Neuroscience-Informed Treatment Development for Adolescent Alcohol Use
NCT03238300
October 19, 2021

**PROTOCOL TITLE: Neuroscience-Informed Treatment Development for Adolescent Alcohol Use**

**PRINCIPAL INVESTIGATOR: Lindsay M. Squeglia, Ph.D.**

## Objectives / Specific Aims

Adolescence marks a key period in the development of problematic alcohol use, with approximately 15% of 18 year olds meeting criteria for an alcohol use disorder. Decreasing drinking at an early age could offer substantial advantages for neurocognitive and psychosocial outcomes and could protect against the development of habitual drinking. Efforts to prevent or diminish adolescent heavy drinking have been only modestly effective, with up to 86% of youth returning to substance use within 12 months following treatment. Inadequate treatment for adolescents is an important public health concern given that alcohol use during this time is highly predictive of long-term problematic drinking. While several medications have been efficacious in treating adult alcohol dependence, pharmacotherapy research focused on adolescent alcohol use has been sparse. Evaluation of alternative and more efficacious treatments for adolescent alcohol users is warranted.

N-acetylcysteine (NAC) is an over-the-counter antioxidant supplement with glutamatergic properties that has shown promise in treating marijuana dependence in adolescents. In a double-blind randomized placebo-controlled study, youth in the NAC group had more than double the odds of negative urine cannabinoid tests during treatment compared to the placebo group. Preclinical findings suggest NAC works through restoring glutamate homeostasis disrupted by addiction, a finding replicated across multiple substances of abuse. No published clinical trials to date have examined the efficacy of NAC on alcohol use, despite glutamate's established role in alcohol use disorders. This is surprising, given previous promising NAC findings in marijuana-dependent adolescents and the safety, tolerability, and affordability of this over-the-counter supplement.

This K23 application proposes to examine the effects of NAC on adolescent glutamatergic systems and drinking behaviors in a randomized, double-blind, placebo-controlled within-subjects crossover study. In counterbalanced order, 55 participants will receive a 10-day course of NAC 1200 mg twice daily and a subsequent 10-day course of matched placebo twice daily, separated by a washout period. Human laboratory and imaging procedures will be conducted after each course of medication treatment. Functional magnetic resonance imaging (fMRI) will examine alcohol reactivity and magnetic resonance spectroscopy (MRS) will examine glutamate levels in the anterior cingulate after NAC versus placebo.

**Aim 1: Quantify the effect of NAC versus placebo on alcohol cue reactivity in adolescent heavy drinkers.** Using fMRI, we will determine the effect of NAC on alcohol cue reactivity.
*Hypothesis:* Heavy drinking youth with show decreasing alcohol cue reactivity from baseline in key reward regions while on NAC compared to placebo.

**Aim 2: Quantify the effect of NAC versus placebo on glutamate levels in adolescent heavy drinkers and non-drinkers.** Using MRS, we will determine the effect of NAC versus placebo on modulating anterior cingulate glutamate levels in heavy alcohol-using adolescents and non-alcohol using adolescents.
*Hypothesis:* Heavy drinking youth will show increasing levels of glutamate from baseline in the anterior cingulate while on NAC compared to placebo.

**Exploratory Aim: Examine the effect of NAC on adolescent drinking.** Given the short dosing periods (~10 days), we do not expect dramatic changes in drinking behavior in heavy alcohol-using adolescents. However, drinking days and drinks per drinking day will be assessed during the NAC, placebo, and washout periods. Given the findings with marijuana dependent adolescents, we anticipate a potential decrease in alcohol use while participants are on NAC versus placebo.

## Background

### A. SIGNIFICANCE

**Alcohol use during adolescence is problematic and has long-term consequences.** Adolescence is a key period in the development of alcohol use disorders, with nearly 15% of youth meeting diagnostic

criteria for an alcohol use disorder by age 18 (Swendsen et al. 2012). Heavy adolescent alcohol use is related to serious psychosocial problems, including comorbid psychopathology (Deas and Thomas 2002; Rowe et al. 2004), poorer academic success (Kristjansson et al. 2013), and detrimental neurocognitive consequences (Squeglia et al. 2014). Furthermore, binge drinking patterns initiated during adolescence hold into adulthood (Degenhardt et al. 2013) and significantly increase risk for subsequent adult alcohol use disorders and related problems (Hingson et al. 2006a; b).

**Existing adolescent substance use treatments are inadequate.** Decreasing substance use at this early stage could have significant long-term implications; however, efforts to prevent or decrease heavy alcohol use during adolescence have only been modestly effective (Jensen et al. 2011; Tripodi et al. 2010), with up to 86% of youth returning to some alcohol or drug use at least once within 12 months following treatment (Chung and Maisto 2006; Tanner-Smith et al. 2013; Winters et al. 2009). While several medications have been efficacious in treating adult alcohol dependence, pharmacotherapy research focused on adolescent alcohol use has been sparse (Miranda et al. 2014). This interferes with treatment, as safety and efficacy of medications for adolescents cannot be inferred from adult studies (Bridge et al. 2007). Evaluation of alternative and more efficacious treatments for adolescent alcohol users is warranted.

**N-acetylcysteine has potential for reducing adolescent alcohol use.** Based on preclinical findings, glutamate has emerged as a potential pharmacotherapeutic target in the treatment of addictions (Kalivas 2009; Kalivas and Volkow 2011). N-acetylcysteine (NAC) is an over-the-counter antioxidant supplement with glutamatergic properties that has shown promise in treating addictive disorders (Deepmala et al. 2015) by restoring glutamate homeostasis disrupted by addiction, a finding replicated across multiple substances of abuse (McClure et al. 2014; Olive et al. 2012). In a double-blind randomized placebo-controlled study of marijuana-dependent youth, those in the NAC group had greater than double the odds of negative urine cannabinoid tests during treatment compared to the placebo group (Gray et al. 2012). No published clinical trials to date have examined the efficacy of NAC on alcohol use in any age group.

