Neuropsychopharmacology www.nature.com/npp

ARTICLE

Sex differences in amphetamine-induced dopamine release in the dorsolateral prefrontal cortex of tobacco smokers

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Sex differences exist in the neurochemical mechanisms underlying tobacco smoking and smoking-related behaviors. Men tend to smoke for the reinforcing effects of nicotine, whereas women tend to smoke for stress and mood regulation, and have a harder time maintaining long-term abstinence. The mesolimbic dopamine (DA) system drives the reinforcing effects of tobacco smoking, whereas the mesocortical DA system—including the dorsolateral prefrontal cortex (dlPFC)—is critical for stress-related cognitive functioning and inhibitory control. This study is the first to investigate dlPFC $D_{2/3}$-type receptor (D_2R) availability and amphetamine-induced cortical DA release in smokers and nonsmokers. Forty-nine subjects (24 tobacco smokers (12 females) and 25 sex- and age-matched nonsmokers) participated in two same-day [^{11}C]FLB457 positron emission tomography (PET) scans before and 3-hours after amphetamine administration (0.4–0.5 mg/kg, PO). D_2R availability (non-displaceable binding potential; BP_{ND}) was measured pre- and post-amphetamine. The percent fractional change in BP_{ND} ($\%\Delta BP_{ND}$) between pre- and post-amphetamine, an index of DA release, was compared between male and female smokers and nonsmokers. Smokers showed significantly lower dlPFC D_2R availability ($BP_{ND} = 0.77 \pm 0.05$) than nonsmokers ($BP_{ND} = 0.92 \pm 0.04$), $p = 0.016$, driven by males. Female smokers showed significantly less amphetamine-induced DA release in dlPFC ($\%\Delta BP_{ND} = 1.9 \pm 3.0\%$) than male smokers ($\%\Delta BP_{ND} = 14.0 \pm 4.3\%$), $p < 0.005$, and female nonsmokers ($\%\Delta BP_{ND} = 9.3 \pm 3.3\%$), $p < 0.005$. This study shows that in the prefrontal cortex, smokers have lower D_2R availability than nonsmokers and that female vs. male smokers have a blunted amphetamine-induced DA release. These findings demonstrate that tobacco smoking differentially affects the mesocortical DA system in men vs. women, suggesting a potential target for gender-specific treatments.

Neuropsychopharmacology (2019) 44:2205–2211; https://doi.org/10.1038/s41386-019-0456-y

INTRODUCTION

Tobacco smoking is the world's leading cause of preventable death [1]. Deaths related to the use of combustible tobacco products greatly exceed those from alcohol, firearms, AIDS, and all other drugs including opioids, combined [2–6]. Tobacco smoking is largely driven by the reinforcing effects of nicotine—the primary addictive chemical in tobacco cigarettes. Sex differences have been documented both in the reinforcing effects of nicotine and in tobacco smoking treatment. Men experience greater nicotine-induced reinforcement than women [7, 8]. Men also respond better than women to nicotine-replacement therapies (NRTs) [9]—the first line of treatment for smoking cessation. Women are more reinforced by smoking cues [10], report greater psychological withdrawal [11], tend to relapse to smoking in response to stress [12, 13], and have a harder time maintaining long-term abstinence [14]. To treat female tobacco smokers more effectively, it is important to understand the molecular mechanisms underlying sex/gender-based behavioral differences.

Nicotine binds to and activates nicotinic acetylcholine receptors, which, in turn, facilitates DA release in striatal and cortical brain regions [15, 16] via the mesolimbic and mesocortical pathways, respectively. The mesolimbic ("reward") DA pathway drives the reinforcing effects of tobacco smoking, while the mesocortical ("goal-directed") DA pathway—including the dlPFC—is critical for inhibitory control [17], which is compromised by stress [18, 19]. In both non-human primates and humans, stress impairs prefrontal cortex (PFC) functioning [18, 20]. Systematic nicotine exposure alters medial PFC–ventral tegmental area (mPFC–VTA) coupling, particularly connections from the dlPFC [18]. Thus, stress-induced impairment of PFC function could contribute to sex differences in smoking-related behaviors.

It is well known from PET brain imaging studies that individuals with drug and alcohol use disorders, including tobacco smoking, have significantly lower D_2R availability in various subregions of the striatum compared to healthy controls [21–27]. Importantly, lower striatal D_2R availability has only been shown in male [21, 28], but not female tobacco smokers [28]. Sex differences in D_2R availability have also been found in the midbrain [29], where DA neurons originate, and in the ventral striatum specifically, in response to smoking a cigarette [30].

