

Sex Differences in Midbrain Dopamine D2-Type Receptor Availability and Association with Nicotine Dependence

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Women differ from men in smoking-related behaviors, among them a greater difficulty in quitting smoking. Unlike female smokers, male smokers have lower striatal dopamine D2-type receptor availability (binding potential, BP_{ND}) than nonsmokers and exhibit greater smoking-induced striatal dopamine release. Because dopamine D2-type autoreceptors in the midbrain influence striatal dopamine release, a function that has been linked to addiction, we tested for sex differences in midbrain dopamine D2-type receptor BP_{ND} and in relationships between midbrain BP_{ND}, nicotine dependence and striatal dopamine D2-type receptor BP_{ND}. Positron emission tomography was used with [¹⁸F]fallypride to measure BP_{ND} in a midbrain region, encompassing the substantia nigra and ventral tegmental area, in 18 daily smokers (7 women, 11 men) and 19 nonsmokers (10 women, 9 men). A significant sex-by-group interaction reflected greater midbrain BP_{ND} in female but not male smokers than in corresponding nonsmokers ($F_{1, 32} = 5.089, p = 0.03$). Midbrain BP_{ND} was positively correlated with BP_{ND} in the caudate nucleus and putamen in nonsmokers and female smokers but not in male smokers and with nicotine dependence in female but not in male smokers. Striatal BP_{ND} was correlated negatively with nicotine dependence and smoking exposure. These findings extend observations on dopamine D2-type receptors in smokers and suggest a sex difference in how midbrain dopamine D2-type autoreceptors influence nicotine dependence.

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INTRODUCTION

Cigarette smoking is the leading preventable cause of premature death in the United States and has been causally linked to diseases of nearly all organs in the body, diminished health, and adverse effects during fetal development (National Center for Chronic Disease, 2014). Nicotine, an essential component of tobacco products that promotes their compulsive use (Stolerman and Jarvis, 1995), interacts with nicotinic acetylcholine receptors in the brain, mimicking the action of acetylcholine and enhancing dopamine release and neurotransmission in pathways that mediate reward (for reviews, see Balfour, 2015; Cosgrove *et al*, 2015; Subramaniam and Dani, 2015). Sex differences are seen in responses to nicotine with respect to reinforcement and reward in humans (Perkins, 2009) and nicotine self-administration by animals (Jensen *et al*, 2015) and in the

ability of smokers to quit (Pogun and Yazarbas, 2009). Moreover, there are sex differences in the success of smoking-cessation therapy, with female smokers being less likely than males to achieve long-term abstinence (Cepeda-Benito *et al*, 2004; Perkins and Scott, 2008; Rose and Behm, 2014). To some extent, these findings may reflect sex differences in the regulation of central dopaminergic neurotransmission (Becker, 1999; Pilotte *et al*, 1984).

Sex differences have also been observed in studies of the effects of nicotine on cerebral glucose metabolism, where application of the nicotine patch reduced metabolic activity in women to eliminate sex differences observed in placebo conditions (Fallon *et al*, 2005). In addition, dopamine D2-type receptor availability, measured as binding potential (BP_{ND}), is lower in the caudate nucleus and putamen of male smokers as compared with nonsmokers (Fehr *et al*, 2008), but a parallel difference is absent in females (Brown *et al*, 2012). Sex differences in the time course of smoking-induced dopamine release have also been reported, with women responding consistently in the ventral striatum and women responding faster than men in the dorsal putamen (Cosgrove *et al*, 2014). Finally, women show faster nicotine accumulation in the brain when smoking than men, and menthol

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accelerates brain nicotine accumulation in men but not in women (Zuo *et al*, 2015).

Given that smoking-induced dopamine release differs between men and women, it is reasonable to consider the possibility of sex differences in the substantia nigra and ventral tegmental area (SN-VTA) within the midbrain (Gardner and Ashby, 2000), where striatal dopaminergic neurons originate. In the SN-VTA, dopamine D2-type receptors are predominantly somatodendritic autoreceptors, which inhibit dopamine release in terminal fields of the dopaminergic neurons (Khan *et al*, 1998; Sesack *et al*, 1994). Evidence of this type of regulation is seen in the negative correlation of dopamine D2-type receptor BP_{ND} in the SN-VTA (midbrain BP_{ND}) with a measure of striatal dopamine release following amphetamine administration (Buckholtz *et al*, 2010). Given the aforementioned sex differences in dopamine D2-type receptor BP_{ND} in the caudate nucleus and putamen (striatal BP_{ND}) and smoking behavior, we expected sex differences in midbrain BP_{ND} that may differ by smoking status. Further, we hypothesized that midbrain BP_{ND} is negatively related to nicotine dependence and influences the association of cigarette smoking with striatal BP_{ND}.

