Neuropsychopharmacology (2013) 38, 1548–1556

www.neuropsychopharmacology.org

Treatment for Tobacco Dependence: Effect on Brain Nicotinic Acetylcholine Receptor Density

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Cigarette smoking leads to upregulation of brain nicotinic acetylcholine receptors (nAChRs), including the common $\alpha_4\beta_2$* nAChR subtype. Although a substantial percentage of smokers receive treatment for tobacco dependence with counseling and/or medication, the effect of a standard course of these treatments on nAChR upregulation has not yet been reported. In the present study, 48 otherwise healthy smokers underwent positron emission tomography (PET) scanning with the radiotracer 2-FA (for labeling $\alpha_4\beta_2$* nAChRs) before and after treatment with either cognitive-behavioral therapy, bupropion HCl, or pill placebo. Specific binding volume of distribution (V_S/f_P), a measure proportional to $\alpha_4\beta_2$* nAChR density, was determined for regions known to have nAChR upregulation with smoking (prefrontal cortex, brainstem, and cerebellum). In the overall study sample, significant decreases in V_S/f_P were found for the prefrontal cortex, brainstem, and cerebellum of −20 (±35), −25 (±36), and −25 (±31)%, respectively, which represented movement of V_S/f_P values toward values found in non-smokers (mean 58.2% normalization of receptor levels). Participants who quit smoking had significantly greater reductions in V_S/f_P across regions than non-quitters, and correlations were found between reductions in cigarettes per day and decreases in V_S/f_P for brainstem and cerebellum, but there was no between-group effect of treatment type. Thus, smoking reduction and cessation with commonly used treatments (and pill placebo) lead to decreased $\alpha_4\beta_2$* nAChR densities across brain regions. Study findings could prove useful in the treatment of smokers by providing encouragement with the knowledge that decreased smoking leads to normalization of specific brain receptors.
Neuropsychopharmacology (2013) **38,** 1548–1556; doi:10.1038/npp.2013.53; published online 20 March 2013

Keywords: tobacco dependence; nicotine; nicotinic acetylcholine receptor; bupropion; cognitive-behavioral therapy; positron emission tomography

INTRODUCTION

In the United States, roughly 19% of adults (~46 million) smoke cigarettes (CDC, 2009). The majority of these smokers (62.7%) are screened for tobacco dependence when visiting a physician, and a substantial percentage receives either counseling (20.9%) or medication (7.6%) to aid in smoking cessation (Jamal *et al*, 2012). Current first-line treatments for smoking include counseling with cognitive-behavioral therapy (CBT) and bupropion HCl (Zyban), as well as nicotine replacement therapies (such as the patch, gum, and lozenge) and varenicline (Chantix) (Fant *et al*, 2009; Jamal *et al*, 2012). Although the efficacy of these treatments is extensively documented (Agboola *et al*, 2010; Cahill *et al*, 2011), changes in brain nicotinic acetylcholine receptors (nAChRs) with a standard course of these treatments have not yet been reported.

Cigarette smoking leads to upregulation of nAChRs in the human brain, including the common $\alpha_4\beta_2$* nAChR subtype (Whiting and Lindstrom, 1988). Human postmortem tissue studies show that chronic smokers have increased numbers of $\alpha_4\beta_2$* nAChRs compared with non-smokers (Benwell *et al*, 1988; Breese *et al*, 1997) and that former smokers (>1 year abstinent) have nAChR densities similar to non-smokers (Breese *et al*, 1997). Many laboratory animal studies also demonstrate upregulation of nAChRs in response to chronic nicotine administration (eg, Marks *et al*, 2011; Zhang *et al*, 2002).

The functional significance of nAChR upregulation has been extensively studied (Govind *et al*, 2009; Lester *et al*, 2009; Quick and Lester, 2002), and although the exact significance of this upregulation is not fully known, laboratory studies indicate that nicotine exposure leads to increased receptor function and sensitivity to nicotine. Increased receptor number and function may be due to nicotine administration resulting in increased trafficking of nAChRs to the cell surface, increased receptor assembly

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Received 12 December 2012; revised 10 February 2013; accepted 12 February 2013; accepted article preview online 21 February 2013

and/or maturation, or other mechanisms (Govind *et al*, 2009).

Previous brain imaging studies of human smokers, including a recent one by our group, have used positron emission tomography (PET) or single photon emission computed tomography to demonstrate upregulation of nAChRs in smokers compared with non-smoking controls in brain regions other than the thalamus (Brody *et al*, 2013; Cosgrove *et al*, 2009; Mamede *et al*, 2007; Mukhin *et al*, 2008; Staley *et al*, 2006; Wullner *et al*, 2008). In follow-up scanning, nAChR upregulation in smokers was found to normalize to levels of non-smokers when participants were given contingency management to maintain abstinence for roughly 3 (Mamede *et al*, 2007) to 12 (Cosgrove *et al*, 2009) weeks. However, to our knowledge, no one has yet reported the effects of commonly used, standard first-line treatments for cigarette smoking on nAChR density.