**NAC has potential for reducing neuroinflammation associated with alcohol use.** Recent evidence from human and animal research has converged to support the role of alcohol in promoting deleterious adaptations in inflammatory signaling cascades. Specifically, alcohol appears to induce a number of pro-inflammatory effects throughout the CNS and periphery, including upregulating pro-inflammatory cytokines such as IL-1B, IL-6 and TNFα, and increasing high-mobility group protein B1 (HMGB1), which is a chromatin protein that regulates gene transcription, and is produced by immune cells in response to an immune challenge (i.e., alcohol exposure) and endogenous pro-inflammatory cytokines (Yu et al., 2006). One recent human post-mortem study found that earlier age of drinking onset is associated with increased expression of HMGB1 in the orbitofrontal cortex (Vetreno et al., 2013). Binge-like ethanol administration in adolescent rats increased the inflammatory cytokines TNFα and IL-1β (Pascual et al., 2014), and another recent rodent study found that intermittent ethanol treatment in adolescent mice was associated with learning deficits and increased perseverative behavior in adulthood, as well as increased frontal cortical HMGB1 (Vetreno and Crews, 2012). Further, alcohol-induced HMGB1 signaling is associated with induction of the inflammatory cytokine IL-1β in the brain (Crews et al., 2013). Uncontrolled activation of such inflammatory mediators (as may be the case in heavy or chronic alcohol exposure) is associated with increased neuroimflammation, reduced neuroprotection and neuronal repair, and increased neurodegeneration (Guerri and Pascual, 2013). One hypothesized consequence of such disrupted immune signaling is neuronal cell death in frontal control regions of the brain, consistent with the deficits in inhibitory control observed over the course of AUDs (Li et al., 2009). Thus, understanding the role of inflammatory signaling in adolescent alcohol users may have significant implications for understanding the behavioral sequelae of alcohol use and potentially improving treatments. Further, given that NAC is an anti-oxidant and has therapeutic potential in terms of reducing inflammation (Zafarullah et al., 2003), measuring levels of inflammatory markers before and after NAC treatment could provide added insight in terms of informing the development of future treatment options for adolescent drinkers.

**NAC has potential for improving gut dysbiosis.** Accumulating evidence suggests that NAC may have the potential to reshape microbiome structure and benefit gut health. Several studies have shown that NAC exhibits an antibiofilm effect towards harmful bacteria and promotes the growth of beneficial bacteria suggesting a curative effect on microbial imbalances (Zheng et al. 2019, Xu et al. 2014, Eroshekno et al. 2017). In addition to this, NAC reduces oxidative stress and inflammation, two markers that have been reported as elevated in patients with AUD (Hou et al. 2015, Kamal et al. 2020, Ozaras et al. 2003). In fact, one study showed that pretreatment with NAC blocked oxidative stress marker proteins and prevented binge alcohol-induced gut leakiness in mice (Abdelmegeed et al. 2013). Collection of salivary microbiome may be a favorable alternative to classic stool collection as some studies have reported that the oral microbiome closely represents the diversity and composition of the upper gastrointestinal tract (Kodukula et al. 2017), displays changes with alcohol consumption (Fan et al. 2018), and displays some of the same genera with stool samples of AUD participants (Ames et al. 2020). The collection of salivary microbiota will allow for the investigation into the relationship between microbiota and adolescent alcohol use as well as provide feasibility data for future studies.

**NAC is safe and tolerable for adolescents**. Part of the appeal of NAC is its long-established safety record in children and adults, with FDA approval since 1963. NAC has been used safely for several decades in children and adults, often at doses greatly exceeding those proposed for our study (Mucomyst Package Insert, 2004). A meta-analysis of NAC studies found that this supplement was well tolerated, with generally mild side-effects, most commonly gastrointestinal adverse effects that did not require treatment interruption (Grandjean et al. 2000). Systemic allergic reactions to NAC have been observed, but only with intravenous administration (Bailey and McGuigan 1998). Reflecting its safety profile, NAC is available over-the-counter as a supplement, which further increases its potential acceptability and accessibility.

**Summary.** Given the safety profile of NAC and preclinical evidence of its actions on the glutamatergic system and reversing addiction pathology, it is a strong candidate medication targeting adolescent heavy alcohol use. Thus, the primary aim of this study is to examine the effects of NAC on alcohol cue reactivity and glutamatergic functioning in heavy alcohol-using adolescents, and obtain preliminary data for estimating sample sizes needed to adequately power subsequent clinical trials. Understanding the biological mechanisms of behavioral change is fundamental in making substantive advances in the field of adolescent addiction treatment.

## B. INNOVATION

**1. NAC's effect on adolescent drinking has not been examined.** Despite the success of NAC increasing abstinence rates two-fold in marijuana-dependent adolescents (Gray et al. 2012), there are no studies examining the effect of NAC on adolescent alcohol use, which is the most commonly used substance during adolescence. Effective interventions during adolescence could have substantial long-term implications by reducing acute and enduring negative social, academic, and cognitive consequences related to heavy teen drinking (Squeglia et al. 2014).

