HHS Public Access

Author manuscript

*Drug Alcohol Depend*. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

*Drug Alcohol Depend*. 2015 November 1; 156: 234–242. doi:10.1016/j.drugalcdep.2015.09.021.

# The effects of *N*-Acetylcysteine on frontostriatal resting-state functional connectivity, withdrawal symptoms and smoking abstinence: A double-blind, placebo-controlled fMRI pilot study*

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## Abstract

**BACKGROUND—**Chronic exposure to drugs of abuse disrupts frontostriatal glutamate transmission, which in turn meditates drug seeking. In animal models, *N*-acetylcysteine normalizes dysregulated frontostriatal glutamatergic neurotransmission and prevents reinstated drug seeking; however, the effects of *N*-Acetylcysteine on human frontostriatal circuitry function and maintaining smoking abstinence is unknown. Thus, the current study tested the hypothesis that *N*-Acetylcysteine would be associated with stronger frontostriatal resting-state functional connectivity (rsFC), attenuated nicotine withdrawal and would help smokers to maintain abstinence over the study period.

**METHODS—**The present study examined the effects of *N*-Acetylcysteine on frontostriatal rsFC, nicotine-withdrawal symptoms and maintaining abstinence. Healthy adult, non-treatment seeking smokers (*N*=16; mean (SD) age 36.5±11.9; cigs/day 15.8±6.1; yrs/smoking 15.7±8.9) were randomized to a double-blind course of 2400 mg *N*-Acetylcysteine (1200 mg b.i.d.) or placebo over the course of 3 ½ days of monetary-incentivized smoking abstinence. On each abstinent day, measures of mood and craving were collected digitally and participants attended a lab visit in

*Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:...

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**Conflicts of interest statement**

No conflict declared

**Contributors**

Brett Froeliger was the Principle Investigator and designed the experimental paradigm. Patrick McConnell conducted data analysis and participated in manuscript preparation. Neringa Stankeviciute conducted participant recruitment, data collection and provided feedback on the manuscript. Erin McClure contributed to the development of study procedures. Peter Kalivas provided feedback on the experimental design and study concepts. Kevin Gray was the study Physician and Co-Investigator, and contributed to the development of the manuscript. All authors have approved the manuscript.

The authors report no conflicts of interest.

order to assess smoking (i.e., expired-air carbon monoxide [CO]). On day 4, participants underwent fMRI scanning.

**RESULTS—**As compared to placebo ($n$=8), smokers in the *N*-Acetylcysteine group ($n$=8) maintained abstinence, reported less craving and higher positive affect (all $p$'s <.01), and concomitantly exhibited stronger rsFC between ventral striatal nodes, medial prefrontal cortex and precuneus—key default mode network nodes, and the cerebellum [$p$<.025; FWE]).

**CONCLUSIONS—**Taken together, these findings suggest that *N*-Acetylcysteine may positively affect potentially dysregulated corticostriatal connectivity, help to restructure reward processing, and help to maintain abstinence immediately following a quit attempt.

### Keywords

accumbens; cingulate; cigarette; nicotine; rsfc; glutamate

## 1. INTRODUCTION

Nicotine addiction is the leading cause of preventable premature death in the USA (CDC, 2002), costing nearly $200 billion each year (AHA et al., 2010). Despite the relative effectiveness of current first-line medications for promoting smoking abstinence (Cahill et al., 2013; Gonzales et al., 2006; Jorenby et al., 2006; Oncken et al., 2006), most quit attempts result in relapse (Cahill et al., 2013). Therefore, further research examining new medications for treating the neuropathophysiology of nicotine addiction is needed in order to help initiate and maintain smoking abstinence and prevent relapse.

Chronic drug abuse produces neuroplasticity in frontostriatal (i.e., medial prefrontal cortex: mPFC, nucleus accumbens: NAcc) glutamatergic circuitry, which subserves compulsive drug seeking and the loss of adaptive behavioral responding to changing environmental contingencies (Kalivas, 2009). In animal models, chronic nicotine exposure—the primary psychoactive component of tobacco (Stolerman and Jarvis, 1995)—is shown to impair functioning of the glial glutamate transporter (GLT-1) in the NAcc (Gipson et al., 2013). Impaired GLT-1 function decreases the rate of glutamate elimination from the extracellular space, thereby augmenting the spillover of synaptically-released glutamate during reinstated drug seeking (Baker et al., 2003; Berglind et al., 2009; Pierce et al., 1996). Increased release of synaptic glutamate, derived from mPFC (e.g., anterior cingulate cortex; ACC) synapses in the NAcc core, stimulates extrasynaptic glutamate receptors which cause the rapid, transient synaptic potentiation required for reinstatement of nicotine seeking (Gipson et al., 2013). Consistent with findings from animal models, human positron emission tomography (PET) research using a glutamate receptor ligand reveals that, as compared to both nonsmokers and former cigarette smokers, current smokers have elevated glutamate receptor occupancy in the mPFC (Akkus et al., 2013). This observation potentially resulted from reduced glutamate receptor density or changes in affinity of the glutamate binding site, either of which may be a result of smoking or a predisposing factor in nicotine addiction (Akkus et al., 2013). Further, cue-induced craving is associated with greater functional magnetic resonance imaging (fMRI) blood-oxygenation-level-dependent (BOLD) response in the ventral striatum/NAcc (Bell et al., 2014; Jasinska et al., 2014) and mPFC (McClernon et al., 2009;

Wilson and Sayette, 2014); critically, the magnitude of smoking cue-reactivity in the mPFC predicts relapse (Janes et al., 2010).