Another aspect of commonly used treatments that has not yet (to our knowledge) been studied with brain imaging is prediction of treatment response. The most replicated predictor of smoking cessation outcome is severity of dependence on cigarettes, which includes number of cigarettes per day (Batra *et al*, 2008; Dale *et al*, 2001; Hymowitz *et al*, 1997; Japuntich *et al*, 2011; Kozlowski *et al*, 1994; Paluck *et al*, 2006; Westman *et al*, 1997). Greater severity of dependence has been associated with poorer outcome for group psychotherapy (Kozlowski *et al*, 1994), bupropion HCl (Dale *et al*, 2001; Paluck *et al*, 2006), and nicotine patch (Batra *et al*, 2008; Westman *et al*, 1997), as well as in naturalistic settings with no specific treatment (Hymowitz *et al*, 1997). Other factors, such as self-efficacy/self-confidence (Gwaltney *et al*, 2005; Haaga and Stewart, 1992; Li and Froelicher, 2008; Schnoll *et al*, 2003; Shiffman *et al*, 2000), desire to quit (Wiggers *et al*, 2005), low negative affect (Shiffman *et al*, 2007), absence of depression (Japuntich *et al*, 2007), little craving response to cues (Waters *et al*, 2004), low anger (Al'Absi et al., 2007), slow nicotine metabolism (Schnoll *et al*, 2009), and absence of lapses during early treatment (Kenford *et al*, 1994) have also been found to predict quit status. Thus, clinical factors have been extensively studied for their value in predicting treatment response, with greater severity of specific symptoms being linked to less likelihood of quitting.

In the present examination of a relatively large sample of PET scans from smokers, we sought to: (1) determine the effects of first-line treatments for cigarette smoking (group CBT and bupropion HCl) on $\alpha_4\beta_2$* nAChR density, with the primary study hypothesis being that movement toward normalization of $\alpha_4\beta_2$* nAChR densities occurs with decreased nicotine exposure from smoking from before to after treatment, (2) explore associations between reduced nAChR density with treatment and smoking-related symptoms, and (3) explore whether pre-treatment nAChR levels predict response to these treatments.

MATERIALS AND METHODS

Participants and Screening Methods

Forty-eight otherwise healthy adult smokers underwent PET scanning before and after treatment. Participants were recruited using the same methodology as in our previous reports (Brody *et al*, 2011; Brody *et al*, 2013). The central inclusion criteria were current nicotine dependence and smoking 10–40 cigarettes per day. Exclusion criteria were pregnancy, use of a medication or history of a medical condition that might affect the central nervous system at the time of scanning, or any history of mental illness or substance abuse/dependence. In addition to the 48 participants with full data sets, 14 participants enrolled in the study and underwent pre-treatment PET scanning, but their data were used only for the examination of prediction of treatment response either because they did not complete treatment ($n = 12$) or because they did not have a usable second PET scan due to scanner malfunction ($n = 2$). Thus, 110 PET scans were used for the data analysis.

During an initial visit, screening data were obtained to verify participant reports and characterize smoking history. Rating scales were the Smoker's Profile Form (containing demographic variables and a detailed smoking history), Fagerström Test for Nicotine Dependence (FTND) (Heatherton *et al*, 1991), Hamilton Depression Rating Scale (Hamilton, 1967), Hamilton Anxiety Rating Scale (Hamilton, 1969), and Beck Depression Inventory (Beck and Steer, 1996). An exhaled carbon monoxide (CO) level was determined using a MicroSmokerlyzer (Bedfont Scientific, Kent, UK) to verify smoking status (CO $\geqslant$8 parts per million (ppm)). A breathalyzer (AlcoMatePro) test, urine toxicology screen (Test Country I-Cup Urine Toxicology Kit), and urine pregnancy test (for women of childbearing potential; Test Country Cassette Urine Pregnancy Test) were obtained to support the participant's report of no current alcohol or drug dependence and no pregnancy. This study was approved by the local Institutional Review Board (IRB) and Radiation Safety Committee, and participants provided written informed consent.

Abstinence Period and PET Protocol

Approximately 1 week after the initial screening session, participants underwent PET scanning with the same general abstinence and 2-FA bolus-plus-continuous-infusion PET protocol as in previous studies (Brody *et al*, 2009; Brody *et al*, 2011; Brody *et al*, 2013). Participants began smoking/nicotine abstinence two nights before the pre- and post-treatment PET sessions and were monitored as described previously, so that nicotine from smoking would not compete with the radiotracer for receptor binding during scanning. On the day of PET scanning, participants arrived at the PET center at 1100 hours, reported on their abstinence, and had an exhaled CO measurement (a level of $\leqslant$4 ppm was considered as consistent with smoking abstinence). At 1200 hours, bolus-plus-continuous-infusion of 2-FA was initiated, with 147 MBq of 2-FA administered as an intravenous bolus in 5 ml saline over 10 s. This same amount of 2-FA was also diluted in 60 ml saline, and 51.1 ml was infused over the next 420 min (7.3 ml/h) by a computer-controlled pump (Harvard model 22, Harvard Instruments, Natick, MA). After initiation of the bolus-plus-continuous-infusion, participants remained seated for the next 4 h to allow the radiotracer to reach a relatively steady state in brain. At 1600 hours, PET scanning commenced and continued for 3 h, with a 10-min break after 90-min of scanning. Scans were acquired as a series of 10-min frames.