Although previous PET studies have investigated the mesolimbic DA pathway, the mesocortical DA system remains largely

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Sixteen of the participants in this study took part in the following clinical trial: imaging extrastriatal dopamine release in tobacco smokers and nonsmokers; NCT02348385.

Received: 18 February 2019 Revised: 31 May 2019 Accepted: 19 June 2019
Published online: 3 July 2019

unexplored in tobacco smokers. This is due, in part, to the limitations of widely available radiotracers, such as [^{11}C]raclopride, which are best for measuring striatal D_2R availability. The development of the radiotracer [^{11}C]FLB457, a high-affinity D_2R radiotracer [31, 32], provides a tool to measure extrastriatal (i.e. PFC) D_2R availability. Due to its high-affinity and long equilibration time, [^{11}C]FLB457 is not suitable for analysis of D_2R availability in striatum. D_2R radiotracers can also be used with drug challenges, i.e., amphetamine, to measure changes in synaptic DA [33] levels and infer DA system function.

To examine sex differences in D_2R availability and DA function in the mesocortical pathway, we used [^{11}C]FLB457 and PET imaging. The goals of this study were to measure cortical D_2R availability and amphetamine-induced cortical DA release in male and female tobacco smokers and nonsmokers. The dlPFC was our *a priori* region-of-interest (ROI) because of its primary role in the mesocortical pathway, executive control, and stress-related cognitive function and impairment, in animal and human studies [18, 34, 35]. We hypothesized that smokers compared to nonsmokers would have lower dlPFC D_2R availability, consistent with findings in the striatum [21, 28, 30]. Further, since stress compromises PFC functioning [20] and female smokers are more likely to relapse to smoking in response to stress [12, 36], we hypothesized that female smokers would show a smaller magnitude amphetamine-induced dlPFC DA response compared to male smokers and to female nonsmokers.

MATERIALS AND METHODS

Subjects

Twenty-five tobacco smokers (12 female) and twenty-five age- and sex-matched healthy controls/nonsmokers (12 female) participated in two same-day [^{11}C]FLB457 PET scans, one scan before ('baseline'), and the second scan 3-h after amphetamine administration (0.4–0.5 mg/kg, PO). One female smoker had her amphetamine administration and subsequent scan 5 days after the baseline scan. One male smoker was excluded due to a cerebellar abnormality. All subjects also underwent structural magnetic resonance imaging (MRI) required to delineate anatomical information [37]. A subset ($n = 16$, 10 males) of the smokers were included in a previous study testing different hypotheses [38]. Written informed consent for all study procedures, approved by the Yale Human Investigation Committee and the Yale-New Haven Hospital Radiation Safety Committee, was obtained from all subjects prior to participation. The study adhered to the Protection of Human Subjects of Research and Ethical Principles and Guidelines.

Subject screening procedures included a physical exam, electrocardiogram, blood tests, and urine toxicologies. Subjects had no history of significant major medical disorders and did not meet DSM-IV criteria for current or past psychiatric or substance abuse diagnosis (except nicotine dependence for smokers). Smokers were required to have been smoking cigarettes daily for at least one year (Table 1). Current tobacco smoking status was confirmed by spirometer carbon monoxide (CO) levels >10 parts per million (ppm) and urine cotinine—the primary metabolite of nicotine—levels >150 ng/ml on intake day. On scan day, smokers were required to be overnight abstinent, verified by CO levels <11 ppm or ≤50% of their intake level. All female subjects were required to have a negative pregnancy test on intake day and PET scan day prior to radiotracer administration. Menstrual cycle phase was not controlled and use of hormonal contraception was not exclusionary. Plasma samples were collected in all subjects on the day of the PET scan prior to the first scan for analysis of sex steroid hormone levels: follicle-stimulating hormone (FSH), estradiol, progesterone and testosterone.