Resting-state functional connectivity (rsFC) fMRI has emerged as an effective method for examining systems-level functional connectivity [Biswal et al., 1995; i.e., fluctuating BOLD activation in distributed neural networks (Shmuel and Leopold, 2008)]. Recently, combined human rsFC and magnetic resonance spectroscopy (MRS) evidence has been reported linking mPFC glutamate concentrations to rsFC in frontostriatal circuitry via positive correlation (Duncan et al., 2013). Interestingly, elevated mPFC glutamate concentrations have also been associated with mental imagery (Huang et al., 2015); when considering the role of imagery in drug craving (Taylor et al., 2000), this finding offers a plausible mechanism through which elevated mPFC glutamate concentrations may contribute to frontostriatal desynchronization. Indeed, other rsFC studies have revealed that frontostriatal rsFC is weaker among drug-dependent populations, including nicotine dependence (Hong et al., 2009), opiate addiction (Ma et al., 2010) and polysubstance abuse (Motzkin et al., 2014). Thus, a convergence of evidence from animal models and human research stresses the need for a principled investigation of novel glutamatergic pharmacotherapies for treating frontostriatal circuitry in an effort to prevent smoking relapse (Reissner and Kalivas, 2010).

*N*-acetylcysteine, a cysteine prodrug that regulates intra- and extra-cellular glutamate, holds promise as a medication to normalize frontostriatal function and prevent relapse. *N*-Acetylcysteine exerts antioxidant properties via activation of the cystine-glutamate exchanger (Dringen et al., 2001; Lewerenz et al., 2013) and, in animal models, is shown to restore GLT-1 function that has been down-regulated by chronic use of addictive drugs (Baker et al., 2003). Preclinical models show that *N*-Acetylcysteine blocks reinstated drug-seeking (Reichel et al., 2011) and restores glutamatergic synapses in the NAcc (Kupchik et al., 2012; Moussawi et al., 2009). Clinically, *N*-Acetylcysteine is shown to be safe and well-tolerated (LaRowe et al., 2006; Mardikian et al., 2007; McClure et al., 2014b) and some evidence suggests that *N*-Acetylcysteine may treat frontostriatal glutamate-mediated behavior. In a MRS study of cocaine-dependent individuals (Schmaal et al., 2012), acute administration of *N*-Acetylcysteine normalized elevated glutamate levels in the mPFC (i.e., dACC). Further, *N*-Acetylcysteine has been shown to significantly attenuate withdrawal-induced craving for cocaine (LaRowe et al., 2006), and in an open label trial, reduce marijuana use and craving in adolescent marijuana users (Gray et al., 2010). In spite of the nascent database on *N*-Acetylcysteine's potential role in treating substance-use disorders (SUDs; McClure et al., 2014b) and compulsive behaviors (Berk et al., 2013) more broadly, only a few studies have reported —with mixed findings—the effects of *N*-Acetylcysteine on smoking behavior (Knackstedt et al., 2009; Prado et al., 2015; Schmaal et al., 2011). Nevertheless, the majority of research conducted in animal models has been performed in the context of reinstatement paradigms, suggesting that *N*-Acetylcysteine may be most effective under conditions of abstinence. Indeed, the administration of *N*-Acetylcysteine to abstinent smokers significantly attenuates perceived reward from smoking following an ad-lib smoking period (Schmaal et al., 2011). Taken together, the literature suggests that *N*-Acetylcysteine may be most effective in treating frontostriatal circuitry function under conditions of abstinence, and thus may help to prevent relapse; however, the effects of *N*-

Acetylcysteine on systems-level neural function in humans and long-term smoking behavior remains unknown. Hence, further research is needed to examine the clinical efficacy of *N*-Acetylcysteine for smoking cessation. The present study aimed to test the hypothesis that administering *N*-Acetylcysteine to nicotine-dependent smokers, while abstinent from cigarettes, would be associated with stronger rsFC in the frontostriatal pathway; modulate frontostriatal mediated behaviors, including craving and positive mood; and help to prevent lapse/relapse over the course of a 3 ½ day study period—a timeframe representative of greatest relapse vulnerability (Westman et al., 1997).

## 2. MATERIALS AND METHODS

### 2.1. Participant Characteristics, Recruitment & Screening Procedures

Seventeen adult nicotine-dependent smokers were recruited from the community, met all inclusion/exclusion criteria, and completed all aspects of the study. Smokers were included if they were generally healthy, smoked ≥ 10 cigarettes/day of a brand delivering >.05 mg of nicotine according to the standard Federal Trade Commission method, for at least 2 years, were not using any nicotine products other than cigarettes—including e-cigarettes— and were not immediately interested in quitting smoking. Smokers were required to have an afternoon expired-air carbon monoxide (CO) level ≥ 10 ppm during the screening visit and report at least moderate nicotine dependence (FTND ≥ 3: see below). Exclusion criteria included: use of carbamazepine and/or nitroglycerin within the past 14 days; current/history of a serious health or psychiatric disorder; use of medications altering CNS functioning; a positive urine drug screen; any condition making MRI research unsafe; and among females, a positive urine pregnancy test. All participants read and signed a Medical University of South Carolina (MUSC) Institutional Review Board (IRB) approved informed consent form. All procedures were approved by the MUSC IRB. Participants completed a screening visit where they provided biological samples, underwent a medical evaluation, completed surveys and trained in a mock scanner. One participant was excluded from the analyses for not following task instructions on the questionnaires and during the fMRI scanning protocol, thus resulting in a final *N*=16 (Table 1).