PET scans were obtained using the Philips Gemini TruFlight (Koninklijke Philips Electronics N.V., Eindhoven, the Netherlands), a fully three-dimensional PET-CT scanner, which was operated in non-TOF mode. Reconstruction was done using Fourier rebinning and filtered back projection, and scatter and random corrections were applied. The mean spatial resolution (FWHM) for brain scanning is 5.0 mm (transverse) by 4.8 mm (axial). 2-FA was prepared using a published method (Dolle *et al*, 1998). A magnetic resonance imaging (MRI) scan of the brain was obtained for each participant within a week of PET scanning with the same specifications as in our previous report (Brody *et al*, 2011). Blood samples were drawn during PET scanning for determinations of free, unmetabolized 2-FA and nicotine plasma levels, as described previously (Brody *et al*, 2013).

Symptom Rating Scale Administration

In addition to baseline ratings cited above, symptom rating scales were obtained at the beginning and end of treatment and at both PET scanning sessions. Before and after the treatment, participants were asked to rate on an analog scale how rewarding a cigarette would be and how a cigarette would taste (0 = none/bad to 10 = very much/great). During the PET sessions, the Urge to Smoke (UTS) (Jarvik *et al*, 2000) craving scale (an analog scale with 10 craving-related questions rated on a scale of 0–6), Profile of Mood States (POMS) (McNair *et al*, 1988), and Shiffman–Jarvik Withdrawal Scale (SJWS) (Shiffman and Jarvik, 1976) were administered.

Treatment for Tobacco Dependence

Within a week of the initial PET session, participants were randomly assigned to treatment with CBT, bupropion HCl sustained release formula, or matching pill placebo. These treatments were continued through the second PET scanning session at 11 weeks, with all the participants instructed to have a target quit date of 2 weeks after initiating treatment. Participants receiving pill treatments did not receive concomitant psychotherapy, in order to isolate the effects of the treatment types.

Participants randomized to CBT had weekly 60-min group psychotherapy sessions, using standard clinical techniques (Abrams *et al*, 2003). Psychotherapy consisted of education about smoking addiction, withdrawal, and relapse prevention; recognizing danger situations (triggers) that could lead to relapse; developing new coping skills, such as avoiding triggers, coping with negative affective states, reducing overall stress, and distracting attention from smoking using thought-stopping techniques; developing lifestyle changes; and social support (Carmody, 1990; Fiore *et al*, 2000). Participants had exhaled CO levels monitored at each session and were encouraged to taper off cigarettes. The manualized psychotherapy sessions were performed on a rotating basis by a study psychotherapist (SS) and the PI.

Participants randomized to receive bupropion HCl or matching pill placebo were treated in a double-blind manner. A research pharmacist distributed packets of medication/placebo to a study physician (ALB or MSM). These packets were identified by a numeric code recorded by the pharmacist. Film-coated bupropion HCl SR and placebo were obtained from the Biomedical Research Institute of New Mexico (Albuquerque, NM). Placebo ingredients were inert and the same as those found in bupropion HCl SR tablets. Participants were started on 1 pill per day (150 mg pills for bupropion) on the day following the first PET scanning session for 3 days, with the dosage increased to 1 pill orally twice per day thereafter. All participants receiving pill treatment were advised of potential benefits and side effects of bupropion HCl when given their study medication/placebo and met with a study physician weekly for medication management visits (15 min). During these visits, titration of dosage, review of side effects, and monitoring of cigarette usage took place, as well as the measurement of exhaled CO. Participants in the bupropion HCl and placebo groups were instructed to take the twice daily dosing through the second PET session.

For all the participants, quit status was defined as a self-report of $\geqslant 7$ days of continuous abstinence from smoking and an exhaled CO level of $\leqslant 8$ ppm at the time of the follow-up PET session.

PET Image Analysis

After decay and motion correction, each participant's PET scan(s) were co-registered to their MRI using PMOD version 2.9 (http://www.pmod.com/technologies/). Regions of interest (ROIs) were drawn on MRI using PMOD and transferred to the co-registered PET. ROIs were the prefrontal cortex, brainstem, and cerebellum, which were chosen for three reasons. First and most importantly, previous reports indicate that these ROIs (and most brain regions other than the thalamus) have upregulation of nAChR densities in cigarette smokers (Brody *et al*, 2013; Mamede *et al*, 2007; Mukhin *et al*, 2008; Staley *et al*, 2006; Wullner *et al*, 2008). Second, these ROIs have a range of 2-FA-binding levels from moderate to high (Brody *et al*, 2006; Brody *et al*, 2013; Kimes *et al*, 2008; Mukhin *et al*, 2008), which eliminates the issues of examining the highest nAChR density region (thalamus) as an experimental variable (because it does not have upregulation of nAChRs in smokers), and ROIs with very low nAChR density (eg, corpus callosum), which may have very small differences between groups or conditions. And third, the use of a limited number of regions maintained power for the central statistical analysis of the study, which includes a correction for number of ROIs studied. Representative slices of the prefrontal cortex (middle frontal gyrus) were drawn bilaterally, while the brainstem and cerebellum were drawn as whole structures. ROI placement was visually inspected for each PET frame in order to minimize the effects of co-registration errors and movement; this procedure was repeated if there was a noticeable problem.

