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Reduced β_2^* -Nicotinic Acetylcholine Receptor Availability in Smokers with Schizophrenia

Deepak Cyril D'Souza, M.D.^{1,2,3}, Irina Esterlis, Ph.D.^{1,3}, Michelle Carbutto, B.A.^{1,2,3}, Maegan Krasenics, B.A.^{1,2,3}, John Seibyl, M.D.^{1,4}, Frederic Bois, Ph.D.^{1,3}, Brian Pittman, M.S.^{2,3}, Mohini Ranganathan, M.D.^{1,2,3}, Kelly Cosgrove, Ph.D.^{1,3}, and Julie Staley, Ph.D.^{1,2,3}

¹Psychiatry Service, VA Connecticut Healthcare System, West Haven, CT, USA

²Abraham Ribicoff Research Facilities, Connecticut Mental Health Center, New Haven, CT, USA

³Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA

⁴Institute for Neurodegenerative Disorders, New Haven, T 06510, USA

Introduction

Individuals with schizophrenia have the highest rates of comorbid cigarette smoking (58–88%) compared with the general population (~ 23%)(1-4). The association between schizophrenia and tobacco smoking is stronger even after controlling for confounds such as institutionalization, socioeconomic status and medications (4-6). Smokers with schizophrenia smoke more cigarettes and favor stronger cigarettes suggesting that they are more dependent on nicotine than other smokers reviewed in (4). They also appear to extract more nicotine from cigarettes than smokers without mental illness as suggested by some (7, 8) but not all studies (9). The odds that smokers with schizophrenia will become heavy smokers are significantly greater than those of smokers from the general population reviewed in(4). Furthermore, a number of studies suggest that the risk for developing to schizophrenia is associated with higher rates of smoking (4, 5, 10). Finally, smoking cessation rates for schizophrenia patients are significantly lower than the general population and, to a lesser extent, in smokers with other mental illnesses reviewed in(4).

Nicotine, the primary addictive chemical in tobacco smoke, initiates its effects in the brain through nicotinic acetylcholine receptors. Nicotinic acetylcholine receptors containing β_2 subunits are some of the most abundant in the brain, have the highest affinity for nicotine, and are the most common subtype in the striatal reward pathways. The β_2^* -nicotinic acetylcholine receptors are a critical neural substrate mediating the primary reinforcing effects of nicotine in the brain (11). Repeated exposure to nicotine or smoking has been consistently shown, in preclinical, post-mortem human and human imaging studies, to result in the upregulation of nicotinic acetylcholine receptors, i.e., increased availability,

Correspondence: Deepak Cyril D'Souza Psychiatry Service 116A, VA Connecticut Healthcare System, 950 Campbell Avenue, West Haven, CT 06516 Phone: (203) 932-5711 x2594 Fax: (203) 937-4860 deepak.dsouza@yale.edu.

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throughout the brain (12-19). The increases in β_2^* -nicotinic acetylcholine receptor binding sites following chronic nicotine treatment has been shown to result from an increase in the number of nicotinic acetylcholine receptors containing β_2 subunit proteins (20).

Post mortem evidence suggests alterations in β_2^* -nicotinic acetylcholine receptors in schizophrenia (21, 22). Smokers with schizophrenia showed lower numbers of β_2^* -nicotinic acetylcholine receptors compared to comparison smokers in several brain regions including the hippocampus, cortex, and striatum (21). Furthermore, there was no difference in numbers of β_2^* -nicotinic acetylcholine receptors in these same regions between smokers and nonsmokers with schizophrenia (21), suggesting a failure to upregulate in smokers with schizophrenia. Finally, there is a link between the genes that encode the α_4 and β_2 subunits of the nicotinic acetylcholine receptor and heavy smoking in individuals with schizophrenia (23).

The availability of β_2^* -nicotinic acetylcholine receptors *in vivo* can be measured with [^{123}I]-5-IA-5380 ([^{123}I]-5-IA) and single photon emission computed tomography (SPECT). ([^{123}I]-5-IA binds to β_2 -containing nicotinic acetylcholine receptors at the interface between the α_4 and β_2 subunits. While it has been traditionally thought that nicotinic acetylcholine receptors containing two α_4 and three 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 , β_3) may combine with the α_4 and β_2 subunits and they may also play a role in upregulation of the receptor (24). Therefore, to allow for the possibility of other β_2 containing subunit combinations, e.g., $\alpha_4\beta_2\alpha_5$, we denote this as β_2^* - nicotinic acetylcholine receptor to identify these as β_2 -containing nicotinic acetylcholine receptors. [^{123}I]-5-IA has low nonspecific binding (25), acceptable dosimetry in human subjects with high brain uptake (26, 27), and high test-retest reproducibility (28). Imaging with [^{123}I]-5-IA SPECT in nonhuman primates and human subjects results in a binding pattern that is consistent with the established regional distribution of the β_2^* -nicotinic acetylcholine receptors and is highest in the thalamus and intermediate throughout the cortex and cerebellum (29). Using [^{123}I]-5-IA SPECT we have previously shown significantly higher β_2^* -nicotinic acetylcholine receptor availability in the striatum, cerebellum and throughout the cortex in recently abstinent smokers compared to nonsmokers (30) and that it takes 6-12 weeks for β_2^* -nicotinic acetylcholine receptor availability to normalize to nonsmoker comparison levels (18).

The primary goal of this study was to compare β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia relative to comparison smokers. Based on post-mortem findings (21, 22), smokers with schizophrenia were hypothesized to exhibit lower β_2^* -nicotinic acetylcholine receptor availability relative to age and sex-matched healthy comparison smokers. A secondary goal of the study was to examine the relationship between β_2^* -nicotinic acetylcholine receptor availability, the symptoms of schizophrenia and tobacco smoking craving and withdrawal.

Methods

Regulatory approvals

This study was conducted with the approval of the institutional review boards of the Yale University School of Medicine and the Veterans Affairs Connecticut Healthcare System. The use of the radioactive isotope was approved by the Yale Radiation Safety Committee. Magnetic Resonance Imaging (MRI) was performed with the approval of the Yale Magnetic Resonance Research Center. The radiotracer was administered under IND 61,156.

Subjects

Tobacco smokers with a diagnosis of DSM-IV schizophrenia (31) and nicotine dependence were compared to age and gender matched comparison smokers selected from a group of healthy tobacco smokers who were being studied concurrently. The comparison smokers were medically and psychiatrically healthy and did not have any lifetime diagnosis of substance abuse or dependence (excluding nicotine and caffeine).

Consent process in smokers with schizophrenia

Before signing the consent form, subjects had several meetings with study staff where detailed information was provided (see supplemental section) to ensure that they understood the study and were suitable candidates. Subjects were required to pass a questionnaire about the key risks of the study. Parents and family and non-research clinicians were involved in the process when available. The patient's primary clinician (i.e. non-research clinician) was required to assent to patient's participation.

Screening procedures in smokers with schizophrenia

Subjects underwent a medical examination by a research physician to exclude any major, unstable medical issues or neurological disorders as described in the supplemental section.

