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BRIEF REPORT

CINP

Sex differences in striatal dopamine D_2/D_3 receptor availability in smokers and non-smokers

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Abstract

In previous research, nicotine-dependent men exhibited lower putamen D_2/D_3 dopamine-receptor availability than non-smokers (Fehr *et al.* 2008), but parallel assessments were not performed in women. Women and men (19 light smokers, 18 non-smokers) were tested for differences due to sex and smoking in striatal D_2/D_3 dopamine-receptor availability, using positron emission tomography with [^{18}F]fallypride. Receptor availability was determined using a reference region method, in striatal volumes and in whole-brain, voxel-wise analysis. Significant sex × smoking interactions were observed in the caudate nuclei and putamen. *Post-hoc t* tests showed that male smokers had significantly lower D_2/D_3 dopamine-receptor availability than female smokers (−17% caudate, −21% putamen) and male non-smokers (−15% caudate, −16% putamen). Female smokers did not differ from non-smokers. Whole-brain analysis demonstrated no statistically significant voxels or clusters. These results suggest that low receptor availability may confer vulnerability to nicotine dependence or that smoking selectively affects D_2/D_3 receptor down-regulation in men but not women.

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Introduction

Pre-clinical studies have demonstrated sex differences in the brain after chronic nicotine exposure (Pogun & Yararbas, 2009). For example, after repeated i.v. nicotine administration, sex-related differences in nicotinic acetylcholine receptors (nAChRs) and in the dopamine transporter were observed in rats. Male but not female rats showed increased nAChR density in the cortex and hippocampus (Koylu *et al.* 1997) and female but not male rats showed increased dopamine-transporter density in the nucleus accumbens (Harrod *et al.* 2004). Possibly related is the fact that activation of pre-synaptic nAChRs by nicotine stimulates the release of dopamine from mesolimbic dopamine neurons, an effect that may mediate the rewarding effects of nicotine (Paterson & Nordberg, 2000).

In human studies, women and men differ in several features of nicotine dependence. Women become addicted to nicotine faster, make fewer attempts to quit, remain abstinent for shorter periods, relapse more often than men, are more sensitive to smoking cues, have less success in quitting (Pogun & Yararbas, 2009) and suffer from worse negative mood symptoms and cravings following acute abstinence (Xu *et al.* 2008). Male smokers also have lower dopamine-transporter availability in the striatum when compared to male non-smokers (Yang *et al.* 2008).

Differences between the sexes in the dopamine system may provide insight regarding these sex-related differences associated with smoking. For example, women have a higher dopamine-synthesis rate than men in the striatum (Laakso *et al.* 2002),

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greater dopamine-transporter availability in the striatum and diencephalon (Staley *et al.* 2001) and greater dopamine release in the globus pallidus and cortex following D-amphetamine challenge (Riccardi *et al.* 2008). Previous studies, however, have not examined sex differences in dopamine biomarkers *in vivo* as related to smoking.

Motivated by previously published findings that showed lower dopamine-receptor availability in male smokers when compared to male non-smokers (Fehr *et al.* 2008), we used positron emission tomography (PET) and [^{18}F]fallypride to examine the effects of smoking status on striatal D_2/D_3 dopamine-receptor availability in men and women who were daily smokers and in a comparison non-smoker group.

Method and materials

Participants

This study was approved by the University of California, Los Angeles Office of the Human Research Protection Program. Participants were between the ages of 19 and 50 yr and were recruited from the Greater Los Angeles community using newspaper advertisements. After a thorough explanation of the study, the participants provided written informed consent and then completed a Structured Clinical Interview for DSM-IV, a medical history and a physical examination. Laboratory tests on blood and urine samples were performed to screen for pregnancy and other factors related to eligibility. Participants were excluded for any history of an Axis I psychiatric diagnosis (other than nicotine dependence in smokers) and for current use of any medication or a current or past medical condition that could affect the central nervous system. Nineteen individuals who smoked 4–14 cigarettes/d [10 men 36.6 (8.2); nine women 36.4 (8.6)] and 18 age-matched non-smokers [nine men 37.0 (8.6); nine women 31.6 (9.7)] completed the study. Values are given as mean age (S.D.). All of the 18 female participants were premenopausal and five of them reported using oral contraceptives.

PET scanning

[^{18}F]Fallypride (a high-affinity D_2/D_3 dopamine-receptor radioligand) was synthesized in the Cyclotron Facility of the Greater Los Angeles Veterans Affairs Healthcare System as previously reported (Mukherjee *et al.* 1999; Riccardi *et al.* 2008) and was used with PET to measure D_2/D_3 dopamine-receptor availability in the caudate nucleus and putamen of participants in the two groups. Each smoker was studied about 1 h after he or she smoked a cigarette.