On PET scan day, all subjects completed mood and other relevant questionnaires including the Beck's Depression Inventory (BDI) [39], the State/Trait Anxiety Inventory (STAI) [40], and Barratt Impulsiveness Scale Version11 (BIS-11) [41]. Tobacco smokers completed measures of tobacco smoking behavior including the Fagerström Test for Nicotine Dependence (FTND) [42], Minnesota

Table 1. Group demographics

	NS ($N = 25$) Mean ± SE	S ($N = 24$) Mean ± SE	NS v. S *P*-value	FS ($N = 12$) Mean ± SE	MS ($N = 12$) Mean ± SE	FS v. MS *P*-value	FNS ($N = 12$) Mean ± SE	FNS v. FS *P*-value	MNS ($N = 13$) Mean ± SE	MNS v. MS *P*-value
Demographic										
Age (years)	29.3 ± 1.8	34.0 ± 1.9	0.08	32.8 ± 2.8	35.3 ± 2.8	0.52	30.3 ± 2.9	0.55	28.4 ± 2.4	0.07
Weight (kg)	74.4 ± 2.7	77.5 ± 2.8	0.44	70.3 ± 2.4	84.7 ± 4.1	0.01*	66.6 ± 3.1	0.36	81.7 ± 3.2	0.57
Smoking measures										
Cigarettes/day				12.8 ± 1.5	13.6 ± 1.5	0.70				
Years smoked				13.5 ± 2.0	16.1 ± 1.9	0.36				
FTND				4.6 ± 0.53	6.1 ± 0.9	0.15				
CO level				10.0 ± 1.4	8.8 ± 1.3	0.51				
Cotinine level				5.4 ± 0.5	6.0 ± 0.0	0.27				
MNWQ				9.75 ± 1.90	5.58 ± 1.38	0.09				
QSU				43.3 ± 3.37	36.8 ± 5.06	0.29				
Self-Report Mood Scales										
STAI state	26.2 ± 1.4	32.9 ± 2.2	0.01*	35.8 ± 2.8	30.0 ± 3.3	0.19	24.6 ± 1.6	0.002*	27.6 ± 2.3	0.55
STAI trait	27.7 ± 1.38	28.9 ± 2.09	0.64	31.9 ± 2.31	25.8 ± 3.37	0.15	28.1 ± 1.66	0.20	27.4 ± 2.19	0.70
BDI	0.9 ± 0.5	3.0 ± 0.8	0.03*	3.1 ± 0.9	2.8 ± 1.3	0.88	0.8 ± 0.6	0.049*	0.9 ± 0.8	0.22
BIS-11	53.8 ± 1.96	57.1 ± 2.17	0.28	59.2 ± 2.98	54.9 ± 3.15	0.33	54.0 ± 2.47	0.19	53.7 ± 3.10	0.79

Mean ± SE shown
BDI Beck's Depression Inventory, *BIS-11* Barratt Impulsiveness Scale 11, *CO* carbon monoxide, *FNS* female nonsmokers, *FS* female smokers, *FTND* Fagerström's Test for Nicotine Dependence, *MNS* male nonsmokers, *MNWQ* Minnesota Nicotine Withdrawal Scale, *MS* male smokers, *NS* nonsmokers, *QSU* Questionnaire of Smoking Urge, *STAI* State/Trait Anxiety Inventory, *S* smokers, *SE* standard error of the mean
*Significant at $p < 0.05$

Nicotine Withdrawal Scale (MNWQ) [43], and Questionnaire of Smoking Urge (QSU) [44]. CO and urine cotinine were collected on PET scan day.

Amphetamine administration

Amphetamine (0.4–0.5 mg/kg PO) was administered 150–180-minutes prior to the second scan. Amphetamine levels in plasma have been shown to peak during this timeframe following oral amphetamine administration [32]. Peak amphetamine levels relate to peak extracellular DA [45] and provide maximum sensitivity for us to detect differences in BP_{ND} between pre- and post-amphetamine conditions. Blood samples to measure plasma amphetamine concentrations were collected at $t = 0$, 60, 120, 180, 225, and 270-minutes relative to time of amphetamine administration. Out of 49 subjects, two (one male smoker and one female smoker) received a dose of 0.5 mg/kg. All others received 0.4 mg/kg as previously discussed [38, 46]. Mean amphetamine dose did not differ between smokers and nonsmokers (Table 1). Male and female smokers did not differ in mean amphetamine dose per bodyweight.