Specific binding volume of distribution (designated as V_S/f_P, based on standard nomenclature (Innis *et al*, 2007)) was calculated for each region and used for the central study analyses, because this value is proportional to $\alpha_4\beta_2$* nAChR density. V_S/f_P values were determined for each participant as the difference between total binding volume of distribution (V_T/f_P) and the nondisplaceable volume of distribution corrected for the free fraction of plasma 2-FA (V_{ND}/f_P), such that $V_S/f_P = V_T/f_P - V_{ND}/f_P$. V_T/f_P values were determined

from the 17 10-min PET frames and is defined as the ratio $C_T/(C_P \cdot f_P)$, where C_T is the mean total decay-corrected concentration of 2-FA in the ROIs, $(C_P \cdot f_P)$ is the mean decay-corrected concentration of free 2-FA in plasma, and f_P is the fraction of free (unbound) 2-FA in plasma. V_{ND}/f_P values were based on data from previously published findings by our group (Brody *et al*, 2011; Brody *et al*, 2006). In addition, for scans in which participants had a measurable plasma concentration of nicotine ($\geqslant 0.2$ ng/ml), V_S/f_P values were corrected for nicotine levels at the time of scanning using the following equation: $V_S/f_P = (V_S/f_P)_{obs}/(1 - I/IC_{50})$, where $(V_S/f_P)_{obs}$ is the observed value of specific binding volume of distribution, I is the plasma nicotine level at the time of scanning, and IC_{50} is the plasma nicotine concentration resulting in 50% reduction in V_S/f_P. The IC_{50} value of 0.87 ng/ml used here was previously reported by our group (Brody *et al*, 2006).

Statistical Analysis

Means ($\pm$ SDs) were determined for demographic, rating scale, and smoking-related variables for the entire study sample and study subgroups based on treatment type. Baseline data were compared among subgroups using analyses of variance for continuous data and Chi-Square tests for categorical data to determine if groups were comparable on these items before treatment. For verifying the effect of treatment on smoking-related variables, repeated-measures analyses of covariance (ANCOVA) were performed, with the smoking-related variable (ie, cigarettes per day and exhaled CO levels) as the repeated measure, treatment type as a between-group variable, and age as a nuisance covariate (as the subgroups differed in age and previous research indicates that nAChR densities decline with age (Brody *et al*, 2013; Mitsis *et al*, 2007; Rogers *et al*, 1998)). All ANCOVA results are presented as Greenhouse–Geisser corrected values.

For evaluating changes in $\alpha_4\beta_2$* nAChR density with treatment, an overall multivariate repeated-measures analysis of covariance (MANCOVA) was performed using V_S/f_P values for the three ROIs pre- and post-treatment as repeated measures, treatment subgroup (CBT, bupropion, or placebo) and quit status as between-subject factors, and age as a nuisance covariate. This MANCOVA controls for Type 1 error for a multivariate-dependent variable, here V_S/f_P values for the ROIs. Follow-up ANCOVAS were performed for each brain region separately with the same variables as for the overall MANCOVA. For examining associations between decreases in V_S/f_P values and reductions in cigarettes per day, Pearson Product Moment Correlation coefficients were determined between these variables.

For exploratory analyses, Pearson Product Moment Correlation coefficients were determined between V_S/f_P values and subjective symptom rating scales (analog cigarette taste and reward scales, UTS craving scale, SJWS, and POMS; uncorrected for multiple comparisons). For exploring pre-treatment predictors of treatment response, binary logistic regression was used, as in previous studies (Dale *et al*, 2001; Japuntich *et al*, 2011; Schnoll *et al*, 2003; Westman *et al*, 1997), with quit status as the dependent variable and pre-treatment smoking-related (cigarettes per day and cigarette-related rating scales) and PET V_S/f_P data as the covariates (uncorrected for multiple comparisons). For these exploratory analyses, data from participants who did not complete treatment or did not have a usable second scan were included and non-completers were considered to be non-quitters (in accordance with the recent recommendations (Hughes *et al*, 2003; West *et al*, 2005) and use (Rigotti *et al*, 2009) of this classification). Statistical tests were performed using PASW/SPSS Statistics version 19.0 (SPSS, Chicago, IL).

