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Abstract

Background: Identifying the biological basis of smoking cessation success is of growing interest. The rate of nicotine metabolism, measured by the nicotine metabolite ratio, affects multiple aspects of nicotine dependence. Fast nicotine metabolizers tend to smoke more, experience more withdrawal and craving, and have lower cessation rates compared with slow metabolizers. The nicotine metabolite ratio predicts treatment response, and differences in brain activation between fast metabolizers and slow metabolizers have been reported in fMRI studies. As reinforcing/rewarding effects of tobacco are associated with dopamine transmission, the purpose of the present study was to study the dopaminergic system in human smokers based on their nicotine metabolite ratio.

Methods: The first aim of the study was to explore if there were differences in D_2 and D_3 receptor binding between fast metabolizers and slow metabolizers during abstinence. The second aim was to explore smoking-induced dopamine release in both groups. Participants underwent 2 [^{11}C]-(+)-PHNO PET scans: one scan during abstinence and the other after smoking a tobacco cigarette. Subjective measures were recorded and blood was drawn for measurement of nicotine and cotinine levels.

Results: During abstinence, slow metabolizers (n = 13) had lower [^{11}C]-(+)-PHNO binding potential than fast metabolizers (n = 15) restricted to the D_2 regions of the associative striatum and sensorimotor striatum. After smoking a cigarette, [^{11}C]-(+)-PHNO binding potential was decreased in the limbic striatum and ventral pallidum, suggestive of increases in dopamine, but there were no nicotine metabolite ratio differences.

Conclusions: Further studies are required to delineate if differences in [^{11}C]-(+)-PHNO binding between slow metabolizers and fast metabolizers at abstinence baseline are preexisting traits or induced by prolonged tobacco use.

Keywords: Cigarettes, dopamine, D2, D3, NMR

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Significance Statement

Smoking is a serious public health problem, and it is known that the rate of metabolism of nicotine can influence key smoking characteristics such as the amount smoked and the ability to quit. The aim of the present study was to determine the impact of the rate of metabolism of nicotine on the brain reward system in tobacco smokers. We found that slow metabolizers had fewer dopamine receptors (of the D2-type) than fast metabolizers, but the two groups had a similar dopamine response to smoking a cigarette. Thus, the rate of nicotine metabolism may contribute to dopaminergic signaling in the brain.

Introduction

There is increasing interest in understanding the biological basis of individual differences in smoking characteristics. One biomarker of individual differences is the rate at which nicotine is metabolized, or the nicotine metabolite ratio (NMR) (Dempsey et al., 2004). When stratified by NMR, it has been shown that fast metabolizers (FM) smoke more than slow metabolizers (SM) (Benowitz et al., 2003; Johnstone et al., 2006; Malaiyandi et al., 2006; Mwenifumbo et al., 2007; Schnoll et al., 2009, 2014) and take larger puff volumes (Strasser et al., 2011), suggesting an attempt to titrate smoking. Perhaps due to more cigarette smoking and higher levels of dependence, FM have higher craving (Kaufmann et al., 2015) and reward (Sofuoglu et al., 2012) and greater withdrawal (Rubinstein et al., 2008). Consistent with these findings, FM and SM also differ in response to both placebo and active smoking cessation treatments, with SM showing greater success in quitting (Lerman et al., 2006, 2015; Patterson et al., 2008; Schnoll et al., 2009; Chenoweth et al., 2013, 2016; Vaz et al., 2015; Ebbert et al., 2016).

Studies have begun to delineate differences in brain responses in SM vs FM. In 2 fMRI studies, FM had greater neural response to smoking cues than did the SM (Tang et al., 2012) (Falcone et al., 2016). It was also found that, in smokers, those with the faster nicotine metabolizer genotype had higher brain activation in the anterior cingulate and ventral striatum; no genotype group differences were observed among nonsmokers (Li et al., 2017). Although these studies are informative, dopamine (DA) is a final common path in addiction (Di Chiara et al., 1992), and the effects of NMR on baseline DA receptor levels and on DA transmission after a smoking challenge are currently unknown.

PET imaging provides a noninvasive means to measure neurotransmitter levels and receptors. [^{11}C]-(+)-PHNO (Wilson et al., 2005) allows for the measurement of DA D_2 and D_3 receptors, but also provides a more sensitive measure of DA fluctuations compared with the traditionally used [^{11}C]-raclopride (Shotbolt et al., 2012). An advantage of PET imaging with [^{11}C]-(+)-PHNO is the ability to measure not only $D_{2/3}$ receptors (as with traditional [^{11}C]-raclopride), but to explore the expression of D_2 vs D_3 receptors, based on a regional signal analysis approach (Kiss et al., 2011; Le Foll et al., 2014a). In an elegant study by Tziortzi et al., gradients of binding to D_3 or D_2 receptors were demonstrated, with 100% of the signal obtained from the substantia nigra (SN) being attributed to D_3 (Tziortzi et al., 2011). By contrast, the entire signal from striatal regions was due to the D_2 receptor. The ventral pallidum (VP; 75%), globus pallidus (GP; 65%), and ventral/limbic striatum (LST; 50%) provide intermediate D_3 fractions.