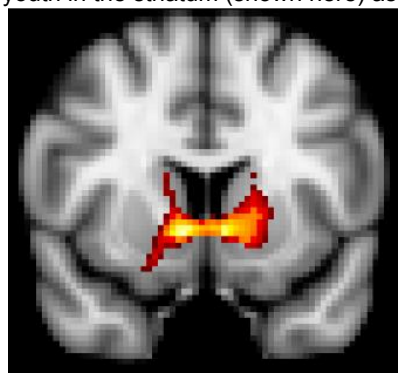
**2. Magnetic resonance spectroscopy is an innovative neuroimaging technique that will help elucidate NAC's mechanism of action.** Further, we will be using innovative neuroimaging techniques including magnetic resonance spectroscopy (MRS) to understand the neurological underpinnings of NAC's mechanism of action. With this knowledge, we will be well positioned to develop refined circuit-specific treatment strategies for adolescents with substance use disorders.

Given NAC's accessibility as an over-the-counter supplement, safety and tolerability with youth, and low cost, this medication holds great promise as a potential treatment option for substance using adolescents.

## C. PRELIMINARY STUDIES

**NAC may reduce co-occurring alcohol use in marijuana-dependent adolescents.** To date, no published clinical trials have examined the effect of NAC on alcohol use. Therefore, secondary analyses were performed on an adolescent marijuana dependence NAC clinical trial dataset (Gray et al. 2012) to examine the effect of NAC or placebo on co-occurring alcohol use over the 8-week treatment course. Participants (ages 15-21; N=116) interested in marijuana dependence treatment were randomized to 1200 mg of NAC twice daily (same dosing proposed in this study) or matched placebo for 8 weeks. Of the 116 participants randomized in the study, 89 (77%) returned for at least one study treatment visit and had recorded alcohol use data (even if abstinent from alcohol use) and were included in the longitudinal analysis. Of those 89, 45 were randomized to receive NAC and 44 to placebo. Out of the 89 participants with study data available, 77 (87%) reported at least one drink during the 30 days prior to study entry and 69 (77%) noted at least one drink within a week of study entry; 36 (80%) in the NAC group and 33 (75%) in the placebo group ($p$=0.572). There were no differences between study groups with respect to age, race, gender, use characteristics, or psychiatric comorbidities. Less marijuana use [based on urine cannabinoid tests (UCT) and creatinine adjusted cannabinoid levels (CC)] was associated with less alcohol use in the NAC treated group but not in the placebo treated group [UCT: interaction t86=2.44, p=0.016]. In other words, participants who reduced marijuana use while using NAC also had a significant reduction in alcohol use; this was not the case in the placebo condition. This suggests that NAC may be effective in reducing adolescent alcohol use.

**Figure 1.** Robust activation was elicited from alcohol, compared to neutral, stimuli in heavy drinking youth in the striatum (shown here) as



**Heavy-drinking youth display robust activation in reward regions during the Alcohol Cue Reactivity Task.** In a pilot study using the same fMRI alcohol cue reactivity task proposed for this application (Schacht et al. 2011), 11 heavy drinking youth displayed robust activation in several brain regions including the anterior cingulate, insula, striatum, and amygdala during alcohol vs. non-alcohol cue trials (see Figure 1), which is highly consistent with activation patterns seen in adult studies (Schacht et al. 2013a). Understanding the role of NAC on reducing cue reactivity as well as glutamatergic levels (via MRS) will be the primary aims of this application.

## Intervention to be studied

### PHARMACOLOGICAL INTERVENTION

This double-blind crossover trial will compare NAC (1,200mg twice daily) and placebo. If assessment procedures reveal that a participant meets inclusion criteria and none of the exclusion criteria, the participant will be randomized to each condition for 10 days in a counterbalanced order. There will be a minimum 11-day washout period between conditions to allow for the clearance of NAC (Holdiness 1991). Medication response and tolerability/adverse events will be assessed at each clinic visit. Medication videos will be uploaded by participants to track medication compliance. Reminders to take medication will be sent via email or text message to increase compliance. Primary mentor Dr. Gray has a current IND for using NAC with adolescent substance users (#78,927).

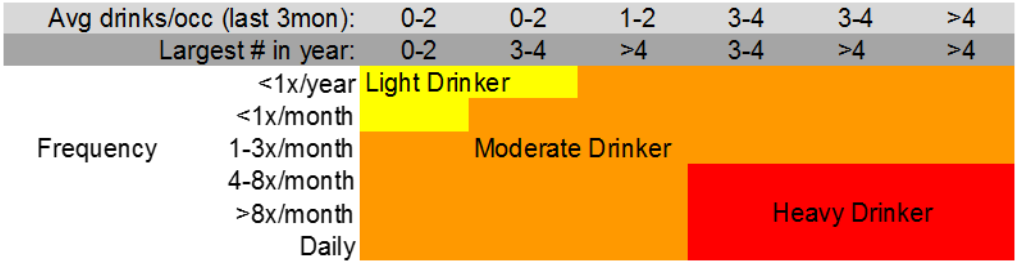
Study personnel will review medication logs and data from the Medication Event Monitoring System (MEMSCap™) that track when the pill bottles are opened, as well as perform pill counts throughout treatment to monitor medication adherence. Medication tolerability and effects will be systematically assessed. Participants will be encouraged to contact study personnel between visits to address any immediate concerns regarding adverse effects. If a participant experiences intolerable medication-related adverse effects at any point during the study, a dose reduction to 600 mg twice daily may be undertaken. The dosage may be increased back to 1200 mg twice daily at the discretion of the medical clinician. However, if a participant is unable to tolerate the reduced dose, medication will be discontinued, and the participant will continue to come in for study visits.

## Inclusion and Exclusion Criteria/ Study Population

Individuals responding to recruitment materials or otherwise referred to the study will be pre-screened on

the phone or in person to ascertain preliminary eligibility status. They may have completed the YC Intake Protocol (PRO# 94743) prior to the formal study screening. A series of questions will determine preliminary eligibility, and formal screening appointments will be scheduled for those who meet these eligibility criteria. The comprehensive assessment will include a medical history and physical examination, urine drug testing, semi-structured psychiatric diagnostic interview, and detailed assessment of alcohol and other substance use history. No information obtained during the pre-screening will be used as research data. All study related procedures will take place on the MUSC campus within the designated office areas of the Addiction Sciences Division and the Center for Biomedical Imaging.