Inclusion/Exclusion Criteria for Smokers with Schizophrenia

Only subjects with a primary diagnosis of schizophrenia were included. Diagnosis was confirmed by Structured Clinical Interview for DSM-IV conducted by a research assistant and evaluation by research psychiatrist. Subjects with a diagnosis of substance abuse within the past month or substance dependence (with the exception of nicotine or caffeine) within the past 6 months prior to screening evaluation were excluded. Smoking status was confirmed by plasma cotinine levels > 150 ng/mL, urine cotinine levels > 100 ng/mL, and carbon monoxide levels > 11 parts per million at baseline. Nicotine dependence was evaluated using the Fagerström Test for Nicotine Dependence (32). Subjects were also screened to ensure clinical stability, which included a chart review and review of medications and evaluation by a research psychiatrist. Subjects who were deemed clinically unstable as evidenced by recent psychiatric hospitalization or emergency room visits, increase in clinic visits due to psychiatric symptoms, homicidality, suicidality, and/or grave disability were excluded. Subjects needed to be taking stable doses of antipsychotic medications for at least 12 weeks. Benzodiazepines were permitted on a PRN basis. Subjects taking tricyclic antidepressants, anticholinergics or selective serotonin reuptake inhibitors were excluded due to some evidence that these drugs may interfere with [¹²³I]5-IA binding.

Subjects were screened for any MRI exclusions, such as ferrous metal in the body, cardiac pacemaker or severe claustrophobia. If a potential subject had any experience working with metal or had any eye injury involving metal, orbital x-rays were performed.

Smoking Cessation

Subjects were helped to abstain from smoking for at least 5 days prior to the SPECT scan day using brief behavioral counseling based on Clinical Practice Guidelines and contingency management. Approximately 1 week of tobacco smoking abstinence is required for nicotine to clear from the brain in order to not interfere with [¹²³I]5-IA binding (19, 30). Eligible smokers with schizophrenia were hospitalized on the Clinical Neuroscience Research Unit (CNRU) of the Connecticut Mental Health Center a smoke-free unit, for the purposes of initiating, maintaining and confirming nicotine abstinence. Subjects were not allowed to use any drugs that could facilitate smoking abstinence including nicotine replacement products (NRTs), bupropion or varenicline. Prior to abstaining from nicotine, subjects were advised

about the risks and benefits of quitting smoking and told that they would be monitored daily to ensure abstinence. A trained member of the research team met with subjects daily to counsel on strategies to cope with withdrawal symptoms and approaches to quit long-term. Counseling was paired with contingency management: subjects were paid \$25 for the first day of inpatient abstinence, and payments were escalated by \$25 every inpatient day as described in greater detail in the supplemental section. If subjects were able to complete the inpatient abstinence period prior to the scan, they were eligible to receive \$800. Abstinence from smoking or any other nicotine products was confirmed by daily monitoring of breath carbon monoxide measurements and dipstick-urinary cotinine levels [NicAlert, (Nymox)]. At the end of the study subjects were provided with referrals to obtain smoking cessation treatment if they expressed a desire to quit smoking long-term.

Nicotine craving and withdrawal were evaluated using the Tiffany Urge to Smoke Questionnaire (33) and Minnesota Nicotine Withdrawal Questionnaire (34), respectively, at intake, during smoking cessation, and on SPECT scan day. Positive, negative, and general symptoms of psychosis were measured by the Positive and Negative Syndrome Scale (PANSS) (35) and the Scale for the Assessment of Negative Symptoms (SANS) (36). Depressive symptoms were assessed by the Montgomery-Asberg Depression Scale (MADRS) (37) and involuntary movements by the Abnormal Involuntary Movements Scale (AIMS)(38).

SPECT Scanning

After achieving abstinence for at least 5 days, subjects participated in one [^{123}I]5-IA SPECT scan as described previously (39). [^{123}I]5-IA was prepared by iododestannylation to give a product with radiochemical purity of >90%. Blood samples were collected and analyzed as described previously (40). In the hour preceding the radiotracer administration, all subjects received a 0.6-g saturated solution of potassium iodide or potassium perchlorate tablet (for subjects allergic to shell fish) to protect their thyroid. [^{123}I]5-IA was administered (~10mCi, IV) using the bolus plus constant infusion paradigm with a B/I ratio of 7.0 h. SPECT images were acquired using the three-headed PRISM 3000XP (Picker, Cleveland, Ohio) equipped with low energy ultra high-resolution (LEUHR) fan beam collimators. In order to perform non-uniform attenuation correction, a simultaneous emission transmission protocol (STEP) was acquired with a 20mCi [^{57}Co] line source at 5.5 h. Three emission scans (30 min each) were obtained between 6-8 h of infusion (time necessary for radiotracer to achieve equilibrium in the brain). Plasma samples were collected at the midpoint of second scan to quantify total free parent in plasma (40) so that inter-individual differences in metabolism and protein binding of [^{123}I]5-IA can be corrected (41).

MRI Scan

Each subject participated in one MRI scan prior to SPECT scanning. MRI was performed on a Signa 1.5T system (General Electric Co, Milwaukee, Wisconsin). Axial images were acquired parallel to the anteroposterior commissural line with an echo time of 5 milliseconds, repetition time of 24 milliseconds, matrix 256×192 , number of excitations of 1, field of view of 24 cm, and 128 contiguous slices with a thickness of 1.3 mm.

SPECT Image Processing

SPECT emission images were analyzed as described previously (39). SPECT emission images were filtered using a 3-dimensional (3D) Butterworth filter (order 10, cutoff frequency 0.24 cycle/pixel) and reconstructed using a filtered back projection algorithm with a ramp filter on a 128×128 matrix to obtain 50 slices with a pixel size of $2.06 \times 2.06 \times 3.56$ mm in the x-, y-, and z-axes. MRIs were coregistered to the SPECT images to provide an

anatomical guide for placement of the regions of interest using Medx (version 3.4) software (Medical Numerics, Inc., MD). A standard 3D volume of interest (VOI) was generated for each region and transferred to the coregistered SPECT image to determine regional radioactive densities. Regions of interest (ROIs) chosen were those known to contain β_2^* -nicotinic acetylcholine receptors and included frontal, parietal, anterior cingulate, temporal and occipital cortices, thalamus, striatum (an average of caudate and putamen), and cerebellum. The average of two raters is used for all analyses with interrater variability being <10% between raters across regions.

Regional β_2^* -nicotinic acetylcholine receptor availability was determined by V_T/f_p ((regional activity (kilobecquerel[kBq]/cc)/plasma free parent (kBq/mL)) (42), a highly reproducible outcome measure (39). V_T/f_p corrects for possible differences in radiotracer metabolism and plasma protein binding between groups and subjects, and is referred to as β_2^* -nicotinic acetylcholine receptor availability because only receptors that are “available” to be bound by the radiotracer are measured. Receptors that are already occupied (i.e., by residual nicotine, by a pharmacologically active metabolite [cotinine or nornicotine], or by endogenous neurotransmitter [acetylcholine]) are not available. Therefore, receptor availability is not a measure of all receptors. 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 subjects or comparison groups.