PET imaging has also been used to study smoking-induced change in DA. Studies with [^{11}C]-raclopride showed smoking-induced changes in binding potential (BP_{ND}) (Brody et al., 2004, 2009, 2010). In some studies, changes in DA were limited to subjects that had a hedonic response, as measured with a 10-point scale of subjective ratings while in the scanner (Barrett et al., 2004). In our previous study (Le Foll et al., 2014b), we demonstrated a good magnitude of change in [^{11}C]-(+)-PHNO BP_{ND} due to smoking (approximately 12%) in the LST and VP using [^{11}C]-(+)-PHNO. Further, it is also known from PET studies that DA D_2 receptor availability is lower in the striatum of people who are nicotine dependent (Fehr et al., 2008), similar to other drugs of abuse (Volkow et al., 1993; Martinez et al., 2004). By comparison, D_3 receptor levels (in the SN) are reportedly higher in drug dependence (Boileau et al., 2012). It would be of interest to determine whether fast metabolizers and slow metabolizers have different levels of basal D_2 and D_3 receptors and whether the response to smoking a cigarette is different.

The purpose of the present study was: (1) to measure differences in [^{11}C]-(+)-PHNO binding at abstinence baseline in FM vs SM; and (2) to measure smoking-induced differences in [^{11}C]-(+)-PHNO binding in these groups. Prior to conducting this study, preliminary analyses were conducted on our previous study (Le Foll et al., 2014b). Based on these results, it was hypothesized that SM metabolizers will show greater decreases in [^{11}C]-(+)-PHNO binding after smoking. It was further hypothesized, based on these preliminary results, that SM would have lower levels of basal D_2 receptors in the striatum, and higher D_3 receptor levels (in the SN), than FM.

Methods

Participants

All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board and the University of Toronto and complied with the 1975 Helsinki Declaration (5th revision, 2000). Participants were recruited from the community, provided written informed consent, and participated in a comprehensive screening interview. All met the following criteria: (1) Males and females of any ethnic origin 18 years of age or older; (2) No use of medication for smoking cessation in the previous month; (3) Smokers who are nontreatment seekers (smoking status verified by expired CO and the presence of nicotine and cotinine in plasma); (4) No DSM diagnoses or other drug dependence; (5) No medical conditions requiring immediate investigation or treatment; (6) Not pregnant; (7) No regular use of any therapeutic or recreational psychoactive drug use that may interfere with PET scanning; (8) No exposure to radiation in the last 12 months exceeding permissible limits for participants participating in research; (9) No current use of medication that may interfere with [^{11}C]-(+)-PHNO; (10) No PET or MRI scanning contraindications; (11) (Not having any clinical condition, drug sensitivity, or prior therapy that, in the investigator's opinion, makes the participant unsuitable for the study; (12) No current use of antidepressants that may inhibit CYP2A6 or impact responses to nicotine. After initial determination of eligibility, those that qualified as FM (NMR > 0.47) or SM (NMR < 0.23) were enrolled in the study. Data from 10 (7 FM and 3 SM) participants were included from a previous study (Le Foll et al., 2014b).

Procedure

Participants were recruited by word-of-mouth, advertisements in local newspapers, social media, through posters, and from referral from other studies. After an initial phone screen, eligibility was assessed after obtaining signed informed consent. In the current study, after confirmation of eligibility, participants underwent 2 PET scans after being asked to refrain from smoking for a period of 12 (Le Foll et al., 2014b) or 48 hours of abstinence from smoking. All efforts were made to conform to the 48-abstinence time line. However, there were unforeseen circumstances. For some participants, there were delays in scanning, so the actual abstinence was longer; for others, the participants requested to refrain from smoking for longer than 48 hours. For some, the scans had to be rescheduled at the last minute so the abstinence was shorter than 48 hours (i.e., about 24 hours; range of abstinence period: 12–144 hours). In all cases, abstinence was verified with expired CO levels below 10 ppm. Participants were then escorted to a room where they either smoked their preferred cigarette (smoking condition) or relaxed (abstinence condition). The order of these sessions was counterbalanced. During each PET session, participants were screened for use of recreational drugs and given a pregnancy test if applicable. The cigarette was smoked with the use of a smoking topography device (CReSS, Borgwaldt KC). Measures taken were: average flow (milliliters-per-second), number of puffs, puff volume (milliliters), puff duration (seconds), and inter-puff interval (seconds). Questionnaires (Visual Analog Scale [VAS], Tobacco Craving Questionnaire [TCQ], Minnesota Nicotine Withdrawal Scale [MNWS], Questionnaire on Smoking Urges [QSU]) were administered at baseline and at the completion of the 90-minute PET scan. The participants visited the negative pressure room between 26 and 74 minutes prior to the start of the PET scan. Blood was taken for determination of nicotine and cotinine levels at the start of each scan.

