

β_2 -Nicotinic Acetylcholine Receptor Availability During Acute and Prolonged Abstinence From Tobacco Smoking

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Context: Available levels of nicotinic acetylcholine receptors containing the β_2 subunit (β_2^* -nAChR) are higher in recently abstinent tobacco smokers compared with participants who never smoked. Variations in β_2^* -nAChR availability during the course of abstinence may be related to the urge to smoke, the extent of nicotine withdrawal, and successful abstinence.

Objective: To examine changes in β_2^* -nAChR availability during acute and prolonged abstinence from tobacco smoking and to determine how changes in β_2^* -nAChR availability were related to clinical features of tobacco smoking.

Design: Tobacco smokers participated in up to 4 iodide 123-labeled 5-iodo-A-85380 ($[^{123}\text{I}]5\text{-IA}$) single-photon emission computed tomography (SPECT) scans during abstinence at 1 day ($n=7$) and 1 ($n=17$), 2 ($n=7$), 4 ($n=11$), and 6 to 12 ($n=6$) weeks. Age-matched nonsmokers participated in a single $[^{123}\text{I}]5\text{-IA}$ SPECT scan. All participants completed 1 magnetic resonance imaging study.

Setting: Academic imaging center.

Participants: Tobacco smokers ($n=19$) and an age-matched nonsmoker comparison group ($n=20$).

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

Results: Compared with nonsmokers, β_2^* -nAChR availability in the striatum, cortex, and cerebellum of smokers was not different at 1 day of abstinence, was significantly higher at 1 week of abstinence, and was not different at 4 or at 6 to 12 weeks of abstinence. In smokers, β_2^* -nAChR availability was significantly lower in the cortex and cerebellum at 6 to 12 weeks compared with 1 week of abstinence. In addition, cerebellar β_2^* -nAChR availability at 4 weeks of abstinence was positively correlated with craving on the day of the SPECT scan.

Conclusions: These data suggest that higher β_2^* -nAChR availability persists up to 1 month of abstinence and normalizes to nonsmoker levels by 6 to 12 weeks of abstinence from tobacco smoking. These marked and persistent changes in β_2^* -nAChR availability may contribute to difficulties with tobacco cessation.

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A WEALTH OF EVIDENCE FROM postmortem¹⁻³ and preclinical⁴⁻⁷ studies demonstrates smoking- and nicotine-induced elevations in nicotinic acetylcholine receptors (nAChRs) throughout the brain. We previously demonstrated in vivo higher available levels of nAChR containing the β_2 subunit (β_2^* -nAChR) in recently abstinent tobacco smokers compared with individuals who never smoked (nonsmokers).⁸ Higher β_2^* -nAChR availability in smokers may be due to a variety of molecular changes, including increased assembly of α_4 and β_2 subunits in the endoplasmic reticulum,⁹ increased transport of receptors to the membrane,¹⁰ decreased receptor turn-

over,¹¹ and/or the presence of nicotine-promoting intracellular maturation of the $\alpha_4\beta_2$ -nAChR to a high-affinity conformation.¹² Higher β_2^* -nAChR availability is thought to functionally reflect higher numbers of desensitized receptors.^{13,14} In addition, smoking 1 cigarette led to receptor occupancy of more than 88%,¹³ suggesting that smokers maintain saturation of β_2^* -nAChRs during the day. Thus, a chronic tobacco smoker who maintains persistently elevated nicotine levels may experience repeated cycles of nAChR activation and desensitization in the course of each day.¹⁴

Preclinical studies have demonstrated that nAChR levels return to control levels after termination of nicotine expo-

sure but with great variability in the time course.¹⁵⁻¹⁷ A postmortem human study indicated that individuals who had quit smoking at least 2 months before their death (range, 2 months to 30 years) had nicotine binding levels similar to those of control nonsmokers.² A recent *in vivo* study in a small number of male smokers demonstrated a trend for a normalization of the β_2^* -nAChR availability by 21 days of smoking cessation.¹⁸ However, these data need to be confirmed in a larger, more heterogeneous population during prolonged abstinence.

The exact subunit combination of nAChR that upregulates in response to nicotine is emerging. The nAChRs that contain α_4 and β_2 subunits^{19,20} are the most abundant nAChRs in the brain, and nicotine demonstrates the highest affinity for these receptors.²¹ In recent years, evidence has emerged that the β_2^* -nAChRs are a critical neural substrate mediating the effects of nicotine in the brain. Much of this information has been derived from studies in β_2 knockout mice. These studies have demonstrated that the β_2 subunit is critical for self-administration,²² conditioned place preference,²³ discriminative stimulus and taste aversion,²⁴ dopamine release,^{22,25,26} dopamine-dependent locomotor activation,²⁷ and enhancement of incentive aspects of motivation²⁸ of nicotine. Studies in wild-type animals further confirm that the β_2 subunit is critical to the reinforcing properties of nicotine.²⁹ The β_2 subunit also determines the sensitivity to nicotine³⁰⁻³² but does not play a critical role in nicotine withdrawal symptoms in rodents.³³ Nicotine-induced increases in nAChR, termed *upregulation*, are conferred by a specific microdomain that is in the β_2 subunit,³⁴ and thus this upregulation is confined to nAChRs that contain the β_2 subunit.^{4,19,31,35-38}

Availability of β_2^* -nAChR can be measured *in vivo* with the radiotracer iodide 123-labeled 5-iodo-A-85380 (¹²³I]5-IA) and single-photon emission computed tomography (SPECT). The nicotinic agonist [¹²³I]5-IA binds with high affinity to the nicotine-binding site on nAChRs that contain the β_2 subunit.³⁹ This ligand demonstrates low nonspecific binding⁴⁰ and has acceptable dosimetry in humans with high brain uptake^{41,42} and good test-retest reproducibility.⁴³ Because [¹²³I]5-IA is administered at a trace dose (<1% occupancy) for SPECT, it does not interfere with receptor and cell function. Imaging with [¹²³I]5-IA SPECT in nonhuman primates and humans results in a binding pattern that is consistent with the established regional distribution of β_2 -nAChR and is highest in the thalamus and intermediate throughout the cortex and cerebellum.^{43,44}

The primary goal of the present study was to evaluate the time course of change in β_2^* -nAChR availability during prolonged abstinence by using [¹²³I]5-IA SPECT. A secondary goal was to explore the relationships between β_2^* -nAChR availability and behavioral features of tobacco smoking and withdrawal. We hypothesized that during acute abstinence (ie, at 1 day of withdrawal), β_2^* -nAChR availability would be lower compared with 1 week of abstinence because of the presence of nicotine in the brain, which would block radiotracer binding. Consistent with our previous study,⁸ we hypothesized that, compared with control nonsmokers, β_2^* -nAChR availability would be higher at 1 week of withdrawal and then

would progressively decline during the course of prolonged abstinence.