Statistical Analyses

Statistical analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC) and SPSS version 16.0 (SPSS Inc. Headquarters, Chicago, IL). Independent t-tests were used to examine demographic variables (e.g., smoking characteristics) between diagnostic groups. Multivariate analysis of variance (MANOVA) models examined differences in receptor availability (V_T/f_p) between patients and comparison smokers in brain regions (frontal and parietal cortices, striatum, hippocampus and thalamus) chosen a priori based on the post-mortem literature.

PANSS, MADRS, SANS and AIMS data in smokers with schizophrenia were examined descriptively using means, standard deviations and graphs. Each outcome was tested for normality using Kolmogorov-Smirnov test statistics and normal probability plots. The outcomes were approximately normal and assessed using linear mixed models with time (baseline day and scan day) included as a within-subjects explanatory factor. Regional β_2^* -nicotinic acetylcholine receptor availability was correlated with smoking characteristics (packs per day, Fagerström score, years smoked, craving Tiffany [desire/relief], withdrawal), in both groups and measures of schizophrenia (PANSS, SANS) and depression (MADRS) in smokers with schizophrenia with Spearman's rho correlation coefficients. All results were considered statistically significant at $P < 0.05$.

RESULTS

Fourteen smokers with schizophrenia were enrolled but two were not scanned; one failed to achieve smoking abstinence and one could not tolerate inpatient hospitalization. Demographics of the remaining 12 are presented in table 1. Though both male and female smokers were recruited, the only female subject who enrolled was unable to remain abstinent for the scan. The period of smoking abstinence varied from 5-7 days (5.57 ± 0.65) due to scan and inpatient scheduling constraints. An additional subject was not included in the analysis because his β_2^* -nicotinic acetylcholine receptor availability data was >3 SD

above the mean. Data from the remaining 11 subjects were compared to age and gender-matched comparison smokers who were selected from a sample of subjects who were being concurrently scanned with [^{123}I]5-IA SPECT (Table 2). At intake (table 2), smokers with schizophrenia reported greater nicotine dependence (Fagerström score; 6.3 ± 2.1 vs. 4.2 ± 3.0) and smoked a greater number of cigarettes per day than comparison smokers (21.9 ± 8.4 vs. 16.9 ± 4.9), although these did not reach statistical significance (Fagerström score: $t(20)=1.96$, $p=0.06$, cigarettes per day: $t(20)=1.71$, $p=0.10$).

Confirmation of Smoking Abstinence

Breath carbon monoxide ($F(1, 10)= 26.35$, $p = 0.0004$) and urine cotinine levels ($F(1, 10)= 87.91$, $p < 0.0001$) declined over time, confirming that all smokers with schizophrenia were abstinent from tobacco smoking for at least 5 days prior to scanning (supplemental Fig. 1). Furthermore, both plasma and urine cotinine levels declined over time and there were no differences between smokers with schizophrenia and comparison smokers in either urine [$(F(1, 20)= 0.22$, $p = 0.65)$, time ($F(1, 12)= 252.3$, $p < 0.0001$), group $\times$ time ($F(1, 20)= 0.08$, $p = 0.78)$] or plasma cotinine [group ($F(1, 17)= 0.71$, $p = 0.41$), time ($F(1, 17)= 36.5$, $p < 0.0001$), group $\times$ time ($F(1, 17)= 1.25$, $p = 0.28)$] levels (supplemental figure 2).

β_2^* -nicotinic acetylcholine receptor availability

There was a significant omnibus MANOVA effect of group (Hotelling's Trace = 1.09, $p=0.025$). Smokers with schizophrenia had significantly lower β_2^* -nicotinic acetylcholine receptor availability in the parietal (22%, $F(1, 20)=4.7$; $p=.04$) and frontal (26%, $F(1, 20)=9.4$, $p=.006$) cortices and thalamus (21%, $F(1, 20)=5.5$, $p=.03$). However, there were no significant differences in striatum (17%; $F(1, 20)=2.9$, $p=0.10$) and hippocampus (13%; $F(1, 20)=1.6$, $p=0.22$) (Figure 1; table 3).

Smoking Behaviors—In smokers with schizophrenia, there were no statistically significant changes in scores on the Tiffany desire for smoking subscale ($F(6, 54)= 0.72$, $p = 0.63$), the Tiffany relief from smoking subscale ($F(6, 54)= 1.23$, $p = 0.3$) or the Minnesota Questionnaire for smoking withdrawal ($F(6, 54)= 0.2$, $p = 0.97$) from the baseline state (smoking as usual) to the day of the SPECT scan (supplemental figure 3). On the day that subjects were scanned, smokers with schizophrenia reported greater desire to smoke cigarettes ($t = -2.97$, $p = 0.01$) and greater expected relief from smoking ($t = -2.82$, $p = 0.01$) (supplemental figure 4). There were no significant differences ($F=.85$, $p=.37$) in withdrawal symptoms in smokers with schizophrenia (4.6 ± 4.9) and comparison smokers (2.7 ± 4.8). All but one smoker with schizophrenia resumed smoking within minutes of completing all the study procedures.

Depression, schizophrenia symptoms, negative symptoms and dyskinesia

There were no statistically significant changes in total PANSS scores ($F(1,10)= 2.14$, $p = 0.17$), MADRS scores ($F(1,10)= 0.58$, $p = 0.46$) or SANS scores ($F(1,10)= 0.14$, $p = 0.72$) (supplemental figure 5, supplemental table 1) between baseline (smoking as usual state) and the day of the SPECT scan (5 or more days of smoking abstinence) among smokers with schizophrenia. Furthermore, while there were no changes in PANSS positive symptoms ($F(1,10)= 2.91$, $p = 0.12$), there was a reduction in PANSS negative symptoms ($F(1,10)= 5.71$, $p = 0.038$) and an increase in PANSS general symptoms ($F(1,10)= 6.1$, $p = 0.033$) with smoking abstinence. Total AIMS scores increased significantly over time ($F(1,10)= 5.71$, $p = 0.038$).

Relationship between β_2^* -nicotinic acetylcholine receptor availability and nicotine dependence

There were no significant correlations between the number of cigarettes smoked per day or nicotine dependence and β_2^* -nicotinic acetylcholine receptor availability in either smokers with schizophrenia or comparison smokers.

Relationship between β_2^* -nicotinic acetylcholine receptor availability and negative symptoms

There were significant negative correlations between β_2^* -nicotinic acetylcholine receptor availability in the parietal cortex, frontal cortex, thalamus, striatum and hippocampus, and negative symptoms of schizophrenia assessed after smoking abstinence (figure 2). This was evident with both the negative symptoms subscale of the PANSS and the SANS (table 4). Finally, negative symptoms assessed at baseline also negatively correlated with β_2^* -nicotinic acetylcholine receptor availability in the parietal cortex (Pearson's $r = -0.61$, $p = 0.046$). There were negative correlations with the frontal cortex, temporal cortex, striatum and thalamus (Pearson's $r = -0.57$ to -0.52) but these relationships did not reach statistical significance ($p = 0.05$ to 0.09).