Questionnaires consisted of a the 32-item QSU (Tiffany and Drobes, 1991), which can be separated into two factors (QSU1: desire to smoke for the pleasurable effects of the cigarette; QSU2: relief of negative affect), and the TCQ, a 12-item scale (Singleton et al., 2003) with 4 factors (TCQ1: relief from withdrawal symptoms or negative mood; TCQ2: anticipation of positive outcomes from smoking; TCQ3: lack of control over tobacco use; TCQ4: intent and planning to smoke for positive outcomes). Also included were the MNWS, an 8-item scale assessing the degree of withdrawal and a 21-item Visual Analog Scale to determine changes in emotional reactivity (VAS1: I feel anxious; VAS2: I feel irritable; VAS3: I feel alert; VAS4: I feel restless; VAS5: I feel an increase of energy; VAS6: I feel an increase in my speed of thinking; VAS7: I have a craving for cigarettes; VAS8: I feel hungry; VAS9: I feel unhappy and unwell; VAS10: I feel impatient; VAS11: I feel sleepy; VAS12: I feel tense; VAS13: I feel dizzy; VAS14: I have difficulty in concentrating; VAS15: I feel frustrated; VAS16: I feel angry; VAS17: I feel depressed; VAS18: I have a headache; VAS19: I have gastrointestinal disturbances; VAS20: My last cigarette was completely different; VAS21: My last cigarette tasted the best).

Determination of NMR

Nicotine, cotinine, and 3'hydroxycotinine were assessed by LC-MS/MS as previously described; limits of quantification were 0.1 ng/mL whole blood for each compound. NMR, which is highly reproducible across time and laboratory, was calculated as the ratio of 3'hydroxycotinine/cotinine (St Helen et al., 2012; Tanner et al., 2015). This study used the same range of NMR as previously seen (Tang et al., 2012), resulting in the lowest tertile, slow metabolizers, with NMRs < 0.23, and the faster tertile, fast metabolizers, with NMRs > 0.47.

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) PET/CT camera system, which measures radioactivity in 81 brain sections with a reconstructed pixel size of 1.07x 1.07 x 2.00 mm 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]-(+)-PHNO (duration of the bolus injection approximately 2 minutes). Emission data were reconstructed by 2D filtered back projection to yield dynamic images with fifteen 1-minute frames and fifteen 5-minute frames. The emission scan lasted for 90 minutes. 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 ~370 ± 40 MBq (approximately 10 ± 1 mCi) of [^{11}C]-(+)-PHNO was injected as a bolus into an antecubital vein.

MRI Image Acquisition

Subjects underwent standard proton density weighted brain 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 x 192; FOV, 22 x 16.5 cm) to aid region of interest delineation of the PET images.

PET Image Analysis

Region of Interest (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).

Binding Potential

[^{11}C]-(+)-PHNO specific binding potential (BP_{ND}) was estimated in each ROI using the simplified reference tissue method (Lammertsma and Hume, 1996) (SRTM), with cerebellar cortex (excluding vermis) as reference region. Parameter estimation was performed using PMOD (version 2.8.5; PMOD Technologies Ltd). The change in [^{11}C]-(+)-PHNO BP_{ND} from abstinence baseline to smoking condition was calculated as:

% Change in [^{11}C]-(+)-PHNO = ((BP_{ND}Smoking-BP_{ND}Abstinence)/BP_{ND}Abstinence)*100.

Data Analyses

[^{11}C]-(+)-PHNO BP_{ND} at abstinence baseline was analyzed using a repeated-measures ANOVA (SPSS 24) (2 groups x 6 ROIs). ROIs with significant group differences in [^{11}C]-(+)-PHNO BP_{ND} at abstinence baseline were further investigated for relationship with plasma cotinine and nicotine with Pearson's

Product-Moment Correlation. Changes in [^{11}C]-(+)-PHNO BP_{ND} after smoking were analyzed with a mixed condition (2 levels; abstinence and smoking) x ROI (6 levels; SN, VP, GP, LST, AST, SMST) x group (2 levels; FM, SM [between-subjects factor]) ANOVA. ROIs with significant effects of condition were correlated with objective measures and smoking topography values using Pearson's Product-Moment Correlation. Percent (%) change in BP_{ND} ((BP_{ND}Smoking-BP_{ND}Abstinence)/BP_{ND}Abstinence)*100) was entered into an ANOVA (ROI (6) x group) investigating group differences in smoking-induced DA release between FM and SM. Group differences in smoking topography were analyzed with *t* tests. Subjective measures were analyzed with ANOVAs. Throughout, sphericity in repeated-measures ANOVAs was evaluated with the Mauchley's test, and the Geisser-Greenhouse correction was applied.