RESULTS

Baseline Demographic and Rating Scale Data

At baseline, the study sample was middle-aged (42.8 ($\pm$ 13.7) years old), mostly male (72.9%), and roughly half Caucasian (54.2%), with some college education (14.5 ($\pm$ 1.9)) and minimal anxiety/depressive symptoms

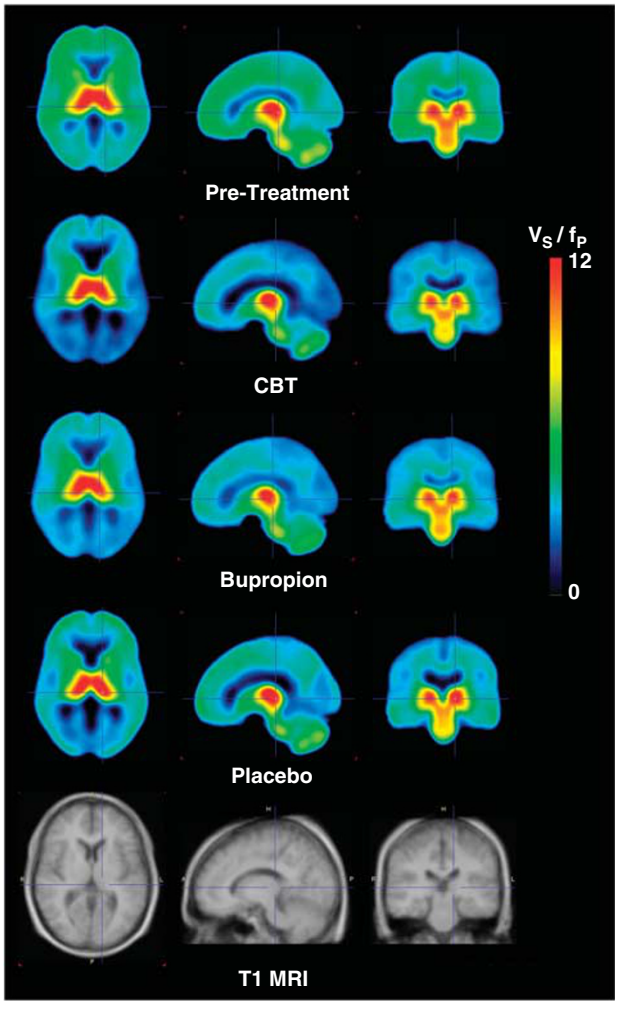

Figure 1 Mean positron emission tomography (PET) images from the study sample demonstrating decreased 2-FA binding from pre- to post-treatment for the three treatment groups. Top row consists of pre-treatment scans ($n = 48$), followed by the post-treatment mean images for the cognitive-behavioral therapy (CBT; $n = 16$), bupropion HCl ($n = 18$), and pill placebo ($n = 14$) treatment groups (rows 2–4, respectively). Mean PET images were spatially normalized to a group mean magnetic resonance imaging (MRI) scan (bottom row). V_S/f_P = specific binding volume of distribution.

(Table 1). The sample smoked roughly one pack of cigarettes per day on average (19.6 (±4.0)) and was moderately nicotine-dependent (FTND −4.2 (±2.3)).

Study subgroups based on assigned treatment type did not differ on any demographic or rating scale scores other than age, which was higher for the CBT-treated subgroup (51.6 (±12.3)) than for the bupropion- (35.5 (±11.6)) or placebo- (42.1 (±12.5)) treated subgroups (F (2, 45) = 7.5, $P = 0.002$). For this reason, age was used as a nuisance covariate in all between-group analyses.

Effects of Treatment on Smoking-Related Variables

As expected, treatment for tobacco dependence was associated with a decrease in number of cigarettes per day (F (1, 44) = 13.7, $P = 0.001$), with the study sample having a 54 (±38)% reduction. A significant interaction between time from pre- to post-treatment and subgroup based on treatment type was also found (F (2,44) = 6.4, $P = 0.004$), with the CBT-treated subgroup having a greater reduction in cigarettes per day (−76 (±30))% than the bupropion-treated (−38 (±35))% or placebo-treated (−50 (±41))% subgroups. Treatment was also associated with decreased CO levels (F (1,44) = 5.4, $P < 0.05$), with no significant between-group effect (Table 1). Eleven participants met criteria for having quit smoking at the end of treatment ($n = 7$ for CBT, 1 for bupropion, and 3 for placebo).

Effects of Treatment on $\alpha_4\beta_2$* nAChR density

For the overall MANCOVA examining effects of treatment on $\alpha_4\beta_2$* nAChR density, both changes in V_S/f_P from pre- to post-treatment (F (1,41) = 5.6, $P = 0.02$) and differences between quitters and non-quitters (F (1,41) = 5.8, $P = 0.02$) were significant, with no significant effect of treatment type (F (2,41) = 1.2, NS). In the overall study sample, treatment was associated with decreases in V_S/f_P in the prefrontal cortex, brainstem, and cerebellum by −20 (±35), −25 (±36), and −25 (±31)%, respectively (Figure 1, Table 2). Mean post-treatment V_S/f_P values for the prefrontal cortex, brainstem, and cerebellum were 4.1, 7.2, and 5.5, respectively, which demonstrated movement toward V_S/f_P values from non-smokers by 3.0, 5.5, and 4.1, respectively, from a separate study (Brody *et al*, 2013). Thus, treated smokers had 54.2, 60.5, and 60.0% normalization of nAChR levels for the three regions, respectively.

