

Sex Differences in Availability of β_2^* -Nicotinic Acetylcholine Receptors in Recently Abstinent Tobacco Smokers

Kelly P. Cosgrove, PhD; Irina Esterlis, PhD; Sherry A. McKee, PhD; Frederic Bois, PhD; John P. Seibyl, MD; Carolyn M. Mazure, PhD; Suchitra Krishnan-Sarin, PhD; Julie K. Staley, PhD†; Marina R. Picciotto, PhD; Stephanie S. O'Malley, PhD

Context: Sex differences exist in the reinforcing effects of nicotine, smoking cessation rates, and response to nicotine therapies. Sex differences in availability of nicotinic acetylcholine receptors containing the β_2 subunit (β_2^* -nAChRs) may underlie differential nicotine and tobacco smoking effects and related behaviors in women vs men.

Objectives: To examine β_2^* -nAChR availability in male and female smokers vs nonsmokers and to determine associations among β_2^* -nAChR availability, tobacco smoking characteristics, and female sex steroid hormone levels.

Design: Male (n=26) and female (n=28) tobacco smokers participated in an iodide 123-labeled 5-iodo-A-85380 ($[^{123}\text{I}]5\text{-IA}$) single-photon emission computed tomography (SPECT) imaging session at 7 to 9 days of abstinence. Age-matched male (n=26) and female (n=30) nonsmokers participated in a $[^{123}\text{I}]5\text{-IA}$ SPECT imaging session. All participants completed a magnetic resonance imaging study.

Setting: Academic imaging center.

Participants: Tobacco smokers (n=54) and age- and sex-matched nonsmokers (n=56).

Main Outcome Measure: The $[^{123}\text{I}]5\text{-IA}$ SPECT images were converted to equilibrium distribution volumes and were analyzed using regions of interest.

Results: The β_2^* -nAChR availability was significantly higher in male smokers compared with male nonsmokers in striatum, cortex, and cerebellum, but female smokers did not have higher β_2^* -nAChR availability than female nonsmokers in any region. In women, β_2^* -nAChR availability in the cortex and cerebellum was negatively and significantly correlated with progesterone level on the SPECT imaging day. In female smokers on imaging day, the progesterone level was positively and significantly correlated with depressive symptoms, craving for a cigarette, and nicotine withdrawal.

Conclusions: The regulatory effects of nicotine in the brain (ie, tobacco smoking-induced upregulation of β_2^* -nAChRs) seem to be distinctly different between men and women, and female sex steroid hormones likely have a role in this regulation. These findings suggest an underlying neurochemical mechanism for the reported behavioral sex differences. To treat female smokers more effectively, it is critical that nonnicotinic-mediated medications should be explored.

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Author Affiliations:

Department of Psychiatry, Yale University School of Medicine (Drs Cosgrove, Esterlis, McKee, Bois, Mazure, Krishnan-Sarin, Staley, Picciotto, and O'Malley), and Institute for Neurodegenerative Disorders (Dr Seibyl), New Haven, and Department of Psychiatry, Veterans Affairs Connecticut Healthcare System, West Haven (Drs Cosgrove, Esterlis, Bois, and Staley).
†Deceased.

NICOTINE THERAPIES (NTs) (eg, nicotine patch, gum, lozenge, and inhaler, as well as the nicotinic partial agonist therapy varenicline tartrate) are the most widely used treatments to help individuals quit smoking. Men respond better to NT than women,¹ and women often have a more difficult time quitting smoking than men.²⁻⁶ Perkins⁷ demonstrated that men are better able than women to identify nicotine in a nasal spray and to discriminate nicotine from placebo, indicating that men are better able to detect the interoceptive cues of nicotine. Consistently, women experience greater craving relief than men from

a denicotinized tobacco inhaler,⁸ highlighting the role sensory cues have in nicotine dependence for women. These results are consistent with findings showing that women are more reinforced than men by the nonnicotine-conditioned stimuli that are strongly associated with smoking, whereas men are more reinforced than women by the nicotine per se in cigarettes and in NT.⁹

Results of preclinical investigations generally support the clinical findings showing sex differences in response to nicotine, suggesting an underlying biological sexual dimorphism. For example, female mice responded more to the locomotor stimulating^{10,11} and conditioned reward-

Table 1. Demographics and Tobacco Smoking Characteristics of Male and Female Nonsmokers and Smokers

Characteristic	Men, Mean (SD)		Women, Mean (SD)	
	Nonsmokers (n = 26)	Smokers (n = 26)	Nonsmokers (n = 30)	Smokers (n = 28)
Age, y	34.8 (11.7)	33.8 (11.7)	31.3 (11.7)	36.0 (10.0)
Fagerström Test for Nicotine Dependence score	...	4.7 (2.3)	...	5.8 (2.0)
Cigarettes per day, No.	...	16.2 (5.5)	...	18.4 (5.9)
Years smoked	...	13.6 (8.0)	...	17.4 (10.5)
Level at intake				
Cotinine, ng/mL	<15	220.7 (163.0)	<15	223.0 (132.2)
Nicotine, mg/L	<0.004	0.0107 (6.4)	<0.004	0.0118 (7.9)
Carbon monoxide, ppm	3.2 (2.5)	20.9 (10.0)	2.5 (2.8)	23.1 (8.6)
Level on imaging day				
Cotinine, ng/mL	<15	22.8 (31.1)	<15	31.8 (64.0)
Nicotine, mg/L	<0.004	<0.004	<0.004	<0.004
Carbon monoxide, ppm	1.6 (1.9)	3.4 (2.2)	2.3 (2.4)	2.8 (1.6)

SI conversion factors: To convert cotinine level to nanomoles per liter, multiply by 5.675; to convert nicotine level to micromoles per liter, multiply by 6.164.

ing properties of nicotine,¹² whereas male mice responded more to pharmacological properties of nicotine by titrating their consumption to available nicotine dose.¹² The molecular mechanisms underlying these sex differences are not understood, although it is clear that behaviors, as well as the primary reinforcing effects of nicotine, are mediated by nicotinic acetylcholine receptors containing the β_2 subunit (β_2^* -nAChRs).¹³⁻¹⁶