Results

Participant Characteristics

In total, 15 FM and 13 SM completed the study (7 FM and 3 SM from the previous study; Le Foll et al., 2014b). Table 1 presents demographic information. There were no group differences in age, gender, cigarettes per day (CPD), Fagerstrom Test of Nicotine Dependence (FTND), pack-years, CO levels at baseline, cotinine at baseline, or cotinine + 3'hydrozycotinine at baseline (the last 2 measures were based on 8 FM and 10 SM). The relatively greater number of Asian smokers with slow NMRs is consistent with the higher frequency of reduced/null activity variant alleles in Asians (Benowitz et al., 2002). There were no group differences in the time from smoking to the start of the scan or between mass injected, corrected activity or specific activity between the smoking and abstinence PET scans. The area under the curve for cerebellar Time Activity Curves was not different between groups or condition. All participants tested negative for drugs of abuse on the days of the PET scans (with the exception of one who tested positive for MDMA on the abstinence day) and had a CO reading of <10 ppm upon arrival. There were no group differences in plasma nicotine, cotinine, or CO at either PET scan or in average flow, number of puffs, puff volume, puff duration, or inter-puff interval (Table 1).

Baseline Abstinence

A group x ROI ANOVA revealed no significant interaction and no effect of group; only an effect of ROI was revealed ($F(5, 130) = 89.343$, $P_{GG} < .001$; partial eta squared: 0.775). Since we had a priori hypotheses about group differences in the striatum and D3-rich areas (SN), data were further analyzed with planned comparisons investigating group differences for each ROI. This analysis revealed significant differences in the AST ($P = .028$) and SMST ($P = .024$) with SM having lower binding in both regions. See Figure 1. Correlations of BP_{ND} at abstinence baseline with either cotinine or nicotine levels revealed no significant correlations for the AST (cotinine SM: $r^2 = -.497$, $P = .084$; nicotine SM: $r^2 = -.253$, $P = .405$; cotinine FM: $r^2 = -.014$, $P = .960$; nicotine FM: $r^2 = .394$, $P = .146$) or SMST (cotinine SM: $r^2 = .008$, $P = .978$; nicotine SM: $r^2 = .144$, $P = .638$; cotinine FM: $r^2 = .212$, $P = .447$; nicotine FM: $r^2 = .375$, $P = .168$).

Table 1. Subject Characteristics.

	SM	FM	P value
NMR	.17 ± .02	.65 ± .05	<.001
Males	8	5	-
Asian	6	1	-
Caucasian	4	11	-
Black	2	1	-
Hispanic	1	2	-
Age	37.5 ± 3.8	34.5 ± 2.7	.528
Years of education	14.2 ± .7	15.5 ± .6	.184
Cigarettes per day	11.6 ± 1.2	14.9 ± 2.0	.164
Cotinine levels (ng/mL)	10.9 ± 2.3	10.7 ± 2.3	.788
Cotinine + 3 hydroxycotinine (ng/mL)	200.1 ± 49.8	299.8 ± 90.1	.322
Fagerstrom test of nicotine dependence	4.4 ± .6	6.0 ± 1.0	.201
Pack-years	16.5 ± 4.3	12.1 ± 2.4	.363
CO level (ppm)	15.3 ± 2.4	12.9 ± 1.7	.422
Time between smoking and scan (min)	40.1 ± 3.5	46.5 ± 4.3	.272
Average Flow (mL/s)	36.8 ± 2.7	36.0 ± 3.3	.863
Number of puffs	15.5 ± 1.1	16.3 ± 1.5	.656
Puff volume (ml)	55.1 ± 4.3	59.3 ± 8.9	.696
Puff duration (s)	1.6 ± .1	2.2 ± .5	.289
Inter-puff interval (s)	19.4 ± 1.9	17.6 ± 2.0	.525
	Abstinence	Smoking	P value
Mass injected (μg)	2.3 ± .1	2.1 ± .1	.054
Corrected activity (mCi)	9.1 ± .3	9.2 ± .2	.75
Specific activity (mCi/μmol)	1078.2 ± 66.9	1193.6 ± 70.4	.254

	Abstinence			Smoking		
	SM	FM	P value	SM	FM	P value
Plasma nicotine (ng/mL)	1.4 ± 0.6	1.0 ± .3	.62	9.9 ± 1.1	8.04 ± 1.2	.273
Plasma cotinine (ng/mL)	76.9 ± 22.1	66.7 ± 15.9	.704	86.1 ± 21.3	56.5 ± 10.4	.29
CO level (ppm)	3.5 ± .6	3.5 ± .6	.931	4.3 ± .7	3.1 ± .5	.194

P values represent the results of *t* tests.