**Figure 2.** Drinking classification. Only **heavy drinkers** will be recruited for this proposal.




**Inclusion criteria:** Participants (*N*=105; 50% female) will be between the ages of 15-19 and may or may not use alcohol. The age of interest corresponds to a time when many adolescents initiate and escalate heavy binge drinking (Johnston et al. 2015), and is therefore the optimal time to intervene. All participants in the alcohol-using group must meet criteria for heavy drinking, based on quantity and frequency of drinking (Squeglia et al. 2011; Squeglia et al. 2012) (see Figure 2). Binge drinking criteria will be used to classify heavy drinking youth, as opposed to alcohol dependence diagnosis, as diagnostic criteria may not be optimal in capturing problematic drinking in youth (Winters et al. 2011). This criteria will be used for the recruitment for all alcohol using participants. Inclusion criteria for non-alcohol-using group: has used alcohol <10 times in their life and has never had a binge drinking episode (4+ females, 5+ males), <5 lifetime experiences with marijuana and none in the past three months; <5 lifetime cigarette use; and no history of other intoxicant use.

**Exclusionary criteria:** To maximize our ability to detect effects of interest and minimize potential confounds, we will use rigorous screening criteria. Exclusion criteria for the alcohol-using group will include: not having a parent to consent (for those under age 18); history of alcohol treatment or treatment-seeking; current DSM-5 diagnosis of moderate or severe substance use disorder other than alcohol or cannabis (American Psychiatric Association 2013); positive urine toxicology screen for narcotics, amphetamines, sedatives, hypnotics, or opiates (not prescribed by a doctor); alcohol withdrawal (> 10 on the Clinical Institute Withdrawal Assessment for Alcohol (Sullivan et al. 1989); medical conditions or medications that contraindicate taking NAC; current use of N-acetylcysteine or any supplement containing N-acetylcysteine (must agree not to take any such supplement throughout study participation);medical history of severe asthma (uncontrolled with medication); history of a serious medical, psychiatric, or neurological problem that could affect neural response, brain development, or study participation, including diabetes, seizure disorder, and severe head injury with loss of consciousness; history of learning disability, pervasive developmental disorder, or other condition requiring special education; current use of psychoactive medications that affect cerebral blood flow; non-correctable visual or hearing problems; non-fluent in English; MRI contraindications (e.g., braces, claustrophobia, irremovable metal implants or piercings); (for females) pregnancy or refusal to use reliable methods of birth control; refusal of blood draw, abstinence from alcohol for >14 days before participation, and use of alcohol <12 hours before scanning (confirmed with breathalyzer). While cigarette and marijuana use will not be exclusionary, we will exclude any participants who are daily users of cannabis or tobacco. Exclusionary criteria for the control group will include all of the above exclusionary criteria except for abstinence from alcohol for >14 days before participation.

**Rationale for vulnerable populations (children and alcohol users).** 45 participants in the alcohol-using group will be between the ages of 15 and 19 and heavy alcohol users. We will include one control group of 10 non-alcohol using youth between the ages of 15-19 to test the effect of NAC on glutamate levels in non-alcohol users. We will also include a second control group of 30 non-alcohol using youth

and a second alcohol using group of 20 youth between the ages of 15-19 who will only complete baseline assessments to investigate potential neural and microbiome differences within binge drinking adolescents as compared to non-drinking controls. Creation of alcohol-focused interventions during adolescence could have substantial long-term implications by reducing acute and long-term negative social, academic, and cognitive consequences related to heavy teen drinking and by reducing the rate of youth transitioning from heavy drinking to more problematic alcohol dependence. While participants in this study are not treatment seeking, all participants will be given treatment referrals at the conclusion of the study.

**Diverse Population:** In this cohort, approximately 50% of the participants will be female, and we aim to recruit participants to approximate the racial and ethnic composition of Charleston County.

**Number of Subjects**

We will recruit 105 15-19 year-olds (50% female).

**Setting**

Visits will take place at MUSC in the Institute of Psychiatry and the Biomedical Imaging MRI research facility at 30 Bee St (for those visits requiring an MRI). If a participant is unable to attend or complete a visit due to unexpected conflict (e.g., transportation issues, travel, University closings), arrangements may be made to remotely complete as much of the visit procedures as possible to maintain data collection and study engagement.

**Recruitment Methods**

**Recruitment and process for obtaining informed consent.** Adolescents will be recruited from the Charleston area. We have received approval from the Charleston County School District to recruit through local high schools. Study staff will make initial contact with school leadership to arrange classroom presentations for high school students. We will also canvas flyers in the Charleston area, although not on the school grounds or college campuses. We will also recruit through paid online advertisement sources such as Facebook, Instagram, Craigslist, etc. The fliers and advertisements include IRB-approved materials that describe the research opportunity, basic inclusion criteria, monetary compensation, and study contact information. Interested youth or parents call our office and verbal consent/assent is obtained from the parents and teens respectively (or simply consents for 18 year olds). The project is described in more detail. The teen is screened with basic questions approved for telephone administration by our IRB. If the teen remains eligible and interested, HIPAA and informed consent (parents and youth over age 18) or assent (youth under age 18) forms are reviewed.

