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Neural Basis of Smoking-Induced Relief of Craving and Negative Affect: Contribution of Nicotine

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Keywords:	nicotine, insula, striatum
Abstract:	Smoking-induced relief of craving and withdrawal promotes continued cigarette use. Understanding how relief is produced, and the role of nicotine in this process, may facilitate development of new smoking-cessation therapies. As the US Food and Drug Administration considers setting a standard for reduced nicotine content in cigarettes to improve public health, knowledge of how nicotine contributes to relief also can inform policy. We assessed effects of nicotine using resting state functional MRI and behavioral assessments of craving and negative affect. Twenty-one young (18-25 years old) daily smokers underwent overnight abstinence on 4 days. On each of the following mornings, they self-rated their cigarette craving and negative affect, and underwent resting-state fMRI before and after smoking a cigarette that delivered 0.027, 0.110, 0.231, or 0.763 mg nicotine. Functional connectivity between the anterior insula and anterior cingulate cortex (ACC), and between the nucleus accumbens and orbitofrontal cortex (OFC), was assessed. Smoking reduced craving, negative affect and nucleus accumbens-OFC connectivity irrespective of nicotine dose, with positive correlations of the effects on behavioral and connectivity measures. Only the highest nicotine dose (0.763 mg) reduced right anterior insula-ACC connectivity; this reduction was positively correlated with the behavioral effects of the 0.763-mg dose only. While nicotine-based therapies may act on right anterior insula-ACC functional circuits to facilitate smoking cessation, non-nicotine (e.g., the conditioned, sensorimotor) aspects of smoking may promote cessation by reducing OFC-accumbens connectivity to alleviate withdrawal.

Neural Basis of Smoking-Induced Relief of Craving and Negative Affect: Contribution of Nicotine

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Abstract

Smoking-induced relief of craving and withdrawal promotes continued cigarette use. Understanding how relief is produced, and the role of nicotine in this process, may facilitate development of new smoking-cessation therapies. As the US Food and Drug Administration considers setting a standard for reduced nicotine content in cigarettes to improve public health, knowledge of how nicotine contributes to relief also can inform policy. We assessed effects of nicotine using resting state functional MRI and behavioral assessments of craving and negative affect. Twenty-one young (18-25 years old) daily smokers underwent overnight abstinence on 4 days. On each of the following mornings, they self-rated their cigarette craving and negative affect, and underwent resting-state fMRI before and after smoking a cigarette that delivered 0.027, 0.110, 0.231, or 0.763 mg nicotine. Functional connectivity between the anterior insula and anterior cingulate cortex (ACC), and between the nucleus accumbens and orbitofrontal cortex (OFC), was assessed. Smoking reduced craving, negative affect and nucleus accumbens-OFC connectivity irrespective of nicotine dose, with positive correlations of the effects on behavioral and connectivity measures. Only the highest nicotine dose (0.763 mg) reduced right anterior insula-ACC connectivity; this reduction was positively correlated with the behavioral effects of the 0.763-mg dose only. While nicotine-based therapies may act on right anterior insula-ACC functional circuits to facilitate smoking cessation, non-nicotine (e.g., the conditioned, sensorimotor) aspects of smoking may promote cessation by reducing OFC-accumbens connectivity to alleviate withdrawal.

Key Words: craving, insula, nicotine, smoking, striatum, withdrawal

Introduction

As a leading contributor to preventable disease and death worldwide, smoking causes an estimated 540,000 deaths per year in the U.S. alone (Carter et al., 2015). Abstinence-induced cigarette craving and negative affect can promote relapse in abstinent smokers (Killen and Fortmann, 1997; Shiffman and Waters, 2004), and smoking a cigarette can rapidly reverse these symptoms (e.g., Bell et al., 1999). Understanding the neural basis of how resumption of smoking during a lapse produces relief of withdrawal symptoms may help in the design of new smoking-cessation therapies.

Although nicotine itself can alleviate tobacco withdrawal, the sensorimotor stimuli, taste and smell linked to the smoking experience also contribute to relief (Rose et al., 2000), perhaps through conditioned responses to these stimuli. Cigarettes delivering very low doses of nicotine (0.02 - 0.09 mg) reduce craving and negative affect as much as conventional cigarettes (which deliver ~ 0.95 mg nicotine; CDC, 2010) (Butschky et al., 1995; Eid et al., 2005; Rose and Behm et al., 2004), presumably by providing the non-nicotine components of smoking. In this study of the neural mechanisms of smoking-induced relief of craving and negative affect during abstinence, one objective was to identify the contribution of nicotine *per se*. Another goal was to provide empirical data regarding potential acute effects of reducing nicotine content in cigarettes, as the US Food and Drug Administration is considering policy to set a maximum level of nicotine content in cigarettes to render them minimally addictive (Gottlieb and Zeller, 2017). A simulation model with inputs derived from empirical evidence has predicted that reducing the nicotine yield of cigarettes to 0.4 mg would reduce the number of smokers in the U.S. by ~13 million within five years (Apelberg et al., 2018).

This prediction is based on findings from studies predominantly of adult smokers; information on how a reduction in the nicotine content of cigarettes would affect young smokers is warranted. Compared to older smokers, young smokers generally smoke fewer cigarettes per day and are less nicotine-dependent (White et al., 2009). Nonetheless, almost half of all adult smokers begin smoking daily by age 18, and 85% before age 21 (Institute of Medicine, 2015). Because myelination and synaptic pruning in the frontal lobes continue into adolescence (Giedd et al., 1999) and the third decade of life (Sowell et al., 2001), smoking during the period of late adolescence to emergent adulthood can alter the trajectory of brain development. We therefore tested young smokers (18-25 years old), to help development of novel approaches to allay the transition to greater nicotine dependence, and also provide evidence as to how young smokers respond to reductions in the nicotine content of cigarettes.