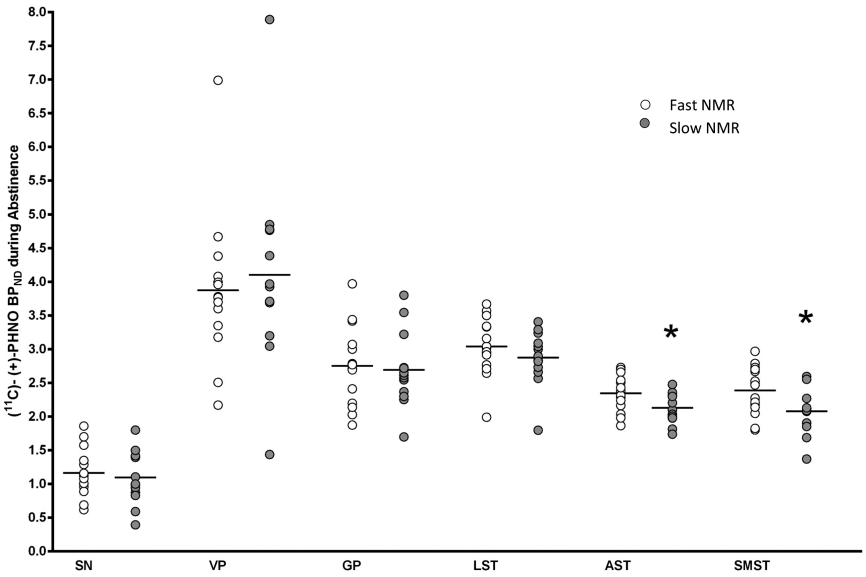

Figure 1. Binding potential (BP_{ND}) measured at abstinence baseline in participants with fast nicotine metabolism ratios (NMRs) (open symbols) or slow NMRs (dark symbols) in regions of interest (ROIs) (presented in order of D_3 fraction: SN: substantia nigra; VP: ventral pallidum; GP: globus pallidus; LST: ventral/limbic striatum; AST: associative striatum; SMST: sensorimotor striatum). *$P < .05$, fast NMR different from slow NMR.

Difference Between Abstinence and Smoking Conditions

A condition (2 levels, abstinence and smoking) x group (2 levels) x ROI (SN, GP, VP, LST, AST, SMST) ANOVA revealed a significant ROI x condition interaction ($F(5, 130) = 8.301$, $P_{GG} = .001$; partial eta squared: 0.242) with no effects of group (3-way interaction: $F(5, 130) = .361$, $P_{GG} = .702$; partial eta squared = .014), suggesting that the effects of smoking were different in the various ROIs but that the FMs were not different from the SMs (Figure 2). Indeed, analysis of the percent change in BP_{ND} from abstinence to smoking condition with comparisons on the effect of group for each ROI revealed no significant effects (group x ROI interaction: $F(5, 130) = .710$, $P_{GG} = .617$; partial eta squared = .124; Figure 2; SN: $P = .726$; VP: $P = .145$; GP: $P = .899$; LST:$P = .544$; AST: $P = .335$;SMST: $P = .354$). Follow-up analyses of the significant condition x group interaction with comparisons on the effect of condition for each ROI revealed that the changes in [^{11}C]-(+)-PHNO BP_{ND} from abstinence to smoking were significant in the LST ($P < .001$) and VP ($P = .001$), suggesting that smoking increased DA levels in those areas. Two participants had VP values that were more than 2 SDs above the mean. Removal of the 2 participants with high [^{11}C]-(+)-PHNO BP_{ND} levels in the VP did not change the results (condition x ROI: $F(5, 120) = 8.149$, $P_{GG} = .001$; partial eta squared = 0.253; condition x ROI x group: $F(5, 120) = 1.185$, $P_{GG} = .314$, partial eta squared = .047; effect of condition: $P = .003$).

The change in [^{11}C]-(+)-PHNO BP_{ND} in the LST was correlated with the time between smoking and the start of the scan ($r^2 = .415$, $P = .028$) and the number of puffs ($r^2 = -.446$, $P = .017$) (Figure 3) but not with nicotine or cotinine assessed just before smoking scan. The change in [^{11}C]-(+)-PHNO BP_{ND} in the VP was significantly correlated with the inter-puff interval ($r^2 = .376$, $P = .049$, Figure 3); this correlation was no longer significant when the data from the participants with the 2 extreme values in the VP were removed. No correlations with changes in [^{11}C]-(+)-PHNO BP_{ND} in either the LST or VP were found with nicotine levels taken before smoking scan (LST: $r^2 = -.237$, $P = .225$; VP: $r^2 = -.190$, $P = .334$) or cotinine levels taken before smoking scan (LST: $r^2 = -.283$, $P = .144$; VP: $r^2 = -.334$, $P = .082$). Further, nicotine levels were not correlated with the time between smoking and the scan ($r^2 = -.285$, $P = .142$)

Questionnaires

Questionnaire data from after the PET scans were analyzed with condition (abstinence, smoking) x group (FM, SM) ANOVAs and revealed a significant interaction for TCQ1 ($F(1, 26) = 5.155$, $P = .032$; follow-up analyses revealed no differences in the direction of effect of condition). Effects of condition were revealed for TCQ3 ($F(1, 26) = 6.182$, $P = .02$), MNWS ($F(1, 26) = 0.021$), QSU2 ($F(1, 26) = 13.894$, $P = .001$), VAS1 $F(1, 26) = 11.252$, $P = .002$), VAS2 ($F(1, 26) = 15.396$, $P = .001$), VAS4 ($F(1, 26) = 15.064$, $P = .001$), VAS 7 ($F(1, 26) = 13.834$, $P = .001$), VAS10 ($F(1, 26) = 10.894$, $P = .003$), VAS12 ($F(1, 26) = 6.282$, $P = .019$), VAS14 ($F(1, 26) = 5.418$, $P = .028$), VAS15 ($F(1, 26) = 7.651$, $P = .010$), and VAS16 ($F(1, 26) = 5.677$, $P = .025$).