Participants who quit smoking had greater reductions in V_S/f_P across regions than non-quitters (for prefrontal cortex, brainstem, and cerebellum: −25, −38, and −42% for quitters and −19, −21, −20% for non-quitters), respectively. Furthermore, correlations were found between reductions in cigarettes per day and decreases in V_S/f_P for two of the three ROIs studied ($r = 0.33$, $P = 0.02$ for the brainstem, $r = 0.37$, $P = 0.01$ for the cerebellum, and $r = 0.18$, $P = 0.22$ for the prefrontal cortex).

For completeness, a statistical analysis with the same general structure as above was performed for V_S/f_P values for the thalamus (the region without significant smoking-induced upregulation of nAChRs). In this analysis, change in V_S/f_P from pre- to post-treatment, difference between quitters and non-quitters with treatment, and effect of treatment type were not significant (F (1,41) = 3.7, F (1,41) = 0.1, and F(2,41) = 0.3, respectively, all *P*-values NS).

Table 1 Demographics and Rating Scale Scores for Overall Study Sample ($n = 48$) and Subgroups Based on Treatment Type

Variable	Study sample ($n = 48$)	Cognitive-behavioral therapy subgroup ($n = 16$)	Bupropion-treated subgroup ($n = 18$)	Placebo-treated subgroup ($n = 14$)
Age	42.8 (±13.7)	51.6 (±12.3)[a]	35.5 (±11.6)	42.1 (±12.5)
Gender (% female)	27.1	18.8	38.9	21.4
Ethnicity (% Caucasian)	54.2	37.5	55.5	71.4
Education (years)	14.5 (±1.9)	14.4 (±2.5)	14.3 (±1.4)	14.9 (±2.0)
Hamilton Anxiety Rating Scale	2.1 (±2.6)	3.0 (±3.4)	1.4 (±2.0)	1.9 (±2.0)
Hamilton Depression Rating Scale	1.9 (±2.3)	2.4 (±3.2)	1.4 (±1.7)	2.0 (±1.8)
Beck Depression Inventory	1.8 (±2.0)	2.1 (±2.6)	1.5 (±1.5)	2.0 (±2.6)
Fagerström Test for Nicotine Dependence	4.4 (±2.0)	5.3 (±2.3)	3.9 (±1.5)	4.0 (±2.1)
Quit attempts lifetime	2.7 (±2.5)	2.9 (±2.4)	2.2 (±2.3)	3.1 (±2.9)
Cigarettes per day pre-treatment	19.6 (±4.0)	20.4 (±4.5)	18.7 (±4.2)	19.9 (±3.1)
Cigarettes per day post-treatment	9.2 (±8.0)[b]	5.4 (±6.8)[c]	12.0 (±7.7)	10.0 (±8.5)
Percentage of change in cigarettes per day with treatment	−54.2 (±38.1)	−76.0 (±29.6)	−38.2 (±35.0)	−49.9 (±41.0)
Exhaled CO pre-treatment	17.7 (±9.1)	17.3 (±7.2)	18.1 (±9.3)	17.6 (±11.1)
Exhaled CO post-treatment	8.4 (±8.9)[d]	5.6 (±5.0)	9.6 (±9.9)	9.9 (±10.9)
Percentage of change in exhaled CO with treatment	−51.0 (±39.1)	−59.3 (±39.6)	−50.7 (±40.7)	−42.1 (±37.2)

All values are presented as mean (±SD) or percentages.
[a]$P < 0.01$ between subgroups (ANOVA).
[b]$P = 0.001$ for change in cigarettes per day for the study sample from pre- to post-treatment.
[c]$P < 0.01$ for the between-subgroups change in cigarettes per day from pre- to post-treatment.
[d]$P < 0.05$ for change in exhaled carbon monoxide (CO) from pre- to post-treatment for the study sample.

Table 2 Specific Binding Volumes of Distribution (V_S/f_P) in Brain Regions of Interest Pre- and Post-Treatment

Brain region	V_S/f_P values—smoker group ($n=48$)	V_S/f_P values—CBT-treated smokers ($n=14$)	V_S/f_P values—bupropion-treated smokers ($n=18$)	V_S/f_P values—placebo-treated smokers ($n=16$)
Pre-treatment prefrontal cortex	5.4 (±2.3)	5.8 (±2.0)	5.3 (±3.0)	5.2 (±1.6)
Post-treatment prefrontal cortex	4.1 (±1.8)	4.0 (±1.7)	4.0 (±2.0)	4.3 (±1.8)
Percentage of Δ prefrontal cortex	−20 (±36)*	−28 (±28)	−18 (±40)	−15 (±39)
Pre-treatment brainstem	9.8 (±3.5)	10.4 (±2.5)	9.8 (±4.5)	9.4 (±3.0)
Post-treatment brainstem	7.2 (±2.9)	6.8 (±2.0)	7.6 (±3.1)	7.1 (±3.7)
Percentage of Δ brainstem	−25 (±26)**	−32 (±22)	−19 (±22)	−25 (±35)
Pre-treatment crebellum	7.6 (±2.7)	8.5 (±2.0)[a]	7.3 (±3.6)	7.0 (±1.8)
Post-treatment cerebellum	5.5 (±2.4)	5.4 (±1.5)	5.7 (±2.6)	5.6 (±3.1)
Percentage of Δ cerebellum	−25 (±31)	−33 (±25)	−21 (±26)	−21 (±42)

All values are mean ± SD; *$P\leqslant 0.05$, **$P\leqslant 0.01$, repeated-measures analyses of covariance for the study sample from pre- to post-treatment with V_S/f_P for the brain regions as the repeated measures, treatment group and quit status as between-subject factors, and age as a covariate.
[a]$P\leqslant 0.05$ for cerebellar V_S/f_P value differences before treatment between CBT-treated and placebo-treated smokers (uncorrected). No other between-group differences were significant.