Scans were acquired on an EXACT HR+PET Scanner (Siemens, Germany) in 3D mode with an in-plane resolution full-width at half-maximum (FWHM) 4.6 mm, axial resolution FWHM of 3.5 mm and an axial field of view of 15.52 cm. The participants were placed in the supine position with the brain centred in the transaxial field of view. A 7-min transmission scan was acquired before each emission scanning session using a rotating ^{68}Ge/^{68}Ga rod source to measure and correct for attenuation. PET dynamic data acquisition was initiated with a bolus injection of the radiotracer ($\sim$5 mCi$\pm$5%, specific activity $\geq$1 Ci/μmol) and emission data were acquired in two 80-min scanning sessions, separated by a 20-min break. The specific activities for each group were as follows: males (non-smokers 3.63$\pm$3.46 Ci/μmol; smokers 8.69$\pm$5.94 Ci/μmol); females (non-smokers 5.08$\pm$3.65 Ci/μmol; smokers 5.75$\pm$4.36 Ci/μmol).

PET image processing

Data were reconstructed using ECAT v7.3 Ordered Subset Expectation Maximization (OSEM; Hudson & Larkin, 1994), chosen for its superior resolution and noise properties compared to filtered back projection. Reconstructed PET images were combined into 16 images, each representing an average of 10-min dynamic frames of data. These 16 images were motion-corrected using FSL McFLIRT (Jenkinson *et al.* 2002). After motion-correction, PET images were co-registered to each participant's structural magnetic resonance imaging (MRI) using a six-parameter rigid-body linear transformation computed using the Automated Registration Tool (ART) software package (Ardekani *et al.* 1995).

Binding potential (BP_{ND}) determinations were made in the PMOD kinetic-analysis program (version 3.1; PMOD Technologies Ltd, Switzerland) using the SRTM2 model (Wu & Carson, 2002). We first estimated k_2', the rate parameter for transfer of the tracer from the reference tissue to the plasma. The caudate nucleus and putamen were defined as volumes of interest (VOIs) using an automated segmentation program (FSL FIRST; Oxford University, UK) that provides a 3D binary mask for these regions in native space (Fig. 1). Reference VOIs were drawn on the cerebellar hemispheres (left and right, excluding the vermis); the cerebellum was used as the reference tissue due to its low D_2/D_3 dopamine-receptor availability relative to the striatum (Mukherjee *et al.* 2002).

Time-activity curves for these VOIs (and for every image voxel) were extracted from motion-corrected, co-registered PET images within PMOD. These

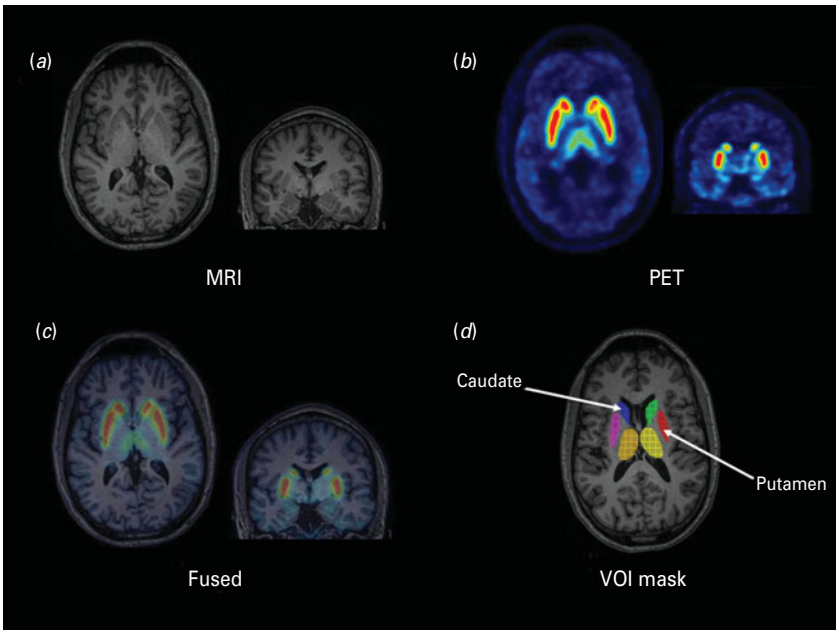

Fig. 1. Corresponding magnetic resonance imaging (MRI) and positron emission tomography (PET) scans together with the fused image (co-registered PET to MRI). The volume-of-interest (VOI) mask, created in FSL FIRST, shows caudate and putamen.