Discussion

This report provides the first *in vivo* evidence of lower β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia relative to comparison smokers during early abstinence. The magnitude of group differences range from 13-25% (effects size of 0.32-0.08) with the largest differences in descending order observed in the frontal cortex, parietal cortex, thalamus, striatum and hippocampus. These *in vivo* findings are consistent with the post-mortem findings of lower β_2^* -nicotinic acetylcholine receptors in smokers with schizophrenia compared to comparison smokers (21, 22, 43), except for thalamus which failed to show significant group differences in post-mortem studies. Importantly, this study demonstrates an inverse correlation between negative symptoms and β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia, i.e., those individuals with lower β_2^* -nicotinic acetylcholine receptor availability had greater negative symptoms.

Lower β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia may reflect altered receptor functionality. Specifically, higher β_2^* -nicotinic acetylcholine receptor availability in tobacco smokers likely represents greater numbers of desensitized and inactivated nicotinic acetylcholine receptors (44, 45). The lower β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia may be related to abnormal desensitization or turnover of nicotinic acetylcholine receptors. It should be noted that nicotine-induced desensitization is one of the major mechanisms for nicotine addiction (46-48). Since maintained nicotinic acetylcholine receptor desensitization may be important for relieving nicotine withdrawal in human smokers (49), the reduced β_2^* -nicotinic acetylcholine receptors availability observed in smokers with schizophrenia might explain why they are more likely to be addicted to nicotine.

The reduced β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia may also reflect a deficit in nicotine-induced upregulation of high affinity nicotinic acetylcholine receptors as suggested by post-mortem studies. However, that conclusion cannot be drawn from the current study. Future *in vivo* studies comparing schizophrenic smokers to schizophrenic non-smokers and ex-smokers will need to be conducted.

Underlying differences in the β_2 gene (CHRNA2) may also explain lower nicotinic receptor availability in smokers with schizophrenia. In a family-based association study of a

Canadian sample, CHRNA4 combined with the α_4 gene (CHRNA4) may be linked to schizophrenia (50). However, there was no significant association between CHRNA4 and CHRNA4 and CHRNA4, and schizophrenia in a Japanese population (51). CHRNA4 and CHRNA4 have also associated been with smoking among schizophrenics (23, 52).

Relationship between Negative Symptoms and β_2^* -nicotinic acetylcholine receptor availability

The relationship between β_2^* -nicotinic acetylcholine receptor availability and negative symptoms is specific and robust. Those individuals with lower β_2^* -nicotinic acetylcholine receptor availability had greater negative symptoms. This relationship was consistent across two separate measures of negative symptoms: the SANS and PANSS. There was no such relationship with depression or positive symptoms. The current finding of a negative correlation between β_2^* -nicotinic acetylcholine receptor availability and negative symptoms is consistent with the observation in some studies that the heaviest smokers with schizophrenia had the lowest number of negative symptoms (53). The literature on the relationship between nicotine dependence and symptoms is mixed; one study reported a link to both negative and positive symptoms, four found associations with one type of symptoms but not the others (53-56), and another four did not find any significant relationship (57-60). Smoking high nicotine cigarettes has been shown to decrease negative symptoms more than denicotinized cigarettes and these effects are specific, since no effects were observed on positive symptoms, depression or anxiety (61). Nicotine, which has been shown to increase burst firing of dopaminergic neurons and dopamine release (62-64), may reduce negative symptoms by correcting the cortical hypodopaminergia that possibly mediates negative symptoms (65). Thus, the present finding of a negative association between receptor availability and negative symptoms may contribute to understanding the high smoking rates in schizophrenia and the relationship between smoking and negative symptoms in this population. Finally, in the absence of any approved treatments for negative symptoms the results of this study should provide the impetus to test treatments based on a β_2^* -nicotinic acetylcholine receptor mechanism for negative symptoms.

Effect of Smoking Cessation on Symptoms

There was a small increase in dyskinesia which is not considered clinically significant and is well within the margin of test-retest variability of the Abnormal Involuntary Movements Scale (AIMS). Smoking cessation and short-term abstinence had no significant effects on positive symptoms, negative symptoms or depression ratings. The data from this small sample do not support the “self-medication” hypothesis according to which individuals with schizophrenia smoke to alleviate positive and negative symptoms and depression. Nevertheless, most smokers with schizophrenia resumed smoking immediately after completing the SPECT scan. Perhaps they relapsed to smoking to “self-medicate” feeling states or needs that are distinct from positive, negative symptoms or mood states, or that their drive to smoke is different from comparison smokers.

Relationship of β_2^* -nicotinic acetylcholine receptor availability to baseline smoking

Similar to observations in “healthy” smokers (30), there were no significant correlations between the number of cigarettes smoked per day or nicotine dependence and β_2^* -nicotinic acetylcholine receptor availability in several brain regions in smokers with schizophrenia.

Alternative explanations—There are several other explanations for the reduced β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia. It is possible that antipsychotic treatment, which the two groups were not matched for, may contribute to the group differences in β_2^* -nicotinic acetylcholine receptor availability. However, the region-

specific reduction in β_2^* -nicotinic acetylcholine receptor availability observed in the current *in vivo* study argues against a generalized effect of antipsychotic exposure. Furthermore, preclinical studies suggest that chronic exposure to clinically relevant doses of several antipsychotics do not affect high-affinity nicotinic receptors in the brain (66). Similarly, human post-mortem studies suggest that the lower β_2^* -nicotinic acetylcholine receptor numbers observed in smokers with schizophrenia are most likely independent of antipsychotic exposure. Ongoing *in vivo* studies comparing medicated and unmedicated smokers with schizophrenia will conclusively address this issue.

Smokers with schizophrenia and comparison smokers may differ in metabolism of nicotine and its metabolite, cotinine. However, analysis of plasma and urine nicotine and cotinine levels on the SPECT scan day, which is an indication of nicotine clearing the brain (30), did not reveal significant differences in these levels between the two groups.

Endogenous acetylcholine may compete with the [123 I]5-IA radioligand binding at the receptor site. In fact, increased endogenous acetylcholine after a high dose of the acetylcholinesterase inhibitor physostigmine to nonhuman primates resulted in a ~15% displacement of [123 I]5-IA in the thalamus (67). However, the findings of lower receptor number in post-mortem study where acetylcholine is washed out, i.e., not a confound, argues against the explanation that endogenous acetylcholine may be lowering receptor availability in this study (21).

It is unlikely that the reduced β_2^* -nicotinic acetylcholine receptors availability in smokers with schizophrenia is due to a change in receptor affinity. Breese et al. conducted Scatchard analyses in their post mortem and suggested that the changes in nicotinic acetylcholine receptor levels were due to changes in total receptor numbers (B_{max}) and not to a change in receptor affinity (K_d)(21).