Discussion

The purpose of the present study was to investigate differences in DA receptor levels at abstinence baseline between FM and SM and also to determine whether differences exist between FM and SM in changes in DA levels after smoking a cigarette. It was found that, at abstinence baseline, SMs had lower DA D_2 receptor levels in the AST and SMST than FMs, with no group differences in the D3 region of the SN. After smoking a cigarette, decreases in [^{11}C]-(+)-PHNO BP_{ND}, corresponding to increases in DA levels, were seen in the LST and VP in both the FMs and SMs, with no group differences based on NMR status. The amount of change in [^{11}C]-(+)-PHNO BP_{ND} in the LST was correlated with the time between smoking and the scan and the number of puffs taken on a cigarette (but not cotinine or nicotine levels), while change in [^{11}C]-(+)-PHNO BP_{ND} in the VP was correlated with inter-puff interval.

In the present study, SMs had lower [^{11}C]-(+)-PHNO BP_{ND} in the D_2 regions of the AST and SMST at abstinence baseline. These differences were not attributable to plasma nicotine or cotinine levels, which did not differ between groups and did not correlate with BP_{ND} in these ROIs, and were observed in the

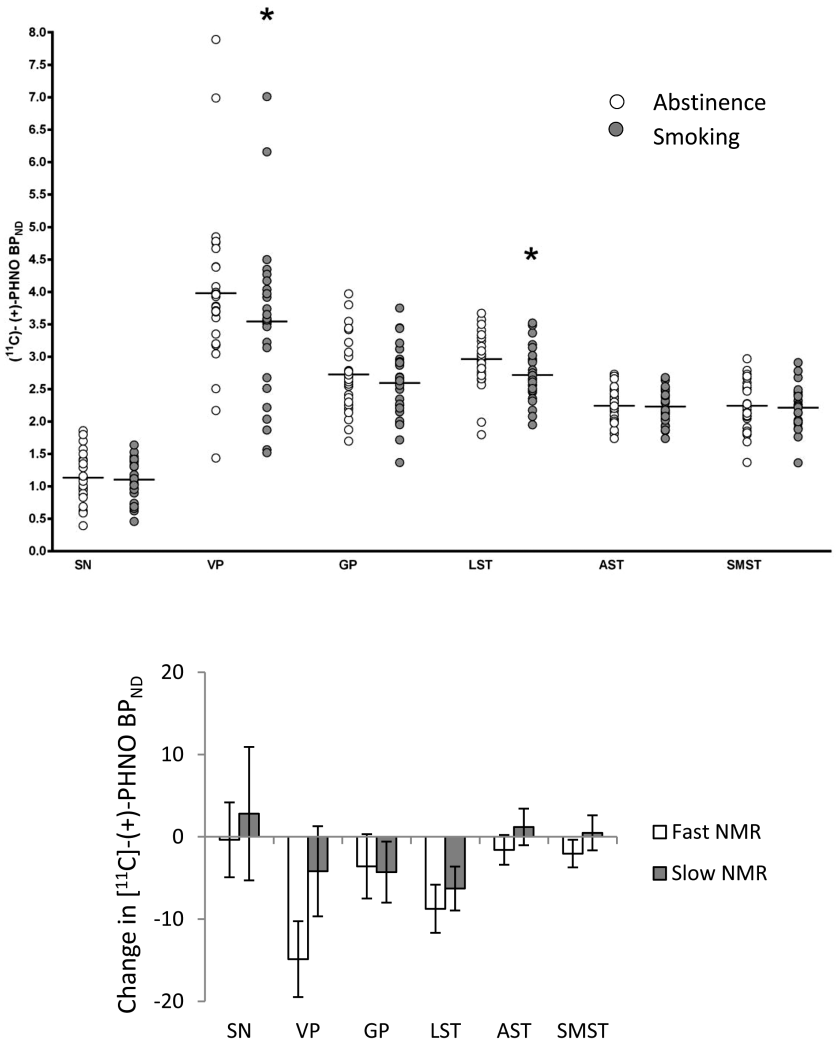

Figure 2. Top: Binding potential (BP_{ND}) measured at abstinence baseline (open symbols) or after smoking a preferred cigarette (dark symbols) in regions of interest (ROIs). *P < .05, abstinence different from smoking. **Bottom**: Change in [^{11}C]-(+)-PHNO BP_{ND} between abstinence baseline and smoking condition in ROIs. Open symbols are the fast metabolizers (FM) and closed symbols are the slow metabolizers (SM). No differences were found between the FMs and SMs. ROIs are presented in order of D_3 fraction: SN: substantia nigra; VP: ventral pallidum; GP: globus pallidus; LST: ventral/limbic striatum; AST: associative striatum; SMST: sensorimotor striatum.