The primary reinforcing effects of nicotine are mediated by the β_2^* -nAChR. These receptors have a high affinity for nicotine and nicotinic agonists and have been consistently shown in preclinical^{17,18} and postmortem¹⁹ human studies to upregulate (ie, increase in number) in response to nicotine. We measure β_2^* -nAChR availability using the radiotracer iodide 123-labeled 5-iodo-A-85380 (¹²³I]5-IA) and single-photon emission computed tomography (SPECT). This tracer is a high-affinity nicotinic agonist radioligand that binds to the nicotine-binding site on β_2^* -nAChRs,²⁰ with excellent test-retest reproducibility.²¹ Higher β_2^* -nAChR availability has been demonstrated in smokers compared with nonsmokers using SPECT and positron emission tomography brain imaging,²²⁻²⁴ which likely reflects tobacco smoking-induced upregulation of β_2^* -nAChRs. Sex differences in β_2^* -nAChR number have also been examined. Results of preclinical studies in nicotine-naive rodents are conflicting, with one study²⁵ demonstrating sex differences in β_2^* -nAChR number and another study²⁶ showing no sex difference in β_2^* -nAChR number. Our group previously found no differences in β_2^* -nAChR availability between male and female nonsmokers.²⁷ Preclinical studies^{25,28} report greater nicotine-induced upregulation of nAChRs in male vs female rodents compared with each group's same-sex controls; however, this has not been examined in humans to date. Understanding sex differences in the underlying neurochemistry involved in tobacco smoking dependence may pave the way for development of novel treatment medications.

In the present study, we hypothesized that sex differences in β_2^* -nAChR availability may underlie differential nicotine and tobacco smoking effects and related behaviors in women vs men. The primary objective was to

examine sex differences in β_2^* -nAChR availability in smokers compared with nonsmokers. A secondary goal of the study was to determine associations among β_2^* -nAChR availability and behavioral and physiological variables, such as tobacco smoking characteristics (craving and withdrawal, as well as depression) and sex steroid hormone levels (estradiol and progesterone in women), in smokers and nonsmokers.

METHODS

PARTICIPANTS

Participating in a [¹²³I]5-IA SPECT imaging session and a magnetic resonance imaging study were the following 2 groups of men and women (**Table 1**): (1) Fifty-two men comprised 26 nonsmokers (age range, 21-58 years) and 26 smokers (age range, 18-57 years). (2) Fifty-eight women comprised 30 nonsmokers (age range, 19-56 years) and 28 smokers (age range, 18-50 years). Smokers underwent imaging at 7 to 9 days of abstinence based on previous studies^{22,23} demonstrating that 7 to 9 days of abstinence are necessary for nicotine to clear from the brain so that nicotine will not compete with the radiotracer at the binding site.

This study was approved by the Yale University School of Medicine Human Investigation Committee, the West Haven Veterans Administration Human Subjects Subcommittee, and the Radiation Safety Committee, Yale University. Participants provided written informed consent and were recruited by word of mouth, posters, and television and newspaper advertisements. Eligibility was determined by a medical examination, including a physical examination, electrocardiogram, serum chemistries, thyroid function studies, complete blood cell count, urinalysis, and urine toxicological screening. Participants had no history of significant medical illness or major head trauma. The Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders* was administered to rule out Axis I disorders except for nicotine dependence in tobacco smokers.

SMOKING ASSESSMENTS

Tobacco smokers had to have smoked 10 or more cigarettes daily for at least 1 year, confirmed by plasma cotinine levels

exceeding 150 ng/mL, urine cotinine levels exceeding 100 ng/mL, and carbon monoxide levels exceeding 11 ppm at intake (to convert cotinine level to nanomoles per liter, multiply by 5.675). Smokers were helped to quit smoking using clinical practice guidelines and contingency management^{29,31} as described previously.²³ Participants were not permitted to use any NT or medication throughout the study. Nonsmoker status (defined as <100 lifetime cigarettes and none in the previous 2 years) was confirmed by plasma cotinine levels of less than 15 ng/mL, urine cotinine levels of less than 100 ng/mL, and carbon monoxide levels of less than 8 ppm at intake and on SPECT imaging day. On imaging day, abstinent smokers had plasma cotinine levels of less than 15 ng/mL, urine cotinine levels of less than 100 ng/mL, and carbon monoxide levels of less than 8 ppm. Plasma nicotine and cotinine levels were measured as previously described.²² Urine cotinine levels were measured using cotinine test strips (Accutest NicoMeter [Jant Pharmacal] or NicAlert [Nymox Pharmaceutical]). Women had a negative pregnancy test result during screening and before radiotracer injection on imaging day. Menstrual cycle phase was not controlled, and hormonal contraception was not exclusionary. Blood samples were obtained on imaging day to determine sex steroid hormone levels, including estradiol and progesterone.

QUESTIONNAIRES

In smokers, nicotine dependence severity was assessed at intake using the Fagerström Test for Nicotine Dependence,³² and craving and nicotine withdrawal symptoms were assessed using the Urge to Smoke Questionnaire (QSU)³³ and the Minnesota Nicotine Withdrawal Scale (MNWS),³⁴ respectively, at intake and on imaging day. The QSU yields 2 factors, the intent or desire to smoke (QSU-Intent) and the relief of negative affect and withdrawal (QSU-Relief). In smokers and nonsmokers, symptoms of depression were assessed at intake and on imaging day using the Beck Depression Inventory (BDI).

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging studies were obtained using a 1.5-T camera (Siemens Sonata) in a standard orientation (echo time of 5-7 milliseconds, repeat time of 24 milliseconds, 256 × 192-pixel matrix, 1 excitation, 30-cm field of view, and 124 contiguous sections with 1.2-mm thickness). The data were used for coregistration to the SPECT images.