Imaging data acquisition

The high-affinity $D_{2/3}$ radioligand [^{11}C]FLB457 was synthesized as previously described [37, 38]. [^{11}C]FLB457 was injected intravenously as a bolus over 1-min by a computer-controlled pump (Harvard Apparatus, Holliston, MA, USA), and emission data were collected for 90 min. PET data were acquired using an ECAT EXACT HR + (Siemens/CTI, Knoxville, TN, USA). A 6-min transmission scan was acquired prior to the emission scan for attenuation correction. Subjects participated in an MRI on a 3T whole-body scanner (Trio, Siemens Medical Systems, Erlangen, Germany) on average ±13 days relative to the PET scan day. Structural T1 MRI was acquired for anatomical localization of the PET ROIs.

Imaging data processing and analysis

Sinograms were reconstructed with filter-back projection (FBP) with all corrections (attenuation, normalization, scatter, randoms, and deadtime) into a sequence of 27 frames: 6 × 30-s; 3 × 1-min; 2 × 2-min; 16 × 5-min. Motion correction was performed on dynamic image data by registering each frame to a summed early frame (e.g. the first 10-min of data) using a six-parameter mutual information algorithm [47] (FMRIB's Linear Image Registration Tool, FMRIB Software library, Version3.2). PET summed images were smoothed at 3 × 3 × 3 voxel FWHM Gaussian filter. Image dimensions and voxel size were 128 × 128 × 63 and 2.06 × 2.06 × 2.43 mm^3, respectively. The final reconstructed image resolution was ~6 mm full-width at half maximum.

Each MR image was normalized to Montreal Neurological Institute (MNI) space [48] using an affine linear plus nonlinear registration (Bioimage Suite 2.5, http://www.bioimagesuite.org/index.html), to extract ROIs from the automated anatomic labeling (AAL) template, as previously described [37]. The ROIs were then mapped from AAL space to PET space via the two transformations (e.g., PET-MR and MR-AAL template) to compute time activity curves in ROIs. All ROIs were bilateral summations and were defined using the AAL template including the dlPFC which was defined by combining the frontal superior, frontal mid, and frontal inferior triangularis corresponding to Brodmann's areas 9 and 46 [49]. Primary analyses focused on the *a priori* dlPFC ROI (Fig. S1). Exploratory analyses were conducted on additional extrastriatal ROIs with a measurable [^{11}C]FLB457 specific signal (defined as $BP_{ND} > 0.5$) [50], which included amygdala, anterior cingulate cortex, hippocampus, midbrain, occipital cortex, parietal cortex, temporal cortex, and thalamus.

PET data were fitted with the simplified reference tissue model (SRTM) [51, 52] using the cerebellum as a reference region to estimate BP_{ND} (an index of D_2R availability that is proportional to the number of available binding sites) as previously validated and described [37, 38]. The cerebellum reference region was gray matter masked and excluded the cerebellar vermis because of its low levels of $D_{2/3}R$ expression. This reference region approach is sensitive to detecting amphetamine-induced DA release with [^{11}C]FLB457 in extrastriatal regions, as validated in our prior study [37], which included 6 (4M, 2F) smokers from the current study. The $\%\Delta BP_{ND}$ between pre- and post-amphetamine was calculated as:

$$\%\Delta BP_{ND} = \left(1 - \left[\frac{BP_{ND}(\text{Post\_amphetamine})}{BP_{ND}(\text{Baseline})}\right]\right) \times 100$$

Outcome measurements were estimated in defined ROIs with a measurable [^{11}C]FLB457 specific signal (listed above) for primary and exploratory analyses.

Statistics

Student's *t*-tests were used to evaluate basic demographics (i.e., age, weight), mood questionnaires, and smoking characteristics between groups. Linear regressions were performed to examine potential associations between [^{11}C]FLB457 BP_{ND} and $\%\Delta BP_{ND}$ values with clinical correlates of tobacco smoking including cigarettes smoked per day, years smoked and, FTND, MNWQ, QSU, and BIS-11 scores. These were corrected for multiple comparisons. Exploratory analyses (*t*-tests and linear regressions) were conducted on the hormone data to examine group differences and associations with BP_{ND} and $\%\Delta BP_{ND}$ values (Table S1). Hormone analyses were not corrected for multiple comparisons because it was an exploratory aim.

To compare group differences in baseline dlPFC D_2R availability, [^{11}C]FLB457 BP_{ND} values were statistically analyzed using univariate analysis of variance (ANOVA) with sex and smoking status as between-subjects factors. The main effect of smoking status was examined to test the *a priori* hypothesis that smokers have lower dlPFC D_2R availability compared to nonsmokers. To address our *a priori* hypothesis, we conducted pairwise *F*-tests within the ANOVA model to compare male smokers to male nonsmokers and female smokers to female nonsmokers. All main effects and interactions were tested with appropriate post-hoc contrasts within the model.