Limitations: Whether these findings are a result of schizophrenia, smoking or some interaction between the two cannot be confirmed unless nonsmoking schizophrenia patients are studied. In post mortem studies (21), β_2^* -nicotinic acetylcholine receptor availability in smokers with schizophrenia and never smokers with schizophrenia differed only in the cortex. Furthermore, while nicotine dose (cigarettes smoked per day) was not correlated with availability of β_2^* -nicotinic acetylcholine receptors, the limited range of nicotine exposure (heavy smoking) in the current sample was not ideal to establish the relationship between dose and receptor availability. Future studies that include subjects with a wider range of nicotine exposure might be useful in determining whether there is any relationship between nicotine dose and receptor availability. Other study limitations include the lack of a psychiatric comparison group and the medication status of smokers with schizophrenia. Future work includes imaging an unmedicated cohort of subjects. Lastly, whether these changes are present at the onset of the illness or develops with time is not known. Subjects early in the course of their illness will need to be studied.

Conclusions

In conclusion, this study provides the first *in vivo* evidence to our knowledge of reduced β_2^* -nicotinic acetylcholine receptor availability and an inverse correlation between β_2^* -nicotinic acetylcholine receptor availability and negative symptoms in smokers with schizophrenia. Given the absence of any effective treatments for negative symptoms, the findings of this study suggest that medications targeting the β_2^* -nicotinic acetylcholine receptor system may be effective. Future studies will need to be conducted in unmedicated schizophrenia patients, nonsmoking schizophrenia patients, first episode psychosis and prodromal patients to clarify the effects of antipsychotics and stage of illness on nicotinic

acetylcholine receptor availability and to determine whether there are reductions in nicotine-induced upregulation of nicotinic acetylcholine receptors in schizophrenia as suggested by post-mortem data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Dalack GW, Healy DJ, Meador-Woodruff JH. Nicotine dependence in schizophrenia: clinical phenomena and laboratory findings. *Am J Psychiatry*. 1998; 155(11):1490–501. [PubMed: 9812108]
2. Hughes J, Hatsukami D, Mitchell J, Dahlgren L. Prevalence of smoking among psychiatric outpatients. *Am J Psychiatry*. 1986; 143:993–997. [PubMed: 3487983]
3. Ziedonis DM, George TP. Schizophrenia and nicotine use: report of a pilot smoking cessation program and review of neurobiological and clinical issues. *Schizophr Bull*. 1997; 23(2):247–54. [PubMed: 9165635]
4. de Leon J, Diaz FJ. A meta-analysis of worldwide studies demonstrates an association between schizophrenia and tobacco smoking behaviors. *Schizophr Res*. 2005; 76(2-3):135–57. [PubMed: 15949648]
5. de Leon J, Becona E, Gurpegui M, Gonzalez-Pinto A, Diaz FJ. The association between high nicotine dependence and severe mental illness may be consistent across countries. *J Clin Psychiatry*. 2002; 63(9):812–6. [PubMed: 12363123]
6. Diwan A, Castine M, Pomerleau CS, Meador-Woodruff JH, Dalack GW. Differential prevalence of cigarette smoking in patients with schizophrenic vs mood disorders. *Schizophr Res*. 1998; 33(1-2): 113–8. [PubMed: 9783351]
7. Strand JE, Nyback H. Tobacco use in schizophrenia: a study of cotinine concentrations in the saliva of patients and controls. *Eur Psychiatry*. 2005; 20(1):50–4. [PubMed: 15642444]
8. Williams JM, Ziedonis DM, Abanyie F, Steinberg ML, Foulds J, Benowitz NL. Increased nicotine and cotinine levels in smokers with schizophrenia and schizoaffective disorder is not a metabolic effect. *Schizophr Res*. 2005; 79(2-3):323–35. [PubMed: 15961287]
9. Bozikas VP, Niopas I, Kafantari A, Kanaze FI, Gabrieli C, Melissidis P, Gamvrula K, Fokas K, Karavatos A. No increased levels of the nicotine metabolite cotinine in smokers with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005; 29(1):1–6. [PubMed: 15610938]
10. Kelly C, McCreadie RG. Smoking habits, current symptoms, and premorbid characteristics of schizophrenic patients in Nithsdale, Scotland. *Am J Psychiatry*. 1999; 156(11):1751–7. [PubMed: 10553739]

11. Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JP. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*. 1998; 391(6663):173–7. [PubMed: 9428762]
12. Breese C, Marks M, Logel J, Adams C, Sullivan B, Collins A, Leonard S. Effect of smoking history on [3H]nicotine binding in human postmortem brain. *J Pharmacol Exp Therap*. 1997; 282:7–13. [PubMed: 9223534]
13. Marks M, Romm E, Gaffney D, Collins A. Nicotine-induced tolerance and receptor changes in four mouse strains. *J Pharmacol Exp Ther*. 1986; 237:809–819. [PubMed: 3712280]
14. Marks M, Pauly J, Gross S, Deneris E, Hermans-Borgmeyer I, Heinemann S, Collins A. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J Neurosci*. 1992;2765–2784. [PubMed: 1613557]
15. Wonnacott S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. *Trends Pharmacol Sci*. 1990; 11:216–219. [PubMed: 2200178]
16. Flores C, Rogers S, Pabreza L, Wolfe B, Kellar K. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regulated by chronic nicotine treatment. *Mol Pharmacol*. 1992; 41:31–37. [PubMed: 1732720]
17. Schwartz R, Kellar K. Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo. *Science*. 1983; 220:214–216. [PubMed: 6828889]
18. Cosgrove KP, Batis J, Bois F, Maciejewski PK, Esterlis I, Kloczynski T, Stiklus S, Krishnan-Sarin S, O'Malley S, Perry E, Tamagnan G, Seibyl JP, Staley JK. beta2-Nicotinic acetylcholine receptor availability during acute and prolonged abstinence from tobacco smoking. *Arch Gen Psychiatry*. 2009; 66(6):666–76. [PubMed: 19487632]
19. Staley J, Cosgrove K, Ellis S, Al-Tikriti M, Jatlow P, Picciotto M, Baldwin R. [¹²³I]5-IA-85380 SPECT imaging of β_2 -nicotinic acetylcholine receptors in nonhuman primate brain: effects of chronic nicotine administration. *Nic Tobacco Res*. 2005 Submitted.
20. Marks M, McClure-Begley T, Whiteaker P, Salminen O, Brown R, Cooper J, Collins A, Lindstrom J. Increased Nicotinic Acetylcholine Receptor Protein Underlies Chronic Nicotine-induced Up-Regulation of Nicotinic Agonist Binding Sites in Mouse Brain. *J Pharmacol Exp Ther*. 2011
21. Breese C, Lee M, Adams C, Sullivan B, Logel J, Gillen K, Marks M, Collins A, Leonard S. Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. *Neuropsychopharmacology*. 2000; 23:351–364. [PubMed: 10989262]
22. Durany N, Zochling R, Boissl K, Paulus W, Ransmayr G, Tatschner T, Danielczyk W, Jellinger K, Deckert J, Riederer P. Human postmortem striatal alpha4beta2 nicotinic acetylcholine receptor density in schizophrenia and Parkinson's syndrome. *Neurosci Lett*. 2000; 287:109–112. [PubMed: 10854724]
23. Voineskos S, De Luca V, Mensah A, Vincent JB, Potapova N, Kennedy JL. Association of alpha4beta2 nicotinic receptor and heavy smoking in schizophrenia. *J Psychiatry Neurosci*. 2007; 32(6):412–6. [PubMed: 18043764]
24. Kuryatov A, Onksen J, Lindstrom J. Roles of accessory subunits in alpha4beta2(*) nicotinic receptors. *Mol Pharmacol*. 2008; 74(1):132–43. [PubMed: 18381563]
25. Horti AG, Koren AO, Lee KS, Mukhin AG, Vaupel DB, Kimes AS, Stratton M, London ED. Radiosynthesis and preliminary evaluation of 5-[123/125I]iodo-3-(2(S)-azetidylmethoxy)pyridine: a radioligand for nicotinic acetylcholine receptors. *Nucl Med Biol*. 1999; 26(2):175–82. [PubMed: 10100216]
26. Fujita M, Seibyl JP, Vaupel DB, Tamagnan G, Early M, Zoghbi SS, Baldwin RM, Horti AG, Kore NA, Mukhin AG, Khan S, Bozkurt A, Kimes AS, London ED, Innis RB. Whole-body biodistribution, radiation absorbed dose, and brain SPET imaging with [123I]5-i-A-85380 in healthy human subjects. *Eur J Nucl Med Mol Imaging*. 2002; 29(2):183–90. [PubMed: 11926380]
27. Ueda M, Iida Y, Mukai T, Mamede M, Ishizu K, Ogawa M, Magata Y, Konishi J, Saji H. 5-[123I]Iodo-A-85380: assessment of pharmacological safety, radiation dosimetry and SPECT imaging of brain nicotinic receptors in healthy human subjects. *Ann Nucl Med*. 2004; 18(4):337–44. [PubMed: 15359928]
28. Staley JK, van Dyck CH, Weinzimmer D, Brenner E, Baldwin RM, Tamagnan GD, Riccardi P, Mitsis E, Seibyl JP. 123I-5-IA-85380 SPECT measurement of nicotinic acetylcholine receptors in