were fit using the simplified reference tissue model (SRTM; Lammertsma & Hume, 1996) to provide a whole-brain estimate of k_2', taken as a volume-weighted average, which was used for both the VOI and voxel-wise analyses. The VOI and voxel-wise time-activity curves were fit identically using the SRTM2 model (Wu & Carson, 2002) holding k_2' fixed, in PKIN and PXMOD respectively, yielding striatal BP_{ND} measurements and voxel-wise maps.

BP_{ND} was calculated as $BP_{ND} = R_1 * k_2'/k_{2a} - 1$, where $R_1 = K_1/K_1'$, the ratio of tracer-delivery parameters for the tissue of interest and reference tissue, and k_{2a} is the effective rate parameter for transfer of tracer from the tissue of interest to the plasma. The voxel-wise maps were registered into standard space (i.e. Montreal Neurological Institute avg152 template) using non-linear registration procedures (Ardekani *et al.* 2005) prior to statistical analysis using Statistical Parametric Mapping 8.

MRI scanning

Each participant received a 1.5T T1-weighted 3D volumetric MRI scan (Siemens Sonata) for co-registration with the PET scan to identify the VOIs. A whole-brain magnetization prepared rapid gradient echo (MPRAGE) sequence was used with the following parameters: slice thickness = 1 mm; 160 slices, TR = 1900 ms; TE = 4.38 ms; flip angle = 15; field of view = 256 mm.

Clinical measures

Participants were administered the Fagerström Test of Nicotine Dependence [FTND (smokers only); Heatherton *et al.* 1991], the State-Trait Anxiety Inventory (STAI; Spielberger & Vagg, 1984) and the Beck Depression Inventory-II (BDI; Steer *et al.* 1999).

Data analysis

Group differences in age, education and clinical measures were tested by independent-samples *t* test. Multivariate analysis of covariance (MANCOVA), using age as a covariate, was used to assess the effects of sex, smoking status and the interaction between them on mean BP_{ND} in the caudate and putamen. *Post-hoc* two-way analyses of variance and *t* tests were performed separately to assess sex differences in BP_{ND} in the smoker and non-smoker groups.

Results

There were no significant differences in age, education or scores on the BDI or STAI among the four groups (male and female smokers and non-smokers; Supplementary Table S1). There were no significant differences in specific activity, injected mass or fallypride dose among the four groups. Among the smokers, there were no significant sex differences in exposure to smoking, measured in pack years [values

Table 1. (*a*) Two-way multivariate analysis of covariance (MANCOVA) showed a significant sex × smoking status interaction and two-way analyses of covariance (ANCOVAs) for data of both the caudate and putamen showed significant sex × smoking status interactions; (*b*) *t* tests showed significant effects of sex in the smoking group and smoking status in male smokers and non-smokers, in both caudate and putamen

(*a*)

	Non-smokers (mean±S.D.)		Smokers (mean±S.D.)	
Region	F ($n=9$)	M ($n=9$)	F ($n=9$)	M ($n=10$)
Caudate	17.7±3.6	18.0±3.3	18.3±3.3	14.9±1.9
Putamen	20.9±4.0	21.3±3.4	22.8±3.6	18.0±2.6
	F	*p*		
MANCOVA (Wilks' λ)				
Sex	2.36	0.11		
Smoking status	2.62	0.09		
Sex × smoking interaction	4.24	**0.02**		
2-way ANCOVA				
Putamen				
Sex	2.84	0.1		
Smoking status	0.01	0.94		
Sex × smoking interaction	7.93	**0.01**		
Caudate				
Sex	1.41	0.24		
Smoking status	0.59	0.45		
Sex × smoking interaction	5.86	**0.02**		

(*b*)

t tests	*t*	*p*
Non-smokers M/F		
Caudate	−0.18	0.86
Putamen	−0.2	0.84
Smokers M/F		
Caudate	2.86	**0.01**
Putamen	3.39	**0**
Smokers *vs.* non-smokers F/F		
Caudate	−0.39	0.7
Putamen	−1.05	0.31
Smokers *vs.* non-smokers M/M		
Caudate	2.59	**0.02**
Putamen	2.33	**0.03**

M, Male; F, female.
Bold values meet statistical significance of $p < 0.05$.

are mean (S.D.)] [men: 240 (135); women: 250 (120)], nicotine dependence, measured on the FTND [men: 3.3 (1.5); women: 3.3 (1.5)]; and cigarettes smoked per day [men: 13 (7.4); women: 13 (6.8)].