To compare differences in amphetamine-induced DA release, a repeated-measures ANOVA featuring time (pre- vs. post-amphetamine) as a within-subjects factor with sex and smoking status as between-subjects factors was performed on dlPFC BP_{ND} values. To address our *a priori* hypothesis, we conducted pairwise *F*-tests within the ANOVA model to compare pre- vs. post-amphetamine BP_{ND} measures between female smokers and male smokers, and between female smokers and female nonsmokers. All main effects and interactions were tested with appropriate post-hoc comparisons, within the model. Each exploratory ROI was examined with a separate but identical repeated-measures ANOVA statistical model post-hoc and were not corrected for multiple comparisons.

RESULTS

Smoking and mood characteristics

Twenty-four smokers and 25 nonsmokers were included in the final analysis (Table 1). On average, smokers smoked 13.2 ± 1.0 cigarettes per day for 14.8 ± 1.4 years and had FTND scores of 5.3 ± 0.5, indicating moderate dependence levels. Male and female smokers were matched for age, cigarettes smoked per day, years of smoking, and FTND scores. Overnight abstinent smokers had significantly higher STAI state ($p = 0.01$) and BDI scores ($p = 0.03$) compared to nonsmokers. Female smokers had significantly higher STAI state ($p = 0.002$) and BDI scores ($p = 0.049$) compared to female nonsmokers, while male subgroups were comparable ($p > 0.22$). All subgroup comparisons in Table 1 showed no differences in STAI trait and BIS-11 scores. Male and female smoker subgroups did not differ on MNWQ and QSU

smoking-related measures. There were no significant relationships between questionnaire scores and D_2R availability, or amphetamine-induced DA release.

PET scan parameters

On average, pre- and post-amphetamine scans did not differ in injected activity, injected mass, or injected mass per bodyweight. Nonsmokers received lower injected activity, but higher injected mass, than smokers for baseline [^{11}C]FLB457 scans ($p \leq 0.02$). Male nonsmokers compared to male smokers had a lower injected activity and higher injected mass for baseline [^{11}C]FLB457 scans ($p \leq 0.04$). These mass differences might have mitigated as opposed to accentuated our group differences (see Supplemental Information). There were no significant differences in injected activity or injected mass between male and female smokers, for baseline or post-amphetamine [^{11}C]FLB457 scans (Table S1).

Amphetamine administration and plasma levels

Amphetamine levels in plasma were not different at any time points between smokers and nonsmokers, or between male smokers and female smokers. Amphetamine levels increased following administration [38] and peaked at the start of the PET scan (150–225-min post-amphetamine; Figure S2).

Hormone levels

We found no significant differences in hormone levels (FSH, estradiol, progesterone, testosterone) between groups—females vs. males, smokers vs. nonsmokers or female vs. male smokers (Table S2), except, male smokers had higher testosterone levels than female smokers (17.1 ± 6.70 and 1.97 ± 0.30, respectively $p =$ 0.047, uncorrected). There were no significant relationships between hormone levels and D_2R availability or amphetamine-induced DA release in females, female smokers, or female nonsmokers.

D_2R availability

D_2R availability was measured in the baseline scan, prior to amphetamine administration. *A priori* planned contrast testing revealed a main effect of smoking status; dlPFC D_2R availability was significantly lower in smokers ($BP_{ND} = 0.77 \pm 0.05$) than nonsmokers ($BP_{ND} = 0.92 \pm 0.04$), $p = 0.016$ (Fig. 1). *A priori* planned contrast testing also revealed that male smokers had a significantly lower dlPFC D_2R availability ($BP_{ND} = 0.69 \pm 0.06$) than male nonsmokers ($BP_{ND} = 0.90 \pm 0.05$), $p = 0.009$, while female smokers had lower, but not significantly different dlPFC D_2R availability ($BP_{ND} = 0.85 \pm 0.06$) compared to female nonsmokers ($BP_{ND} = 0.95 \pm 0.08$), $p = 0.29$. The main effect of sex ($p = 0.10$) and the interaction between sex and smoking status ($p = 0.40$) were not significant. There were no baseline differences in D_2R availability between groups in exploratory ROIs (Table S3).

