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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; years/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 3½ days of monetary-incentivized smoking abstinence. On each abstinent day, measures of mood and craving were collected and participants attended a lab visit in 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 dysregulated corticostriatal connectivity, help to restructure reward processing, and help to maintain abstinence immediately following a quit attempt.

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

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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). Therefore, 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, reduced 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 and 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 3½ 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

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