Positron emission tomography (PET) was performed using [¹⁸F]fallypride, a radiotracer with high affinity for D2-type receptors (Mukherjee *et al*, 2002). In addition to the primary assessment of the interaction between sex and smoking status on midbrain BP_{ND}, relationships of midbrain BP_{ND} with striatal BP_{ND}, where smokers exhibit lower BP_{ND} than nonsmokers (Brown *et al*, 2012; Fehr *et al*, 2008), and with nicotine dependence were tested.

MATERIALS AND METHODS

Participants

All procedures were approved by the University of California Los Angeles Office for the Protection of Research Subjects. The participants were 18–55 years of age and were either daily smokers (5–20 cigarettes per day; 11 men and 7 women) or nonsmokers (< 5 cigarettes in their lifetimes; 10 men and 9 women). Participants were recruited through internet and local newspaper advertisements, and the participants were compensated for their time.

After receiving a complete explanation of the study procedures, each participant provided written, informed consent. Exclusion criteria (smokers and nonsmokers) were as follows: use of any psychotropic medication or substance except for nicotine and caffeine (in beverages) or meeting DSM-IV criteria for current or lifetime abuse of either alcohol or marijuana; central nervous system, cardiovascular, pulmonary, hepatic or systemic disease; human immunodeficiency virus seropositive status; pregnancy; lack of English fluency; MRI contraindications; or a current DSM-IV Axis-I diagnosis other than nicotine dependence, measured using the Structured Clinical Interview for DSM-IV. All participants were deemed physically healthy, on the basis of history and medical examination, and were right-handed, as indicated by the Edinburgh Handedness Questionnaire.

Demographics and Smoking-Related Ratings

The participants were characterized with regard to age and sex, as well as features of smoking exposure, including

number of cigarettes smoked per day, years of smoking, pack-years, and nicotine dependence, as measured by the Fagerström Test for Nicotine Dependence (FTND). Information was also obtained regarding other substances used in the past month (eg, marijuana, alcohol), and abstinence was verified by urine toxicology at intake and immediately before the PET scans. Exhaled carbon monoxide (CO) was measured using a Micro⁺Smokerlyzer (Bedfont Scientific, Kent, UK) to verify smoker or nonsmoker status at intake.

PET and MRI Scanning

Participants were instructed to refrain from smoking in the 2 h before PET scanning, which was performed with [¹⁸F]fallypride. Data were acquired using a Siemens ECAT EXACT HR+ scanner, which has an in-plane resolution full-width at half-maximum (FWHM) of 4.6 mm, axial resolution FWHM of 3.5 mm, and an axial field of view of 15.52 cm in the three-dimensional scanning mode. A transmission scan was conducted using a rotating ⁶⁸Ga/⁶⁸Ge rod source for attenuation correction. Images were reconstructed using the ECAT v7.3 Ordered Subset Expectation Maximization method (6 iterations, 16 subsets) with a Gaussian postreconstruction filter for each 1-min frames.

Participants were placed in the supine position for scanning, with the head positioned and secured with plastic tape to avoid movement during the scan. Emission data were collected for 80 min after the intravenous injection of [¹⁸F]fallypride (5.14 ± 0.30 mCi, specific activity 6.89 ± 5.23 Ci/μmol). Participants were then removed from the scanner for a 20-min break after which they returned to the scanner and were repositioned. Emission data were then collected for a second 80-min period.

Structural MRI scans of the brain were acquired on a 1.5 T Siemens Sonata tomograph for co-registration with PET images and definition of volumes of interest (VOIs; see below for details). A high-resolution, sagittal, T1-weighted, 3-dimensional, volumetric scan was acquired using a whole-brain magnetization-prepared rapid acquisition with gradient echo (MPRAGE) sequence (TR = 1900 ms, TE = 4.38 ms, flip angle = 15, field of view = 256 × 256 × 160, 160 slices, thickness = 1 mm).