[¹²³I]5-IA SPECT IMAGING

Participants received a 0.6-g saturated solution of potassium iodide to protect their thyroid from possible exposure to radioactive iodide before radiotracer administration. The [¹²³I]5-IA was synthesized as previously described³⁵ and was administered as a bolus to constant infusion at a ratio of 7.0 for 8 hours. Participants were injected with equivalent doses of a bolus (mean [SD], 147 [25] MBq) and constant infusion (mean [SD], 25 [5] MBq/h). Three consecutive 30-minute emission images and one 15-minute simultaneous transmission and emission image were obtained between hours 6 and 8 of the [¹²³I]5-IA infusion using a SPECT camera (PRISM 3000 XP; Picker). The axial resolution (full width at half maximum) was 12.2 mm, measured using a [¹²³I] line source in water in a cylindrical phantom. Blood samples were obtained before injection and at the beginning and end of the emission imaging sessions for analysis of plasma total parent and free fraction of parent tracer in plasma (free fraction). The chemical fate of [¹²³I]5-IA after injection was assessed in plasma as previously described.³⁵

IMAGE ANALYSIS AND OUTCOME MEASURES

Images were reconstructed and analyzed as previously described, including a nonuniform attenuation correction.²¹ Using commercially available software (MEDx, version 3.4; Medical Numerics, Inc), magnetic resonance images were coregistered to the SPECT images to provide an anatomical guide for placement of the regions of interest. Regions of interest were those known to express β_2^* -nAChRs and included frontal, parietal, anterior cingulate, temporal, and occipital cortices, as well as thalamus, striatum (mean of the caudate and putamen), and cerebellum.

The outcome measure V_T/f_P (regional activity divided by free plasma parent between 6 and 8 hours) was used to correct for possible differences in radiotracer metabolism or plasma protein binding between groups and participants. Specifically, V_T/f_P equals [¹²³I]5-IA uptake in a region of interest divided by free plasma parent (both in kilobecquerels per milliliter).³⁶ We refer to V_T/f_P as β_2^* -nAChR availability because we are measuring receptors that are available to be bound by radiotracer. Receptors that are already occupied (eg, by residual nicotine, a pharmacologically active metabolite [cotinine or nornicotine], or by endogenous neurotransmitter [acetylcholine]) are not available. The V_T/f_P value is proportional to the binding potential in milliliters per gram (B_{max}/K_D), which is proportional to the receptor number (B_{max}) at equilibrium, given the assumptions that there is no change in affinity (K_D) and that nondisplaceable (nonspecific and free) uptake does not differ between participants. As described previously,²¹ there is no appropriate reference region for this radiotracer because of the wide expression of β_2^* -nAChRs in the brain.

STATISTICAL ANALYSIS

Statistical analyses were conducted using commercially available software (SPSS, version 16.0; SPSS, Inc). Independent *t* test was used to examine demographic variables between groups. Multivariate analysis of variance models were first used to examine differences in β_2^* -nAChR availability (V_T/f_P) by sex and smoking status in 4 brain regions (the mean cortex [mean of frontal, parietal, anterior cingulate, temporal, and occipital cortices], thalamus, striatum, and cerebellum). The cortical regions were averaged together because of the high intercorrelations between these regions. Based on the previous finding that β_2^* -nAChR availability decreases with age,³⁷ we initially used age as a covariate, but because there was no effect on β_2^* -nAChR availability, age was dropped from further analysis. To further examine differences between male and female smokers vs male and female nonsmokers (4 groups), analyses of variance were conducted for each of the 8 brain regions. Using Pearson product moment correlation coefficients, correlational analyses within each group were assessed for associations among the following: β_2^* -nAChR availability, depression (BDI score) at intake and on imaging day, female sex steroid hormone (estradiol and progesterone) levels on imaging day, and tobacco smoking characteristics (years smoked, cigarettes per day, MNWS score, QSU-Intent and QSU-Relief scores, and Fagerström Test for Nicotine Dependence score). Because of multiple comparisons, statistical significance for the correlations was set at $P \leq .01$.

RESULTS

CLINICAL POPULATION

Fifty-two men (26 nonsmokers and 26 smokers) and 58 age-matched women (30 nonsmokers and 28 smokers) participated in the study. Included in this sample are par-

Table 2. Clinical Characteristics of Male and Female Tobacco Smokers at Intake and at 7 to 9 Days of Abstinence

Measure	Score at Intake, Mean (SD)		Score at 7-9 d of Abstinence, Mean (SD)	
	Men	Women	Men	Women
Minnesota Nicotine Withdrawal Scale	6.0 (5.4)	6.5 (7.3)	3.5 (4.2)	5.0 (5.1)
Urge to Smoke Questionnaire				
Intent	8.9 (3.6)	9.6 (3.1)	6.6 (4.3) ^a	6.8 (4.3) ^a
Relief	7.0 (4.4)	8.3 (5.6)	5.9 (4.4)	6.0 (4.3) ^a
Beck Depression Inventory	3.9 (4.1)	4.8 (5.4)	2.9 (3.8)	4.0 (4.5)

^aSignificantly different from score at intake ($P < .05$).

Table 3. Availability of Nicotinic Acetylcholine Receptors Containing the β_2 Subunit Throughout the Brain in Male and Female Nonsmokers and Tobacco Smokers^a

Region	Men, Mean (SD)				Women, Mean (SD)			
	Nonsmoker V_T/f_p	Smoker V_T/f_p	% Difference	P Value	Nonsmoker V_T/f_p	Smoker V_T/f_p	% Difference	P Value
Thalamus	120.8 (38.2)	132.5 (33.1)	12	.20	141.6 (33.0)	124.5 (29.0)	-17	.04
Striatum	62.0 (22.4)	78.4 (21.4)	16	.007	71.9 (9.6)	69.6 (21.0)	-2	.43
Parietal cortex	44.3 (15.0)	57.3 (15.1)	14	.004	51.2 (11.8)	54.5 (15.7)	1	.70
Frontal cortex	48.9 (16.6)	62.5 (17.8)	13	.009	57.0 (12.8)	58.2 (17.7)	3	.77
Anterior cingulate	50.4 (14.1)	65.7 (18.2)	15	.002	55.7 (11.5)	61.4 (15.2)	6	.24
Temporal cortex	55.1 (16.2)	69.2 (17.2)	14	.004	60.1 (12.4)	64.1 (14.7)	4	.50
Occipital cortex	48.8 (14.7)	65.4 (16.2)	17	<.001	53.4 (11.3)	61.1 (15.2)	8	.10
Cerebellum	59.1 (16.7)	75.7 (18.9)	17	.001	64.8 (14.2)	66.0 (18.2)	1	.09

Abbreviation: V_T/f_p , iodide 123-labeled 5-iodo-A-85380 uptake in a region of interest divided by free plasma parent (both in kilobecquerels per milliliter).