A brain region of primary interest with respect to tobacco withdrawal and smoking cessation is the anterior insula. Smokers with insula damage are more likely than those with damage outside the insula to quit smoking (Naqvi et al., 2007). The urge to smoke is positively correlated with glucose metabolism in the anterior insula of adult smokers (Brody et al., 2002), and is negatively correlated with right ventral anterior insula thickness in adolescent smokers (Morales et al., 2014). In sated young smokers, connectivity of the right anterior insula with the ACC is correlated negatively with pack-year exposure and nicotine dependence (Li et al., 2017). Smoking reduces connectivity between these regions in young smokers, and this effect is positively correlated with relief of craving (Bi et al., 2017). The extent to which nicotine contributes to effects of smoking on connectivity of the anterior insula with the ACC is not currently known.

Circuitry linking the orbitofrontal cortex (OFC) and nucleus accumbens may also be relevant to tobacco withdrawal and its relief. Smoking-induced dopamine release in the ventral striatum is

correlated with the reduction of abstinence-induced negative affect (Brody et al., 2009). Further, ventral striatal dopamine release, measured after methylphenidate administration, is correlated with glucose metabolism in the OFC (Volkow et al., 2007), which is itself positively correlated with the magnitude of the urge to smoke (Brody et al., 2002). Together, these results indicate that connectivity between the ventral striatum and the OFC may modulate tobacco withdrawal. It may be that rather than nicotine itself, the non-nicotine aspects of smoking decrease withdrawal by altering connectivity between these two regions. For example, the OFC contributes to associative learning (e.g., Gallagher et al., 1999; Schoenbaum and Roesch, 2005), and activity within the ventral striatum and OFC represents predictions of reward magnitude (see Diekhof et al., 2012 for a review). Therefore, the learned or conditioned predictions of reward that arise from the sight of the cigarette, or the physical action of smoking, may provide relief by altering connectivity between the ventral striatum and the OFC. This study evaluates this possibility, and the potential contribution of nicotine.

The effects of smoking cigarettes delivering less than conventional doses of nicotine on resting-state functional connectivity in the brain have not been tested before. Here, in young, we tested effects of a range of nicotine doses (0.027, 0.110, 0.231 and 0.763 mg) on associations of cigarette craving and negative affect with resting-state functional connectivity of the anterior insula with the ACC, and of the nucleus accumbens with the prefrontal cortex. Because cigarettes delivering <0.1 mg nicotine alleviate withdrawal symptoms to a similar extent as conventional cigarettes (i.e., Butschky et al., 1995; Eid et al., 2005; Rose and Behm et al., 2004), it was predicted that smoking would relieve craving and negative affect regardless of nicotine dose. It was expected that this relief would be positively correlated with reductions in connectivity within the two neural circuits tested. Although the hypotheses tested pertained to connectivity of the anterior insula and

nucleus accumbens only, connectivity of the posterior insula and caudate and putamen were also examined to test selectivity of observed effects on functional connectivity.

Materials and Methods

Participants (Table 1)

Here, we provide an assessment of a subset of participants from a larger sample from which behavioral results are already published (Faulkner et al., 2017), in which forty-six daily smokers were recruited from the greater Los Angeles area. Inclusion criteria were: age 18-25 years old and smoking ≥5 cigarettes per day for ≥1 year. Exclusion criteria were: positive urine test for illicit drugs (including marijuana), self-report of marijuana use >6 times per month and alcohol use >10 days per month assessed using a comprehensive substance abuse inventory developed in-house, *DSM-IV* psychiatric disorders including substance dependence (other than nicotine dependence), history of neurological injury or disease, pregnancy, and use of mentholated or electronic cigarettes. Of the 46 participants recruited, 24 underwent MRI scanning; three participants were excluded due to excessive head motion (max-min translation >2.5 mm), leaving data from 21 participants in the final analyses. Each participant received a detailed explanation of the protocol, and gave written, informed consent as approved by the Institutional Review Boards of UCLA and University of Toronto at an initial intake session.

General Experimental Design

This study employed a within-subjects, double-blind design across four testing sessions (inter-session interval, mean=2.74, SD=2.42 days). The order of nicotine doses (0.027, 0.110, 0.231 or 0.763 mg) was randomized between subjects. In each testing session, participants were administered the Urge to Smoke (UTS) Scale and the Positive and Negative Affect Schedule (PANAS) (*see Questionnaire Measures, below*). Testing occurred in the morning after overnight abstinence from smoking (~12 h), and again immediately after ad-libitum smoking of one of four research cigarettes; thus, measurements were taken from each participant at eight time points (four pre-smoking and four post-smoking over four non-consecutive days). Research cigarettes were non-mentholated and delivered 0.027, 0.110, 0.231 and 0.763 mg nicotine. Considering that typical commercial cigarettes deliver ~ 0.95 mg [CDC, 2010], we tested a range of very low (0.027 mg) to moderately high (0.763 mg) nicotine doses. Smoking abstinence was verified by measuring CO concentrations in expired air (<10 ppm used to denote abstinence) using a portable monitor (coVita, Haddonfield, NJ). The UTS and PANAS were administered on average 9.72 (SD = 4.47) min after smoking, and participants underwent a resting-state scan on average 42.13 (SD = 7.82) min after smoking. At the beginning of the session participants also completed the Fagerström Test for Nicotine Dependence (FTND), used to determine their level of nicotine dependence. All data were collected at the Semel Institute for Neuroscience and Human Behavior at the University of California, Los Angeles.

Cigarettes

Research cigarettes, manufactured by 22nd Century Group Inc. (Spectrum – Clarence, NY), were provided by the National Institute on Drug Abuse, and delivered 0.027, 0.110, 0.231 or 0.763 mg nicotine, determined by the International Organization for Standardization Method. They were non-mentholated and did not vary in any other tobacco constituents or in ventilation.

Smoking Topography

A clinical Research Support System topography monitor (Borgwaldt KC, Richmond, VA) was used to record the number of puffs per cigarette, and the average volume, intensity and duration of each puff. Smoking topography data were collected from 13 of the 21 participants who were included in the final analyses.

Questionnaire Measures

The Fagerström Test for Nicotine Dependence (FTND), a 10-item questionnaire (Fagerström, 2012), was used to quantify the level of nicotine dependence. Scores on this questionnaire range from 0 (no dependence) to 10 (high dependence). The Urge to Smoke (UTS) scale, a 10-item questionnaire (Jarvik et al., 2000), was used to quantify cigarette craving. Scores on this questionnaire range from 0 (no craving) to 7 (high craving). The Positive and Negative Affect Schedule (PANAS) is a questionnaire that contains 30 items, 15 of which

quantify positive affect, and 15 of which quantify negative affect (Watson et al., 1988). Scores on this questionnaire range from 0 (low positive or negative affect) to 5 (high positive or negative affect).