PET Data Processing

Image processing and VOIs. Reconstructed PET data consisting of 160 1-min frames were combined into 16 images, each containing data averaged over 10 min. FSL MCFLIRT (FMRIB Centre, Department of Clinical Neurology, University of Oxford, Oxford, UK) was used to correct for head motion (Jenkinson *et al*, 2002). The images were then co-registered to the MPRAGE image using a six-parameter, rigid-body spatial transformation (FSL FLIRT).

The primary question of interest, regarding the potential interaction between sex and smoking status on midbrain BP_{ND}, was addressed using a midline SN-VTA VOI (see Figure 1). Because of its small size and variability in positioning, the SN-VTA VOI was drawn using a data-driven approach according to a published procedure (Zald *et al*, 2010). An average PET image was created using all co-registered 10-min frames of emission data acquired. Regions containing the SN-VTA were hand-drawn on six

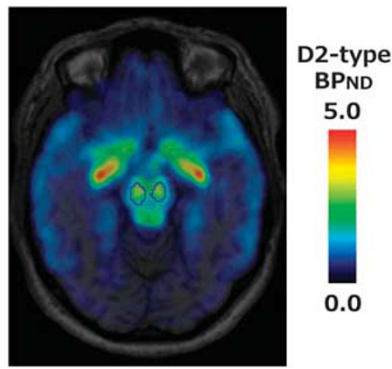


Figure 1 SN-VTA VOI in an individual subject. Volume of interest (VOI) in the midbrain, encompassing the substantia nigra and ventral tegmental area (SN-VTA), is shown with a blue line on the fusion image of the structural MRI and the binding potential (BPND) map of a single subject.

successive *z*-axis slices (1-mm thickness) in MNI152 space. The resulting VOI was transformed to native space using FSL FNIRT. The voxels of this VOI were sequentially selected in order of signal intensity, starting from the voxel with the maximum signal intensity until the volume of selected voxels reached 756 voxels, each of dimensions 1 mm³ to form the final SN-VTA VOI.

Striatal VOIs were also created for modeling of the PET data and for further investigation as described below. VOIs of the caudate nucleus and putamen were derived from individual MPRAGE images using autosegmentation procedures in FSL FIRST (Patenaude *et al*, 2011). The cerebellum, which has a very low level of specific binding for [¹⁸F]fallypride (Mukherjee *et al*, 2002), was used as the reference region. VOIs were manually created for the right and left cerebellar hemispheres in MNI152 space, combined into a single VOI, and transformed into native space with FSL FNIRT.

Modeling of PET data. Time-activity data within VOIs were extracted from motion-corrected, co-registered PET images and imported into PMOD Kinetic Modeling (PKIN) (PMOD Technologies, Zurich, Switzerland). The simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996) was used to estimate *k*2', the rate parameter for the transfer of the radiotracer from the reference-region tissue compartment to the plasma, for the striatal VOIs.

The VOI time-activity curves were refit using the SRTM2 model (Wu and Carson, 2002) and PKIN, holding fixed the *k*2' value derived from the striatal VOIs using SRTM. BPND, an index of receptor availability, was then calculated as $BPND = R1 \cdot k2' / k2a - 1$, where $R1 = K1 / K1'$ is the ratio of tracer-delivery parameters from plasma to tissues in the target region and reference region, and *k*2a is the single-compartment rate parameter for transfer from the target-region tissue compartment to plasma.

Statistical Analyses

Differences in demographic data between groups (nonsmokers, smokers) and in smoking-related measures, including FTND, pack-years, and number of cigarettes per day, between males and females in the smoker group were evaluated by unpaired Student's *t*-tests.

ANCOVA was used to evaluate the main effects of sex and subject group (nonsmokers *vs* smokers), as well as the interaction between sex and group on midbrain BPND. Age was included in the model as a covariate of no interest, chosen beforehand in consideration of evidence for age-related decline in dopamine-receptor density in the brain (Kaasinen *et al*, 2000). After identifying a sex-by-group interaction effect, separate ANCOVAs were performed for each group with sex as the single factor and age as a covariate of no interest.