- human brain by the constant infusion paradigm: feasibility and reproducibility. *J Nucl Med.* 2005; 46(9):1466–72. [PubMed: 16157529]
29. Chefer SI, Horti AG, Lee KS, Koren AO, Jones DW, Gorey JG, Links JM, Mukhin AG, Weinberger DR, London ED. In vivo imaging of brain nicotinic acetylcholine receptors with 5-[123I]iodo-A-85380 using single photon emission computed tomography. *Life Sci.* 1998; 63(25):PL355–60. [PubMed: 9870715]
 30. Staley JK, Krishnan-Sarin S, Cosgrove KP, Krantzler E, Frohlich E, Perry E, Dubin JA, Estok K, Brenner E, Baldwin RM, Tamagnan GD, Seibyl JP, Jatlow P, Picciotto MR, London ED, O'Malley S, van Dyck CH. Human tobacco smokers in early abstinence have higher levels of beta2* nicotinic acetylcholine receptors than nonsmokers. *J Neurosci.* 2006; 26(34):8707–14. [PubMed: 16928859]
 31. First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM-IVTR Axis I Disorders- Non-patient Edition. American Psychiatric Association; Washington,DC: 2002.
 32. Heatherton T, Kozlowski L, Frecker R, Fagerstrom K. The Fagerstrom test for nicotine dependence: a revision of the Fagerstrom tolerance questionnaire. *Brit J Addiction.* 1991; 86:1119–1127.
 33. Tiffany S, Drobes D. The development and initial validation of a questionnaire on smoking urges. *British Journal of Addictions.* 1991; 86:1467–1476.
 34. Hatsukami DK, Hughes JR, Pickens RW, Svikiel D. Tobacco withdrawal symptoms: an experimental analysis. *Psychopharmacology (Berl).* 1984; 84(2):231–6. [PubMed: 6438682]
 35. Kay SR, Opler LA, Lindenmayer JP. The Positive and Negative Syndrome Scale (PANSS): rationale and standardisation. *British Journal of Psychiatry - Supplementum.* 1989; (7):59–67.
 36. Andreasen NC. The Scale for the Assessment of Negative Symptoms (SANS): conceptual and theoretical foundations. *Br J Psychiatry Suppl.* 1989; (7):49–58. [PubMed: 2695141]
 37. Montgomery SM. Depressive symptoms in acute schizophrenia. *Prog Neuropsychopharmacol.* 1979; 3(4):429–33. [PubMed: 400999]
 38. Guy, W. ECDEU Assessment Manual for Psychopharmacology. Dept. of Health EaW. , editor. Washington DC: 1976.
 39. Staley J, Dyck Cv, Weinzimmer D, Brenner E, Baldwin R, Tamagnan G, Riccardi P, Mitsis E, Seibyl J. Iodine-123-5-IA-85380 SPECT Measurement of Nicotinic Acetylcholine Receptors in Human Brain by the Constant Infusion Paradigm: Feasibility and Reproducibility. *J Nucl Med.* 2005; 46:1466–1472. [PubMed: 16157529]
 40. Zoghbi S, Tamagnan G, Fujita M, Baldwin RM, Amici L, Tikriti MA, Seibyl J, Innis R. Measurement of plasma metabolites of (S)-5-[123I]iodo-3-(2-azetidylmethoxy)pyridine (5-IA-85380), a nicotinic acetylcholine receptor imaging agent, in nonhuman primates. *Nucl Med Biol.* 2001; 28:91–96. [PubMed: 11182569]
 41. Cosgrove K, Mitsis E, Bois F, Frohlich E, Tamagnan G, Krantzler E, Perry E, Maciejewski P, Epperson C, Allen S, O'malley S, Mazure C, Seibyl J, Dyck Cv, Staley J. 123I-5-IA-85380 SPECT imaging of nicotinic acetylcholine receptor availability in nonsmokers: effects of sex and menstrual phase. *Journal of Nuclear Medicine.* 2007; 48:1633–40. [PubMed: 17873128]
 42. Innis R, Cunningham V, Delforge J, Fujita M, Gjedde A, Gunn R, Holden J, Houle S, Huang S, Ichise M, Iida H, Ito H, Kimura Y, Koeppe K, Knudsen G, Knuuti J, Lammertsma A, Laruelle M, Logan J, Maguire R, Mintun M, Morris E, Parsey R, Price J, Slifstein M, Sossi V, Suhara T, Votaw J, Wong D, Carson RE. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *Journal of Cerebral Blood Flow & Metabolism.* 2007; 27:1533–1539. [PubMed: 17519979]
 43. Durany N, Zochling R, Boissl KW, Paulus W, Ransmayr G, Tatschner T, Danielczyk W, Jellinger K, Deckert J, Riederer P. Human post-mortem striatal alpha4beta2 nicotinic acetylcholine receptor density in schizophrenia and Parkinson's syndrome. *Neurosci Lett.* 2000; 287(2):109–12. [PubMed: 10854724]
 44. Wonnacott S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. *Trends Pharmacol Sci.* 1990; 11(6):216–9. [PubMed: 2200178]