Behavioral Analyses

The effect of smoking on responses on the UTS and PANAS were assessed using linear mixed models with time (pre-/post-smoking) and nicotine dose (4 levels) added as separate factors, and participant as a random-effect variable. We recently showed that fast metabolizers of nicotine (determined by a group median split) report nicotine dose-dependent reductions in cigarette craving but slow metabolizers do not (Faulkner et al., 2017); therefore, we included the nicotine metabolite ratio (NMR) (dichotomous based on the group median split) as a separate factor to control for this variable (see section below for description of how the NMR is calculated). Further, because we have shown that sex can also influence responses to smoking reduced-nicotine cigarettes (Faulkner et al., 2018), we included sex as a separate factor to also control for this variable. Behavioral data were analyzed using the Statistical Package for Social Scientists (SPSS Inc., Chicago, IL). The distributions of all data were normal, non-skewed with no excess kurtosis.

Nicotine Metabolite Ratio

Blood samples (4 ml) were drawn for assay of the nicotine metabolite ratio (NMR) at the first testing session only. The NMR (ratio of 3'-hydroxycotinine to cotinine) in plasma was

determined using Liquid Chromatography-Tandem Mass Spectrometry (Tanner et al., 2015). Analyses were conducted at the University of Toronto.

fMRI Data Pre-processing

Resting-state images were acquired over 5 min using a 3-T Siemens AG Trio MRI system while participants viewed a black screen (152 T2*-weighted echoplanar images; repetition time = 2 s; echo time = 30 ms; slice thickness = 4 mm; flip angle = 90°; matrix: 64 x 64; field of view = 192 mm). A T2-weighted matched-bandwidth anatomical scan and a T1-weighted magnetization-prepared rapid-acquisition gradient echo scan (MPRAGE) were acquired for registration purposes with the MPRAGE used for spatial registration to standard space.

Image analysis was performed using FSL 5.0.9 (www.fmrib.ox.ax.uk/fsl). The image time-course of the resting state fMRI data was first realigned to compensate for small head movements (Jenkinson et al, 2002). All non-brain matter was removed using FSL's brain extraction tool. Time-series statistical analysis was carried out using FMRIB's Improved Linear Model, with local autocorrelation correction (Woolrich et al, 2001) after high-pass temporal filtering (Gaussian-weighted LSF straight line fitting, with sigma = 50 s).

Motion cleaning and noise reduction was performed using a 32-parameter linear regression model (Satterthwaite et al., 2013) that included the 6 motion parameters (3 translational dimensions along X, Y and Z axes and 3 rotational dimensions: "pitch", "roll" and "yaw") combined with the time-series from the lateral ventricles (i.e., cerebral spinal fluid) and white matter to provide 8 parameters, the temporal derivatives of these parameters and the quadratic of all parameters, resulting in 32 parameters in total. In addition, we calculated

framewise displacement (FD) with root-mean-squared matrix calculation (using FSL's 'fsl_motion_outliers' tool) to obtain the average rotation and translation parameter differences across images. Time points that exceeded acceptable FD thresholds were included as 'spike' regressors in the model (Power et al., 2012; 2013). A fixed threshold for all subjects was determined by calculating the standard deviation (SD) of FD across all data and computing the following equation: 0.25mm + 2 * SD (Satterthwaite et al., 2013). The mean number of images regressed out was 6.45 (SD = 6.06; range 0-28). The time series at each voxel of the resultant residuals from the regression model were then scaled and normalized at each voxel: [(residuals - mean)/standard deviation] + 100.

Registration of contrast images was conducted through a three-step procedure; EPI images were first registered to the matched-bandwidth high-resolution structural image, then to the MPRAGE structural image, and finally into standard space [Montreal Neurological Institute (MNI) 152 template] space, using 12-parameter affine transformations (Jenkinson and Smith, 2001). Registration from MPRAGE structural images to standard space was further refined using FNIRT nonlinear registration (Andersson et al, 2007a). Resulting registered contrast images were smoothed using a 5 mm full-width at half-maximum Gaussian kernel.

fMRI Data Analysis

Based on prior literature, it was expected that connectivity of the anterior but not posterior insula with the ACC would be related with features of tobacco use disorder (e.g., Bi et al., 2017; Li et al., 2017). The anterior insula has been dichotomously split into the dorsal and ventral anterior insula based on structural and functional homogeneity (e.g., Picard et al., 2013). We therefore

defined six insula seeds from Deen et al (2011); the left and right dorsal anterior insula (number of voxels per seed = 245, total volume per seed = 1960 mm^3), left and right ventral anterior insula (number of voxels per seed = 306, total volume per seed = 2448 mm^3) and the left and right posterior insula (number of voxels per seed = 625, total volume per seed = 5000 mm^3) (*Figure. S1*). Three striatal seeds were defined from the Harvard-Oxford Probabilistic Atlas; the bilateral nucleus accumbens (number of voxels = 125, total volume = 1000 mm^3) the bilateral caudate (number of voxels = 884, total volume = 7072 mm^3) and the bilateral putamen (number of voxels = 1490, total volume = 11920 mm^3) (*Figure S1*). Finally, both the bilateral ACC and prefrontal cortex region of interest masks (used to constrain analyses to the ACC and prefrontal cortex, respectively) were defined from the Harvard-Oxford Atlas; the prefrontal cortex mask included the frontal orbital cortex, frontal medial orbital cortex and frontal pole (as defined by this atlas) (*Figure S2*).

To determine connectivity of each seed during each session (i.e. pre- and post-smoking), the average time series from each seed region was extracted from the scaled and normalized pre- and post-smoking images; these time series were then entered as a single explanatory variable in a voxel-wise general linear model with the relevant (pre- or post-smoking) image as the dependent variable. This resulted in one first-level connectivity map per testing session, per seed, that were submitted for higher-level analyses.