METHODS

PARTICIPANTS

Nineteen healthy tobacco smokers (9 men and 10 women; mean [SD] age, 41.1 [9.0] years; 13 white American, 5 African American, and 1 Hispanic American) and 20 age-matched healthy control participants (9 men and 11 women; mean [SD] age, 42.4 [9.8] years; 10 white American, 7 African American, 2 Hispanic, and 1 Asian American) participated in this study. Smokers participated in up to 4 [¹²³I]5-IA SPECT scans and 1 magnetic resonance imaging (MRI) study. Participants were grouped by the following abstinence times (mean [SD]): 1 day (1.0 [0] days; n=7), 1 week (7.7 [1.4] days; n=17), 2 weeks (17.9 [3.0] days; n=7), 4 weeks (30.5 [4.3] days; n=11), and 6 to 12 weeks (69.0 [23.5] days; n=6). Participants were grouped as described because of the difficulty in retaining participants who remained abstinent for long periods and because of the challenges in having participants complete time-consuming (eg, >8 h/d) scans on multiple days. Participants were scanned during a range of days at each time point because of scheduling constraints. Nonsmoker controls (n=20) participated in 1 [¹²³I]5-IA SPECT scan and 1 MRI study.

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. The use of the radiotracer [¹²³I]5-IA was approved by the US Food and Drug Administration. Participants were recruited from the community by word of mouth, posters, and newspaper advertisements. Eligibility was determined as follows. All participants underwent a medical examination by a study physician (E.P.) to exclude any major medical issues or neurological disorders. This included a physical examination, electrocardiography, serum chemistry evaluations, thyroid function studies, complete blood cell count, urinalysis, and urine toxicological screening. Participants were given structured interviews using the Structured Clinical Interview for DSM-IV to rule out any Axis I disorder except for nicotine dependence. All tobacco smokers had to smoke at least 10 cigarettes per day for at least 1 year. Smoking status was confirmed by plasma cotinine levels of more than 150 ng/mL, urine cotinine levels of more than 100 ng/mL (to convert to nanomoles per liter, multiply by 5.675), and carbon monoxide levels of more than 11 ppm on the day of intake. Smokers were helped to quit smoking using clinical practice guidelines and contingency management.⁴⁵⁻⁴⁷ Briefly, contingency management is a behavioral therapy in which reinforcement is provided contingent on a successful response. In this study, monetary reinforcement was provided contingent on abstinence from smoking measured by urine cotinine and breath carbon monoxide levels. Breath carbon monoxide and urine cotinine levels were monitored daily for the first 8 days of smoking cessation and a minimum of twice weekly thereafter. Participants were instructed that they could not use any form of nicotine replacement therapy or medication throughout the study. All controls were nonsmokers (defined as having smoked <100 cigarettes in their lifetime) and had no history of significant medical illness or major head trauma. Nonsmoking status 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 11 ppm on the day of intake and the day of the scan. All women were required to have a negative pregnancy test result during the screening process and before radiotracer injection on each study day. Plasma nicotine and co-

tinine levels were measured as previously described.⁸ Urine cotinine levels were measured using cotinine test strips (Accutest NicoMeter [Jant Pharmacal Corporation, Encino, California] or NicAlert [Nymox Pharmaceutical Corporation, Hasbrouck Heights, New Jersey]).

The severity of nicotine dependence was assessed at intake with the Fagerström Test for Nicotine Dependence,⁴⁸ and craving and nicotine withdrawal symptoms were assessed with the Urge to Smoke Questionnaire (QSU)⁴⁹ and the Minnesota Nicotine Withdrawal Scale,⁵⁰ respectively, at baseline (ie, on the day of their intake before quitting smoking) and on each day they underwent [¹²³I]5-IA SPECT scans. The QSU has 2 main factors, the intention/desire to smoke (QSU-Intent) and relief of negative affect and withdrawal (QSU-Relief).

MRI STUDIES

The MRI studies were obtained on a 1.5-T camera (Siemens AG, Munich, Germany) in a standard orientation (echo time, 5-7 ms; repetition time, 24 ms; 256 × 192 matrix; number of signals acquired, 1; field of view, 30 cm; 124 contiguous sections with 1.2-mm thickness) and were used for coregistration to the SPECT images to provide an anatomical guide for placement of regions of interest.

[¹²³I]5-IA SPECT SCANS

All participants received a 0.6-g saturated solution of potassium iodide (to protect their thyroid from possible exposure to radioactive iodide) in the hour before radiotracer administration. The radiotracer [¹²³I]5-IA was synthesized as previously described⁵¹ and administered as a bolus to constant infusion at a ratio of 7.0 for 8 hours. Participants were injected with mean (SD) equivalent doses of a bolus and constant infusion as follows: control non-smokers (157.9 [14.6] MBq and 22.8 [2.1] MBq/h, respectively) and smokers abstinent 1 day (134.5 [24.6] MBq and 20.5 [3.8] MBq/h, respectively), 1 week (154.3 [15.9] MBq and 22.2 [2.6] MBq/h, respectively), 2 weeks (159.4 [5.6] MBq and 23.3 [0] MBq/h, respectively), 4 weeks (158.9 [7.4] MBq and 22.6 [1.7] MBq/h, respectively), and 6 to 12 weeks (141.5 [30.5] MBq and 21.9 [3.5] MBq/h, respectively). Three consecutive 30-minute emission scans and one 15-minute simultaneous transmission and emission protocol scan were obtained from hours 6 to 8 of the [¹²³I]5-IA infusion on a SPECT camera (PRISM 3000 XP; Picker, Cleveland, Ohio). The SPECT camera is a 3-headed camera equipped with a low-energy, ultrahigh-resolution fan-beam collimator (photopeak window, 159 keV ± 10%; matrix, 128 × 128) with a uniform sensitivity across the field of view. A cobalt 57-distributed source was measured with each experiment to control for day-to-day variation in camera sensitivity. The axial resolution (full width at half maximum) is 12.2 mm, measured with a [¹²³I] line source in water in a cylindrical phantom. Blood was drawn before injection and at the beginning and end of the emission scans for analysis of plasma total parent and free fraction of parent tracer in plasma (free fraction). The chemical fate of [¹²³I]5-IA after the injection was assessed in plasma as previously described.⁵¹ Briefly, plasma total parent was assessed by acetonitrile protein denaturation, whereas the free fraction was determined by ultrafiltration (Centrifree units; Amicon, Beverly, Massachusetts).