### 2.2. Pharmacological Procedure

In a double-blind, placebo-controlled design, smokers were randomly assigned to receive either 2400 mg *N*-Acetylcysteine (1200 mg b.i.d.), or placebo, daily over the course of 3 ½ days of monetary-incentivized smoking abstinence ($50/day), and participated in an fMRI session on day 4. On study days 1–3, participants attended brief lab visits in order to provide: 1) a biochemical measure of smoking: expired CO; and 2) to assess for any adverse events. Following the fMRI visit, the participant and the researcher conducting the experiment were each asked to independently record which medication they perceived was administered during the study.

### 2.3. Behavioral Assessment and Analyses

**2.3.1 Baseline measures—**Smokers completed the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991). Depressive symptoms were assessed with the Center for Epidemiological Studies-Depression (CES-D) scale (Radloff, 1977). Cognitive

status was assessed with the Cognitive Failures Questionnaire (CFQ; Broadbent et al., 1982).

**2.3.2 Assessment of craving and affect—**Smokers filled out digital surveys, via text messaging, querying craving and mood states randomly throughout each day. State-dependent withdrawal symptoms were measured using the modified version of the Shiffman-Jarvik Withdrawal Questionnaire (SJWQ; Shiffman and Jarvik, 1976). State-dependent mood was measured using the 20-item positive and negative affect schedule (PANAS; Watson et al., 1988). On the fMRI visit, state craving was also assessed using an 8-point Likert Scale querying the subject "How much do you crave a cigarette right now?"; 1 indicating "Not at all" and 8 indicating "Extremely".

**2.3.3 Biochemically confirmed smoking—**At the beginning of each of the 5 visits (screening, abstinence days 1–3, and day 4—fMRI visit), smoking was biochemically assessed via expired breath CO concentration (PPM; (Vitalograph BreathCO Monitor, Lenexa, KS).

### 2.4. Behavioral Analyses

Behavioral analyses were performed with $\alpha = .05$ using Greenhouse-Geisser corrected degrees of freedom. Between-subjects repeated-measures analyses of covariance (RM-ANCOVAs) were performed in SPSS independently for positive (PA) and negative (NA) affect, the SJWQ symptom subscale, and expired-air CO concentration. RM-ANOVA (2×4) for PA/NA and RM-ANCOVA for SJWQ, assessed mean variance across 4 time-points (abstinence days 1, 2, 3 and 4 (fMRI visit)) while controlling for years smoking in the SJWQ analysis. Baseline SJWQ and PANAS data were missing for one Placebo subject so were excluded from the analyses. RM-ANCOVA (2×3) for CO, reported in Table 3.a., excluded day 3 from analysis due to missing CO data from 4 subjects (2/group) and controlled for both baseline CO and for years smoking. Simple main effects of group at each time point were assessed post hoc using *t*-tests. Where significant main effects of group or group x time interactions were observed, variables were further explored via correlational analysis with regard to their relationships with the different resting-state pathways. To control for family-wise error across dependent measures in these correlational analyses, we adjusted the alpha levels for the number of total comparisons for each pathway: 2 subjective (positive affect and craving; $\alpha = 0.025$) and 1 objective (CO; $\alpha = 0.05$). Lastly, to assess state craving on the experimental visit, ANCOVA was used to assess group differences, controlling for years smoking.

### 2.5. Neuroimaging Data Acquisition, Processing and Analyses

**2.5.1 Data Acquisition—**Data were collected on a Siemens Magnetom TrioTim 3T MR scanner (Siemens, Erlangen, Germany) with a 32-channel head coil. A 3D, T1-weighted, multi-planar rapid gradient-echo (MPRAGE) sequence was used to acquire high-resolution ($1mm^3$ voxel) structural images. Next, a 6-min, eyes-closed rsFC scan was acquired using an echo-planar gradient-echo pulse sequence (TR=2000 ms, TE = 30ms, flip angle = 90°; 36 transverse slices, 3.0 mm thickness, 0.58ms gap; voxel size was 3.3mm × 3.3mm × 3.0mm).

**Data processing:** Structural images were pre-processed using the VBM8 toolbox (dbm.neuro.uni-jena.de/vbm8) for SPM12 (www.fil.ion.ucl.ac.uk/spm). Data were preprocessed according to default settings: bias correction; tissue classification/segmentation (Rajapakse et al., 1997); partial volume estimation (PVE; Tohka et al., 2004); denoising/filtering (Manjón et al., 2010; Rajapakse et al., 1997); warping to the DARTEL IXI-550 template in Montreal Neurologic Institute (MNI) space; and resampling to a 1.5 $mm^3$ voxel-size using affine and nonlinear transforms (Ashburner, 2007). Forward-deformation fields were calculated from each subject's skull-stripped and rigid-body registered T1 (PVE) image in order to warp functional data into MNI space. Preprocessing of functional data included: slice time correction and realignment (Friston et al., 1994); motion outlier detection (framewise displacement > 1 mm; www.nitrc.org/projects/artifact_detect) and correction (via interpolation; cibsr.stanford.edu/tools/human-brain-project/artrepair-software.html); coregistration of functional images to PVE image; warping to MNI space using forward deformations and resampling to 1.5$mm^3$ voxel size; and smoothing with a 10$mm^3$ FWHM Gaussian filter. Motion was corrected for ten subjects *(n* = 3, *N*-Acetylcysteine*; n* = 7, Placebo*).* Exclusion threshold for rapid motion was 20% of run length but no subjects exceeded this threshold. Mean volumes corrected did not differ significantly between groups (*t* (14) = 1.4, *p* = .194).