In order to avoid finding an artefactual correlation owing to variation in tracer dose, correlations of midbrain BPND with caudate nucleus and with putamen BPND were tested controlling for whole-brain BPND, evaluated from all voxels with $BPND \geq 1.0$ (see Supplementary Materials and Methods for the voxel-wise analysis). Relationships of BPND in the midbrain, caudate nucleus, and putamen with smoking-related measures (ie, nicotine dependence, cigarettes per day) were evaluated using partial correlation analysis, controlling for age. In analyses involving pack-years, however, age was not controlled for because it was expected to be collinear with pack-years. Correlation coefficient comparisons were performed using Fisher's *r* to *z* transformation.

These statistical analyses were conducted using SPSS IBM 19 (IBM, Armonk, NY, USA). The criterion for statistical significance was $p < 0.05$, two-tailed. Results are shown as mean \pm SD.

RESULTS

Participant Characteristics

Participant characteristics are shown in Table 1. The groups did not differ in age although there was a trend for the smokers (39.1 ± 9.46 (men), 37.9 ± 8.65 (women)) to be older than the nonsmokers (36.2 ± 8.44 (men), 31.6 ± 9.72 (women)) ($p = 0.13$). Smokers exhibited significantly higher CO levels in expired air than nonsmokers during screening for inclusion in the study ($p < 0.001$), with no sex difference (20.6 ± 10.19 p.p.m. (men), 14.4 ± 9.29 p.p.m. (women); $p = 0.22$). Smokers also showed moderate nicotine dependence as indicated by FTND scores, with no sex difference (4.2 ± 2.23 (men), 3.4 ± 2.07 (women); $p = 0.48$). There were also no sex differences in smoking exposure, as indicated by pack-years (15.2 ± 11.49 (men), 11.7 ± 7.67 (women); $p = 0.49$) and in number of cigarettes smoked per day (14.0 ± 4.63 (men), 12.3 ± 3.04 (women); $p = 0.40$). Thirteen smokers met the criteria for current nicotine dependence, whereas none met criteria for any lifetime dependence on other substances, as defined in DSM-IV. Twenty participants (six male smokers, four female smokers, five male and female nonsmokers) reported having used alcohol in the month before study, endorsing alcohol use on 4.2 ± 3.62 days. Two male smokers reported having used marijuana, each reporting use on only 1 day in the month before study.

Interaction of Sex with Smoking Status on Midbrain Dopamine D2-Type BPND

There was a significant interaction between sex and group (smoker, nonsmoker) on midbrain BPND ($F_{1,32} = 5.089$, $p = 0.03$), but no significant main effects of sex or group (sex: $F_{1,32} = 1.156$, $p = 0.29$; group: $F_{1,32} = 0.962$, $p = 0.33$).

Table 1 Characteristics of Research Participants

	Participant group				Group difference <i>p</i> -Value	Sex difference: nonsmoker/smoker <i>p</i> -Value
	Nonsmoker (n = 19)		Smoker (n = 18)			
	Men (n = 10)	Women (n = 9)	Men (n = 11)	Women (n = 7)		
Age (years)	36.2 ± 8.44	31.6 ± 9.72	39.1 ± 9.46	37.9 ± 8.65	0.13	0.28/0.78
CO in expired air (p.p.m.)	1.80 ± 1.62	1.90 ± 1.54	20.6 ± 10.2	14.4 ± 9.29	<0.001	0.90/0.22
Nicotine dependence (FTND score)	NA	NA	4.2 ± 2.23	3.4 ± 2.07	NA	0.48
Pack-years	NA	NA	15.2 ± 11.5	11.7 ± 7.67	NA	0.49
Number of cigarettes/day	NA	NA	14.0 ± 4.63	12.3 ± 3.04	NA	0.40

Abbreviations: CO, carbon monoxide; FTND, Fagerström Test for Nicotine Dependence.

Values shown are means ± s.d. Differences by group and sex were evaluated using unpaired Student's *t*-test.