**Consent Process**

**Circumstances under which consent will be sought and obtained, who will seek it, information provided to prospective subjects, and method of documenting consent:** At study entry, any teen who responds to the recruitment fliers or advertisements, appears interested and eligible, and provides verbal consent/assent, are scheduled for an initial appointment. At the screening visit, which will take place in a private office of a research staff within the Addiction Sciences Division, the complete procedures of the study, as described in the consent/assent forms, are provided to prospective subjects and their parents. Parents/guardians of participants under 18 years old will participate with the adolescent in the screening, evaluation, and informed consent/assent procedure. Participants 18 years and older will be able to provide their own informed consent. If still interested, they sign the HIPAA and consent and assent documents and keep the extra copy provided. Trained research staff members complete online and lab-based training in HIPAA policy, human subjects' research, and management of other research issues. During the consent phase, participants and parents are informed that all information provided is confidential within ethical and legal limits to facilitate disclosure. During the consenting process, parents are told that they will not be informed about their child's substance use and that youth self-report and lab data are confidential, with the exception of any acute safety issues (e.g., suicidality, abuse). The forms are reviewed with the interested parties and filed in a locked file cabinet in

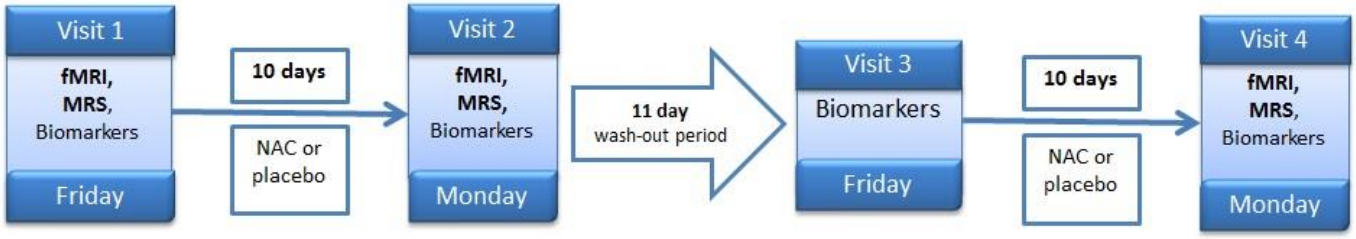
a locked office. Trained research staff members complete online and lab-based training in HIPAA policy, human subjects' research, and management of other research issues.

We may also use several different methods to complete electronic informed consent, if applicable and necessary, that include the following: 1) REDCap electronic consent (e-consent) combined with a video discussion or 2) via MUSC's doxy.me system (teleconsent). As a last resort, we will also email the consent document to the participant and conduct the informed consent via video chat. Participants can then email or mail the signed consent back to the research team if needed. Video chat functionality will only be used if all parties have the capability.

E-consent via REDCap will be saved in a separate informed consent database. All doxy.me signed consent forms will be saved as PDF files within our study records. Using these systems, signatures on the consent form may be obtained electronically via REDCap/doxy.me. In the case that participants mail back hard copies of the consent (in rare instances), those will be stored in locked file cabinets in the offices of research staff.

**Study Design / Methods**

**Overview:** Our primary goal is to assess the effects of NAC versus placebo on alcohol cue reactivity and glutamate levels in adolescent heavy drinkers. A randomized, double-blind, placebo-controlled within-subjects crossover design will be utilized. In counterbalanced order, 55 adolescents will receive a 10-day course of NAC 1200 mg twice daily and a subsequent 10-day course of matched placebo twice daily, separated by 11 days. To allow subjects for flexibility in case of scheduling difficulties, the pill bottle will also include extra doses of medication for each 10-day course. Medication will be prepared and packaged by Pitt St. Pharmacy. Medication will be provided in pharmacy grade bottles. Bottles of medication will use the MEMSCap™, which are child resistant and record a timestamp when the bottle is opened. The MEMSCap system will be used to monitor medication adherence during the trial. Biomarker data will be conducted at baseline and before and after each course of medication treatment and neuroimaging will be collected at baseline and after each treatment trial. Functional magnetic resonance imaging (fMRI) will examine alcohol reactivity, and magnetic resonance spectroscopy will examine glutamate levels in the anterior cingulate, post- NAC and placebo. A secondary goal is to assess the microbiome and neural differences between binge drinking adolescents and non-using controls; therefore, an age- and gender-matched control group (n=30) will be recruited to complete Visit 1, only. We will also include a second alcohol using group of 20 youth between the ages of 15-19 who will only complete baseline assessments to investigate potential neural and microbiome differences within binge drinking adolescents as compared to non-drinking controls. Below describes the detailed procedures for interviews, self-report, neuroimaging, biomarker, and medication administration.




**Consent and interview (30 minutes)**: Following the phone (or in person) screen, eligible adolescents will be invited to participate in a screening visit. Youth will be asked questions regarding demographic and background information, medication use, physical health, neurological history, family history of substance use, and personal substance use history. The youth Structured Clinical Interview (*SCI*; Brown et al. 1994) will ascertain information on psychosocial functioning (e.g., academic/social functioning) and health and medication history. The *Mini International Neuropsychiatric Interview (MINI)*, a structured interview designed to ascertain a current or lifetime history of the major psychiatric disorders in DSM-5 and ICD-10, will be used to assess psychopathology (Sheehan et al. 1998; Sheehan et al. 2010).

**Self-report measures (30 minutes)**: Substance use assessment: Youth typically provide valid self-

reports of alcohol and other drug use (Winters et al. 1990-91), but to maximize accuracy, we will provide a comfortable context for the interview and assure confidentiality of substance use information. Biological samples will be collected to confirm self-reported alcohol use (see below). At baseline (pre-intervention), substance use histories are assessed using a modified *90-day Timeline Follow-back* (Sobell and Sobell 1992) (TLFB) to obtain information on typical use of alcohol, nicotine, marijuana and other illicit drug types, including binge drinking quantity and frequency. For follow-up substance use assessments the TLFB will be administered to obtain daily drinking and other substance use since baseline.