Associations of connectivity with both craving and negative affect during abstinence were examined with two separate group-level general linear models (GLMs) using FSL's FLAME1 with outlier deweighting. The inputs to each GLM were first-level whole-brain fMRI images - one per 'abstinence' session. Thus, each model included 84 inputs; four images for each of the 21 participants. The exploratory variable of interest was either craving or negative affect scores from each session, and one explanatory variable per participant was also included as a dummy variable

to model each participant's intercept (mean). All statistical maps were cluster-corrected for multiple comparisons (voxel height threshold: $Z > 3.09$; cluster significance: $p < 0.05$).

To determine the effects of smoking on connectivity, group-level GLMs were performed, with each model containing single-subject, whole-brain pre-post difference images (one per nicotine dose). These images were created using a fixed effects model in FSL's FEAT, with each pre- and post- smoking session modeled with a 1 and -1 respectively, and combining across sessions via inclusion of the 4 session-specific explanatory variables within a single contrast. Group-level statistical maps were cluster-corrected for multiple comparisons (voxel height threshold: $Z > 3.09$; cluster significance, $p < 0.05$). The effect of nicotine dose on smoking-induced changes in connectivity was examined post-hoc by creating a mask of any significant clusters from the final group level pre-post smoking contrast, then applying this mask to each participant's individual whole-brain connectivity map, extracting the mean connectivity values (parameter estimates) across this mask, and entering them as the dependent variable into separate linear mixed models in SPSS (SPSS Inc. Chicago IL), with nicotine dose, sex and NMR (dichotomous variable based on group median split) as separate factors. Even though our hypotheses pertained to the anterior insula and nucleus accumbens only, we applied Bonferroni corrections for the six insula seeds (right and left dorsal anterior, ventral anterior, and posterior insula), and separately for the three striatal seeds (nucleus accumbens, caudate, putamen).

To determine the relationship of these smoking-induced changes in connectivity with the changes in cigarette craving and negative affect, change scores for craving and negative affect were each added as the dependent variable to separate linear mixed models, in which connectivity values, nicotine dose, NMR (group median split) and sex were all added as

separate factors. Where appropriate, Bonferroni correction for the four nicotine doses was applied.

Results

Baseline Measures (Before Smoking After Overnight Abstinence)

Self-Reports

During abstinence, the mean cigarette craving was 5.09 (SD=1.66), and mean negative affect was 1.34 (SD=0.53). Cigarette craving and negative affect were not significantly correlated during abstinence ($F(1,67) = 0.519$, $p = 0.474$), and there were no differences between the four abstinence sessions in terms of cigarette craving ($F(4,59) = 1.499$, $p = 0.214$) or negative affect ($F(4,59) = 1.821$, $p = 0.137$) (*Figure S3*). There was no effect of sex on cigarette craving ($F(1,17) = 1.265$, $p = 0.275$) or negative affect ($F(1,17) = 2.072$, $p = 0.168$) during abstinence. There was no effect of day on either cigarette craving ($F(4,114) = 1.137$, $p = 0.266$) or negative affect ($F(4,114) = 1.874$, $p = 0.173$).

Resting-State Functional Connectivity

Both cigarette craving and negative affect were correlated with connectivity between the right dorsal anterior insula and two separate, but adjacent, clusters of voxels within the ACC mask (*Figure 1*). There were no relationships between either cigarette craving or negative affect and

connectivity of any of the other insula seeds with the ACC, or with connectivity of the nucleus accumbens with the prefrontal cortex.

Effect of Smoking

Smoking Topography

There was no main effect of nicotine dose on the number of puffs per cigarette ($F(3,30) = 0.697$, $p = 0.562$), average puff volume ($F(3,30) = 0.694$, $p = 0.563$), average puff intensity ($F(3,30) = 1.278$) = $p = 0.300$) or average puff duration ($F(3,30) = 0.532$) = 0.664). There was no main effect of sex on any of these variables (all ps > 0.418), and no nicotine dose-by-sex interactions (all ps > 0.266).

Self-Reports of Withdrawal Symptoms

Smoking reduced cigarette craving (36% reduction; $F(1,154) = 153.392$, $p < 0.001$; *Figure S3*) and negative affect (16% reduction; $F(1,154) = 118.921$, $p < 0.001$; *Figure. S3*). There was no effect of nicotine dose on the smoking-induced relief of either cigarette craving ($F(3,154) = 0.058$, $p = 0.981$) or negative affect ($F(3,154) = 0.636$, $p = 0.593$). There was no main effect of sex on smoking-induced reductions in the urge to smoke ($F(1,17) = 0.052$, $p = 0.823$) or negative affect ($F(1,17) = 1.976$, $p = 0.178$). Further, there was no sex-by-nicotine dose interaction on the reduction in the urge to smoke ($F(3,54) = 1.634$, $p = 0.192$) or negative affect ($F(3,54) = 0.079$, $p = 0.971$).

Resting-state Functional Connectivity – Anterior Insula

Smoking reduced connectivity of the right dorsal anterior insula with a cluster of voxels within the ACC mask defined from the Harvard Oxford Atlas (*Figure 2a)*. The effect of nicotine dose on this smoking-induced reduction was then assessed; connectivity values (parameter estimates) from within the cluster observed in *Figure 2a* were extracted and entered as the dependent variable into a linear mixed model in SPSS, with nicotine dose, sex and NMR added as separate factors. The results of this post-hoc analysis revealed a significant effect of nicotine dose on this smoking-induced reduction, which survived Bonferroni correction for the 6 insula seeds ($F(3,80) = 4.319$, $p = 0.007$; Bonferroni-corrected $p = 0.041$). Specifically, the 0.763-mg dose significantly decreased right dorsal anterior insula connectivity; this effect survived Bonferroni correction for the 4 nicotine doses ($t(20) = 3.914$, $p = 0.001$; Bonferroni-corrected $p = 0.006$). However, the three lower dose cigarettes did not significantly alter this right dorsal anterior insula – ACC connectivity (0.027-mg nicotine cigarette: $t(20) = 0.624$, $p = 0.342$; 0.110-mg nicotine cigarette: $t(20) = -0.451$, $p = 0.622$; 0.231-mg nicotine cigarette: $t(20) = 1.174$, $p = 0.229$).