A two-way MANCOVA (using age as a covariate) showed a significant sex × smoking interaction (Table 1*a*). Separate two-way ANCOVAs for data from the caudate and putamen VOIs showed significant sex × smoking interactions in both regions. There were no significant main effects of smoking status or sex. *Post-hoc* tests showed significant differences, with male smokers having significantly lower D_2/D_3 dopamine-receptor availability than female smokers (−17 and −21%, caudate and putamen, respectively) and male non-smokers (−15 and −16%) (Table 1*a*, *b*). Female smokers did not differ significantly from non-smokers. Among non-smokers, women and men did not differ significantly in D_2/D_3 dopamine-receptor availability.

Voxel-wise analysis on BP_{ND} maps showed clusters in the left temporal lobe for the contrast male non-smokers > male smokers. The analysis indicated significance at the set level (clusters = 11; $p = 0.002$), indicating that the number of clusters in the entire brain that passed the threshold of extent (> 33.05 contiguous voxels that exceeded an uncorrected height threshold of $p < 0.01$) exceeded the mean number (3.95) expected to occur by chance. Neither an individual voxel nor an individual cluster was significant.

Discussion

This study investigated sex differences and D_2/D_3 receptor availability in light smokers and non-smokers using PET and [^{18}F]fallypride. Men who are moderately nicotine-dependent, as determined by FTND scores, had significantly lower D_2/D_3 dopamine-receptor availability in the caudate and putamen when compared to female smokers and male non-smokers. To our knowledge, this is the first report of sex differences in D_2/D_3 dopamine-receptor availability in smokers.

Sex differences have been observed in the dopamine dynamics of healthy volunteers, with women having a higher dopamine synthesis rate in the striatum (Laakso *et al.* 2002) and significantly greater dopamine release in the cortex following an amphetamine challenge, when compared to men (Riccardi *et al.* 2008). Our findings suggest that these sex-related differences in dopamine-system function may mediate the response of the brain to nicotine. As women generally have higher striatal dopamine-transporter availability than men (Staley *et al.* 2001), the absence of evidence for lower D_2/D_3 dopamine-receptor availability associated with smoking in women may reflect more rapid removal of dopamine from the synapse in female than male smokers. However, as nicotine and cotinine metabolism is faster in women than in men (Benowitz *et al.* 2006), sex-related differences in nicotine clearance could affect the smoking × dopamine system interaction.

As reported in a previous study (Fehr *et al.* 2008), male smokers in our study had significantly lower D_2/D_3 dopamine-receptor availability in the striatum than male non-smokers. This finding may reflect down-regulation of D_2/D_3 dopamine receptors in male smokers as a result of chronic nicotine exposure. Our findings do not rule out a difference in D_2/D_3 dopamine-receptor availability that may be present in male smokers before the initiation of smoking. The results may reflect a greater vulnerability of men with lower striatal D_2/D_3 dopamine-receptor availability to become nicotine dependent. Our findings in the left temporal lobe were weak and may serve as a planned comparison for a future study.

Some limitations of the study should be considered. We included only light to moderate smokers who smoked 4–14 cigarettes/d, whereas many of the previous published studies examined males who were highly nicotine dependent and smoked >15 cigarettes/d. The study did not allow differentiation between differences in D_2/D_3 dopamine-receptor density or in intrasynaptic dopamine concentration as underlying factors for the significant differences we found in D_2/D_3 dopamine-receptor availability between the groups. Our sample sizes were small, which limited our ability to find significant differences in D_2/D_3 dopamine-receptor availability in extrastriatal regions between the groups, as was previously shown (Kobiella *et al.* 2010). We also did not control for phases of the menstrual cycle in our female participants. A previous study reported a 12% increase in D_2/D_3 dopamine-receptor availability in the caudate nucleus and putamen during the luteal phase of the menstrual cycle than in the follicular phase in cynomolgus monkeys, demonstrating the need to control for menstrual phase-related variations on PET measures (Czoty *et al.* 2009). Additional studies are, therefore, warranted to explore this finding of sex-related differences in dopamine receptors in smokers.

Note

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/pnp).

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Statement of Interest

Dr Edythe London has tobacco-related research other than that reported here, supported by a contract between Philip Morris USA and UCLA. The authors have no other potential conflicts of interest.

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