IMAGE ANALYSIS AND OUTCOME MEASURES

Images were reconstructed and analyzed as previously described, including a nonuniform attenuation correction⁴³ with 1 exception. Specifically, in participants who underwent more than 1 SPECT scan, the second and subsequent SPECT scans were

coregistered to the same position as the first scan to apply the same region-of-interest template for that subject. The MRIs were coregistered to the SPECT scans to provide an anatomical guide for placement of the regions of interest using commercially available software (MEDx, version 3.4; Medical Numerics, Inc, Maryland). Regions of interest chosen were those known to contain β_2 -nAChRs and included frontal, parietal, anterior cingulate, temporal, and occipital cortices and the thalamus, striatum (an average of caudate and putamen), and cerebellum. Regions of interest were corrected to account for differences in size. Two raters (K.P.C. and J.B.) conducted the analysis. Variability between the raters was less than 12% across the regions of interest. The mean of the analysis from the 2 raters is reported.

The outcome measure of regional activity divided by the free plasma parent between 6 and 8 hours (V_T/f_p) 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 the free plasma parent (both measured in kilobecquerels per milliliter).⁵² We refer to V_T/f_p as β_2^* -nAChR availability because we are only measuring receptors that are "available" to be bound by the radiotracer. Receptors that are already occupied (ie, by residual nicotine, by a pharmacologically active metabolite [cotinine or nornicotine], or by endogenous neurotransmitter [acetylcholine]) are not available. The outcome measure V_T/f_p is proportional to the binding potential (B_{max}/K_D , in milliliters per gram), 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 or comparison groups. As described previously,⁴³ there is no appropriate reference region for this radiotracer, so nondisplaceable [¹²³I]5-IA uptake could not be measured. The measures of total plasma parent, free fraction, and free plasma parent (f_p ; which is defined as total parent × free fraction) were compared between groups to determine differences in radiotracer metabolism or protein binding.

STATISTICAL ANALYSES

Data were analyzed using SAS, version 9.1 (SAS Institute Inc, Cary, North Carolina). We assessed differences in blood measures (total and free parent and free fraction) and regional β_2^* -nAChR availability, V_T/f_p , between participants in the non-smoker control group and each of the abstinent smoker groups at 1 day and 1, 2, 4, and 6 to 12 weeks using 2-sample *t* tests. Differences in regional brain V_T/f_p and behavioral measures of tobacco smoking and withdrawal between participants in the abstinent smoker groups were assessed using repeated-measures mixed-effects regression models with group as a fixed effect and compound symmetry covariance structure across repeated measurements. We evaluated these between-group differences using repeated-measures mixed-effects models to account for the observations between abstinent smoker groups that were not entirely independent, given that some abstinent smokers contribute observations to more than 1 group. For models examining between-group differences for which there was a significant effect of group, we conducted 4 planned post hoc between-group comparisons (ie, between abstinent smokers at 1 week and at 1 day and 2, 4, and 6-12 weeks). For these post hoc tests, *P* values were Bonferroni corrected for multiple comparisons, and statistical significance was considered at $P \leq .00625$. Correlational analyses for the associations between receptor availability and smoking assessments were conducted using SPSS, version 16.0 (SPSS Inc, Chicago, Illinois). Correlations among β_2^* -nAChR availability, V_T/f_p , at each point, and clinical variables (smoking, craving, and withdrawal) were

Table 1. Demographics and Smoking Characteristics of Nonsmoker Control Subjects and Smokers During Acute and Prolonged Abstinence^a

Characteristics	Nonsmokers (n=20)	Smokers, Time Abstinent				
		1 d (n=7)	1 wk (n=17)	2 wk (n=7)	4 wk (n=11)	6-12 wk (n=6)
Age, y	42.4 (9.8)	42.7 (8.2)	41.7 (9.4)	43.4 (11.7)	43.9 (7.5)	38.7 (7.0)
FTND score	0	5.9 (2.8)	5.5 (2.6)	5.4 (2.8)	4.9 (2.6)	6.8 (2.3)
Cigarettes, No./d	0	19.9 (10.2)	19.7 (8.5)	22.7 (7.9)	14.7 (3.6)	20.3 (10.3)
Time smoked, y	0	21.8 (6.3)	19.9 (7.6)	22.0 (7.4)	20.8 (7.7)	21.2 (6.6)
Plasma cotinine level, ng/mL	<15.0	370.3 (185.6)	21.1 (46.3)	<15.0	<15.0	<15.0
Plasma nicotine level, ng/mL	<4.0	3.6 (5.2)	<4.0	<4.0	<4.0	<4.0
Carbon monoxide level, ppm	2.2 (3.1)	12.0 (7.2)	3.2 (2.4)	2.9 (1.9)	2.9 (2.2)	4.2 (3.3)

Abbreviation: FTND, Fagerström Test for Nicotine Dependence.

SI conversion factors: To convert cotinine to nanomoles per liter, multiply by 5.675; nicotine to micromoles per liter, multiply by 6.16×10^{-3} .

^aUnless otherwise indicated, data are expressed as mean (SD).

Table 2. Blood [¹²³I]5-IA Measures of Nonsmoker Control Subjects and Smokers During Acute and Prolonged Abstinence^a

Measure	Nonsmokers (n=20)	Smokers, Time Abstinent				
		1 d (n=7)	1 wk (n=17)	2 wk (n=7)	4 wk (n=11)	6-12 wk (n=6)
Total parent level, kBq/mL	0.33 (0.10)	0.24 (0.03) ^b	0.30 (0.08)	0.31 (0.09)	0.30 (0.09)	0.28 (0.09)
Free parent level, kBq/mL	0.11 (0.04)	0.08 (0.02)	0.11 (0.03)	0.12 (0.04)	0.10 (0.02)	0.10 (0.04)
Free fraction level, kBq/mL	0.34 (0.04)	0.34 (0.07)	0.36 (0.05)	0.40 (0.06) ^b	0.34 (0.05)	0.36 (0.05)

Abbreviation: [¹²³I]5-IA, iodide 123-labeled 5-iodo-A-85380.

^aData are expressed as mean (SD).

^bIndicates significantly different after Bonferroni correction compared with nonsmoker controls.

assessed with Spearman correlation coefficients (ρ). Different slopes for each abstinence point were estimated to illustrate the relationship between significant clinical variables and β_2^* -nAChR availability. Owing to multiple comparisons, statistical significance was considered at $P \leq .01$.

RESULTS

CLINICAL POPULATION

Nineteen tobacco smokers and 20 age-matched nonsmokers were included in the study. Smokers who participated in scans at different times since the last cigarette were equivalent in age, level of nicotine dependence (as assessed by the Fagerström Test for Nicotine Dependence at intake), number of cigarettes smoked per day, and years of smoking (**Table 1**). Plasma cotinine and nicotine and carbon monoxide levels were negligible in nonsmokers and were highest at 1 day of abstinence and decreased over time in smokers, confirming abstinence from smoking (Table 1).