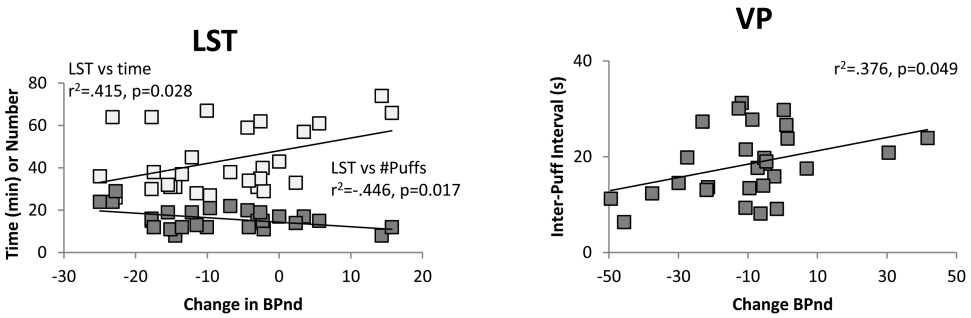

Figure 3. Left: Correlation between change in binding potential (BP_{ND}) in the ventral/limbic striatum (LST) after smoking and time between smoking and the start of the scan (open symbols) and the number of puffs of the preferred cigarette (dark bars). **Right**: Correlation between change in BP_{ND} in the ventral pallidum (VP) after smoking and inter-puff interval.

absence of other group differences in demographic, subjective, or objective variables. These findings are consistent with a previous report of group differences in brain activity at baseline; in this previous report, it was found that those with the faster *CYP2A6* genotype had higher brain activation at resting state, consistent with faster breakdown of nicotine (Li et al., 2017). One possible difference between the NMR groups could be that, due to differences in nicotine elimination kinetics, the intensity of withdrawal may be greater in FM compared with SM (Rubinstein et al., 2008). However, here we did not observe any differences in

withdrawal ratings and we controlled for prolonged abstinence, which allowed for essentially complete elimination of nicotine. Indeed, very low levels, and no difference, in the plasma levels of nicotine between the two groups was observed. In addition, as there were no nonsmokers included in the present study, it remains to be determined whether these changes would also be observed in control subjects (i.e., are these differences pre-existing or induced by tobacco exposure. No group differences by *CYP2A6* genotype in functional connectivity as measured by resting state fMRI were observed among nonsmokers (Li et al., 2017), only among smokers, which argues for a gene x environment interaction. Future studies will be needed in healthy controls and in subjects after prolonged cessation to determine if these changes are persistent or not. Indeed, it is possible that the SM had lower BP_{ND} at abstinence baseline because their receptor levels recover more slowly from abstinence; this is an empirical question for future research.

Another interesting finding is that the relative difference in [^{11}C]-(+)-PHNO BP_{ND} at abstinence baseline between the groups was seen in ROIs in which binding of [^{11}C]-(+)-PHNO is to D_2 receptors, but not in those where binding is to D_3 receptors. There are clear differences in the role and regulation of D_2 vs D_3 receptors (Boileau et al., 2012; Le Foll et al., 2014a). One consistent finding in the literature is that of lower D_2 receptor levels in those with drug dependence (Volkow et al., 1993; Martinez et al., 2004). In addition, it has been shown that lower D_2 receptor levels predict relapse to drug use (Wang et al., 2012). In this context, the present finding is somewhat surprising given that SMs have largely been found to have higher response rates during behavioral counselling and during active treatment in clinical trials (Lerman et al., 2006; Patterson et al., 2008; Ho et al., 2009; Schnoll et al., 2009; Chenoweth et al., 2013, 2016; Vaz et al., 2015; Ebbert et al., 2016). However, a recent prospective study demonstrated the opposite, that people with faster NMRs are more likely to quit (Fix et al., 2017). The authors suggest that one reason for this discrepancy is the difference between the clinical trial situation in previous studies and the prospective ratings in their study. Thus, the lower [^{11}C]-(+)-PHNO BP_{ND} at baseline in the SMs in the current study may be related to poor quit rates in non-treatment seekers. However, greater quitting in *CYP2A6* genotypic SMs vs FMs, using frequencies among current vs former smokers, supports greater success in quitting among SMs (Gu et al., 2000; Schoedel et al., 2004; Chenoweth et al., 2013). Thus the relationship between the lower [^{11}C]-(+)-PHNO BP_{ND} at baseline among SMs smokers and success in quitting smoking requires investigation.