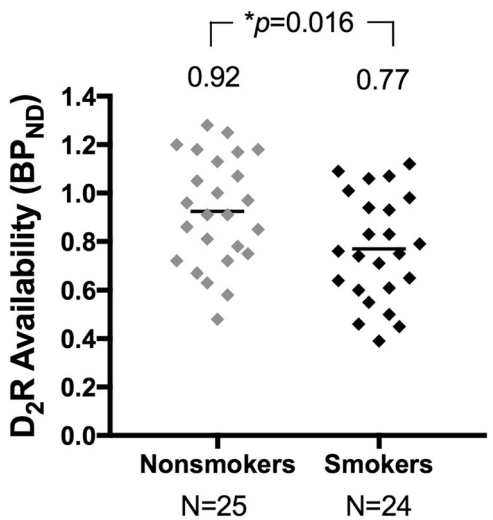

Fig. 1 Baseline dlPFC D_2R availability comparison of smokers and nonsmokers. Smokers (black) showed significantly lower dlPFC D_2R availability than nonsmokers (gray), $p = 0.016$. Group means are shown above data points. BP_{ND} = binding potential

Amphetamine-induced DA release

Planned contrast testing of our *a priori* hypotheses demonstrated that female smokers had significantly less amphetamine-induced DA release in the dlPFC ($\%\Delta BP_{ND} = 1.9 \pm 3.0\%$) compared to male smokers ($\%\Delta BP_{ND} = 14.0 \pm 4.3\%$), $p < 0.005$, and compared to female nonsmokers ($\%\Delta BP_{ND} = 9.3 \pm 3.3\%$), $p < 0.005$; Bonferroni corrected (Fig. 2). Repeated-measures ANOVA also demonstrated a significant effect of time (amphetamine administration; $p <$ 0.0005). Pre- vs. post-amphetamine dlPFC D_2R availability was significantly different within the group of male smokers ($p =$ 0.007), male nonsmokers ($p = 0.0001$), and female nonsmokers ($p = 0.01$), but not in the group of female smokers ($p = 0.37$), suggesting that there was no significant amphetamine-induced DA release in the female smokers. There were also significant main effects of sex ($p = 0.04$) and smoking status ($p = 0.02$). The interaction between time, sex, and smoking status was not significant ($p = 0.10$). In all exploratory ROIs, there was significant amphetamine-induced DA release (pre- vs. post-amphetamine; $p < 0.02$, uncorrected; except the midbrain) and no main effects of sex or smoking status (Table S3).

DISCUSSION

There are two primary findings from this neuroimaging study. First, tobacco smokers had significantly lower D_2R availability in the dlPFC compared to nonsmokers. Although it is well known that tobacco smokers and individuals with other addictive disorders have lower D_2R availability in the striatum [21–26] compared to matched control groups, this is the first study to extend these findings to the PFC. We also found differences by sex in smokers vs. nonsmokers; male smokers had lower dlPFC D_2R availability (DA receptor levels) than male nonsmokers, but female smokers did not differ from female nonsmokers. Second, female

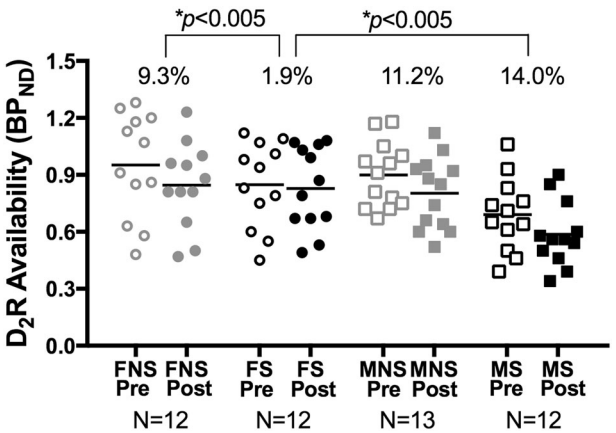

Fig. 2 Baseline and post-amphetamine dlPFC D_2R availability for all subgroups. Female smokers (black circles) showed significantly less amphetamine-induced DA release in the dlPFC than both male smokers (black squares) and female nonsmokers (gray circles). Male nonsmokers are shown in gray squares. Open shapes represent pre-amphetamine data and closed shapes represent post-amphetamine data. Group mean $\%\Delta BP_{ND}$ is shown above data points. BP_{ND} = binding potential, FNS = female nonsmokers, FS = female smokers, MNS = male nonsmoker, MS = male smoker, Pre = pre-amphetamine, Post = post-amphetamine

smokers had significantly lower amphetamine-induced DA release (DA neurotransmission) in the dlPFC compared to male smokers, suggesting that tobacco smoking has no impact on stimulated DA release in males but blunts stimulated DA release in females. In addition, female smokers had significantly less amphetamine-induced DA release compared to female nonsmokers. Taken together, these findings suggest that tobacco smoking differentially affects the mesocortical DA system in males vs. females.