β_2^* -nAChR AVAILABILITY DURING ACUTE AND PROLONGED ABSTINENCE

There were no differences between groups in injected dose, bolus to infusion ratio, or time of scan (data not shown). Concentrations of [¹²³I]5-IA activity in the blood were measured to correct for potential differences between groups in radiotracer metabolism or protein binding. There were significant differences in total parent lev-

els between nonsmokers and smokers abstinent 1 day and in free fraction levels between nonsmokers and smokers abstinent 2 weeks (**Table 2**). There were no differences in total parent or free fraction levels between groups at other time points or between groups at any time point in free parent levels. There was also variability between smokers who participated in multiple scans in changes in β_2^* -nAChR availability over time, with some abstinent smokers showing dramatic changes in β_2^* -nAChR availability ($\leq 48\%$ change in the cortex) and others showing barely any difference (eg, $<5\%$) over time (individual data not shown).

Regional β_2^* -nAChR availability, reflected by V_T/f_P , was compared between nonsmokers and each of the abstinent smoker groups and between the abstinent smoker groups (**Table 3**, **Figure 1**, and **Figure 2**). In smokers abstinent 1 day compared with nonsmokers, V_T/f_P was significantly reduced in the thalamus. In smokers abstinent 1 week compared with nonsmokers, V_T/f_P was significantly higher in the striatum and cerebellum and throughout the cortex. In smokers abstinent 4 weeks compared with nonsmokers, V_T/f_P was significantly higher in the occipital cortex (Table 3 and Figures 1 and 2).

Among the abstinent smoker groups, there were significant between-group differences in V_T/f_P in the thalamus ($F_{4,25} = 4.12$; $P = .01$), parietal cortex ($F_{4,25} = 2.95$; $P = .04$), frontal cortex ($F_{4,25} = 2.88$; $P = .04$), anterior cingulate ($F_{4,25} = 3.75$; $P = .02$), occipital cortex ($F_{4,25} = 3.42$; $P = .02$), and cerebellum ($F_{4,25} = 4.00$; $P = .01$). Compared with smokers abstinent 1 week, those abstinent 1 day had significantly lower V_T/f_P in the thalamus ($t_{25} = -3.51$; cor-

Table 3. β_2^* -nAChR Availability Throughout the Brain in Nonsmoker Control Subjects and Smokers During Acute and Prolonged Abstinence

Region	Nonsmokers, V_T/f_p^a	Smokers, Time Abstinent														
		1 d			1 wk			2 wk			4 wk			6-12 wk		
		V_T/f_p^a	Diff, % ^b	P Value ^c	V_T/f_p^a	Diff, % ^b	P Value ^c	V_T/f_p^a	Diff, % ^b	P Value ^c	V_T/f_p^a	Diff, % ^b	P Value ^c	V_T/f_p^a	Diff, % ^b	P Value ^c
Thalamus	130.6 (22.2)	96.9 (24.4)	-26	.002	138.5 (25.9)	+6	.32	139.2 (35.6)	+7	.46	133.8 (19.3)	+2	.69	115.2 (19.7)	-12	.14
Striatum	70.0 (11.0)	66.5 (13.1)	-5	.49	85.1 (16.8)	+22	.002	80.9 (18.2)	+16	.07	79.5 (12.6)	+14	.04	71.7 (13.3)	+2	.76
Parietal cortex	46.0 (7.8)	44.4 (7.5)	-3	.66	56.6 (9.9)	+23	.001	56.4 (16.2)	+23	.14	52.3 (10.9)	+14	.07	44.5 (9.6)	-3	.71
Frontal cortex	51.0 (8.6)	47.9 (10.8)	-6	.45	61.8 (13.0)	+21	.005	60.9 (15.9)	+19	.16	58.3 (13.8)	+14	.08	49.1 (12.9)	-4	.69
Anterior cingulate	52.5 (7.8)	54.8 (7.7)	+4	.51	67.5 (12.2)	+29	<.001	63.9 (15.1)	+22	.10	61.4 (10.0)	+17	.01	55.3 (11.6)	+5	.50
Temporal cortex	57.7 (8.7)	57.4 (10.4)	-1	.94	70.2 (12.8)	+22	.001	67.5 (15.3)	+17	.048	66.1 (9.7)	+15	.02	58.2 (11.6)	+1	.91
Occipital cortex	54.2 (7.7)	56.3 (11.7)	-4	.59	68.6 (13.4)	+27	.001	66.3 (14.3)	+22	.07	64.1 (10.3)	+18	.005	55.5 (13.9)	+2	.77
Cerebellum	62.9 (8.5)	59.5 (18.4)	-5	.65	77.9 (15.5)	+24	.002	74.0 (17.0)	+18	.14	73.2 (11.5)	+16	.009	60.6 (13.5)	-4	.61

Abbreviations: β_2^* -nAChR, nicotinic acetylcholine receptor containing the β_2 subunit; Diff, difference; [¹²³I]5-IA, iodide 123-labeled 5-iodo-A-85380; V_T/f_p , regional [¹²³I]5-IA uptake/free plasma parent.

^a V_T/f_p is calculated as regional [¹²³I]5-IA uptake divided by free plasma parent. Data are presented as mean (SD).

^bIndicates the percentage of difference from nonsmokers: [(smoker - nonsmoker)/nonsmoker] × 100.

^cP values less than .00625 are significant after Bonferroni correction.

rected $P = .007$) and cerebellum ($t_{25} = -3.02$; corrected $P = .02$). Compared with smokers abstinent 1 week, those abstinent 6 to 12 weeks had significantly lower V_T/f_p in the parietal cortex ($t_{25} = -2.87$; corrected $P = .03$), frontal cortex ($t_{25} = -2.80$; corrected $P = .04$), anterior cingulate ($t_{25} = -3.21$; corrected $P = .01$), occipital cortex ($t_{25} = -3.10$; corrected $P = .02$), and cerebellum ($t_{25} = -3.17$; corrected $P = .02$). There were no significant differences in V_T/f_p between tobacco smokers abstinent 1 week and smokers abstinent 2 or 4 weeks (Table 3 and Figures 1 and 2).

RELATIONSHIP BETWEEN β_2^* -nAChR AVAILABILITY AND CLINICAL FEATURES

There were significant differences in QSU-Intent ($F_{4,25} = 7.41$; $P < .001$) and QSU-Relief scores ($F_{4,25} = 5.63$; $P = .002$), but not in Minnesota Nicotine Withdrawal Scale scores ($F_{4,25} = 0.33$; $P = .86$), between groups of abstinent smokers (Table 4). Specifically, overall group differences in QSU-Intent and QSU-Relief scores were attributable to significantly higher levels of each of these measures on the day of the scan in the smokers abstinent 1 day compared with those abstinent at other points.