**Table 1. Assessment Instruments and Timeline**

| Instrument Name | Purpose/Domain |
|---|---|
| Informed Consent | Obtain informed consent |
| Youth Interview | Characterize sample |
| MacArthur Socioeconomic Scale | Assess socioeconomic status |
| The MINI International Neuropsychiatric Interview | Assess DSM-5 psychiatric disorders |
| Pregnancy Test for Females | Study and neuroimaging eligibility |
| Clinical Institute Withdrawal Assessment of Alcohol-Revised: CIWA | Study eligibility, safety |
| Urine Drug Screen tests: UDS | Study eligibility, assess drug use |
| Vital signs | Medical safety |
| Adverse Events | Monitor AEs and safety |
| Concurrent Medications Form | Monitor medications |
| Medication Adherence Log | Assess medication compliance |
| ***Time Line Follow-Back: TLFB** | **Primary Outcome; alcohol use (amount and frequency)** |
| Adolescent-Obsessive Compulsive Drinking Scale | Assess alcohol craving |
| Breathalyzer | Assess alcohol use (biological) |
| Alcohol Use Disorders Identification Test: AUDIT | Assess drinking behavior & problems |
| Alcohol Dependence Scale: ADS | Assess alcohol use severity |
| Rutgers Alcohol Problem Index: RAPI | Assess alcohol problems |
| Ethyl glucuronide (EtG) | Biomarker of alcohol use |
| Phosphatidylethanol (PEth) | Biomarker of alcohol use |
| IL-6, IL-10, IL-1B, HMGB1 | Biomarkers of neuroinflammation |
| Cannabis Use Disorder Identification Test-Revised: CUDIT-R | Assess marijuana use & problems |
| Marijuana Craving Questionnaire: MCQ | Assess marijuana craving |
| Questionnaire of Smoking Urges: QSU | Assess nicotine craving |
| Fagerstrom Test | Assess nicotine dependence |
| Alcohol Craving Questionnaire | Assess alcohol craving |
| Beck Depression Inventory-II: BDI-II | Measure depression |
| Columbia Suicide Severity Rating Scale | Assess for suicidality |
| State Trait Anxiety Inventory: STAI | Measure anxiety |
| Barratt Impulsiveness Scale | Measure impulsivity |
| Pittsburgh Sleep Quality Index | Assess sleep functioning |
| Risk Behavior Survey: RBS (selected questions) | Assess risky behaviors |
| Pubertal Development Scale: PDS | Assess pubertal development stage |
| Treatment Services Review | Monitor services utilization |
| Penetration of the blind | Assess blinding procedures |
| Diagnostic Interview Schedule for Children: DISC-IV (selected questions) | Assess disruptive disorders |
| D-KEFS Color-Word Interference Test | Measure executive functioning |
| WRAT-3 Reading Subtest | Measure cognitive ability |

| | |
|---|---|
| Cash Choice Task | Measure motivation, inhibition, impulsivity |
| Alcohol Purchase Task (17-item) | Measure alcohol purchasing behavior |
| Beliefs about Medicine Questionnaire | Measure medication attitudes |
| Med Adherence Video Feedback Questionnaire | Measure medication adherence attitudes |
| Microbiome Feasibility Questionnaire | Assess feasibility of stool collection for future studies |

**Biological markers of alcohol use (10 minutes).** Biological samples will be collected to verify self-reported alcohol use. Specifically, blood phosphatidylethanol (B-PEth) (which will detect persistent moderate to heavy drinking in the weeks before testing), urinary Ethylglucuronide (EtG) (which will detect any drinking within 2 to 3 days before testing), and blood neuroinflammation markers: IL-6, IL-10, IL-1B, and HMGB1,which will detect neuroinflammation both before and after NAC treatment will be assayed by the Clinical Neurobiology Lab (CNL) which is a CAP/CLIA certified clinical laboratory directed by Dr. Anton (co-mentor) and the laboratory of Dr.Wei Jiang. Urine EtG is measured by an immunoassay (Microgenics Corp. – Fremont CA) that has excellent correlation with LC-MS methods (Böttcher et al. 2008) and can be positive (> 100 ng/ml) up to 72 hours after alcohol consumption (Helander et al. 2009).

**Saliva Collection (10 minutes).** Saliva will be collected for oral microbiome analysis. All-in-one collection kits will be used for the collection and stabilization of microbial DNA from saliva. DNA extraction and Microbiome 16S rRNA Sequencing will be performed.

**Neuroimaging protocol (1 hour).** Participants will undergo 3 neuroimaging scans (1 scan at baseline and 1 scan after both placebo and NAC conditions).

Mock scanner: On Visit 1, participants will be introduced to the mock scanner to help them become acclimated to the MR environment and give them the opportunity to practice remaining still while in the magnet, which will minimize motion-related confounds in the imaging data.

Scan session: Breath and urine toxicology samples will be collected before every scan. Pre-scan state and craving measures are given. Imaging sessions (~60 minutes total including set-up) consist of:

1. T1-weighted structural: A high-resolution anatomical scan will be acquired, to allow subsequent registration to functional images and region-of-interest (ROI) definition.

**Figure 3.** Alcohol cue reactivity paradigm. BOLD response during alcohol (e.g., beer) vs. neutral (e.g., juice) cues will be the main contrast of interest for Aim 1.

2. The Alcohol Cue Reactivity Task: (Schacht et al. 2011; Schacht et al. 2013b). During the alcohol cue reactivity task, participants are shown pseudorandomly interspersed images of alcoholic (i.e., beer, wine, and hard liquor) and non-alcoholic (e.g., soft drink, juice) beverages, visual control images (i.e., blurred images), and a fixation cross (see Figure 3). A magnetic **fieldmap** will also be acquired to allow geometric unwarping and cost-function masking of EPI images induced by magnetic field inhomogeneities.