Associations between nAChR Densities and Subjective Symptoms: Exploratory Analyses

Correlations were found between change in pre- to post-treatment V_S/f_P values and change in ratings of smoking-related reward for the prefrontal cortex ($r=0.30$, $P<0.05$), brainstem ($r=0.44$, $P<0.01$), and cerebellum ($r=0.39$, $P<0.01$), indicating that diminished nAChR density was associated with a decreased subjective sense of the rewarding properties of smoking. Similarly, V_S/f_P values and ratings of cigarette taste were correlated for the brainstem ($r=0.28$, $P=0.05$), with similar (but nonsignificant) directional associations for the prefrontal cortex ($r=0.18$, NS) and cerebellum ($r=0.26$, $P=0.08$). Associations between nAChR density and UTS craving scores were not significant (r's ranged from -0.19 to -0.11).

For the POMS, negative correlations were found between changes in V_S/f_P values and anger/hostility for the prefrontal cortex ($r=-0.38$, $P<0.01$), brainstem ($r=-0.41$, $P<0.005$), and cerebellum ($r=-0.32$, $P<0.05$), indicating that greater reduction in nAChR densities was associated with increased levels of anger/hostility. No other correlations between V_S/f_P values and other subscales on the POMS or SJWS were found.

Associations between Pre-Treatment Variables and Quit Status: Exploratory Analyses

As expected, smoking fewer cigarettes per day at baseline was associated with a greater likelihood of quitting (Table 3). Similarly, there were statistical trends for lower pre-treatment cigarette-related symptoms (FTND, UTS craving scale, SJWS craving subscale, SJWS physical symptom subscale, and ratings of taste and reward) to be associated with a better chance of quitting. Associations between pre-treatment V_S/f_P values and quit status did not reach significance, but results gave trend-level indications that lower nAChR density in all three brain regions

Table 3 Pre-Treatment Predictors of Quitting Smoking (Binary Logistic Regression Analyses)

Pre-treatment variable	Quitters (mean ± SD)	Non-quitters (mean ± SD)	χ^2	*P*-value
Cigarettes/day	17.8 ± 3.1	20.3 ± 4.0	4.8	0.03
FTND	4.3 ± 1.7	5.3 ± 2.9	2.3	0.13
UTS craving scale score	2.7 ± 1.5	3.4 ± 1.6	2.4	0.12
SJWS craving	31.3 ± 12.7	37.6 ± 10.8	2.7	0.09
SJWS psychological distress	18.1 ± 8.5	17.4 ± 7.6	0.1	0.77
SJWS physical symptoms	8.4 ± 4.7	6.4 ± 3.1	3.1	0.08
SJWS stimulation	10.0 ± 4.8	10.5 ± 5.3	0.1	0.75
SJWS appetite	9.3 ± 3.0	8.6 ± 2.8	0.6	0.46
Taste of a cigarette	5.4 ± 2.3	6.7 ± 2.3	3.0	0.08
Reward of a cigarette	6.2 ± 2.2	7.2 ± 1.9	2.6	0.10
Prefrontal cortex V_S/f_P	4.8 ± 2.0	5.8 ± 2.4	2.0	0.16
Brainstem V_S/f_P	8.5 ± 2.7	10.5 ± 3.7	3.5	0.06
Cerebellum V_S/f_P	6.7 ± 2.2	8.0 ± 2.9	2.4	0.12

Abbreviations: FTND, Fagerström Test for Nicotine Dependence; UTS, Urge to Smoke; V_S/f_P, specific binding volume of distribution.

(prefrontal cortex, brainstem, and cerebellum) were associated with a greater likelihood of quitting.

DISCUSSION

Smoking reduction and cessation with commonly used treatments led to decreases in $\alpha_4\beta_2$* nAChR densities across brain regions. These reductions moved smokers toward normal $\alpha_4\beta_2$* nAChR levels found in non-smokers. No associations between $\alpha_4\beta_2$* nAChR densities and treatment type were found, but significant associations were discovered between the extent of $\alpha_4\beta_2$* nAChR density reduction and the amount of decreased cigarette usage. These results indicate that $\alpha_4\beta_2$* nAChR density is strongly linked to the number of cigarettes smoked per day, but not to the treatment type being administered, and are consistent with our previous study of untreated smokers (Brody *et al*, 2013) and other studies linking nicotine (Marks *et al*, 2011; Yates *et al*, 1995; Zhang *et al*, 2002) and cigarette smoke (Mamede *et al*, 2007; Mukhin *et al*, 2008; Staley *et al*, 2006; Wullner *et al*, 2008) exposure with upregulation of $\alpha_4\beta_2$* nAChRs in brain regions other than the thalamus. Because education about the biological effects of smoking and quitting smoking are standard parts of smoking-cessation psychotherapy (Fiore *et al*, 2008), the additional information found here could prove useful in treatment of smokers by letting them know that brain receptor changes found with regular smoking tend toward normalization with smoking reduction and cessation.