There were significant correlations between regional β_2^* -nAChR availability and clinical features and assessment scores at baseline, eg, at intake before quitting smoking (Table 5). Specifically, baseline QSU-Intent scores correlated negatively with β_2^* -nAChR availability at 1 day of abstinence in the thalamus ($\rho = -0.90$; $P = .006$) and parietal cortex ($\rho = -0.88$; $P = .008$). There were also significant correlations between regional β_2^* -nAChR availability and assessment scores taken at each abstinence point (Table 6). Specifically, a positive correlation was observed between cerebellar β_2^* -nAChR availability and craving on the QSU-Intent ($\rho = 0.74$; $P = .01$) and QSU-Relief ($\rho = 0.74$; $P = .01$) scores at 4 weeks of abstinence. There were no significant correlations between baseline smoking variables (eg, Fagerström Test for Nicotine De-

pendence, number of years smoked, or number of cigarettes smoked per day) with β_2^* -nAChR availability at any point (data not shown).

COMMENT

The present study examined the time course of changes in β_2^* -nAChR availability during acute and prolonged abstinence in tobacco smokers compared with nonsmokers by using [¹²³I]5-IA SPECT. The present findings demonstrate higher β_2^* -nAChR availability in the striatum, cerebellum, and cerebral cortex in tobacco smokers at 1 week of abstinence compared with nonsmokers, but β_2^* -nAChR availability similar to or lower than that of nonsmokers at 1 day and 6 to 12 weeks of abstinence. Although there is not a significant difference between β_2^* -nAChR availability in smokers at 2 and 4 weeks of abstinence compared with nonsmokers, there remains a robust difference, that is, higher β_2^* -nAChR availability in smokers at 2 weeks (16%-23%) and 4 weeks (14%-18%) of abstinence in the striatum, cerebellum, and cortex compared with nonsmokers that does not return to nonsmoker levels until 6 to 12 weeks of abstinence (-4% to 5% difference). There are 2 primary implications to these results. First, at 1 day of abstinence, there is still residual nicotine or a pharmacologically active metabolite of nicotine, such as cotinine or normicotine, present in the brain that interferes with radiotracer binding, thus leading to the appearance of lower β_2^* -nAChR availability. Second, the normalization of the β_2^* -nAChR is prolonged, requiring up to 6 to 12 weeks of abstinence to fully return to nonsmoker levels.

In the smokers abstinent 1 day, the levels of total parent of the radiotracer were significantly lower, but normalized quickly, by 1 week of abstinence. This finding highlights the impact of nicotine or another chemical in tobacco smoke on metabolism, eg, because nicotine was

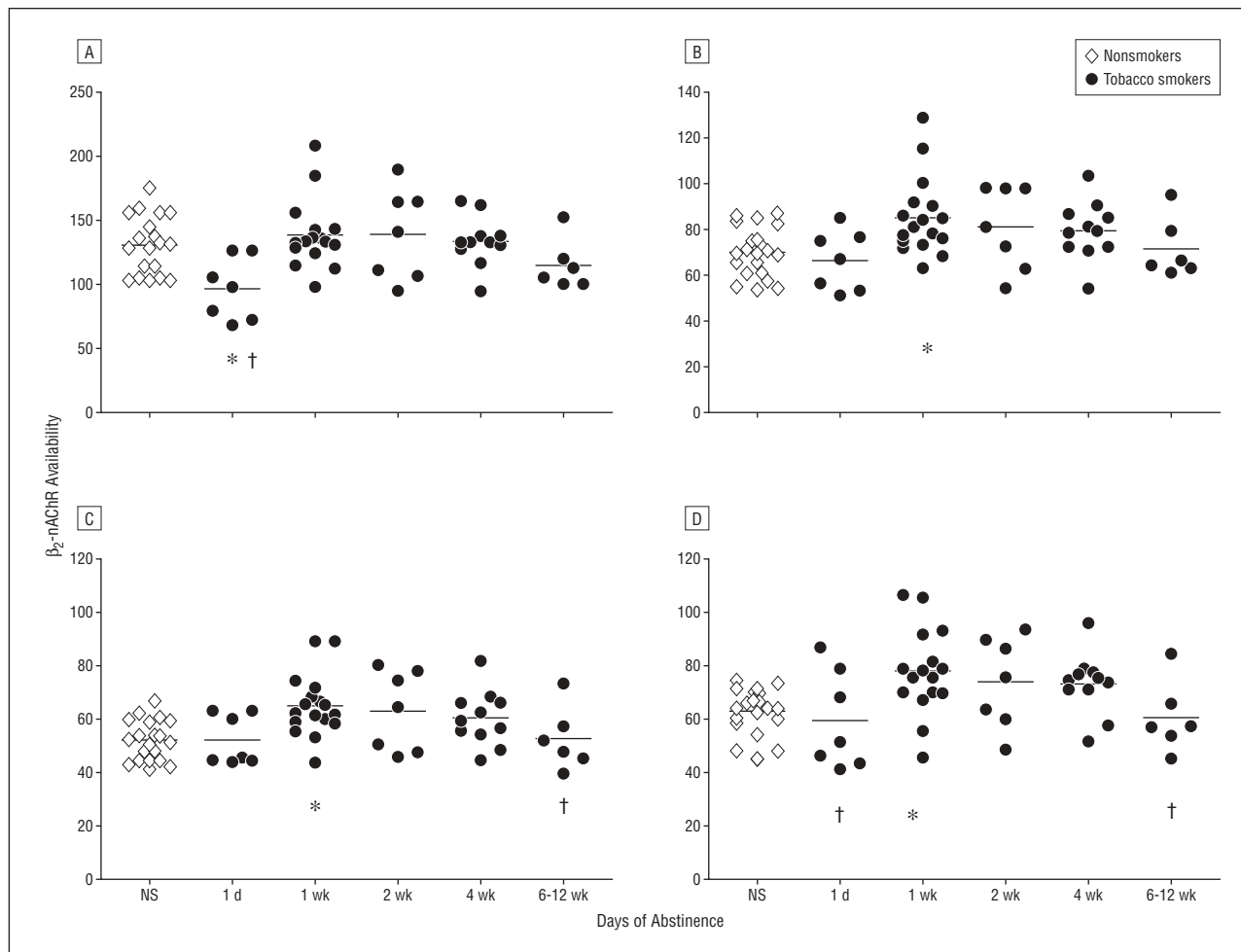


Figure 1. Availability of the nicotinic acetylcholine receptor containing the β_2 subunit (β_2^* -nAChR) measured as regional activity divided by the free plasma parent. Availability is shown in individual nonsmokers (NS) and tobacco smokers at 1 day and 1, 2, 4, and 6 to 12 weeks of abstinence in the thalamus (A), striatum (average of caudate and putamen) (B), cortex (average of cortical regions including parietal, frontal, anterior cingulate, temporal, and occipital cortices) (C), and cerebellum (D). The line in each scatterplot represents the mean value of those participants. *Significant difference from control nonsmokers after Bonferroni correction using 2-sample *t* tests. †Significant difference from smokers abstinent 1 week after Bonferroni correction using planned post hoc between-group comparisons subsequent to the analysis of repeated-measures mixed-effects regression models, including the overall effect of abstinent smoker group.