3. Magnetic Resonance Spectroscopy: The magnetic resonance spectroscopy protocol proposed will be utilizing previously published methods used by Drs. Truman Brown and James Prisciandaro.

**NAC or placebo administration.** This double blind crossover trial will compare NAC (1,200mg twice daily) and placebo. Participants will be randomized to each condition for 10 days in a counterbalanced order, with a minimum 11-day washout period between conditions to allow for the clearance of NAC (Holdiness 1991). Medication response and tolerability/adverse events will be assessed at each clinic visit. Reminders to take medication will be sent via email and/or text message to increase compliance. Participants will also be required to upload videos of themselves taking their morning and evening medication doses. Participants will be trained on how to record and upload these videos and study staff will extract timestamps from these videos to monitor that medication is being taken within the correct time frame.

Participants will be compensated $460 for completing all 5 visits. Also, they can earn up to a $40 bonus ($2 per day while receiving medication) if they take each dose of medication, or provide staff with an

explanation for why the medication was not taken, within a 4 hour requested time window. If they do not take the medication due to adverse side effects, they will need to discuss these side effects with research staff. Participants experiencing adverse side effects interfering with their ability to participate may be taken off the medication. If they are unable to finish the study, they will be prorated for number of completed visits. Participants will also be eligible for a $50 referral bonus for each individual they refer who successfully randomizes into this study. Mileage reimbursement may also be available to participants.

**Data Management**

**Sample Size Determination.** Crossover designs are highly cost-efficient and powerful designs (Wellek and Blettner 2012). Previous research has shown NAC has an effect size of $d$ =1.13 versus placebo on glutamatergic levels in the ACC in cocaine dependent adults in a similar randomized, double-blind, placebo-controlled within-subjects crossover study (Schmaal et al. 2012). With 32 participants, the proposed study will have 85% power to detect such effects ($\alpha$ = 0.05, two-tailed) (Erdfelder et al. 1996). Having 50 heavy drinking participants will allow for 20% participant drop out, which is consistent with the drop out rate at the 4 week mark in Dr. Gray's NAC adolescent marijuana trial, as well as exclusion of participants due to motion in scanner (Gray et al., 2012).

**Analysis and approach.** (1) BOLD response to alcohol vs. non-alcohol cues in the regions of interest (ACC, insula, striatum, amygdala) and (2) glutamate levels in the ACC will be the primary contrasts of interest to test Aims 1 and 2. A generalized linear mixed effect model will examine the effect of NAC vs placebo on alcohol cue reactivity and glutamate. The primary model will contain the main effect of treatment (NAC vs. placebo), as well as day (scan 1 vs. scan 2) and order (NAC vs. placebo) to ensure the crossover design and washout period were successful. During model development, random intercepts will be included to account for variations in baseline response levels for individual participants and random slopes to account for varying responses to treatment. Model fit will be tested using likelihood ratio tests and Akaike Information Criterion values. Baseline levels of clinical and demographic characteristics (sex, age, alcohol use characteristics) will be tested for univariate associations with study outcomes; when associated, these variables will be included in the adjusted model development strategy. Parameter estimates and associated 95% confidence intervals for treatment effects on cue reactivity and glutamate will be reported. Model based parameter estimates across groups (means and standard deviations) will be used to estimate treatment effect sizes, in order to inform power analysis for a subsequent full-scale randomized controlled trial. Power analyses and predetermined statistical analyses will ensure findings are robust and unbiased.

We will also analyze the effects of adolescent binge drinking and sex as a biological variable (SABV), as compared to a non-drinking control group (1:1 sex ratio), to assess the alcohol-related effects within (1) glutamate, GABA, and NAA levels in the ACC, (2) BOLD response to alcohol v. non-alcohol cues in the regions of interest (ACC, insula, striatum, amygdala), and (3) microbiome composition. Two-way analysis of variance (ANOVA) will be used to test the main effects of group and SABV, as well as their interaction (group × SABV). Resulting significant interaction terms will be further tested to assess the effect of group within sex (e.g., difference in glutamate levels between drinking and non-drinking females) and the effect of sex within group (e.g., difference in glutamate levels between male and female heavy drinkers). Clinical and demographic characteristics (age, alcohol use characteristics, GM:BM for MRS data) will be tested for univariate associations with study outcomes; when associated, these variables will be included in an analysis of covariance (ANCOVA). Power analyses and predetermined statistical analyses will ensure findings are robust and unbiased. Summary statistics across groups (means and standard deviations) will be used to estimate SABV and alcohol effect sizes, in order to inform power analysis for a subsequent full-scale studies. SAS will be used for data analysis with a significance level of $\alpha$ = 0.05, corrected for multiple comparisons.

**Biological markers.** Changes in blood phosphatidylethanol (PEth), urinary Ethylglucuronide (EtG), and blood inflammatory marker (IL-6, IL-10, IL-1B, HMGB1) levels from pre- to post-treatment will be explored in relation to participants' change in drinking behaviors. This data can be used to inform power analysis for a subsequent full-scale randomized controlled trial.