Smoking also reduced connectivity of the right ventral anterior insula with the ACC (*Figure S4*); the effects of nicotine dose on this reduction narrowly failed to reach the Bonferroni-corrected threshold for significance ($F(3,80) = 3.852$, $p = 0.013$; Bonferroni-corrected $p = 0.075$). Smoking did not alter connectivity of the right posterior insula, or of any of the left insula seeds.

Post-hoc analyses (i.e. linear mixed models performed in SPSS) revealed that the aforementioned smoking-induced reduction in connectivity of the right dorsal anterior insula with the ACC cluster (shown in *Figure 2a*) was correlated with the reduction in cigarette craving, when collapsed across nicotine dose ($F(1,56) = 11.308$, $p = 0.001$). There was no statistically significant

effect of nicotine dose on this relationship ($F(3,56) = 2.299$, $p = 0.086$). However, when examining each cigarette separately, there was a relationship between the decrease in connectivity and the reduction in cigarette craving due to smoking the 0.763-mg nicotine cigarette; this association survived Bonferroni correction for the four nicotine doses ($r = 0.554$, $p = 0.011$ Bonferroni-corrected $p = 0.043$) (*Figure 2b*). There was no relationship between decreases in connectivity with the reductions in craving after smoking the three lower-dose cigarettes (0.027-mg nicotine cigarette: $r = 0.089$, $p = 0.702$; 0.110-mg nicotine cigarette: $r = -0.150$, $p = 0.516$; 0.231-mg nicotine cigarette: $r = 0.194$, $p = 0.281$).

There was also no relationship between the smoking-induced relief of negative affect and the smoking-induced reduction in connectivity of the right dorsal anterior insula with the cluster shown in *Figure 2a*. Therefore, a subsequent, exploratory analysis was performed to examine whether relief of negative affect was related to reductions in insula connectivity with other voxels within the ACC mask. This post-hoc 'whole-ACC' analysis revealed that the smoking-induced decrease in negative affect correlated positively with the reduction in connectivity of the right dorsal anterior insula with a cluster of voxels within the ACC that was adjacent to the cluster observed in *Figure 2a* (see *Figure* S5). Connectivity values (parameter estimates) were extracted from this cluster and entered into a linear mixed model; results revealed that again, nicotine dose influenced this relationship, which survived Bonferroni correction for the 6 insula seeds ($F(3,54) = 4.353$, $p = 0.008$; Bonferroni-corrected $p = 0.047$). The decrease in connectivity was positively correlated with the alleviation of negative affect due to smoking the 0.763-mg nicotine cigarette only; this association survived Bonferroni correction for the four nicotine doses ($r = 0.554$, $p = 0.009$; Bonferroni-corrected $p = 0.035$). There was no relationship between decreases in connectivity with the reductions in negative affect after smoking the three lower-dose cigarettes

(0.027-mg nicotine cigarette: r = 0.169, p = 0.465; 0.110-mg nicotine cigarette: r = -0.231, p = 0.313; 0.231-mg nicotine cigarette: r = 0.167, p = 0.469).

Resting-state Functional Connectivity – Nucleus Accumbens

Smoking reduced connectivity of the nucleus accumbens with a region within the prefrontal cortex mask, specifically the right OFC (*Figure 3a*). Nicotine dose did not influence this effect (F(3,80) = 0.772, p = 0.513). Smoking did not alter connectivity of the caudate or putamen with any voxels within the prefrontal cortex mask.

The smoking-induced reduction in connectivity of the nucleus accumbens with the right OFC cluster observed in *Figure 3a* was not correlated with the reduction in cigarette craving: F(1,58) = 1.928, p = 0.170), but was positively correlated with the reduction in negative affect (F(1,58) = 4.669, p = 0.039) (*Figure 3b*); nicotine dose did not influence this correlation (F(3,61) = 1.452, p = 0.236). Because the reduction in this connectivity was not correlated with the reduction in cigarette craving, an exploratory 'whole-prefrontal cortex' analysis was performed to examine whether the decrease in craving was related to reductions in connectivity of the nucleus accumbens with other regions in the prefrontal cortex. Results revealed that the smoking-induced relief in cigarette craving was positively correlated with reductions in connectivity between the nucleus accumbens and a cluster of voxels within the right OFC that was adjacent to that shown in *Figure 3* (*Figure S6*). Again, nicotine dose did not influence this relationship (F(3,61) = 0.176, p = 0.912).

Discussion

To help clarify the neural mechanisms by which the nicotine and non-nicotine components of smoking can relieve abstinence-related craving and negative affect, the effects of smoking cigarettes delivering differing doses of nicotine on self-report measures and resting state functional connectivity were examined. Functional connectivity of the right anterior insula with the ACC was reduced by smoking only cigarettes with the highest nicotine dose. Relief of craving and negative affect was correlated with this effect of smoking on insula connectivity. In contrast, smoking relieved craving and negative affect and reduced nucleus accumbens connectivity with the OFC regardless of nicotine dose. The effects of smoking on the behavioral measures and accumbens-OFC connectivity were correlated.

These results suggest distinct neural mechanisms of two different approaches to smoking cessation. Whereas nicotine replacement therapies may provide benefit via changing connectivity between the right anterior insula and ACC, the use of denicotinized tobacco cigarettes may alter connectivity between the nucleus accumbens and OFC by providing the non-nicotine components of smoking.

Since our results with respect to effects of nicotine dose on insula connectivity were unique to the right anterior portion of the insula, they indicate specificity of action in connectivity with the right anterior insula as compared with other insula subregions. This finding is consistent with lateralized effects of the anterior insula observed in features of tobacco use (Bi et al., 2017; Morales et al., 2014; Li et al., 2017), including a greater (albeit non-significantly) likelihood of disruption in cigarette smoking due to removal of cigarette craving after a right than a left insula lesion (Naqvi et al., 2007). Compared to other insula regions, the right anterior insula contains more specialized

'Von Economo' neurons that have connections projecting directly to the ACC (Allman et al., 2010), and are thought to influence interoception and drug craving (Craig et al., 2009).