still present, it may have changed the metabolism of the radiotracer, resulting in lower total parent levels at 1 day of abstinence. Cytochrome P450 (CYP2A6) is primarily responsible for the metabolism of nicotine to its main metabolite cotinine.⁵³ There is evidence that nicotine is metabolized faster in smokers than in nonsmokers, and there are genetically mediated differences in the metabolism of nicotine in smokers.⁵⁴ In addition, nicotine can interfere with the metabolism of other drugs.⁵⁵ Consistently, we expect that [¹²³I]5-IA is metabolized in the liver by enzymes in the cytochrome P450 family, such as CYP2A6, which acts on nicotine, and CYP2B6 and CYP2D6, which catalyze the dealkylation of aromatic ethers.^{56,57} In radiotracer imaging studies, it is imperative that brain uptake is corrected for radiotracer metabolism because differences in metabolism of the radiotracer determine how much radiotracer is available to the brain. That is, fast metabolizers will have less radiotracer available to the brain for a given dose. By using the outcome measure V_T/f_p , we corrected for individual differences in radiotracer metabolism and protein binding.

In the present study, we report a negative correlation between baseline craving scores before smoking cessation and β_2^* -nAChR availability at 1 day of abstinence in the thalamus and parietal cortices. Because receptor availability is defined as receptors that are available to be bound by the radiotracer, at 1 day of abstinence participants with lower receptor availability have more nicotine present in the brain occupying receptors and blocking the radiotracer from binding to the receptor. Thus, we believe that participants who reported high baseline craving likely smoked more cigarettes immediately before their quit day and had lower receptor availability at 1 day of abstinence. However, the experience of craving in the presence of nicotine occupancy of the β_2^* -nAChR is not unusual. Smokers experience craving within 2 hours of their last cigarette, despite continued occupancy of the receptor by nicotine.¹³ Dopamine release has been associated with the feeling of craving,⁵⁸ and nicotine⁵⁹ and cotinine⁶⁰ have been shown to facilitate dopamine release; thus, the prolonged partial occupancy of the receptor by nicotine or cotinine may contribute to the feelings of craving that are reported 2 hours after the last cigarette and throughout the first week of abstinence.

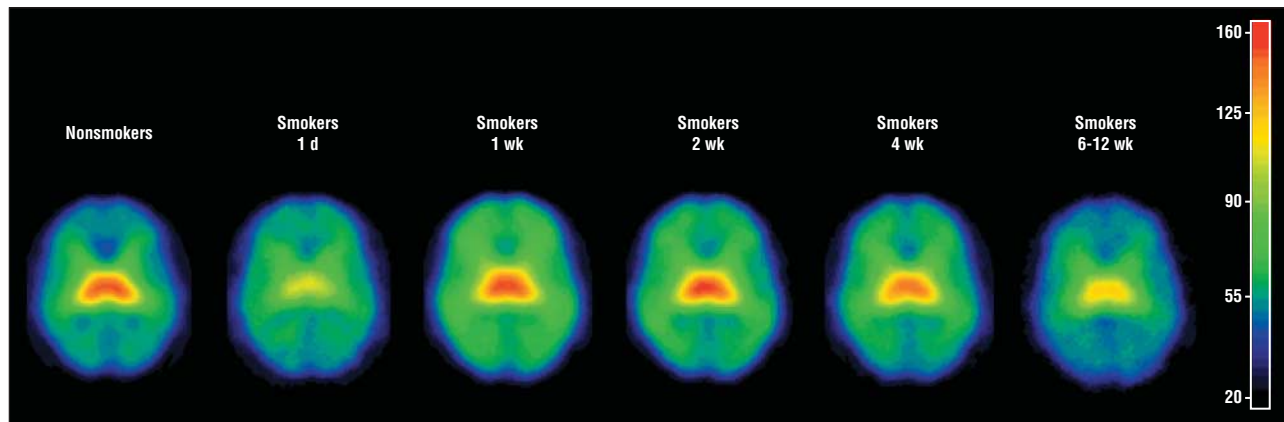


Figure 2. Mean parametric images illustrating the availability of the nicotinic acetylcholine receptor containing the β_2 subunit in nonsmokers and tobacco smokers. Measurements for the smokers were obtained at 1 day and 1, 2, 4, and 6 to 12 weeks of abstinence at similar transaxial levels of the brain. The color scale is shown with red, yellow, green, and blue corresponding to values for regional activity divided by the free plasma parent.

Table 4. Clinical Characteristics of Smokers During Acute and Prolonged Abstinence^a

Measure	Smokers, Time Abstinent				
	1 d (n=7)	1 wk (n=17)	2 wk (n=7)	4 wk (n=11)	6-12 wk (n=6)
MNWS	11.2 (3.2)	10.5 (2.5)	11.7 (3.2)	12.8 (2.8)	12.6 (3.3)
QSU-Intent	26.0 (2.7) ^b	16.3 (2.1)	16.0 (2.7)	12.3 (2.3)	14.8 (2.8)
QSU-Relief	20.1 (2.4) ^b	10.6 (1.8)	10.4 (2.4)	10.2 (2.0)	8.3 (2.5)

Abbreviations: MNWS, Minnesota Nicotine Withdrawal Scale; QSU-Intent, Urge to Smoke Questionnaire intention/desire to smoke; QSU-Relief, QSU relief of negative affect and withdrawal.

^aScores are expressed as least-squares mean (SE).

^bIndicates significantly different after Bonferroni correction compared with smokers at other time points.