^aPercentage difference from nonsmokers is calculated as follows: $([\text{Smoker} - \text{Nonsmoker}] / \text{Nonsmoker}) \times 100$. $P < .006$ after Bonferroni correction is considered significant.

Participants (8 male and 7 female smokers and 10 male and 11 female nonsmokers) from 2 smaller previous studies.^{22,23} Male and female smokers were not matched for smoking characteristics. Plasma cotinine and nicotine and carbon monoxide levels were negligible in nonsmokers and smokers on imaging day, confirming abstinence from smoking, and there were no significant differences between men and women (Table 1). One female smoker had a cotinine level of 328 ng/mL on imaging day, which is responsible for the higher cotinine level in female vs male smokers; however, this was a decrease in the cotinine level from intake, and her nicotine and carbon monoxide levels were also reflective of abstinence from tobacco smoking. In addition, this participant's β_2^* -nAChR levels were within the range of those of other female smokers, so she was not excluded from the study. Male and female smokers did not differ significantly in depressive symptoms (BDI scores), craving for a cigarette (QSU-Intent and QSU-Relief scores), or nicotine withdrawal (MNWS scores) at intake or on imaging day, but there were sex differences between intake and 1 week of abstinence (Table 2). Specifically, scores for men on the QSU-Intent ($P < .05$) and scores for women on the QSU-Intent ($P < .05$) and QSU-Relief ($P < .01$) were significantly lower at 1 week of abstinence compared with intake.

β_2^* -nAChR AVAILABILITY DIFFERENCES BY SEX AND SMOKING STATUS

There were no differences among study groups in injected dose of [¹²³I]5-IA, the ratio of bolus to infusion,

or concentrations of [¹²³I]5-IA activity in the blood. Using multivariate analysis of variance, regional β_2^* -nAChR availability was compared between smokers and nonsmokers as a function of sex (Table 3, Figure 1, and Figure 2). This analysis demonstrated significant omnibus test results for a main effect of smoking status ($F_{4,102} = 60.24$, $P < .001$) and a sex \times smoking status interaction ($F_{4,102} = 2.48$, $P < .05$). Male smokers had significantly higher β_2^* -nAChR availability than male nonsmokers in the parietal cortex ($F_{1,51} = 9.33$, $P = .004$), frontal cortex ($F_{1,51} = 7.48$, $P = .009$), anterior cingulate cortex ($F_{1,51} = 11.01$, $P = .002$), occipital cortex ($F_{1,51} = 15.09$, $P < .001$), temporal cortex ($F_{1,51} = 9.26$, $P = .004$), cerebellum ($F_{1,51} = 12.03$, $P = .001$), and striatum ($F_{1,51} = 7.96$, $P = .007$), but there were no differences in the thalamus ($F_{1,51} = 1.66$, $P = .20$), consistent with a previous study.²² The β_2^* -nAChR availability between female smokers and nonsmokers did not differ significantly in the parietal cortex ($F_{1,57} = 0.15$, $P = .70$), frontal cortex ($F_{1,57} = 0.09$, $P = .77$), anterior cingulate cortex ($F_{1,57} = 1.40$, $P = .24$), occipital cortex ($F_{1,57} = 2.75$, $P = .10$), temporal cortex ($F_{1,57} = 0.47$, $P = .50$), cerebellum ($F_{1,57} = 2.98$, $P = .09$), or striatum ($F_{1,57} = 0.64$, $P = .43$), but female nonsmokers paradoxically had higher β_2^* -nAChR availability than female smokers in the thalamus ($F_{1,57} = 4.37$, $P = .04$) (Table 3, Figure 1, and Figure 2). The β_2^* -nAChR availability in male smokers did not differ from that in female smokers in any region. Finally, there was a trend toward higher β_2^* -nAChR availability in female vs male nonsmokers across regions as follows: parietal cortex ($F_{1,55} = 5.74$, $P = .02$), frontal cortex ($F_{1,55} = 5.74$, $P = .02$), anterior cingulate cor-