In the exploratory analyses, decreases in $\alpha_4\beta_2$* nAChR levels with treatment were associated with decreases in the perceived rewarding properties and taste of cigarettes, along with increased anger/hostility. The findings with reward/taste are strongly supported by studies of laboratory animals in which $\alpha_4\beta_2$* nAChR agonism has been linked with the rewarding properties of nicotine (McGranahan *et al*, 2011), while $\alpha_4\beta_2$* nAChR antagonism has been found to block nicotine's rewarding properties (Tobey *et al*, 2012; Walters *et al*, 2006). Similarly, a study of α_4 mutant knockout mice found that these mice did not have nicotine-elicited increases in dopamine levels (Marubio *et al*, 2003), which is highly consistent with our finding that decreases in $\alpha_4\beta_2$* nAChR levels were associated with diminished reward from cigarettes (presumably mediated at least in part through dopamine release). Additionally, it is noted that nicotine replacement therapy (commonly used for smoking cessation treatment) decreases the rewarding properties of smoking (Levin *et al*, 1994) and would be expected to maintain upregulation of $\alpha_4\beta_2$* nAChRs. Taken together, these findings indicate that the rewarding property of smoking and perceived taste of cigarettes are related to the number of available $\alpha_4\beta_2$* nAChRs, though the exact nature of the relationship between these symptoms and $\alpha_4\beta_2$* nAChR density remains to be verified. As for anger/hostility, we are not aware of studies specifically linking nAChR density with this state; therefore, this finding may represent an epiphenomenon and needs verification by prospective work focusing on this variable.

For the exploratory analyses of pre-treatment predictors of quitting smoking, a lower number of cigarettes per day was associated with a greater likelihood of quitting, which is consistent with previous research (Batra *et al*, 2008; Dale *et al*, 2001; Hymowitz *et al*, 1997; Japuntich *et al*, 2011; Kozlowski *et al*, 1994; Paluck *et al*, 2006; Westman *et al*, 1997). Interestingly, a number of other measures (including brain nAChR levels) that could be thought of as being associated with severity of nicotine dependence had trend-level associations with treatment outcome. Although not reaching significance, FTND scores, craving/withdrawal scores, and reward/taste scores were lower at baseline in those smokers who quit with treatment compared with those who did not quit. Similarly, smokers with lower baseline nAChR densities (presumably the result of less severe nAChR upregulation) showed a trend toward being more likely to quit. The prediction of treatment response with pre-treatment nAChR densities is highly consistent with pharmacogenetic studies, which found associations between response to first-line medications for smoking cessation and genetic variability in neuroreceptors (nAChRs and DRD1 receptors) and nicotine metabolism (Bergen *et al*, 2013; King *et al*, 2012; Lee *et al*, 2012).

A central limitation of the study was sample size. Although this study was relatively large for a PET study of this type (with 110 PET scanning sessions being analyzed), natural variability in treatment response within this small sample likely resulted in the relatively high response rate with pill placebo compared with bupropion (and the unexpectedly high quit rate with CBT). Furthermore, the trend-level findings in several of the exploratory analyses indicate that a larger sample size might provide an enhanced ability to differentiate responses to active *vs* placebo treatment and to elucidate the extent to which nAChR levels can be used to predict treatment response. A larger sample size would also allow for greater power to determine nAChR differences based on sex, as has been reported previously in smokers (Cosgrove *et al*, 2012). Additionally, it should be noted that bupropion HCl acts as a noncompetitive nAChR antagonist (Slemmer *et al*, 2000), which may have affected radiotracer binding or nAChR normalization, though the absence of between-group differences in normalization indicates against a specific effect of bupropion.

In summary, this PET study demonstrated movement toward normalization of nAChR upregulation with standard treatments for smoking. This diminished upregulation was strongly correlated with decreases in smoking levels. Exploratory analyses indicated that decreases in nAChR densities were associated with diminished taste/reward of smoking a cigarette and increased anger/hostility and that pre-treatment clinical and brain imaging findings may result in the ability to predict who will respond to treatment.

ACKNOWLEDGEMENTS

This study was supported by the National Institute on Drug Abuse (ALB (R01 DA20872)), the Tobacco-Related Disease Research Program (ALB (19XT-0135)), and the Department of Veterans Affairs, Office of Research and Development (CSR&D Merit Review Award I01 CX000412 (ALB)). The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

DISCLOSURE

ALB and MAM were previously co-investigators on a grant funded by Pfizer, Inc. that was unrelated to this study. AGM is a co-investigator on a grant from Phillip Morris, Inc. for research unrelated to this study. All the other authors report no biomedical financial disclosures or potential conflicts of interest.

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