We also report a positive correlation between craving on the day of the scan at 4 weeks of abstinence and cerebellar β_2^* -nAChR availability at 4 weeks of abstinence. Thus, individuals with higher cerebellar β_2^* -nAChR availability at 4 weeks of abstinence report a greater urge to smoke on that day. Associations between nicotine and craving have previously been identified in the thalamus^{61,62} and generally in areas associated with emotion and reward and areas with high densities of nAChRs.⁶³ A previous study found associations between craving and regions subserving motor functions, including the primary motor cortex, premotor cortex, and supplementary motor area,⁶⁴ which require input from the cerebellum. In addition, when smokers are told to resist craving actively during cigarette cue exposure, the motor cortex is deactivated.⁶⁵ Our finding of a link between the cerebellum and craving further suggests that craving has a motor component, so that craving for cigarettes may elicit preparation for action or voluntary movement, goal-directed actions (eg, lighting a cigarette or bringing a cigarette up to the mouth), and/or motor imagery that are linked to the motor system.^{66,67} It can be estimated that during the course of 20 years, a smoker who consumed 1 pack per day (assuming 11 puffs on average per cigarette⁶⁸) may perform the action of bringing a lit cigarette to the mouth more than 1.5 million times. Thus, the physical action of smoking a cigarette is likely to be critically tied to craving during abstinence.

In addition to the role of the cerebellum in motor functions, there is increasing interest in the involvement of the cerebellum in cognition. Specifically, activation of the cerebellum has been associated with tasks requiring explicit, episodic memory (eg, recall of autobiographical events).^{69,70} With regard to drug abuse, imaging studies have determined that the cerebellum is activated in response to smoking-related cues⁷¹ and is associated with cue-induced craving in cocaine abusers^{72,73} and recently abstinent alcoholics.⁷⁴ Activity in the cerebellum has also been linked to executive dysfunction in cocaine users.⁷⁵ Together these studies and the present findings highlight the cerebellum as a brain region that is critically linked to craving by both motor and cognitive functions.

We report no significant correlations between nicotine withdrawal and β_2^* -nAChR availability. This is consistent with the preclinical literature suggesting that the β_2^* -subtype does not play a critical role in the physical symptoms of nicotine withdrawal.^{33,76} In this study, participants reported a mild-to-moderate level of nicotine withdrawal symptoms at baseline and over the course of the study; thus, these results require replication in a larger sample with a greater range of nicotine withdrawal symptoms. In addition, we did not obtain significant correlations between β_2^* -nAChR availability at 1 week of abstinence and clinical features. We previously found a negative correlation between the urge to smoke to relieve withdrawal symptoms and β_2^* -nAChR availability in the sensorimotor cortex at approximately 1 week of abstinence.⁸ This discrepancy may be due

Table 5. Correlations Between β_2^* -nAChR Availability and Baseline Assessments

Region	Smokers, Time Abstinent														
	1 d (n=7)			1 wk (n=10) ^a			2 wk (n=7) ^b			4 wk (n=11)			6-12 wk (n=6) ^b		
	MNWS	QSU- Intent	QSU- Relief	MNWS	QSU- Intent	QSU- Relief	MNWS	QSU- Intent	QSU- Relief	MNWS	QSU- Intent	QSU- Relief	MNWS	QSU- Intent	QSU- Relief
Thalamus	0.16	-0.90 ^c	-0.56	0.06	0.29	0.17	-0.31	0.22	-0.34	-0.05	0.83
Striatum	-0.05	-0.76	-0.56	-0.01	0.16	0.17	-0.31	0.17	-0.29	-0.29	0.43
Parietal cortex	0.27	-0.88 ^c	-0.56	-0.15	0.45	-0.20	0.02	-0.03	-0.49	-0.67	0.66
Frontal cortex	0.09	-0.78	-0.49	0.06	-0.31	-0.18	-0.31	0.08	-0.53	-0.43	0.89
Anterior cingulate	-0.22	-0.63	-0.51	0.02	0.46	0.18	-0.20	0.17	-0.35	-0.39	0.77
Temporal cortex	-0.34	-0.67	-0.63	0.07	0.46	0.23	-0.34	0.25	-0.37	-0.25	0.89
Occipital cortex	-0.11	-0.67	-0.32	0.22	0.12	0.59	-0.45	0.48	-0.73	0.05	0.89
Cerebellum	-0.02	-0.85	-0.52	0.11	-0.09	0.12	-0.34	0.64	-0.61	0.21	0.77

Abbreviations: β_2^* -nAChR, nicotinic acetylcholine receptor containing the β_2 subunit; MNWS, Minnesota Nicotine Withdrawal Scale; QSU-Intent, Urge to Smoke Questionnaire intention/desire to smoke; QSU-Relief, QSU relief of negative affect and withdrawal; ellipses, not calculated.

^aBecause of missing QSU baseline data, correlations were calculated in only 10 of 17 participants.

^bBecause of missing QSU baseline data, Spearman correlations could not be calculated.

^c $P \leq .01$ (2-tailed).

to differences in correlational analysis methods, that is, voxel-based analyses in the previous study vs Spearman correlations with regions of interest in the present study. Voxel-based analyses may be more sensitive to detecting significance in smaller brain regions, but that was beyond the scope of the present study. The lack of additional correlations at 1 week of abstinence was discussed previously.⁸

Consistent with our previous study,⁸ we report significantly higher β_2^* -nAChR availability in smokers at 1 week of abstinence in the cortex, striatum, and cerebellum but not in the thalamus compared with nonsmokers. The difference between recently abstinent smokers and nonsmokers in the previous study⁸ was of a greater magnitude (26%-36% in the cerebral cortex and 27% in the striatum) than in the present study (21%-29% in the cerebral cortex and 22% in the striatum). This may be because of the older average age (approximately 5 years) of participants in the current study, because β_2^* -nAChR availability has been shown to decrease with age in nonsmokers.⁷⁷ This study and previous in vivo positron emission tomography⁷⁸ and SPECT^{8,18} studies report no upregulation of thalamic β_2^* -nAChR availability during acute abstinence, which conflicts with the findings of postmortem² and animal^{8,79} studies. In general, this may be because of differences in methods or the higher relative dose of nicotine in the postmortem and animal studies. However, 2 smokers in the present study exhibited increased β_2^* -nAChR availability in the thalamus compared with nonsmokers, highlighting the role of individual differences in receptor regulation.

These findings are also consistent with those of a previous study¹⁸ demonstrating that higher β_2^* -nAChR availability in recently abstinent tobacco smokers compared with nonsmokers is temporary. In the previous study in men, β_2^* -nAChR availability decreased to nonsmoker levels in some participants by 21 days of abstinence. Specifically, compared with nonsmokers, smokers had significantly lower β_2^* -nAChR availability at 4 hours of abstinence, significantly higher β_2^* -nAChR availability at 10 days of abstinence, and similar β_2^* -nAChR avail-

ability at 21 days of abstinence. In addition, they reported significantly lower β_2^* -nAChR availability at 21 days of abstinence compared with 10 days of abstinence. One difference is that the previous study had significantly lower β_2^* -nAChR availability in all regions at 4 hours of abstinence compared with the nonsmokers, whereas the current study reports lower β_2^* -nAChR availability in the thalamus but similar β_2^* -nAChR availability in the striatum and cerebellum and throughout the cortex compared with the nonsmoker group at 1 day of abstinence. This finding is interesting and likely attributable to high levels of residual nicotine or metabolites present in the brain at 4 hours of abstinence (vs approximately 24 hours in the present study), resulting in lower β_2^* -nAChR availability. Also, although the present study did not find a significant decrease, on average, in β_2^* -nAChR availability by 4 weeks of abstinence compared with 1 week of abstinence, in some participants normalization had occurred by this point. The high heterogeneity of the subject population in the present study compared with the previous study in men only¹⁸ likely accounts for the high individual variability with regard to receptor changes during prolonged abstinence. Thus, the present study contributes to the literature with a larger, more heterogeneous subject group, a more prolonged period of abstinence, and additional assessments of behavioral features of tobacco smoking.