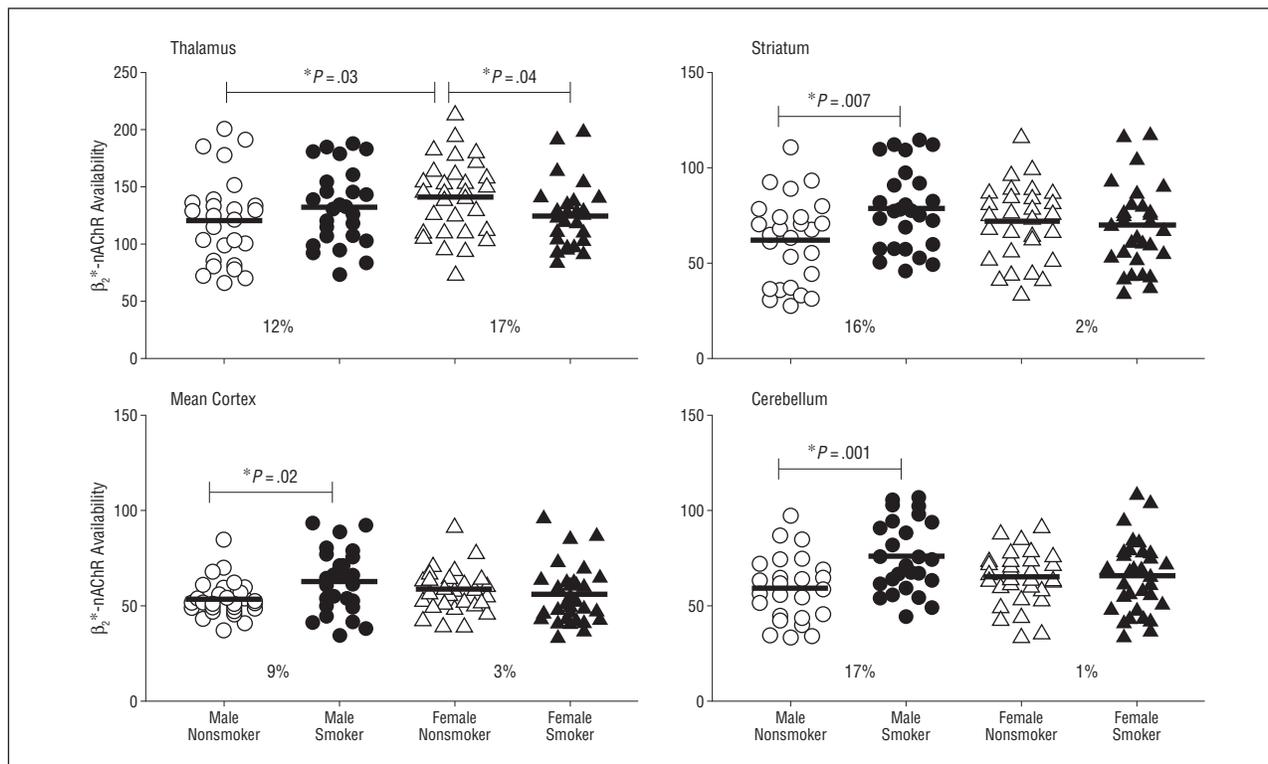


Figure 1. Availability of nicotinic acetylcholine receptors containing the β_2 subunit (β_2^* -nAChR) is shown in individual male nonsmokers, male tobacco smokers, female nonsmokers, and female tobacco smokers in the thalamus, striatum (the mean of the caudate and putamen), the mean cortex (the mean of parietal, frontal, anterior cingulate, temporal, and occipital cortices), and the cerebellum. Smokers underwent single-photon emission computed tomography imaging at 7 to 9 days of abstinence. The line in each scatterplot represents the mean value of those participants. Asterisk indicates significant difference from same-sex control nonsmokers using analysis of variance. Percentage differences are between smokers compared with same-sex nonsmokers.

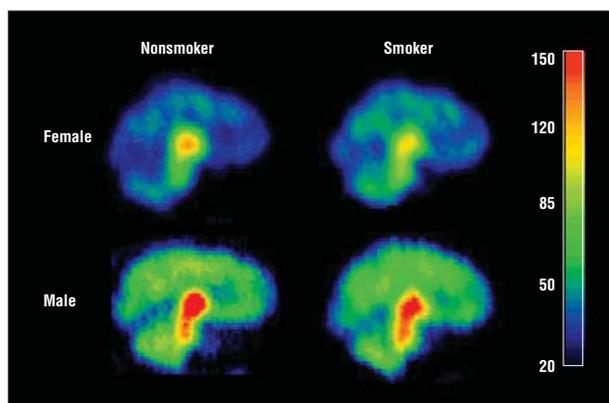


Figure 2. Availability of nicotinic acetylcholine receptors containing the β_2 subunit is shown in a representative female nonsmoker (31 years old), female tobacco smoker (33 years old), male nonsmoker (35 years old), and male tobacco smoker (36 years old) at similar sagittal levels of the brain. Red, yellow, green, and blue correspond to V_t/f_p values (iodide 123-labeled 5-iodo-A-85380 uptake in a region of interest divided by free plasma parent [both in kilobecquerels per milliliter]).

tex ($F_{1,55}=3.12$, $P=.08$), occipital cortex ($F_{1,55}=2.81$, $P=.10$), temporal cortex ($F_{1,55}=2.91$, $P=.09$), cerebellum ($F_{1,55}=1.99$, $P=.16$), striatum ($F_{1,55}=3.08$, $P=.09$), and thalamus ($F_{1,55}=4.79$, $P=.03$) (Table 3 and Figure 1).

In women, when the sample was restricted to ages 18 to 40 years to exclude potential perimenopausal or postmenopausal women, the findings were consistent. Although we obtained information on reproductive status and hormonal therapy, the subsamples were too small

to reliably evaluate β_2^* -nAChR availability by this breakdown.

ASSOCIATION BETWEEN β_2^* -nAChR AVAILABILITY AND CLINICAL CORRELATES

There were no significant correlations in any smoker group (male smokers, female smokers, or smokers as a whole) between regional β_2^* -nAChR availability and tobacco smoking characteristics (years smoked, cigarettes per day, MNWS score, QSU-Intent and QSU-Relief scores, and Fagerström Test for Nicotine Dependence score) at intake or on imaging day. Similarly, there were no significant correlations in any group (male nonsmokers, male smokers, female nonsmokers, female smokers, or smokers or nonsmokers as a whole) between regional β_2^* -nAChR availability and depressive symptoms (BDI score) at intake or on imaging day. There were no significant correlations in smokers between regional β_2^* -nAChR availability and the change in nicotine withdrawal (MNWS score), depressive symptoms (BDI score), or craving for a cigarette (QSU-Intent and QSU-Relief scores) from baseline to imaging day.