**2.5.2 Denoising of functional images—**Modeled data were uploaded into the conn14 toolbox (www.nitrc.org/projects/conn) for denoising and connectivity analyses. Unsmoothed segmented tissue images, along with region of interest (ROI) masks (5mm radius spheres around coordinates of interest, comprising 523.6 $mm^3$) were uploaded into the toolbox. Mean time-courses from the unsmoothed BOLD signal from each ROI were characterized with no additional principal components. Confounds (mean white matter (WM) and cerebrospinal fluid (CSF) signal, and motion) were regressed out of the mean signal for each ROI. Analysis space was set to match the functional images (i.e., 1.5$mm^3$) with an explicit mask generated by skull-stripping the DARTEL IXI-550 template image. A band-pass filter of .008 to .09 Hz with despiking performed after confound regression (no detrending) was used.

**2.5.3 fMRI data analysis—**All fMRI data analyses were performed with conn14 toolbox for SPM12. Hypothesis testing was conducted using seed-to-voxel bivariate correlations with sources comprising L/R.NAcc (±12, 8, −8) (Zink et al., 2003). At the group level, effects of medication were examined with *t*-tests, correcting for multiple comparisons using $p < .025$ FWE at the cluster level ($p < .001$ cluster-forming threshold). Among significant clusters, weighted means from each ROI were extracted and used for descriptive statistics (mean, SD, cohen's *d*).

## 3. RESULTS

### 3.1. Blind Success and Adverse Events

The double-blind procedure used in the study was successful. Neither participants [$X^2$ (2, $N$ = 16) = 1.72, $p$ = .19] nor the researcher [$X^2$ (1, $N$ = 16) =2.28.1, $p$ = .131] were able to identify the condition. A total of 17 adverse events (AEs) (placebo: $n$ = 12; *N*-

Acetylcysteine: $n = 5$) were reported for seven participants (44%). All AEs were mild and consistent with symptoms that accompany smoking withdrawal (Table 2).

### 3.2. Behavioral Measures

**3.2.1 Carbon Monoxide—**A significant group × time interaction ($F(1.83, 21.97) = 3.74$, $p = .043$) was identified; whereas compared to placebo group, the *N*-Acetylcysteine group exhibited significantly lower CO values beginning on abstinence day 2 and continuing to the fMRI visit (Table 3.a).

**3.2.2 Daily self-report measures—**A significant group × time interaction ($F(2.10,29.35) = 3.66$, $p = .036$) was identified for positive affect. As compared to the placebo group, the *N*-Acetylcysteine group reported a non-significant trend for higher positive affect beginning on Abst. Day 2 that was significant on Abst. Days 3 and 4 (Table 3.b). The group × time interaction and main effects of group and time for withdrawal symptoms (SJWQ) (see Table S1[1]) and self-reported negative affect (see Table S2[2]) failed to reach significance (all $p$'s >.3).

**State craving ratings:** The groups exhibited a significant difference in craving rating on the experimental visit ($F$ (1,13) =9.54, $p = .009$); the *N*-Acetylcysteine group reported significantly less craving ($M = 4.3 \pm 1.2$) than the placebo group ($M = 6.4 \pm 1.2$) (Table 3.c).

### 3.3. Effects of N-Acetylcysteine on Bilateral Nucleus Accumbens rsFC

Compared to placebo, smokers who received *N*-Acetylcysteine exhibited stronger rsFC in four striatal pathways: between R.NAcc and left medial prefrontal cortex (mPFC) and bilateral precuneus, and between L.NAcc and bilateral cerebellum and ventromedial prefrontal cortex (vmPFC) (see Table 4; Figure 1). Years smoking was not correlated with connectivity in any of the four pathways, so was not controlled for via regression analyses.

### 3.4. Correlation Analysis of Relations between rsFC, Smoking Behavior and Self-Reported Craving and Mood

#### 3.4.1 NAcc-Medial Prefrontal Pathways

**3.4.1.1 R.NAcc-mPFC Pathway:** R.NAcc-mPFC rsFC was negatively correlated with CO ($r = -.50$, $p = .049$) and approached significant negative correlation with craving ($r = -.48$, $p = .060$) on the fMRI visit. Additionally, rsFC in this circuit positively correlated with positive affect ($r = .69$, $p = .003$) on the fMRI visit. No association between rsFC and negative affect was observed (Fig. 2).

**3.4.1.2 L.NAcc–vmPFC Pathway:** L.NAcc–vmPFC rsFC was negatively correlated with CO ($r = -.69$, $p = .003$) and craving ($r = -.71$, $p = .002$) on the fMRI visit. Additionally, rsFC in this circuit positively correlated with PA ($r = .55$, $p = .028$) on the fMRI visit. No association between rsFC and negative affect was observed (Fig. 2).

[1]Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:...
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**3.4.1.3 R.NAcc-Precuneus Pathway:** R.NAcc-Precuneus rsFC was negatively correlated with CO ($r = -.52$, $p = .037$) and craving on the fMRI visit ($r = -.50$, $p = .050$). Additionally, rsFC in this circuit approached significant positive correlation with positive affect on the fMRI visit ($r = .48$, $p = .061$). No association between rsFC and negative affect was observed (Fig. 2).