Previous preclinical studies demonstrated that, in animals receiving long-term nicotine treatment, nAChR levels returned to levels observed in control animals after termination of nicotine administration, but with variable timing ranging from 1 to 3 weeks.^{15,80,81} The previous human study¹⁸ indicated a return to control levels within 21 days of abstinence, and the present study indicates that, on average, β_2^* -nAChR availability in recently abstinent tobacco smokers does not normalize until 4 to 12 weeks, although this is highly variable between individuals. The differences in the time course changes may result from differences in dosing regimen, chronicity of nicotine administration, route of administration,

Table 6. Correlations Between β_2^* -nAChR Availability and Assessments on Day of Scan

Region	Smokers, Time Abstinent														
	1 d (n=7)			1 wk (n=17)			2 wk (n=7)			4 wk (n=11)			6-12 wk (n=6)		
	MNWS	QSU-Intent	QSU-Relief	MNWS	QSU-Intent	QSU-Relief	MNWS	QSU-Intent	QSU-Relief	MNWS	QSU-Intent	QSU-Relief	MNWS	QSU-Intent	QSU-Relief
Thalamus	-0.72	-0.14	-0.52	-0.12	0.21	-0.03	-0.18	0.06	-0.26	-0.06	0.36	0.54	0.09	0.44	0.40
Striatum	-0.52	0.18	-0.67	-0.31	0.03	-0.15	-0.18	0.60	-0.26	-0.41	0.15	0.32	0.03	0.79	0.39
Parietal cortex	-0.45	0.14	-0.36	-0.28	-0.10	-0.36	0.25	0.46	0.10	-0.60	-0.19	0.23	0.03	0.18	0.13
Frontal cortex	-0.52	0.00	-0.36	-0.36	0.04	-0.17	-0.18	0.06	-0.26	-0.63	-0.16	0.26	-0.03	0.44	0.39
Anterior cingulate	-0.31	0.21	-0.60	-0.01	0.01	-0.08	0.07	0.11	-0.12	-0.34	0.10	0.19	-0.09	0.56	0.39
Temporal cortex	-0.56	0.00	-0.74	0.04	0.12	-0.08	-0.04	0.11	-0.12	-0.33	0.18	0.34	-0.03	0.44	0.39
Occipital cortex	-0.38	-0.25	-0.34	0.01	0.16	0.08	-0.07	0.00	-0.02	-0.25	0.33	0.41	-0.03	0.44	0.39
Cerebellum	-0.74	-0.32	-0.61	-0.23	0.12	-0.05	-0.07	-0.27	0.04	0.14	0.74 ^a	0.74 ^a	-0.09	0.56	0.39

Abbreviations: β_2^* -nAChR, nicotinic acetylcholine receptor containing the β_2 subunit; MNWS, Minnesota Nicotine Withdrawal Scale; QSU-Intent, Urge to Smoke Questionnaire intention/desire to smoke; QSU-Relief, QSU relief of negative affect and withdrawal.

^a $P \leq .01$ (2-tailed).

metabolism between species, specificity of the radioligand, or sex and/or genetic differences in nAChR subunit expression and composition of nicotinic agonist binding sites. However, when taken together, the results consistently highlight a return to control levels or normalization of the β_2^* -nAChR availability after termination of long-term nicotine administration. The preclinical studies support a process of prolonged normalization when we consider that 1 to 3 weeks is substantial in the life span of a rodent. In addition, this prolonged normalization of the receptor availability is in line with the protracted withdrawal symptoms reported by tobacco smokers. For example, although withdrawal symptoms such as anger, anxiety, depression, and difficulty concentrating tend to peak within the first week after smoking cessation, they continue up to 4 weeks after the cessation attempt.⁸² We are currently examining variables that may be associated with the rate of normalization.

One limitation of this study is that [¹²³I]5-IA measures the availability of the β_2 subunit of nAChR, primarily the $\alpha_4\beta_2$ subunit, but other subunits also contribute to the regulation of nAChR by tobacco smoking. The β_2 subunit has been linked primarily to the reinforcing effects of nicotine^{22,28,83} and combines with the α_{3-6} subunits. Different β_2^* subunit combinations appear to be differentially regulated by nicotine, with α_3/α_6 , $\alpha_6\alpha_4\beta_2^*$, and $\alpha_5\alpha_4\beta_2^*$ not upregulating or decreasing in response to nicotine,^{38,84-87} whereas $\alpha_6\beta_2$ (non- α_4) subunits of nAChR increase in response to nicotine.⁸⁷ The nAChR subunit expression varies regionally, and differential regulation of these distinct subunit combinations that are measured by a single nicotinic agonist ligand such as [¹²³I]5-IA will result in regional differences in the degree of upregulation in smokers and the degree of receptor normalization during abstinence. Thus, we are likely measuring the β_2 subunit in combination with α_{3-6} subunits, leaving the in vivo measurement of the β_3 and α_7 subunits for the future, with further radiotracer development. The α_7 and β_3 subunits have been implicated in modulating dopamine release^{14,88} and thus may also play a role in the rewarding properties of tobacco smoke. Although the α_7 subunit does not upregulate in re-

sponse to chronic nicotine use,³¹ it is critically involved along with the β_2 subunit in mediating desensitization/inactivation of the neuronal nAChRs in response to chronic nicotine.⁸⁹

In summary, our data extend the findings of previous studies^{8,18} that showed that the upregulation of the β_2^* -nAChR in recently abstinent tobacco smokers was temporary and could be measured with [¹²³I]5-IA SPECT imaging. Specifically, we demonstrate that the normalization of upregulated β_2^* -nAChR in tobacco smokers during smoking cessation is prolonged. This is consistent with the clinical course of tobacco smoking in which craving, withdrawal symptoms, and risk for relapse are prolonged. The variation between individuals in the magnitude of upregulation and rates of normalization may ultimately be used to delineate subgroups based on genetics, sex, and comorbid mental illness and thus to target treatment medications.

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