ASSOCIATION BETWEEN β_2^* -nAChR AVAILABILITY AND SEX STEROID HORMONE LEVELS IN WOMEN

The mean (SD) estradiol and progesterone levels on imaging day did not differ significantly between female

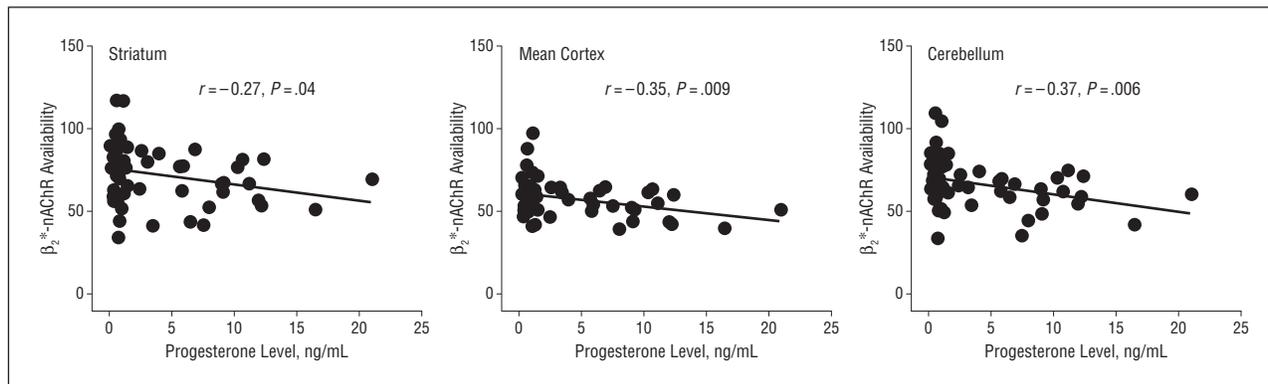


Figure 3. Availability of nicotinic acetylcholine receptors containing the β_2 subunit (β_2^* -nAChR) (V_T/f_p) (iodide 123-labeled 5-iodo-A-85380 uptake in a region of interest divided by free plasma parent [both in kilobecquerels per milliliter]) is shown in individual female tobacco smokers and nonsmokers in the striatum, mean cortex, and cerebellum as a function of the progesterone level on single-photon emission computed tomography imaging day. Significance was determined using Pearson product moment correlation coefficients and is set at $P < .01$. To convert progesterone level to nanomoles per liter, multiply by 3.18.

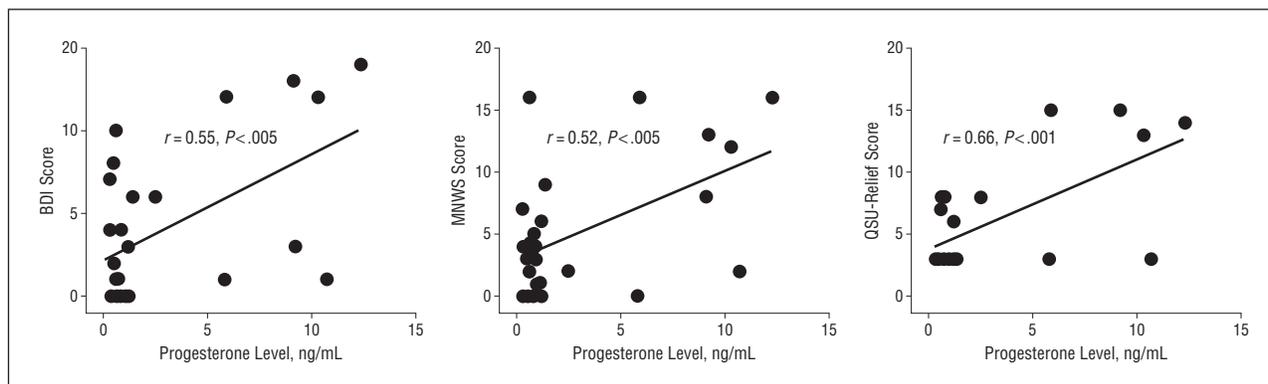


Figure 4. Scores on the Beck Depression Inventory (BDI), Minnesota Nicotine Withdrawal Scale (MNWS), and Urge to Smoke Questionnaire (QSU)-Relief on single-photon emission computed tomography imaging day are shown as a function of progesterone level on imaging day. Significance was determined using Pearson product moment correlation coefficients and is set at $P < .01$. To convert progesterone level to nanomoles per liter, multiply by 3.18.

smokers (79 [58] pg/mL for estradiol and 3 [4] ng/mL for progesterone) and nonsmokers (81 [75] pg/mL for estradiol and 5 [5] ng/mL for progesterone); however, smokers exhibited a more restricted range of both sex steroid hormone levels (32-203 pg/mL for estradiol and 0.3-12.0 ng/mL for progesterone) compared with nonsmokers (32-410 pg/mL for estradiol and 0.3-17.0 ng/mL for progesterone), which is consistent with data showing lower sex steroid hormone levels in smoking vs nonsmoking women (to convert estradiol level to picomoles per liter, multiply by 3.671; to convert progesterone level to nanomoles per liter, multiply by 3.18).³⁸ The β_2^* -nAChR availability was significantly negatively correlated with progesterone level but not with estradiol level or the ratio of estradiol to progesterone on imaging day in female smokers and nonsmokers, such that higher progesterone levels were associated with lower β_2^* -nAChR availability. This was true when female smokers and nonsmokers were combined and when they were examined separately. Specifically, progesterone level and β_2^* -nAChR availability were significantly negatively correlated in the whole group in the cerebellum ($r = -0.37$, $P = .006$) and the mean cortex ($r = -0.35$, $P = .009$), with a trend in the striatum ($r = -0.27$, $P = .04$) and no significant correlations in the thalamus ($r = -0.17$, $P = .22$) (**Figure 3**). When separated by group, progesterone level

and β_2^* -nAChR availability were significantly negatively correlated in nonsmokers in the mean cortex ($r = -0.47$, $P = .01$), and there was a trend for a negative correlation in smokers in the cerebellum ($r = -0.39$, $P = .04$).