**3.4.1.4 L.NAcc–Cerebellum Pathway:** L.NAcc–Cerebellum rsFC was negatively correlated with CO ($r = -.63$, $p = .009$) and craving ($r = -.62$, $p = .011$) on the fMRI visit. Additionally, rsFC in this circuit positively correlated with positive affect ($r = .68$, $p = .004$) on the fMRI visit. No association between rsFC and negative affect was observed (Fig. 2).

### 3.5 Correlations between craving, mood and CO on the fMRI visit

Across all smokers, craving and positive affect were negatively correlated ($r = -.586$, $p = .02$); craving and negative affect were not ($p$'s > .05). CO was negatively correlated with positive affect ($r = -.63$, $p = .009$) and positively correlated with craving ($r = .58$, $p = .018$).

## 4. DISCUSSION

The current study sought to: A) examine the effectiveness of *N*-Acetylcysteine for treating dysregulated frontostriatal circuitry among nicotine-deprived smokers and B) preliminarily evaluate the potential value of using *N*-Acetylcysteine as a relapse-prevention aid. Results from this study revealed that, as compared to placebo, smokers assigned to a 3 ½ day course of *N*-Acetylcysteine exhibited stronger rsFC between NAcc and mPFC/vmPFC (frontostriatal), between NAcc and key nodes of the default mode network (mPFC/Precuneus) and between NAcc and cerebellum. Further, smokers who received *N*-Acetylcysteine reported higher PA and less craving on the fMRI visit and were able to successfully maintain smoking abstinence over the course of the study. While this study is limited due to the cross-sectional nature of the experimental design, these findings suggest that *N*-Acetylcysteine may help to normalize dysregulated frontostriatal circuitry function and restructure reward processing, and may provide a therapeutic benefit for preventing relapse during smoking cessation.

Addiction-related deficits in top-down, prefrontally-mediated cognitive control over affective and motivational responding are well documented (Goldstein and Volkow, 2002). For example, as compared to nonsmokers, smokers exhibit dysregulated prefrontal response during cognitive (Froeliger et al., 2013), affective (Augustus Diggs et al., 2013; Froeliger et al., 2013) and drug-related cue (Augustus Diggs et al., 2013) processing, and withdrawal engenders further disruption in prefrontal function across a broad array of tasks (Azizian et al., 2010; Froeliger et al., 2012a, 2012b, 2012c; Kozink et al., 2010). In addition to diminished prefrontal control, patients with substance use disorders exhibit potentiated BOLD response in the NAcc (which codes reward related signal; Knutson and Gibbs, 2007) to drug cues (Jasinska et al., 2014; Kilts et al., 2004; Kober et al., 2010). Conversely, the flexible deployment of prefrontal cortex is important in modulating ventral-striatal mediated cigarette craving (Kober et al., 2010). Thus, dysregulated cognitive control over motivational responding, contributing to the downward spiral of drug addiction (Garland et

al., 2014), is posited to result from disrupted frontostriatal intra- and extra-cellular glutamate homeostasis (Kalivas, 2009), which may underpin a functional dysconnectivity.

### 4.1. Effects of N-Acetylcysteine on frontostriatal rsFC

Indeed, drug addiction is evidenced by weakened frontostriatal rsFC (Hong et al., 2009; Ma et al., 2010; Motzkin et al., 2014). Of particular relevance to the current study, Hong et al. (2009) reported that nicotine-addiction severity was associated with weaker rsFC between mPFC (i.e., dACC) and ventral striatum during smoking abstinence and that acute nicotine administration failed to improve the strength of connectivity in this circuit. The current findings that *N*-Acetylcysteine administration was associated with stronger rsFC in frontostriatal circuitry—a reversal of the effects reported in Hong et al. (2009)—and attenuated smoking behavior to a biochemically confirmed level indicating abstinence, may suggest a reduction in dependence severity. These findings are consistent with research in animal models demonstrating that *N*-Acetylcysteine normalizes frontostriatal glutamate and prevents reinstated drug seeking. When taken together, the extant literature and current study findings suggest that restoring glutamatergic tone in the frontostriatal pathway may help to prevent smoking relapse and may also be one neurobiological mechanism through which the severity of addiction might be reduced.

Blunted positive affective response to natural environmental rewards is a salient feature of substance use disorders (Koob and Le Moal, 2001), especially when patients with substance use disorders are under conditions of withdrawal (Volkow et al., 1997). Addiction-related neuroplasticity in frontostriatal circuits have been implicated as critical pathways involved in restructuring reward processing, as evidenced by heightened reactivity to conditioned drug cues and attenuated responses to intrinsically positive stimuli (Koob and Le Moal, 2008). While the mPFC is pivotal in directing attention to salient reward-related stimuli (Knutson et al., 2000; Rogers et al., 2004; Rushworth et al., 2007), among patients with substance use disorders, the mPFC codes for cigarette craving (Wang et al., 2007) and is shown to become sensitized to detecting conditioned drug cues (Brody et al., 2002; McClernon et al., 2005). In addition, the NAcc plays a role in detecting changes in the magnitude of perceived reward (Knutson et al., 2001) and risk (Matthews et al., 2004); yet among patients with substance use disorders, accumbens' responsivity to drug cues varies as a function of craving intensity (Goldman et al., 2013; Kufahl et al., 2005; Li et al., 2012; Risinger et al., 2005) and the drug's perceived rewarding effects (Kufahl et al., 2005; Risinger et al., 2005). This restructuring of reward learning entrenches the drug user in a vicious cycle of drug taking that serves to maintain the ongoing use of drugs. Indeed, relapse vulnerability has been attributed to increased incentive salience of drug cues and decreased salience of intrinsically pleasant stimuli (Berridge et al., 2009; Koob and Le Moal, 2001). In the present study, the strength of rsFC in each striatal pathway was negatively correlated with craving, yet positively associated with positive affect. Consistent with the literature (Cook et al., 2004), positive affect and craving were inversely correlated on the fMRI visit while subjects were abstinent from smoking. Given that *N*-Acetylcysteine smokers did not differ from Placebo at baseline with regard to positive affect or craving, these patterns of findings support the hypothesis that *N*-Acetylcysteine facilitates the restoration of control over motivational responding. The theoretical therapeutic mechanism of *N*-Acetylcysteine