Effects of the non-nicotine components of smoking on accumbens-OFC connectivity may be dopamine-dependent inasmuch as consistent pairing of a drug with reward-predicting drug-related paraphernalia (e.g., ashtray, sight of a cigarette) results in dopamine release in the nucleus accumbens and the prefrontal cortex with exposure to these stimuli alone (Di Chiara, 1999). Indeed, compared to smoking a conventional cigarette, smoking a 0.05-mg nicotine cigarette reduces craving to the same extent, although dopamine release in the ventral striatum is less in comparison (Brody et al., 2009). Though not tested here, it is possible that dopamine release in the nucleus accumbens, induced by the non-nicotine components of smoking, can alter activity within the OFC to reduce the withdrawal symptoms measured here. Future studies might test whether denicotinized tobacco or electronic cigarettes may promote cessation by reducing accumbens – OFC connectivity and the associated withdrawal symptoms.

This study has some limitations. The results may not be generalizable to the wider population of smokers because our young smokers had shorter smoking histories and only mild-to-moderate levels of nicotine dependence (e.g., Bi et al., 2017; Li et al., 2017). Further, while the current study was focused on examining connectivity of the insula with the ACC and of the ventral striatum with the OFC, published literature has indicated that nicotine and smoking may relieve withdrawal by altering connectivity between regions other than the ones examined here. For example, Hong et al (2009) report that compared to placebo, nicotine (administered transdermally via patch) downregulated dorsal ACC – striatal circuitry, while Sutherland et al (2013) have shown that nicotine also decreases connectivity between the insula and the amygdala when administered via patch for 17 days. While neither of these studies reports that the nicotine-induced changes in

resting-state functional connectivity of these regions are related to decreases in withdrawal symptoms, Lerman et al (2014) report that smoking-induced reductions in cigarette craving are related to changes in the coupling of large-scale brain networks including the default mode, executive control and salience networks. As such, future research could examine whether nicotine dose influences smoking-induced relief of withdrawal by altering connectivity between regions not examined in the current study. In addition, although a within-subjects design was used, the sample size (N=21) was relatively small and possibly insufficient to detect small nicotine dose effects on connectivity of the left anterior insula or nucleus accumbens. Low statistical power also hindered examination of the influence of nicotine clearance rate. In a study of a larger sample that included participants examined here, fast metabolizers reported nicotine dose-dependent effects on smoking-induced reductions in withdrawal, whereas slow metabolizers did not (Faulkner et al., 2017). Future studies could test whether nicotine-based therapies promote cessation in fast metabolizers by decreasing right anterior insula-ACC connectivity. Another potential limitation is the relatively short scan duration (5 min); however, when resting-state connectivity analyses were performed on data collected over 2 – 11 minutes, average correlation strengths stabilized when ~5 min of data were used (van Dijk et al., 2010). In addition, our results revealed no main effect of day on the strength of connectivity between the right dorsal anterior insula with the ACC, indicating that the correlation strengths of time-courses between these two regions was stable. There are some limitations regarding the nicotine doses of the cigarettes. One is that a full range of nicotine doses extending to that of conventional cigarettes was not studied. In addition, what was observed may be more of a threshold effect rather than a dose effect, as connectivity of the right dorsal anterior insula with the ACC was only reduced by smoking the 0.763-mg cigarette. Finally, one potential limitation concerns possible direct vascular effects of nicotine on blood flow that could

bias the BOLD signal. Nicotine administration increases cerebral blood flow in the striatum, thalamus, insula and prefrontal cortex in rats (Bruijnzeel et al., 2014; Uchida et al., 1997), and increases CBF in the occipital cortex while decreasing CBF in the prefrontal cortex, insula, ACC, amygdala and hippocampus in smokers (Domino et al. 2000; Rowland et al., 2010).

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Author Contributions

EDL, DG, and NEP were responsible for the study concept and design. PF and CC contributed to the acquisition of all data. PF performed the behavioral analysis, and PF and DG performed the fMRI analysis. RFT performed the assay to determine the nicotine metabolite ratio. GH assisted with the behavioral data analyses, and DG assisted the fMRI analyses. EDL, DG, RFT and CC assisted with the interpretation of findings. PF drafted the manuscript. EDL, DG, RFT and NEP provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

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Figure Legends

Figure 1. Correlation of connectivity between the right dorsal anterior insula and the ACC with (A) cigarette craving and (B) negative affect during abstinence. Colored areas denote the region in which connectivity with the right dorsal anterior insula is positively correlated with **(A)** cigarette craving and **(B)** negative affect during abstinence. Voxel height threshold = $Z > 3.09$, cluster significance = $p < 0.05$. Color bars denote range of Z values from activation clusters that survived statistical threshold. Ordinate axis ("y axis") in the scatter plots show mean extracted connectivity (Z) values from each subject's individual spatial map, from within regions surviving statistical threshold in the group level analysis, and the abscissa ("x axis") shows cigarette craving/negative affect scores (values averaged across the four abstinence sessions). Error bars denote ± standard error of the mean across the four abstinent sessions.

Figure 2. Smoking-induced reduction in connectivity of the (A) right dorsal anterior insula and (B) relationship with reduction in cigarette craving. Colored areas denote the region in which smoking reduced connectivity with the right dorsal anterior insula. Voxel height threshold = $Z > 3.09$, cluster significance = $p < 0.05$. Color bar denotes range of Z values from activation clusters that survived statistical threshold. Graph displays the decrease in connectivity due to smoking each cigarette separately, for illustrative purposes only. Values on the y axis are mean extracted connectivity (Z) values from each subject's individual pre-post smoking spatial map, from within regions surviving statistical threshold in the group level analysis. Error bars denote ± SEM. **(B)** Correlation of the smoking-induced reduction in

connectivity between the right dorsal anterior insula with the ACC with the decrease in cigarette craving produced by smoking a cigarette delivering 0.763 mg nicotine. Scatter plot shows mean extracted connectivity (*Z*) values from each subject's pre-post smoking spatial map, from within regions surviving statistical threshold in the group level analysis which revealed that smoking decreased right dorsal anterior insula - ACC connectivity (y axis), and cigarette craving (x axis).