45. Dani JA, Heinemann S. Molecular and cellular aspects of nicotine abuse. *Neuron*. 1996; 16(5): 905–8. [PubMed: 8630247]
46. Hulihan-Giblin BA, Lumpkin MD, Kellar KJ. Acute effects of nicotine on prolactin release in the rat: agonist and antagonist effects of a single injection of nicotine. *J Pharmacol Exp Ther*. 1990; 252(1):15–20. [PubMed: 2299587]
47. Sharp BM, Beyer HS. Rapid desensitization of the acute stimulatory effects of nicotine on rat plasma adrenocorticotropin and prolactin. *J Pharmacol Exp Ther*. 1986; 238(2):486–91. [PubMed: 3016239]
48. Xiao Y, Fan H, Musachio JL, Wei ZL, Chellappan SK, Kozikowski AP, Kellar KJ. Sazetidine-A, a novel ligand that desensitizes alpha4beta2 nicotinic acetylcholine receptors without activating them. *Mol Pharmacol*. 2006; 70(4):1454–60. [PubMed: 16857741]
49. Brody AL, Mandelkern MA, London ED, Olmstead RE, Farahi J, Scheibal D, Jou J, Allen V, Tiongson E, Chefer SI, Koren AO, Mukhin AG. Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. *Arch Gen Psychiatry*. 2006; 63(8):907–15. [PubMed: 16894067]
50. De Luca V, Voineskos S, Wong G, Kennedy JL. Genetic interaction between alpha4 and beta2 subunits of high affinity nicotinic receptor: analysis in schizophrenia. *Exp Brain Res*. 2006; 174(2):292–6. [PubMed: 16636791]
51. Kishi T, Ikeda M, Kitajima T, Yamanouchi Y, Kinoshita Y, Kawashima K, Okochi T, Inada T, Ozaki N, Iwata N. Genetic association analysis of tagging SNPs in alpha4 and beta2 subunits of neuronal nicotinic acetylcholine receptor genes (CHRNA4 and CHRNA2) with schizophrenia in the Japanese population. *J Neural Transm*. 2008; 115(10):1457–61. [PubMed: 18762859]
52. Faraone SV, Su J, Taylor L, Wilcox M, Van Eerdewegh P, Tsuang MT. A novel permutation testing method implicates sixteen nicotinic acetylcholine receptor genes as risk factors for smoking in schizophrenia families. *Hum Hered*. 2004; 57(2):59–68. [PubMed: 15192278]
53. Ziedonis DM, Kosten TR, Glazer WM, Frances RJ. Nicotine dependence and schizophrenia. *Hosp Community Psychiatry*. 1994; 45(3):204–6. [PubMed: 7910577]
54. Uçok A, Polat A, Bozkurt O, Meteris H. Cigarette smoking among patients with schizophrenia and bipolar disorders. *Psychiatry Clin Neurosci*. 2004; 58(4):434–7. [PubMed: 15298658]
55. Patkar AA, Gopalakrishnan R, Lundy A, Leone FT, Certa KM, Weinstein SP. Relationship between tobacco smoking and positive and negative symptoms in schizophrenia. *J Nerv Ment Dis*. 2002; 190(9):604–10. [PubMed: 12357094]
56. Taiminen TJ, Salokangas RK, Saarijärvi S, Niemi H, Lehto H, Ahola V, Syvalahti E. Smoking and cognitive deficits in schizophrenia: a pilot study. *Addict Behav*. 1998; 23(2):263–6. [PubMed: 9573430]
57. Herran A, de Santiago A, Sandoya M, Fernandez MJ, Diez-Manrique JF, Vazquez-Barquero JL. Determinants of smoking behaviour in outpatients with schizophrenia. *Schizophr Res*. 2000; 41(2):373–81. [PubMed: 10708347]
58. Liao DL, Yang JY, Lee SM, Chen H, Tsai SJ. Smoking in chronic schizophrenic inpatients in taiwan. *Neuropsychobiology*. 2002; 45(4):172–5. [PubMed: 12097804]
59. Tang YL, George TP, Mao PX, Cai ZJ, Chen Q. Cigarette smoking in Chinese male inpatients with schizophrenia: a cross-sectional analysis. *J Psychiatr Res*. 2007; 41(1-2):43–8. [PubMed: 16360170]
60. Barnes M, Lawford BR, Burton SC, Heslop KR, Noble EP, Hausdorf K, Young RM. Smoking and schizophrenia: is symptom profile related to smoking and which antipsychotic medication is of benefit in reducing cigarette use? *Aust N Z J Psychiatry*. 2006; 40(6-7):575–80. [PubMed: 16756583]
61. Smith RC, Singh A, Infante M, Khandat A, Kloos A. Effects of cigarette smoking and nicotine nasal spray on psychiatric symptoms and cognition in schizophrenia. *Neuropsychopharmacology*. 2002; 27(3):479–97. [PubMed: 12225705]
62. Grenhoff J, Aston-Jones G, Svensson TH. Nicotinic effects on the firing pattern of midbrain dopamine neurons. *Acta Physiol Scand*. 1986; 128(3):351–8. [PubMed: 3788613]
63. Di Chiara G. Role of dopamine in the behavioural actions of nicotine related to addiction. *Eur J Pharmacol*. 2000; 393(1-3):295–314. [PubMed: 10771025]

64. Marenco S, Carson RE, Berman KF, Herscovitch P, Weinberger DR. Nicotine-induced dopamine release in primates measured with [C-11]raclopride PET. *Neuropsychopharmacology*. 2004; 29(2): 259–268. [PubMed: 14666115]
65. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry*. 1991; 148(11):1474–86. [PubMed: 1681750]
66. Terry AV Jr, Gearhart DA, Mahadik SP, Warsi S, Waller JL. Chronic treatment with first or second generation antipsychotics in rodents: effects on high affinity nicotinic and muscarinic acetylcholine receptors in the brain. *Neuroscience*. 2006; 140(4):1277–87. [PubMed: 16626873]
67. Fujita M, Al-Tikriti MS, Tamagnan G, Zoghbi SS, Bozkurt A, Baldwin RM, Innis RB. Influence of acetylcholine levels on the binding of a SPECT nicotinic acetylcholine receptor ligand [123I]5-I-A-85380. *Synapse*. 2003; 48(3):116–22. [PubMed: 12645036]
68. Tidey JW, Higgins ST, Bickel WK, Steingard S. Effects of response requirement and the availability of an alternative reinforcer on cigarette smoking by schizophrenics. *Psychopharmacology (Berl)*. 1999; 145(1):52–60. [PubMed: 10445372]
69. Roll JM, Higgins ST, Steingard S, McGinley M. Use of monetary reinforcement to reduce the cigarette smoking of persons with schizophrenia: a feasibility study. *Exp Clin Psychopharmacol*. 1998; 6(2):157–61. [PubMed: 9608347]
70. Tidey JW, Rohsenow DJ, Kaplan GB, Swift RM, Reid N. Effects of contingency management and bupropion on cigarette smoking in smokers with schizophrenia. *Psychopharmacology (Berl)*. 2011
71. SRNT. Subcommittee on Biochemical Verification Biochemical verification of tobacco use and cessation. *Nicotine Tob Res*. 2002; 4:149–159. [PubMed: 12028847]
72. Higgins ST, Heil SH, Badger GJ, Mongeon JA, Solomon LJ, McHale L, Bernstein IM. Biochemical verification of smoking status in pregnant and recently postpartum women. *Exp Clin Psychopharmacol*. 2007; 15(1):58–66. [PubMed: 17295585]