(i.e., normalizing frontostriatal glutamatergic neurotransmission) would thus explain the decreased craving and concomitantly increased positive affect observed in *N*-Acetylcysteine-treated smokers during the withdrawal state. However, further research is needed to determine whether *N*-Acetylcysteine-normalized frontostriatal rsFC, and associated positive affect, might translate into normalized striatal BOLD response to natural rewards, and also to what extent this process of reward restructuring may be mediated by the effects of *N*-Acetylcysteine on mPFC glutamate concentrations.

### 4.2. Effects of N-Acetylcysteine on Default Mode rsFC

Precuneus and mPFC BOLD signals, key resting-state default-mode network (DMN) nodes, were also found to correlate more strongly with NAcc as a function of *N*-Acetylcysteine administration, with stronger connectivity predicting reduced self-reported cigarette craving and greater positive affect. These DMN nodes have already been implicated in the pathophysiology of nicotine addiction, both in terms of structural and resting-state functional connectivity (Huang et al., 2014). Specifically, Huang and colleagues demonstrated that connectivity between precuneus and mPFC was increased proportionally with nicotine dependence and withdrawal-induced craving. Given the seed-voxel analytic strategy employed in the present study, we are unable to speak to the relationship between precuneus and mPFC with regard to *N*-Acetylcysteine administration; however, the present findings suggest that addiction-associated alterations in DMN connectivity may in part be mediated through each node's pattern of connectivity with the ventral striatum.

### 4.3. Effects of N-Acetylcysteine on NAcc-Cerebellum rsFC

Here, we report increased rsFC between NAcc and superior, bilateral cerebellum that was associated with the administration of *N*-acetylcysteine, reduced craving and increased positive affect. Task-based fMRI and volumetric VBM analyses have implicated regions of the cerebellum, and its connectivity with the striatum, in various forms of learning such as implicit motor learning (Tzvi et al., 2014) and reversal learning (Moreno-López et al., 2015). The cerebellum plays a role in reactive 'on-line' error correction in movement (Doya, 2000) and motor learning (Bastian, 2006). Smoking cessation can be viewed as a complex learning process – or rather an unlearning process – that involves not only alterations in reward processing and inhibitory control functioning, but also with regard to craving-associated motor programs related to the prediction of smoking reward: such as reaching to one's pocket for a cigarette, or raising one's hand to their lips, etc. Further research is needed to elucidate the role that ventral striatal-cerebellar connectivity may play in motivational processes and drug self-administration.

### 4.4. Summary, Limitations and Future Directions

In sum, the current study findings suggest that *N*-Acetylcysteine may help to treat the neuropathophysiology of nicotine addiction and may prevent relapse, theoretically via alterations in functional communication between top-down control centers and striatal motivational centers. Limitations of the study include a small *N*; a relatively homogenous group of smokers; and a restrictive—yet focused, statistically rigorous, and hypothesis driven—rsFC analytic approach. Critically, although we had *a priori* hypotheses regarding

rsFC between nucleus accumbens and mPFC, we performed whole-brain analyses correcting for multiple-comparisons in a very conservative manner while also providing novel neurobehavioral findings correlating craving and positive affect with *N*-Acetylcysteine-associated changes in frontostriatal, striatal-DMN, and striatal-cerebellar rsFC. These findings alone have exciting clinical implications for *N*-Acetylcysteine-assisted treatment for addictions, given that craving and dysregulated mood are primary precipitants to relapse (Shiffman et al., 1996). In addition, although prior research demonstrates that nicotine addiction severity is associated with weaker rsFC between ACC and ventral striatum, independent of whether nicotine is "on board", the current study design did not allow for dissociating the effects of NAC vs nicotine and/or tobacco on rsFC. Future large-scale studies that assess smoking behavior over an extended time-frame, and potentially in a controlled clinical environment are needed in order to inform clinical significance. Current study findings warrant consideration of using *N*-Acetylcysteine as a means to help a smoker maintain abstinence in conjunction with medications that promote abstinence (McClure et al., 2014a), and/or behavioral interventions that train cognitive restructuring of reward processes (Garland et al., 2014). Finally, protocols that include MRS, to directly assess glutamate concentrations, along with rsFC and task-based BOLD fMRI signal may help to further elucidate the neurobiological and neurocognitive effects of *N*-Acetylcysteine on treating the neuropathophysiology of substance abuse disorders.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

**Role of funding**

This study was supported by NIH/NIDA (R01DA0033459 [BF]; P50DA015369 [PWK]) and pilot funds provided by P30CA138313 [Hollings Cancer Center] to BF.

The authors would like to thank the MUSC Center for Biomedical Imaging (CBI), Taylor Salo at the University of California-Davis for his assistance with computer programming and the Reviewers of this manuscript for providing thoughtful critiques and insightful comments.

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## Highlight

We examine the effects of *N*-Acetylcysteine on maintaining smoking abstinence.

fMRI resting-state connectivity in corticostriatal circuitry was assessed

Relations between neural circuitry function and smoking withdrawal and abstinence was assessed.