In the present study, contrary to our planned hypothesis, no group differences were found between FMs and SMs in the change in [^{11}C]-(+)-PHNO BP_{ND} after smoking. These findings are somewhat surprising given the extensive literature on differences in smoking characteristics between FM and SM (Benowitz et al., 2003; Johnstone et al., 2006; Lerman et al., 2006, 2015; Malaiyandi et al., 2006; Audrain-McGovern et al., 2007; Mwenifumbo et al., 2007; Patterson et al., 2008; Rubinstein et al., 2008; Schnoll et al., 2009, 2014; Strasser et al., 2011; Sofuoglu et al., 2012; Chenoweth et al., 2013; Kaufmann et al., 2015; Vaz et al., 2015; Chenoweth et al., 2016). It is possible that due to our limited sample, we may have been underpowered to detect differences on this response based on NMR. The present findings nevertheless support the results of our previous study (Le Foll et al., 2014b) in which we reported significant elevations of DA in the ventral/limbic striatum (LST) after smoking a cigarette. We also support our previous finding of a relationship between the number of puffs taken on a cigarette and the change in [^{11}C]-(+)-PHNO BP_{ND} in the LST. The present study extends those of our previous report by revealing that the length of time between smoking a cigarette and the start of the PET scan is important in determining the magnitude of effect, and also that inter-puff interval is related to changes in [^{11}C]-(+)-PHNO BP_{ND} in the VP. Thus, important effects of smoking characteristics on changes in DA levels were found, suggesting that smoking affects DA levels. It should be noted, however, that the changes in [^{11}C]-(+)-PHNO BP_{ND} were only marginally associated with nicotine levels ($P = 0.08$), and the time between smoking and scan was not associated with nicotine levels. Thus, although the length of time between smoking and scanning is important, nicotine may not be the only critical variable in determining the elevation of DA levels. Other contributors, such as environmental cues or alternative tobacco constituents could also participate (Tang et al., 2012; Chiuccariello et al., 2013; Falcone et al., 2016).

One finding that is worthy of note is the decrease in [^{11}]-(+)-PHNO BP_{ND} in the VP, corresponding to an increase in DA in this area, after smoking. The VP is an efferent region of the LST and was originally studied for its role as a limbic-interface, within a LST-VP circuit (Mogenson et al., 1993). Since then, it has been posited to have roles in feeding, cue-induced feeding, taste reactivity, maternal behavior, cognition, intracranial self-stimulation, aversion, and, most relevant to the present discussion, drug self-administration (Root et al., 2015). In particular, Berridge and colleagues posit that it is a hedonic "hotspot" (Castro and Berridge, 2014). Although the VP has not been as extensively studied in drug dependence as other brain regions such as the nucleus accumbens of cingulate cortex, the present study adds to the growing literature on the VP by implicating it in smoking.

In the present study, no group differences were found on any demographic variables or on measures of smoking topography. This is in contrast to previous reports of differences in cigarettes per day (Benowitz et al., 2003; Johnstone et al., 2006; Mwenifumbo et al., 2007; Schnoll et al., 2009, 2014) or puff volumes (Strasser et al., 2011). Differences in puff volume may be attributable to the fact that, in the present study, participants were in withdrawal when they smoked their cigarette, while they had only refrained from smoking for one hour in the previous study (Strasser et al., 2011). Indeed, in a study where participants were tested at 12 hours of withdrawal, no differences in smoking topography were observed (Faulkner et al., 2017). Alternatively, the differences may be related to demographic variables in that participants were not required to smoke a minimal number of cigarettes per day, or to have a minimal FTND, for inclusion in this study. The inclusion criteria were intentionally selected to allow for a broader range of participants, but this may have inadvertently diminished some of the baseline differences between groups. However, it should be noted that not all studies found relationships between NMR and CPD or FTND (Ross et al., 2016; Faulkner et al., 2017). Future studies will need to determine the relative contribution of experience, and levels of dependence, on smoking-induced changes in DA, and the interaction of NMR with these changes.

Limitations

This study is not without limitations. First, the present findings rely on relatively small samples. Our sample size determination was based on our preliminary data (Le Foll et al., 2014b). Over the course of the study, it was decided to combine data sets to increase power, and thus the study was terminated near completion to obtain the present sample size. In addition, we could not explore important variables such as gender, which has been

shown to influence many aspects of tobacco smoking (Cosgrove et al., 2014). Our inclusion/exclusion criteria allowed for subjects with different degrees of dependence to be included (which also can be seen as a strength). Due to the complexity of running the experimental procedures, we had also significant variability in the duration of abstinence before the scans or with the time between the smoking cessation and the PET sessions. Those factors could have decreased our statistical power by increasing variability in our outcome measure. Further, although the mass injected was not different between conditions, it approached significance, raising the question as to whether this influenced the results. However, the cerebellar time activity curves were not different between conditions, suggesting that this was not a confounding variable.

Conclusions

The present study demonstrated baseline differences in [^{11}C]-(+)-PHNO BP_{ND} in D_2, but not D_3, regions, between FMs and SMs at abstinence. We also validate in a larger sample our previous findings of a decrease in [^{11}C]-(+)-PHNO BP_{ND} (increase in DA) in the LST and VP after smoking. However, no group differences based on NMR were revealed in changes in [^{11}C]-(+)-PHNO BP_{ND} after smoking a cigarette. Whether differences at abstinence baseline exist between FMs and SMs before exposure to smoking, whether it affects cessation, and the time it may take for those changes to normalize after smoking cessation can be the topic of further studies.

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Statement of Interest

R. F. Tyndale has consulted for Apotex and Quinn Emmanuel on unrelated topics and received funding from GRAND (unrestricted funding support from Pfizer) as well as university and hospital speaker honorariums. Bernard Le Foll has received financial compensation from Bioprojet, GRAND Awards and Allergan.

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