ASSOCIATION BETWEEN SEX STEROID HORMONE LEVELS AND CLINICAL CORRELATES

There were significant correlations between progesterone level on imaging day and clinical correlates on imaging day. Specifically, progesterone level was significantly positively correlated with scores on the BDI ($r = 0.55$, $P < .005$), MNWS ($r = 0.52$, $P < .005$), and QSU-Relief ($r = 0.66$, $P < .001$) (**Figure 4**). Higher progesterone levels were associated with worse depressive symptoms, craving for a cigarette, and nicotine withdrawal.

COMMENT

SEX DIFFERENCES AND β_2^* -nAChR AVAILABILITY

In the present study, we examined sex differences in β_2^* -nAChR availability between smokers and nonsmokers.

We demonstrate significant sex-specific differences in β_2^* -nAChR availability between smokers and nonsmokers. Specifically, male smokers had significantly higher β_2^* -nAChR availability compared with male nonsmokers in the striatum (16%), cerebellum (17%), and cortical regions (range, 13%-17%), but female smokers had similar β_2^* -nAChR availability compared with female nonsmokers (range, 1%-6%) in these areas. This finding is striking given the wealth of literature on tobacco smoking and nicotine-induced upregulation of nAChRs throughout the brain. Specifically, the upregulation, or increased availability, of nAChRs has been widely demonstrated in preclinical rodent studies,^{18,39} in postmortem human studies,^{19,40,41} and in living humans using positron emission tomography²⁴ and SPECT^{22,23} imaging. Although some prior imaging and postmortem investigations have been underpowered to examine sex differences, 2 previous preclinical studies^{25,28} examined sex differences in nicotine-induced nAChR upregulation, determining that male rodents exposed to nicotine compared with nonexposed controls had greater increases in nAChR number than females exposed to nicotine compared with nonexposed females. Consistent with the preclinical studies, our results revealed higher β_2^* -nAChR availability in the brains of male vs female tobacco smokers compared with male and female nonsmokers. Taken together, these data provide a window into the brain chemistry that potentially underlies the multitude of findings of sex differences in tobacco smoking and nicotine-related behaviors in clinical and preclinical studies.

The present finding of a lack of significant upregulation of β_2^* -nAChR availability in the thalamus in male smokers is consistent with receptor imaging,^{23,24} postmortem,¹⁹ and preclinical^{42,43} studies showing that the thalamus is differentially regulated by nicotine compared with other regions. The reasons for this remain unclear but may be due to a ceiling effect. The thalamus has the highest density of β_2^* -nAChRs in the brain, and the number of β_2^* -nAChRs in the thalamus of healthy individuals may already be at a maximal level and not further increased by smoking. In the present study, male smokers and nonsmokers had equivalent thalamic β_2^* -nAChR availability, whereas female smokers had significantly lower thalamic β_2^* -nAChR availability (17%) compared with female nonsmokers. Thalamus was the only region where differences in β_2^* -nAChR availability were found in female smokers compared with nonsmokers. Therefore, not only do female smokers not have higher β_2^* -nAChR availability throughout the brain, but also β_2^* -nAChR availability is significantly lower in the thalamus compared with that in female nonsmokers. This is critical given that the relay of sensory information via thalamocortical connections may be modulated by nicotine exposure.^{44,45} Therefore, sex differences in the effects of tobacco smoking on thalamic β_2^* -nAChR availability may underlie sex differences in cue-induced activation in connecting cortical areas.⁴⁶

In this study, there was a trend for higher β_2^* -nAChR availability in female compared with male nonsmokers. A previous study²⁷ found no differences in β_2^* -nAChR availability between male (n=10) and female (n=19) nonsmokers; however, in the present larger study

of male (n=26) and female (n=30) nonsmokers, women had significantly higher β_2^* -nAChR availability than men in the parietal and frontal cortices and thalamus, with a trend for differences in the other regions. It is unclear whether the lack of a difference in β_2^* -nAChR availability between female smokers vs nonsmokers compared with male smokers vs nonsmokers is because women are beginning at a higher "baseline." The present study cannot address this question because it is unclear whether all women have higher β_2^* -nAChR availability or whether there are initial differences in β_2^* -nAChR availability in women who go on (and do not go on) to become smokers. One preclinical study²⁵ reported that female rodents had higher whole-brain nAChR densities than males, and another study²⁶ reported no difference. Our findings suggest a basal sex difference in nonsmokers, with women having moderately higher β_2^* -nAChR availability than men.

Nicotine from tobacco smoke and nornicotine have been shown to acutely displace β_2^* -nAChR ligands from the receptor.^{47,48} Given acutely (0.06 mg/kg intravenously), cotinine did not displace [¹²³I]5-IA in baboon brain (K.P.C., unpublished data, 2004), and it seems unlikely based on these data that metabolites of nicotine (cotinine and nornicotine) are influencing β_2^* -nAChR availability at 7 to 9 days of abstinence. We chose 7 to 9 days of abstinence based on results of previous studies^{22,23} that suggest this is adequate time for nicotine and its metabolites to clear from the brain and body.

TOBACCO SMOKING CHARACTERISTICS AND β_2^* -nAChR AVAILABILITY

Our group has previously reported associations between β_2^* -nAChR availability and craving for a cigarette. Specifically, recently abstinent smokers (approximately 1 week) with higher β_2^* -nAChR availability reported greater craving to smoke to relieve withdrawal symptoms.²² Consistent with this previous study, herein we found no association between β_2^* -nAChR availability and tobacco smoking characteristics, such as years smoked, cigarettes per day, and nicotine dependence. However, we also did not find an association between β_2^* -nAChR availability and craving for a cigarette at 1 week of abstinence in the smokers as a whole group or when broken down by sex. This is likely because of the much larger sample of smokers in the present study (n=54) vs in the previous study (n=16).²² This suggests that β_2^* -nAChR availability at 1 week of abstinence may not be related to tobacco smoking characteristics, craving for a cigarette, or depressive symptoms in healthy smokers. In women and men, craving was significantly lower at 7 to 9 days of abstinence than before quitting, in keeping with data suggesting withdrawal symptoms that drive craving peak within the first week of quitting and decline by 7 to 9 days of abstinence.⁴⁹ In addition, smokers were helped to remain abstinent using contingency management techniques. The provision of daily support and financial rewards for abstinence likely helped smokers cope with early craving and withdrawal symptoms, allowing these symptoms to resolve with longer abstinence and attenuating relationships between β_2^* -

nAChR availability and tobacco smoking characteristics. Relationships between β_2^* -nAChR availability and clinical correlates may have a more prominent role in smokers with a significant psychiatric comorbidity⁵⁰ or during more acute or prolonged abstinence, as shown previously.²³