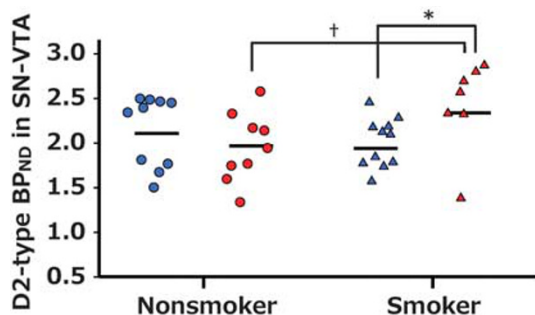


Figure 2 Interaction of sex and smoking status on midbrain D2-type BPND. Plots of individual values of midbrain BPND by sex and group. Horizontal lines show the mean value of each cohort. Interaction effect between sex and smoking status was observed by ANCOVA ($F_{1,32} = 5.089$, $p = 0.03$). *Post-hoc* analyses revealed significantly higher BPND in female than in male smokers ($*p = 0.03$), and a trend for higher BPND in female smokers than in nonsmokers ($†p = 0.09$). BPND values are adjusted for age. Blue circles: male nonsmokers; red circles: female nonsmokers; Blue triangles: male smokers; red triangles: female smokers.

Post-hoc analyses revealed that female smokers had higher midbrain BPND than male smokers (1.74 ± 0.29 (men), 2.18 ± 0.49 (women); $F_{1,15} = 5.482$, $p = 0.03$), but there was no sex difference in midbrain BPND among nonsmokers (1.93 ± 0.38 (men), 1.83 ± 0.42 (women); $F_{1,16} = 0.689$, $p = 0.42$). There also was a trend toward higher midbrain BPND in female smokers than in female nonsmokers ($F_{1,13} = 3.290$, $p = 0.09$), whereas there was no group difference in men ($F_{1,18} = 1.215$, $p = 0.29$) (Figure 2).

Relationship of Midbrain BPND with Nicotine Dependence

Midbrain BPND was negatively correlated with FTND in the sample of all smokers ($r = -0.591$, $p = 0.01$); *post-hoc* analysis revealed that this effect reflected a significant negative correlation of midbrain BPND with FTND in female smokers ($r = -0.989$, $p < 0.001$) but not in male smokers ($r = -0.425$, $p = 0.22$) (Figure 3). The correlation coefficients differed significantly by sex ($z = 3.50$, $p < 0.001$). Other smoking-related measures, such as pack-years ($r = -0.304$, $p = 0.22$)

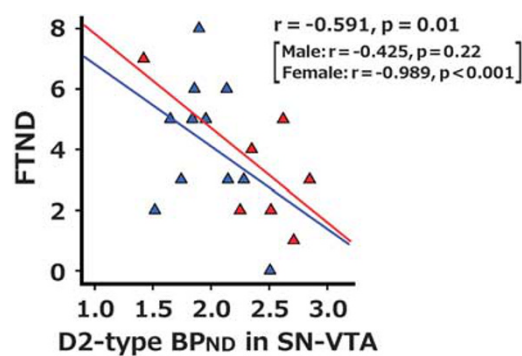


Figure 3 Relationship of midbrain D2-type BPND with nicotine dependence. Midbrain BPND was negatively correlated with FTND in the sample of all smokers ($r = -0.591$, $p = 0.01$). *Post-hoc* analysis in each sex showed significant correlation in female smokers ($r = -0.989$, $p < 0.001$) but not in male smokers ($r = -0.425$, $p = 0.22$). The correlation coefficients differed by sex ($z = 3.50$, $p < 0.001$).

and number of cigarettes smoked per day ($r = -0.284$, $p = 0.27$), were not significantly related to midbrain BPND.

Relationships of Striatal BPND, Midbrain BPND, Nicotine Dependence, and Smoking Exposure

BPND values in both the caudate nucleus and putamen were positively correlated with midbrain BPND in both nonsmokers and smokers of each sex (male nonsmokers: r 's = 0.752 and 0.729; female nonsmokers: r 's = 0.893 and 0.816; female smokers: r 's = 0.776 and 0.813; all p 's < 0.05 uncorrected for multiple comparisons), except for male smokers (r 's = -0.132 and 0.041, p 's > 0.5 uncorrected for multiple comparisons). FTND was significantly negatively correlated with striatal BPND (caudate: $r = -0.556$, $p = 0.02$; putamen: $r = -0.587$, $p = 0.01$). Although correlations between striatal BPND and pack-years did not reach significance in the combined sample (caudate: $r = -0.378$, $p = 0.12$; putamen: $r = -0.414$, $p = 0.09$), the correlations between pack-years and striatal BPND in men (caudate: $r = -0.732$, $p = 0.01$; putamen: $r = -0.727$, $p = 0.01$) but not in women (caudate: $r = -0.186$, $p = 0.69$; putamen: $r = -0.073$, $p = 0.88$) were significant.