The main finding of lower D_2R availability in tobacco smokers vs. controls extends our current understanding of the effect of tobacco smoking on the DA system. Numerous PET studies have demonstrated that individuals with addictive disorders (including tobacco smokers [21, 28, 53], and methamphetamine [24], alcohol [23], cocaine [22, 25], and heroin [26] use disorders) have significantly lower striatal D_2R availability than comparison groups. Here, we show that dlPFC D_2R availability is compromised, and potentially downregulated, by tobacco smoking, but only in males.

Lower dlPFC D_2R availability in smokers vs. nonsmokers was driven by the difference between male smokers and male nonsmokers. We did not find a significant difference in dlPFC D_2R availability between female smokers and female nonsmokers. Two studies previously reported lower striatal D_2R availability in tobacco smokers vs. nonsmokers [21, 28]. Only one included females [28] and similarly reported differences in males (smokers < nonsmokers), but not females (smokers≈nonsmokers). Lower D_2R availability in chronic smokers may be specific to males only, underscoring the importance of including females in these studies.

We found that tobacco smoking differentially alters DA function in males vs. females as indexed by amphetamine-induced DA release. Drug challenge paradigms has been used in many studies of addictive [23, 54] and other psychiatric [55–57] disorders to probe function of the DA system [58]. In this study, amphetamine-induced DA release in the dlPFC was negligible in female smokers compared to both male smokers and to female nonsmokers. Several studies have shown that amphetamine-induced DA release is blunted in individuals with addictive disorders compared to control groups [59], and in individuals with cocaine addiction, the more blunted the DA response, the worse the treatment outcome [22]. These data alone do not address whether this deficit is pre-existing or a consequence of drug use. However, preclinical longitudinal imaging studies, performed pre- and post-chronic cocaine administration, have shown that lower D_2R availability in individuals with substance use disorders may be a consequence of use [60, 61]. While the neurobiological processes underlying a blunted DA response are unclear, these data suggest that blunted DA response is a biomarker of addiction development and treatment resistance.

Although our *a priori* ROI was the dlPFC because of its primary role in the mesocortical pathway, including executive control and stress-related cognitive function and impairment [18, 34, 35], we also examined other ROIs. For all exploratory ROIs, we observed a pattern in D_2R availability between subgroups similar to the dlPFC; however, these differences did not reach statistical significance. These results suggest that the disruption in DA neurotransmission is specific to the dlPFC. For all ROIs (except the midbrain) we also observed a statistically significant amphetamine-induced DA response between pre- and post-amphetamine scans, suggesting that DA neurotransmission is intact in these brain regions in tobacco smokers. For the midbrain ROI, we did not observe any main effects of sex or smoking status at baseline, contrary to one prior study [29], nor did we observe an amphetamine-induced DA response. This study found a sex by smoking status interaction in baseline D_2R availability using a different radiotracer and with a smaller sample size [29]. More research is required to disentangle these conflicting findings.

The mechanisms underlying the differential sex effects of tobacco smoking on the DA system are not established. Both the mesolimbic and mesocortical pathways originate in the VTA of the midbrain. In one prior study, female smokers have higher D_2R availability in the midbrain than female nonsmokers, whereas male smokers and nonsmokers are not different [29]. Midbrain D_2Rs are predominantly inhibitory, and it has been postulated that higher midbrain D_2R availability in female smokers may lead to a suppression of ventral striatal smoking-induced DA release (compared to male smokers) [30]. In males, chronic tobacco smoking and the associated smoking-induced DA release in the ventral striatum likely downregulates D_2R availability in that region. This is supported by the finding that male smokers have less D_2R availability in striatum than male nonsmokers. Our sex-specific finding of lower D_2R availability in the dlPFC of male smokers (but not female smokers) is consistent with striatal findings.