Figure 3. Smoking-induced reduction in connectivity of the nucleus accumbens (A), and relationship with reduction in negative affect (B). Colored areas denote the region in which smoking-induced reductions in connectivity with the nucleus accumbens was reduced by smoking **(A)**. Graph displays the decrease in connectivity due to smoking each cigarette separately, for illustrative purposes only. Values on the y axis are mean extracted connectivity (*Z*) values from each the spatial map of each subject's individual pre- to post-smoking comparison from within regions surviving statistical threshold in the group level analysis. Voxel height threshold = $Z > 3.09$, cluster significance = $p < 0.05$. Color bar denotes range of Z values from activation clusters that survived statistical threshold. Error bars denote ± SEM. **(B)** Correlation of the smoking-induced reduction in nucleus accumbens – OFC connectivity with the decrease in negative affect, collapsed across all testing sessions. Scatterplot shows mean extracted connectivity (*Z*) values from each subject's individual pre-post smoking spatial map, from within regions surviving statistical threshold in the group level analysis (y axis), and the reduction in negative affect scores (x axis) (values averaged across the four testing sessions). Error bars denote ± SEM across the four testing sessions.

Table 1. Participant Characteristics	
Sex	
Participants (Male/Female)	21 (11/10)
Age (years)	22.74 (2.14)
Education (years)	13.39 (1.80)
Ethnicity *(Number of participants)*	
White Caucasian	12
African American	4
Asian American	2
Hispanic	2
Other	1
Cigarette smoking	
Age of first use (years) [a]	16.56 (2.09)
Cigarettes per day [a]	10.82 (4.61)
FTND score [a]	3.30 (1.76)
Substance Use	
Marijuana (days used in the past 30) [a]	1.23 (1.83)
Alcohol (drinks per week) [a]	2.50 (3.38)
Nicotine Metabolite Ratio [a]	0.41 (0.22)

[a] *denotes mean (SD). FTND denotes Fagerström Test for Nicotine Dependence.*

Figure 1

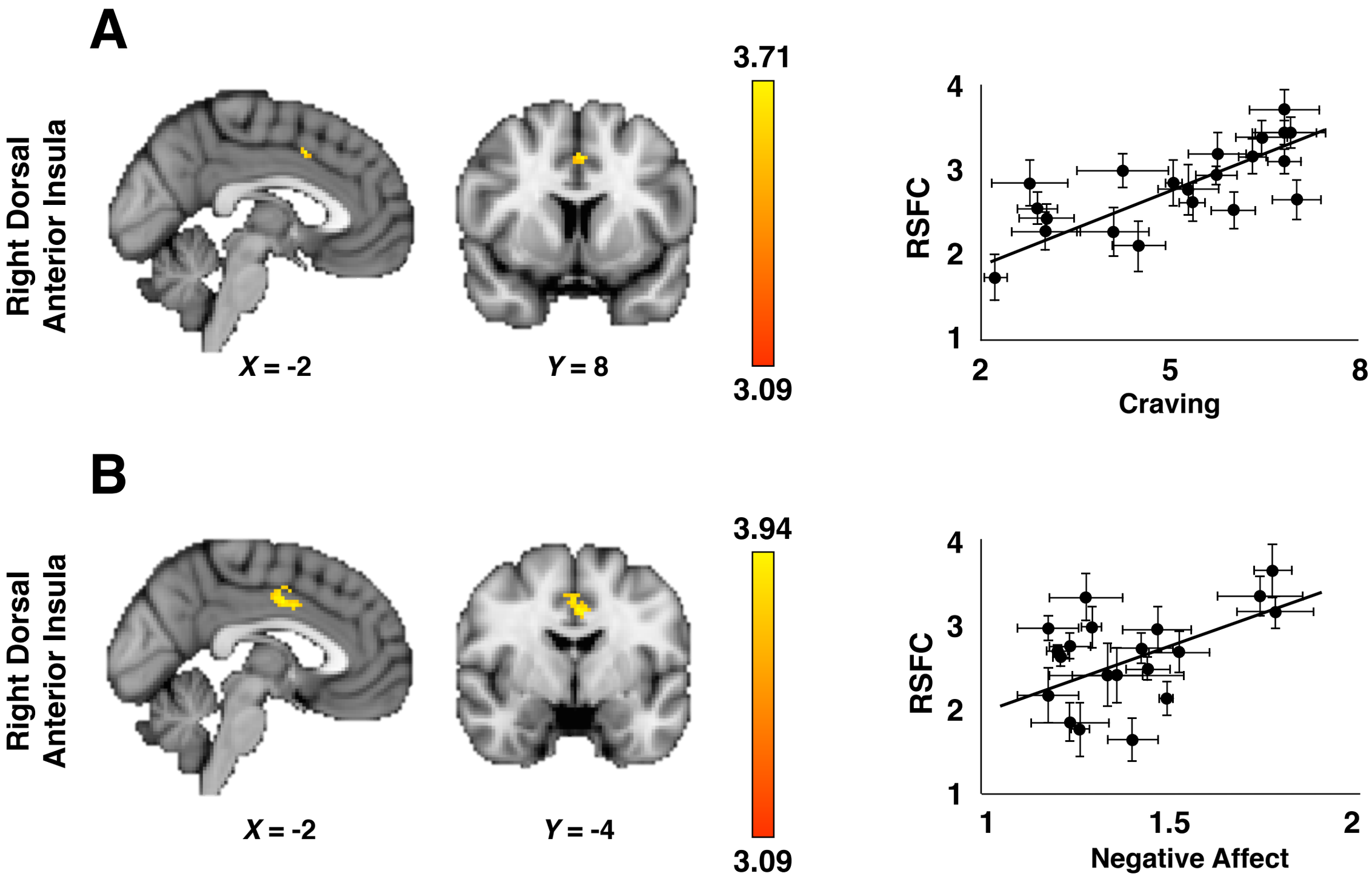

A
Right Dorsal Anterior Insula
X = -2
Y = 8
3.71
3.09
RSFC
Craving
B
Right Dorsal Anterior Insula
X = -2
Y = -4
3.94
3.09
RSFC
Negative Affect