DISCUSSION

The central findings of this study concern midbrain dopamine D2-type receptors and their potential role in sex differences in striatal dopamine signaling in smokers and in clinical features of nicotine dependence. A significant interaction between sex and smoking status on midbrain BP_{ND} was observed, reflecting higher values in female smokers than in male smokers, and a trend for midbrain BP_{ND} to be higher in female smokers than in female nonsmokers but not in male smokers.

Previous studies have consistently reported lower D2-type receptor availability in the striata of individuals with substance-use disorders compared with healthy subjects, and animal studies have suggested that chronic drug exposure contributes to impairment in the dopaminergic system (see Trifilieff and Martinez, 2014; Volkow *et al*, 2011 for reviews). Notably, in rats, chronic nicotine administration using an implanted pump for 14 days produces downregulation of D2-receptor density in the basal ganglia, preferentially in the ventral striatum (Janson *et al*, 1992), and counteracts lesion-induced upregulation of D2-receptor density in the striatum. These effects have been attributed to nicotine-induced dopamine release (Janson *et al*, 1994). Another study demonstrated increased D3-receptor density in the shell of the nucleus accumbens but no significant alterations in D2-receptor density owing to nicotine administration for 5 days in naive rats (Le Foll *et al*, 2003). However, these studies differed in the nicotine administration regimens used. Yet another study suggested that at least five administrations of nicotine are required to evoke dopamine release in naive rats (Cadoni and Di Chiara, 2000).

Dopamine D2-type receptors in SN-VTA are predominantly of the D2S isoform and, as such, have autoinhibitory effects on neuronal excitability and thereby on dopamine release in terminal fields (Beckstead *et al*, 2004; Rice and Patel, 2015). Thus greater midbrain BP_{ND} in female smokers could lead to lower smoking-induced dopamine release and therefore less downregulation of striatal D2-type receptors. Lower striatal D2-type BP_{ND} in male but not in female smokers, as reported previously (Brown *et al*, 2012), may then be paired with higher midbrain BP_{ND} in female but not in male smokers. The view is supported by the significant negative correlation of striatal BP_{ND} with smoking exposure in men but not in women. Moreover, a negative modulatory role of midbrain D2-type autoreceptors on striatal dopamine release is consistent with the positive correlation of midbrain BP_{ND} with striatal BP_{ND} in nonsmokers of both sexes and female smokers but not in male smokers. Thus higher midbrain D2-type BP_{ND} in female but not male smokers would limit striatal dopamine release and potentially resultant downregulation of striatal D2-type BP_{ND} owing to chronic nicotine exposure.

Whereas female smokers exhibited higher midbrain BP_{ND} than female nonsmokers, they also showed a negative correlation of midbrain BP_{ND} with nicotine dependence. The latter observation suggests that high densities of midbrain D2 autoreceptors in women may protect against nicotine dependence by preventing downregulation of D2-type receptors in the striatum. Supporting the view that midbrain autoreceptors protect against drug addiction are studies of mice, in which D2-autoreceptor deletion produced hypersensitivity to cocaine (Bello *et al*, 2011), enhanced cocaine use, and increased dopamine release in the nucleus accumbens

(Holroyd *et al*, 2015). Because smoking-induced striatal dopamine release is related to relief of cigarette craving and withdrawal symptoms during abstinence (Brody *et al*, 2004, 2006; Le Foll *et al*, 2014), lower midbrain BP_{ND} and greater associated smoking-induced dopamine release may be associated with greater negative reinforcement from smoking in men than in women. This effect may confer greater nicotine dependence, as supported by the observation that the magnitude of dopamine release following the use of nicotine gum is positively correlated with nicotine dependence in male smokers (Takahashi *et al*, 2008).