The neurochemical differences reported in the present study may underlie some of the behavioral sex differences reported in the literature [62–65]. For example, female smokers report smoking under stress or to reduce negative mood and tend to relapse in response to stress and negative mood [12, 13]. Under acute stress conditions, DA levels increase in the dlPFC and impair executive functions [20, 66], i.e., working memory and inhibitory control. This stress-induced disruption of the dlPFC may underlie the ability to resist smoking during stress and negative mood, which may lead to stress-induced smoking relapse [67] to manage these symptoms. Previous PET studies in humans have shown that dysregulated DA signaling in striatum is associated with maladaptive behavior such as drug seeking [54] and poorer treatment outcomes [22]. DA signaling in dlPFC is critical for stress management and behavioral disinhibition. We hypothesized that a dysfunctional mesocortical DA system in female smokers could facilitate stress-induced relapse and impede quit attempts. Thus, it is possible that a blunted amphetamine-induced DA response in the dlPFC may explain why women persistently smoke in response to stress. Although the neural mechanism of this blunted DA transmission in female smokers is unknown, it can be mechanistically explained by (1) decreased pre-synaptic DA release [54, 68], (2) reduction in pre-synaptic neuronal stores of dopamine [69], and/or (3) reduced baseline levels of endogenous dopamine [70] as observed in cocaine-dependent individuals. Another example of smoking-related sex difference is that male tobacco smokers are more sensitive to smoking-related rewards and more responsive to NRT than females [63, 71]. Our previous study showed that male smokers have a higher DA response in the striatum (the reinforcement center) in response to a cigarette [30] and the current study showed that female smokers have a blunted DA response to amphetamine (a robust DA probe). This heightened DA response to nicotine in male smokers and lack of a DA response in female smokers may explain why NRTs are more effective in men [22] since NRTs are thought to dampen the nicotine-induced dopaminergic response to smoking and extinguish drug-seeking behavior [72].

This study has several strengths including well-matched groups, a well-validated amphetamine challenge paradigm, and use of a novel high-affinity D_2R radioligand that allowed for measurement of extrastriatal D_2R availability and amphetamine-induced DA release. This study also has some limitations that can be addressed in future studies. Although the sample size allowed for systematic examination of sex differences, the size and makeup of the sample may have made it difficult to detect an influence of sex steroid hormones. The relationship between menstrual phase-related fluctuations in estrogen and/or progesterone on DA has produced mixed results [73–75]. Future studies should examine the relationship between sex steroid hormones and DA, controlling for menstrual cycle phase [76, 77]. It is important to note that the average amphetamine dose (0.38 mg/kg) in the current study is slightly lower than previous studies (0.4–0.5 mg/kg). Future studies should also include objective measurements of stress reactivity and nicotine-related behavior to determine if dlPFC D_2R

availability and amphetamine-induced DA release are differentially associated with stress-related cognitive impairment and behavioral disinhibition, respectively, in male and female smokers.

Much of our understanding of disease and treatment has come from clinical and preclinical studies in males [78]. Literature has shown that these findings do not necessarily translate in women [79]. Studying sex-related differences is essential for disentangling sex-related biological differences and promoting the health of both males and females. The current study identified a biological sexual dimorphism—tobacco smoking impairs mesocortical DA release in female smokers but not male smokers—thus highlighting the importance of including females in research studies. The dlPFC is a critical component of the stress pathway, therefore this sex difference may mediate the effects of stress impeding abstinence from tobacco smoking to a greater degree in women than men. In conjunction with the current findings, the limited efficacy of current dopaminergic and nicotine-replacement treatments—particularly in women—we propose that there is a critical need for the development of gender-specific treatment that target the stress pathway.

FUNDING AND DISCLOSURE

The study was supported by the Office of Research on Women's Health and the National Institutes of Health (P50DA033945, R01DA038832, R01DA14241, K02DA03175, and K01 AA024788). YZ was supported by the Interdepartmental Neuroscience Training Program at Yale University (T32NS4122813), Gruber Science Foundation Fellowship, the National Science Foundation Graduate Research Fellowship Program (NSF-GRFP), and the Neuroimaging Sciences Training Program at Yale University (T32DA022975). The authors declare no competing interests.

ACKNOWLEDGEMENTS

We would like to thank Jon-Michael Anderson, Erin McGovern, Evgenia Perkins, Patrick Worhunsky, and the staff at the Yale PET Center.

ADDITIONAL INFORMATION

Supplementary Information accompanies this paper at (https://doi.org/10.1038/s41386-019-0456-y).

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