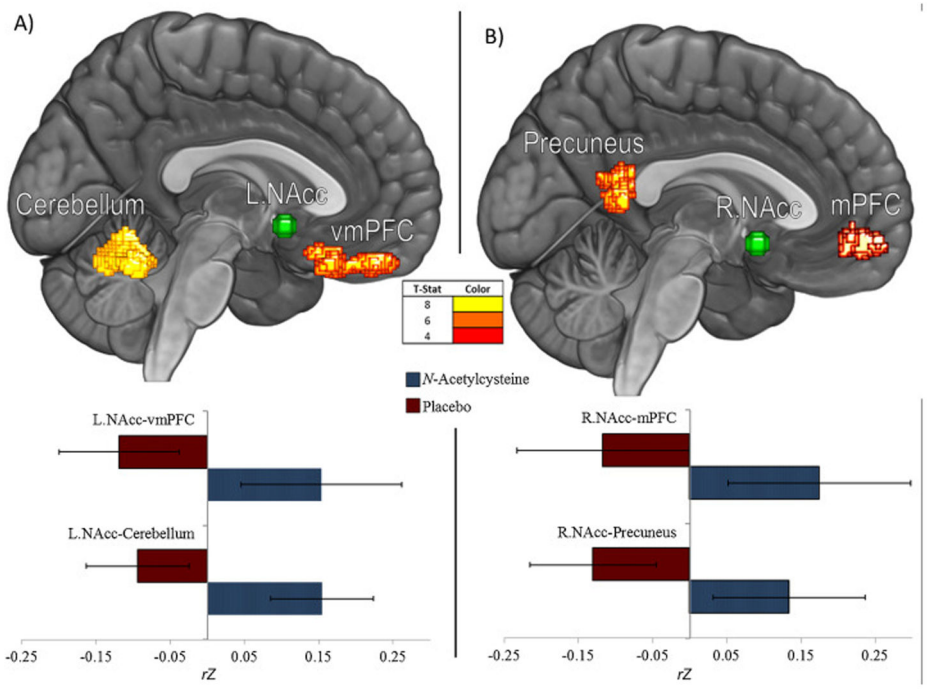


**Figure 1.**

Resting-state functional connectivity (rsFC) in: **A)** corticostriatal circuitry: between right Nucleus Accumbens (NAcc) ROI seed and medial prefrontal cortex (mPFC) and Precuneus; and **B)** left NAcc seed and ventromedial PFC and Cerebellum. As compared to the placebo group, smokers that received *N*-Acetylcysteine exhibited stronger rsFC in pathways A and B ($F<.025$, FWE).