Figure 1. Group Differences in β_2^* -Nicotine Acetylcholine Receptor Availability in Smokers With Schizophrenia and Comparison Smokers^a

Individual Values for Regional Availability of β_2^* -Nicotinic Acetylcholine Receptors in Smokers With Schizophrenia and Comparison Smokers^a

^a Determined with SPECT imaging after 5–7 days of abstinence. V_T is the volume of distribution, and f_p is the free plasma fraction. The horizontal line in each scatter plot represents the group mean value.

^b Significant group difference ($p < 0.05$, MANOVA).

^c Average of the values for the caudate and putamen.

Figure 2. Relationship Between Regional β_2^* -Nicotine Acetylcholine Receptor Availability & Negative Symptoms

Receptor availability was determined with SPECT imaging after 5–7 days of abstinence. V_T is the volume of distribution, and f_p is the free plasma fraction. The line in each scatter plot represents the correlation line of best fit.

^b Significant correlation ($p < 0.05$, Pearson correlation).

^c Average of the values for the caudate and putamen.

Table 1

Demographics of Tobacco Smoking Schizophrenia Subjects

Patient	Age (years)	Cigarettes	FTND	Race	Edu. (years)	Antipsychotic Medication	Dose mg (CPZ eqs)
		per day	Score				
1	60**	18	6	W	15	Aripiprazole 30 mg/day	400
2	53	30	8	W	13.5	Risperdal consta 50 mg IM every 2 weeks	800
3	38	20	8	W	12	Olanzapine 30mg/day	600
4	48	20	4	AA	12	Risperidone 8 mg/day	800
5	25	20	8	AA	GED	Haloperidol decanoate 160mg every 2 weeks	800
6	41	20	7	AA	12	Aripiprazole 30 mg hs	400
7	46	35	3	AA	12.5	Olanzapine 15 mg hs	300
8	42	15	4	AA	9	Risperidone 3mg in the morning, 1mg at noon	400
9	24	10	7	AA	14	Haloperidol decanoate 150mg IM every 4 weeks, Haloperidol PO 1mg/day	800
10	61	40	6	AA	13	Clozapine 300mg/day	300
11	48	20	7	AA	13	Haloperidol 5mg in the morning, 10mg hs	750
12	28	20	8	AA	14	Risperdal consta 25 mg IM every 2 weeks	400
Mean	42.83	22.33	6.33		12.73		562.5
S.D	12.47	8.47	1.77		1.57		214.39

All subjects scanned were male and carried a diagnosis of DSM-IV Schizophrenia Chronic Paranoid Type.

FTND = Fagerstrom Test for Nicotine Dependence; M = male; W = Caucasian; AA = African American; H = Hispanic; Edu. = education; CPS = Chronic Paranoid Schizophrenia; CPZ eqs = chlorpromazine equivalents

** outlier - dropped from the analysis of imaging data

Table 2

Smoking Characteristics of Smokers with Schizophrenia and Comparison Smokers

	Comparison Smokers (n=11)		Schizophrenia Smokers (n=11)	
	Mean	± Std. Dev.	Mean	± Std. Dev.
Age	39	12.3	39	12.6
Years of smoking	16.5	10	23.7	16
# cigarettes/day	16.9	4.9	21.9	8.4
FTND	4.2	2.9	6.3	2.1
CO levels on scan day	4.1	2.4	2.25	1.48*
Days of abstinence	8.0	2.2	5.6	0.7**
Plasma cotinine levels on scan day	23.3	38.7	12.9	9.7

FTND = Fagerstrom Test for Nicotine Dependence, CO = Carbon Monoxide

* Differences: $t(20)=2.18$, $df=20$, $p=0.04$ ** $t(20)=3.45$, $df=20$, $p=0.0025$

Table 3Regional β_2^* -nAChR Availability in Schizophrenia Smokers and Comparison Smokers

Brain region	Comparison Smokers (n=11)		SCZ Smokers (n=11)		p value	Effect size
	mean	± Std.Dev.	mean	± Std.Dev.		
<i>Frontal Cortex</i>	67.85	11.30	50.20	16.25	0.06	0.32
<i>Parietal Cortex</i>	60.83	11.47	47.79	17.18	0.04	0.19
<i>Thalamus</i>	145.00	25.63	114.97	36.26	0.03	0.22
<i>Striatum</i>	85.96	15.89	71.96	23.53	0.10	0.13
<i>Hippocampus</i>	89.50	14.11	77.60	27.60	0.22	0.08

^a Determined with SPECT imaging after 5–7 days of abstinence. V_T is the volume of distribution, and f_p is the free plasma fraction.

^b Average of the values for the caudate and putamen.

Table 4Relationship Between Regional β_2^* - Nicotine Acetylcholine Receptor Availability and Negative Symptoms

Region	PANSS Negative Symptoms Subscale (n=11)				SANS Negative Symptoms (n=11)			
	baseline		Day of SPECT scan ^a		baseline		Day of SPECT scan ^a	
	r	p	r	p	r	p	r	p
Overall cortex	-0.57	0.07	-0.67	0.03	-0.48	0.13	-0.59	0.05
Parietal cortex	-0.61	0.05	-0.71	0.01	-0.5	0.12	-0.56	0.07
Frontal cortex	-0.555	0.08	-0.68	0.02	-0.41	0.21	-0.46	0.16
Anterior Cingulate cortex	-0.53	0.09	-0.64	0.03	-0.46	0.15	-0.59	0.06
Temporal cortex	-0.57	0.07	-0.65	0.03	-0.51	0.11	-0.64	0.04
Occipital cortex	-0.51	0.12	-0.58	0.06	-0.47	0.15	-0.62	0.04
Thalamus	-0.56	0.07	-0.66	0.03	-0.46	0.15	-0.62	0.04
Cerebellum	-0.46	0.15	-0.57	0.07	-0.44	0.18	-0.65	0.03
Striatum	-0.54	0.08	-0.63	0.04	-0.41	0.21	-0.6	0.05
Mean Cortex	-0.57	0.07	-0.67	0.03	-0.48	0.13	-0.59	0.05
Hippocampus	-0.49	0.12	-0.60	0.05	-0.42	0.19	-0.63	0.04
Amygdala	-0.43	0.18	-0.61	0.05	-0.25	0.45	-0.47	0.14

^a After 5–7 days of abstinence.