SEX STEROID HORMONE LEVELS AND β_2^* -nAChR AVAILABILITY

In women, sex steroid hormones, such as progesterone, have been shown to have a critical role in tobacco smoking behaviors, including quit attempts.⁵¹ For example, women had a longer time to relapse when they made their quit attempt in the luteal phase of their menstrual cycle (when progesterone levels are much higher than estradiol levels) than in the follicular phase (when estradiol levels are higher and progesterone levels are minimal),^{52,53} and phase of cycle may influence medication efficacy.⁵³ In a previous study²⁷ among a smaller sample of nonsmokers, no relationship was observed between menstrual cycle phase or sex steroid hormone levels on imaging day and β_2^* -nAChR availability. However, in the present larger study, progesterone level was significantly negatively associated with β_2^* -nAChR availability in female smokers and nonsmokers. Specifically, higher levels of progesterone on imaging day were associated with lower β_2^* -nAChR availability. This indicates that progesterone may be an allosteric modulator of the β_2^* -nAChR and is consistent with preclinical literature that suggests progesterone⁵⁴⁻⁵⁶ and its A-ring metabolites⁵⁵ inhibit nAChRs noncompetitively. Progesterone⁵⁷ and the neurosteroid pregnenolone⁵⁸ also have been shown to block nicotinic receptor function, and progesterone has reduced the urge to smoke.^{59,60} Taken together, these findings suggest that progesterone treatment for smoking cessation should be explored. In addition, progesterone may have inhibitory actions directly or indirectly on the β_2^* -nAChR, and these actions may have a role in the observed sex differences in β_2^* -nAChR availability.

There is a large literature establishing an association of ovarian hormones with mood regulation and affective disorders. Negative affect⁶¹ and depression^{61,62} have been identified as contributing factors to relapse in female smokers. In female smokers herein on the day of SPECT imaging (ie, at 7-9 days of abstinence), higher progesterone levels were associated with worse depressive symptoms, craving to smoke a cigarette, and nicotine withdrawal. These participants had no history or current diagnosis of major depression or other major psychiatric disorders, suggesting that progesterone modulates mood in healthy smokers. Furthermore, although higher progesterone levels were associated with lower β_2^* -nAChR availability in female smokers, there was no association between depressive symptoms, craving to smoke a cigarette, or nicotine withdrawal and β_2^* -nAChR availability in female smokers. Therefore, the association of progesterone with these symptoms is not directly related to β_2^* -nAChR availability. The finding that higher progesterone levels during abstinence are associated with worse craving for a cigarette and worse nicotine withdrawal is somewhat inconsistent with the idea that progesterone

may be therapeutically effective. However, this study is limited in that, although we obtained sex steroid hormone levels on imaging day, we included a heterogeneous group of women, including women taking birth control and menopausal women; therefore, this does not preclude the possibility that exogenously administered progesterone will be an effective therapeutic agent.

LIMITATIONS

There are several limitations to the present study. First, [¹²³I]5-IA measures β_2^* -nAChRs in the brain, and although it has been traditionally thought that nAChRs containing 2 α_4 and 3 β_2 subunits are the primary receptor subunit composition that upregulates in response to nicotine, there is increasing evidence to suggest that other subunits (α_5 , α_6 , and β_3) may combine with the α_4 and β_2 subunits and have a role in upregulation of the receptor.⁶³ Second, we did not obtain sex steroid hormone levels in male participants; therefore, the role hormones have in β_2^* -nAChR availability in men cannot be examined in this study. Third, as previously mentioned, we enrolled a heterogeneous group of women, regardless of menopausal status, gynecological surgical history, or current hormonal contraceptive use. Therefore, we cannot conclude whether endogenous fluctuating gonadal hormone levels underlie the observed sex difference in β_2^* -nAChR availability. Fourth, we did not examine whether sex differences in β_2^* -nAChR availability in smokers are related to differences in time to relapse; we are collecting this information as part of an ongoing study. Fifth, although high test-retest reproducibility of our imaging protocol has been demonstrated,²¹ there are inherent limitations in the ability to correct for motion within SPECT imaging that may contribute to the variability between participants.

CONCLUSIONS

This study highlights the critical need for sex-specific treatment strategies for tobacco smoking. Most available cessation treatments (eg, NTs and varenicline) act at the β_2^* -nAChR; however, clinical investigations consistently demonstrate that men respond more than women to the nicotine in tobacco smoke and that men generally have a better therapeutic response to NT than women.¹ Women also have lower success rates of quitting smoking than men,²⁻⁶ which underscores the need to determine the biological dimorphisms that may drive these behavioral outcomes. The present findings provide a potential biological rationale for these differences. Specifically, the regulatory effects of nicotine in the brain seem to be distinctly different between men and women. Men are more reinforced by the nicotine per se in tobacco smoke, respond better to NT,¹ and have higher β_2^* -nAChR availability compared with male nonsmokers (ie, an upregulation of β_2^* -nAChRs). Compared with male smokers, female smokers are more reinforced by the conditioned cues of smoking⁸ and do not respond as well to NT,¹ and female smokers do not seem, on average, to have higher β_2^* -nAChR availability compared with female nonsmok-

ers. To treat female smokers more effectively, it is critical that nonnicotinic mediated medications should be explored.

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Correspondence: Kelly P. Cosgrove, PhD, Department of Psychiatry, Yale University School of Medicine, 950 Campbell Ave/116A6, West Haven, CT 06516 (kelly.cosgrove@yale.edu).

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