This study is not without limitations, one of which is relatively small number of participants, necessitating the replication of these findings in a larger sample to demonstrate its reproducibility. Another limitation is that menstrual cycle phases of the female participants during testing were not recorded, and this uncertainty may have introduced unexplained variance into the measurements. A trend toward higher binding rate constant k_3 to the D2-type receptor in the caudate nucleus in the luteal phase of the menstrual cycle in healthy women (Wong *et al*, 1988) and higher striatal D2-type BP_{ND} during the luteal phase compared with the follicular phase in cynomolgus monkeys (Czoty *et al*, 2009) have been reported. Whereas one study of human subjects indicated lower D2-type BP_{ND} in posterior putamen in the luteal phase than the follicular phase (Munro *et al*, 2006), another showed no change in putamen D2-type receptor binding with menstrual-cycle phase (Nordstrom *et al*, 1998). Although these results are inconsistent, controlling menstrual-cycle phase in studies of D2-type BP_{ND} is recommended for future studies. Furthermore, sex hormones can influence nicotine metabolism, and in general women metabolize nicotine faster than men (Benowitz *et al*, 2006). This difference, along with other factors that may affect nicotine metabolism, may have global effects on nicotine exposure and D2-type BP_{ND}.

Other limitations reflect those associated with the PET measurement. Participants were studied when abstinent from smoking for at least 2 h but were not required to maintain longer abstinence so as to minimize discomfort from abstinence during the PET procedure, which took 180 min. Because the plasma half-life of nicotine after intravenous infusion or cigarette smoking is approximately 2 h (Benowitz *et al*, 2009), residual nicotine was likely to have been present. Smoking promotes prompt striatal dopamine release, when measured using [¹¹C]raclopride within a bolus-plus-infusion paradigm, presumably in part owing to an acute effect of nicotine (Brody *et al*, 2004; Cosgrove *et al*, 2014). However, no difference in BP_{ND} of any brain region was measured using [¹⁸F]fallypride when comparing data obtained 15 min after smoking with data after 24 h of abstinence (Fehr *et al*, 2008). This result may be a consequence of the extended (3-h) duration of a fallypride scan that was initiated after smoking was completed. It suggests that BP_{ND} measured this way using [¹⁸F]fallypride is not sensitive to tonic intrasynaptic dopamine levels as altered by recent smoking and that any potential confound in this study from recent smoking was minimal. That dopamine depletion with α -methyltyrosine has no effect on [¹⁸F]fallypride BP_{ND} is further evidence that [¹⁸F]fallypride BP_{ND} is minimally affected by individual differences in tonic intrasynaptic dopamine level (Cropley *et al*, 2008). Nevertheless, in future studies, recording CO in exhaled air and the time stamp of

smoking, as well as nicotine and related metabolite levels in blood at the time of scanning, is strongly recommended to address this potentially confounding effect.

Evidence that up to 20% of [¹⁸F]fallypride may be bound to D3 dopamine receptors *in vivo* (Mukherjee *et al*, 2015) and that both D2 and D3 autoreceptors are present in the SN-VTA (Gurevich and Joyce, 1999) precludes making definitive statements regarding either receptor subtype. Moreover, autoradiographic demonstration of greater density of D3 than D2 dopamine receptors in the aged human brain (Sun *et al*, 2012) suggests that the greater SN-VTA BPND seen in female smokers may reflect a greater effect on D3 than D2 receptors. Studies using D3-receptor selective agents support the existence of a mechanism for control of dopamine release by D3 autoreceptors (for a review, see Sokoloff *et al*, 2006), although they may have only a minor functional role (Davila *et al*, 2003). Finally, a causal relationship between smoking and group differences in midbrain BPND in females cannot be claimed. To do so would have required PET assay before the participants initiated smoking. Also, future studies might test for sex differences in midbrain BPND and in smoking-induced dopamine release in the same subject sample.

Despite these limitations, the new finding of a sex difference in midbrain D2-type receptors in smokers may help explain some sex differences in smoking behavior. As described above, higher midbrain BPND in female vs male smokers may protect striatal D2-type receptors from the effects of smoking and may be linked to less severe nicotine dependence. Further, this difference may help explain why female smokers derive less benefit from nicotine replacement therapy than male smokers (Perkins and Scott, 2008). It has been suggested that sex differences in nicotine replacement therapy may be due to a nicotine-induced increase in $\beta 2^*$ nicotinic acetylcholine receptor availability in males but not in females, as shown in human as well as rat studies (Cosgrove *et al*, 2012; Koylu *et al*, 1997). We suggest that, aside from differences in the direct action of nicotine at nicotinic acetylcholine receptors, female smokers may not respond as well as males because nicotine-induced dopamine release is limited by a higher density of midbrain D2 autoreceptors. Future investigations are warranted to link the sex differences observed here with sex differences in smoking behavior, in order to provide optimal treatments for smoking cessation.

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