Figure 2

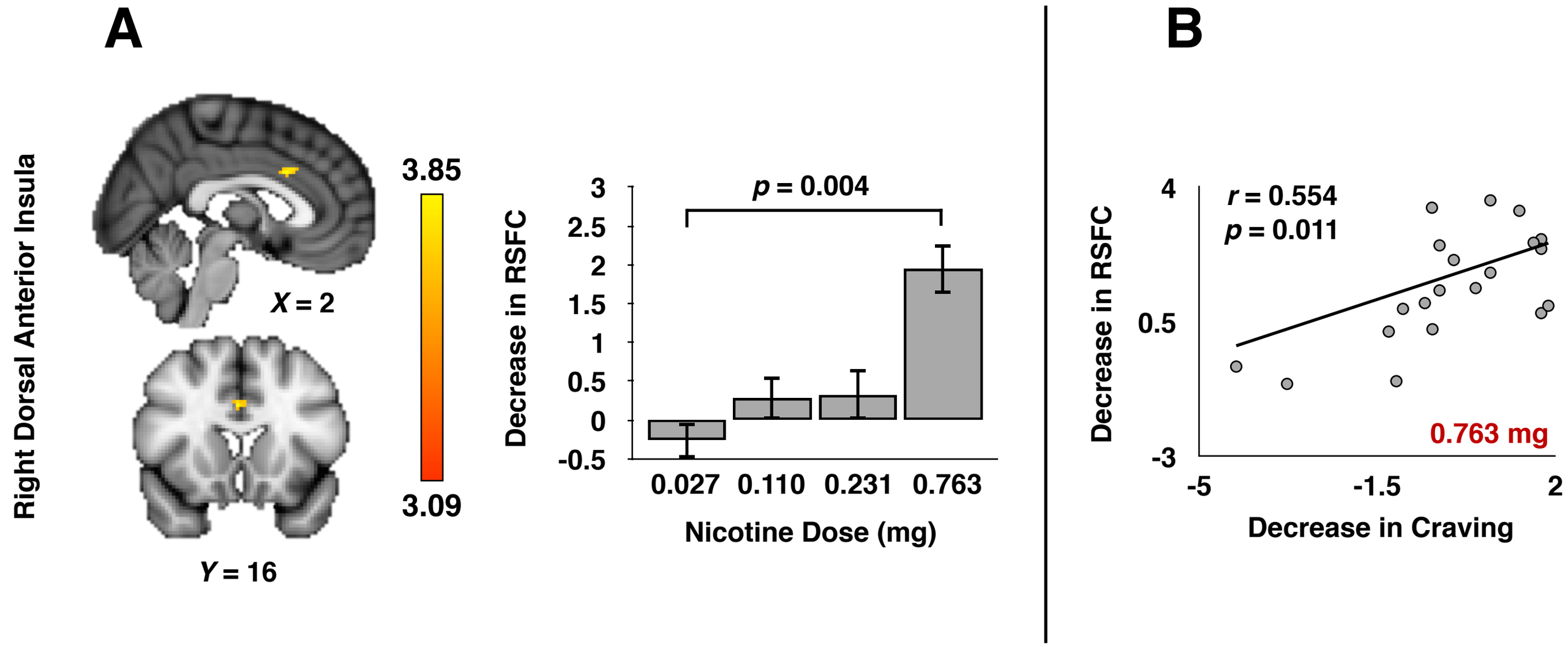
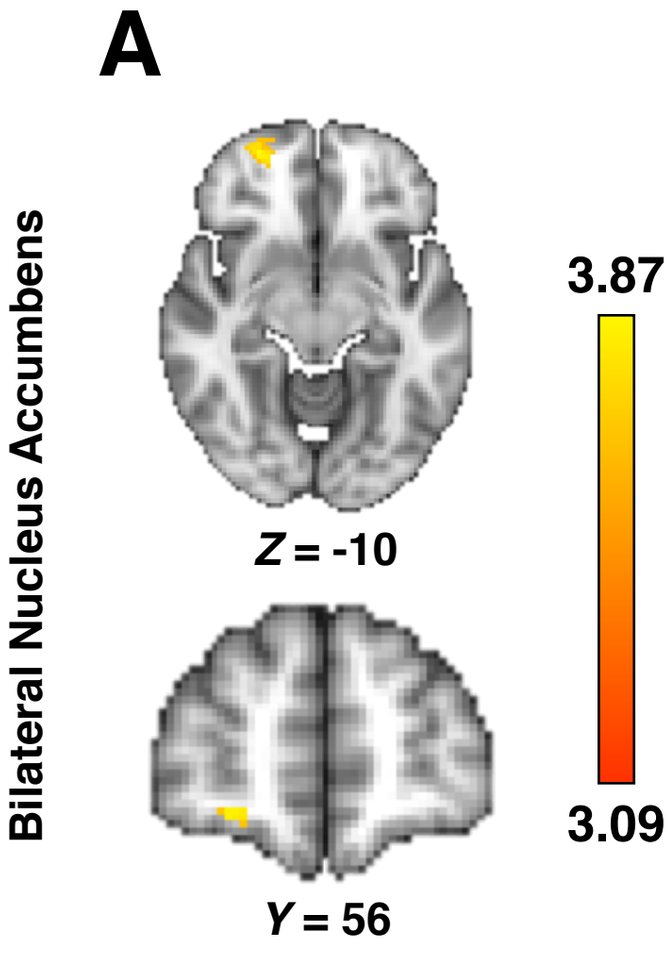

A
Right Dorsal Anterior Insula
X = 2
Y = 16
3.85
3.09
Decrease in RSFC
3
2.5
2
1.5
1
0.5
0
-0.5
p = 0.004
0.027
0.110
0.231
0.763
Nicotine Dose (mg)
B
Decrease in RSFC
4
0.5
-3
r = 0.554
p = 0.011
0.763 mg
-5
-1.5
2
Decrease in Craving

Figure 3


A
Bilateral Nucleus Accumbens
Z = -10
Y = 56
3.87
3.09
Decrease in RSFC
p = 0.513
3
2
1
0
0.027
0.110
0.231
0.763
Nicotine Dose (mg)
B
Decrease in RSFC
2
1
0
-1
-0.3
0
0.3
0.6
0.9
Decrease in Negative Affect