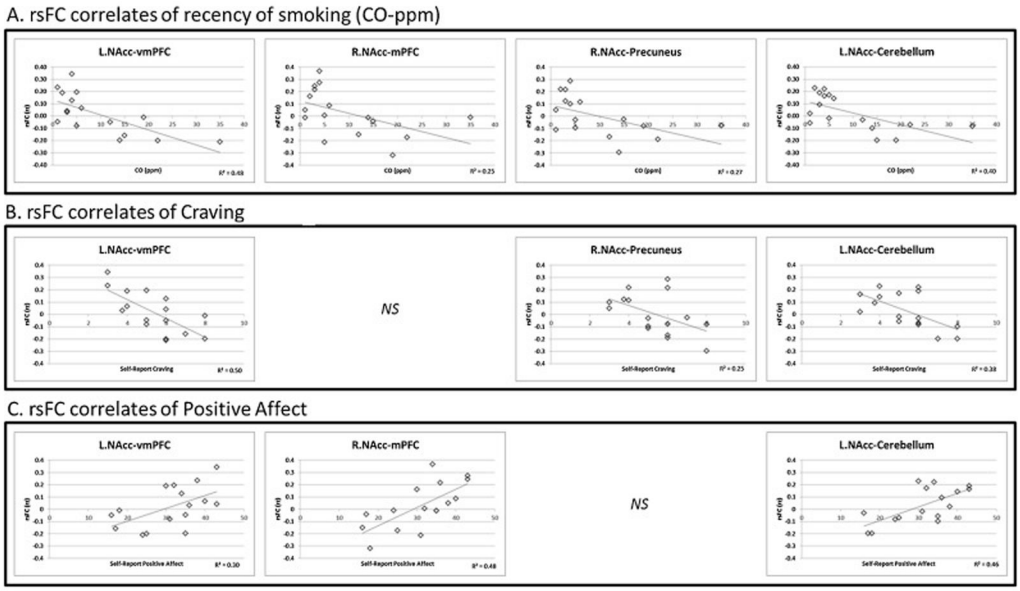


**Figure 2.**

**Table 1**

Subject demographics / Baseline self-report

| | Overall Sample | *N*-Acetylcysteine Group | Placebo Group | $Chi^2$ / *t* | *p* (two-tailed) |
|---|---|---|---|---|---|
| % Female | 31 | 25 | 38 | 0.29 | 0.59 |
| Mean Age | 36.5 (11.9) | 35.0 (14.4) | 38 (9.6) | 0.59 | 0.63 |
| Years of Education | 13.4 (1.9) | 13.75 (2.1) | 13.13 (1.9) | 0.6 | 0.5 |
| Race | | | | 0.25 | 0.61 |
| African Americans (#) | 9 | 4 | 5 | | |
| Caucasians (#) | 7 | 4 | 3 | | |
| *Baseline Clinical Measures* | | | | | |
| Nicotine Dependence (FTND) | 5.8 (1.4) | 5.3 (1.6) | 6.4 (1.1) | 1.6 | 0.12 |
| Years Smoking | 15.7 (8.9) | 13.8 (9.9) | 17.6 (8.0) | 0.86 | 0.4 |
| Average Daily Cigarettes | 15.8 (6.1) | 16.3 (5.3) | 15.4 (7.2) | 0.28 | 0.79 |
| Carbon Monoxide (CO) (Screening) | 25.6 (16.8) | 25.0 (18.4) | 26.3 (16.3) | 0.14 | 0.89 |
| Depressive Symptoms (CESD) | 6.9 (4.9) | 6.5 (5.9) | 7.4 (4.1) | 0.35 | 0.74 |
| Cognitive Failures (CFQ) | 26.9 (16.3) | 31.0 (12.1) | 22.9 (19.6) | 0.99 | 0.34 |

*Note.* Standard deviation reported in parentheses next to mean where applicable.

**Table 2**

Adverse Events

| Group (Total) | *N*-Acetylcysteine (5) | Placebo (12) |
|---|---|---|
| *Event type* | | |
| Reflux | 2 | - |
| Bad Aftertaste | - | 4 |
| Fatigue | - | 3 |
| Dry Mouth | - | 1 |
| Light-Headed | 2 | - |
| Nausea | 1 | 2 |
| Vomiting | - | 1 |
| Irritability | - | 1 |

## Table 3

**Table 3.a. Carbon Monoxide (CO): 2 (Group) x 3 (Time) repeated measures ANCOVA[ab]**

| | GROUP | | | | Overall Model |
|---|---|---|---|---|---|
| | *N*-Acetylcysteine | Placebo | *t* | *p* | *F (p)* |
| Screening | 25.0 (18.4) | 26.3 (16.2) | 0.1 | ns[d] | 3.74 (.043)* |
| Abst. Day 1 | 10.0 (6.0) | 11.75 (8.4) | 0.5 | ns[d] | |
| Abst. Day 2 | 4.4 (3.4) | 13.3 (6.5) | 3.4 | 0.004** | |
| Abst. Day 3[c] | - | - | - | - | |
| Abst. Day 4 (fMRI visit) | 3.5 (1.6) | 15.4 (10.5) | 3.12 | 0.007** | |

**Table 3.b. Positive Affect: 2 (Group) x 4 (Time) repeated measures ANOVA[ab]**

| | GROUP | | | | Overall Model |
|---|---|---|---|---|---|
| | *N*-Acetylcysteine | Placebo | *t* | *p* | *F (p)* |
| Screening | 32.8 (6.3) | 34.7 (7.7) | 0.5 | ns[d] | 3.66 (0.036)* |
| Abst. Day 1 | 33.5 (6.5) | 29.8 (9.2) | 0.9 | ns[d] | |
| Abst. Day 2 | 33.9 (6.6) | 28.6 (7.1) | 1.5 | ns[d] | |
| Abst. Day 3 | 33.3 (5.4) | 26.9 (6.6) | 2.1 | 0.050* | |
| Abst. Day 4 (fMRI visit) | 37.0 (4.9) | 25.1 (7.8) | 3.6 | 0.003** | |

**Table 3.c. State craving on fMRI Visit**

| | GROUP | | | |
|---|---|---|---|---|
| | *N*-Acetylcysteine | Placebo | *t* | *p* |
| Abst. Day 4 (fMRI visit) | 4.3 (1.2) | 6.4 (1.2) | 3.4 | 0.004** |

*Note*. Standard deviation reported in parentheses next to mean where applicable.

[a] Controlling for baseline value during screening (for CO model only) and years of smoking; F-statistic is for Group x Time Interaction

[b] Greenhouse-Geisser corrected degrees of freedom

[c] Time point excluded due to missing data from 4 subjects

[d] $p > .05$

* $p < .05$

** $p < .01$

*Note*. Standard deviation reported in parentheses next to mean where applicable.

## Table 4

Main effect of condition on resting-state functional connectivity with the nucleus accumbens

| **Pattern of Group Contrast:** | ***N*-Acetylcysteine > Placebo** | | | |
|---|---|---|---|---|
| **Seed:** | **Left Nucleus Accumbens** | | **Right Nucleus Accumbens** | |
| Correlated Brain Region(s) | Cerebellum | vmPFC[c] | Precuneus | mPFC[c] |
| Peak MNI Coordinates [x,y,z] | −10, −60, −22 | 10, 24, −26 | 8, −51, 8 | −9, 50, −9 |
| Cluster Size ($mm^3$) | 3375 | 1954 | 1563 | 1384 |
| *N*-acetylcysteine[a] | 0.15 (.07) | 0.15 (.11) | 0.13 (.10) | 0.17 (.12) |
| Placebo[a] | −0.09 (.07) | −0.12 (.08) | −0.13 (.09) | −0.11 (.12) |
| *t*- statistic | 8.72 | 6.73 | 6.53 | 4.83 |
| $p_{cluster}$ FWE | 0.000 | 0.005 | 0.010 | 0.017 |
| cohen's *D*[b] | 0.95 | 0.88 | 0.87 | 0.84 |

*Note*. Standard deviation reported in parentheses next to mean where applicable.

All correlations significant at p < .001 cluster-forming and p < .05 FWE cluster-level

[a] group mean cluster *rZ* value

[b] cohen's *D* calculated based on *N*-Acetylcysteine and Placebo group means

[c] denotes ventromedial and medial prefrontal cortices respectively