**Interpretation of results and future studies.** We believe the proposed study is important and will inform future treatment options for adolescent substance use. Given the promising findings from marijuana-using adolescents (Gray et al. 2012), it is important to examine the effect of NAC on alcohol cue reactivity and glutamatergic systems, as modifying these neural systems via pharmacotherapy could decrease alcohol use during adolescence. However, some limitations exist. We may find that NAC does not affect alcohol cue reactivity or glutamate levels compared to placebo, which in itself would be important information and clarify the underlying mechanism of NAC. Regardless of NAC effects, understanding how varying levels of drinking affect glutamate will be important in informing future targeted therapies. The majority of the preclinical NAC literature was performed on male rodents; sex could play a factor in the response to the treatment and will be explored in analyses, as we will be collecting data on an equal number of male and female participants. NAC has the potential to significantly advance treatment options for adolescent heavy drinkers and could provide a safe, tolerable, accessible, and affordable option for adolescents seeking alcohol treatment.
The long-term goals of this work are to determine if NAC is a promising treatment for adolescent heavy alcohol use, which is consistent with the trans-NIH initiative to identify neurally-informed novel substance use treatments for adolescents.

**Provisions to Monitor the Data to Ensure the Safety of Subjects**

(a) **Trial Management:** All aspects of the study will be run through the MUSC Department of Psychiatry and Behavioral Sciences, where the PI holds her faculty appointment.

(b) **Data Management and Analysis**: Data will be collected using standardized paper or electronic forms and will only be identified with the study's ID of the participant. The codes linking the name of the participant to the study ID will be kept confidential in a secured cabinet by the PI. Collected forms will be securely transported to the PI's data entry center. Research assistants will enter data in REDCap (Research Electronic Data Capture), a secure, web-based application designed exclusively to support data capture for research studies. REDCap provides: 1) an intuitive interface for data entry (with data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R); 4) procedures for importing data from external sources; and 5) advanced features, such as branching logic and calculated fields. These procedures are effective in minimizing data entry errors (e.g., missing or errant data). When possible, participant direct entry into REDCap (rather than paper forms) will be utilized.

(c) **Regulatory Issues:** The protocol will be registered on the clinical trials registry (clinicaltrials.gov). All serious adverse events will be reported to the MUSC Institutional Review Board (IRB) within 24 hrs. Follow-up of all serious adverse events will be reported as well. All adverse events are reviewed weekly by the PI and yearly by both the DSMB and the IRB. Any significant actions taken by the local IRB, including significant protocol changes, will be relayed to NIAAA. We anticipate the serious adverse event rate to be extremely low. If monitoring indicates otherwise, we will convene a special meeting of the DSMB.

(d) **Trial Safety:** The potential risks and benefits and methods to minimize these risks are outlined above. Guidelines have been developed for managing and reporting of adverse events (AEs), including serious adverse events (SAE; defined as any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others). Drs. Squeglia and Gray will serve as the Program Managers for AEs. If an AE is non-serious (self-limited with no intervention needed), no further action will be necessary. However, in the case of a serious, unresolved event, an AE follow-up log will be completed within 24 hours of the start of the SAE. Relevant information will be recorded on SAE Notification Form. The complete report (SAE Notification Form, Concomitant Medication Log, and AE Log) will be reviewed with Dr. Gray, and Dr. Squeglia will notify the IRB, Data and Safety Monitoring Board (DSMB), and NIH about the

SAE. Additionally, Dr. Squeglia will communicate summary reports of DSMB discussion of the SAE, or any deliberations of IRB regarding the review of the SAE or the trial itself, to NIH. If the event is "Serious, Unexpected and Associated" (an SAE is considered unexpected if it is not described in the Package Insert), Dr. Squeglia will complete Food and Drug Administration (FDA) Form 3500A and will forward it to the FDA with Dr. Gray's assistance. Dr. Squeglia also will inform the IRB and the study participants (and parents/guardians, as appropriate) about the SAE. In all of these reviews and reports, strict patient confidentiality will be maintained.

AEs will be coded on a weekly basis using Medical Dictionary for Regulatory Activities (MedDRA) rules and entered into a database. For each weekly study meeting, Dr. Squeglia or research staff will prepare a summary of all AEs, including their severity and presumed relation to study medication. Drs. Squeglia and Gray will review this at their weekly meetings (or before if more urgent).

Study procedures will follow as much as possible the FDA's Good Clinical Practice Guidelines. We will encourage participants (and parents/guardians as appropriate) to notify their physicians that a) they are in a research study evaluating N-acetylcysteine, and b) the physician should contact the PI directly if the physician has any questions.

The research assistants will be instructed not to reveal whether a person is a participant in the study and will report to the PI any outside requests for information about a participant or any breaches in confidentiality. All requests by participants' physicians and other medical providers will be referred directly to the PI.

(f) **Trial Efficacy:** The Data and Safety Monitoring Board may request a blinded interim efficacy report (blinded to the PI and research team) for review while the trial is ongoing. Final (fully unblinded) analyses will occur after all participants have completed all visits.

(g) **Data and Safety Monitoring Plan Administration:** The PI will be responsible for monitoring the trial and will examine the database regularly for missing data, unexpected distributions or responses, and outliers. The PI will prepare the weekly adverse event database immediately prior to the weekly meetings with Dr. Gray to a) see if any particular MedDRA categories are being endorsed more frequently than anticipated, and b) determine if any adverse event symptom checklist scores are higher than expected. A DSM report will be filed with the IRB and NIAAA on a yearly basis, unless greater than expected problems occur. The report will include participant characteristics, retention and disposition of study participants, quality assurance issues and reports of adverse events, significant/unexpected adverse events and serious adverse events.

**Data and Safety Monitoring Board:** We will create a DSMB, comprised of multidisciplinary faculty with expertise in pharmacotherapy and behavioral treatment trials (youth and/or addiction-related). The DSMB will meet annually (more frequently as needed for emergent situations) to review any AEs related to the study, as well as review any data management related errors. The board may be called at any point if needed for serious adverse events, etc. Modification will be made in the procedures and/or the protocol if necessary based on the findings of the board.

## Risks to Subjects

Questionnaires and interviews are all non-invasive and, as such, involve minimal physical risk to participants.

In order to address any concerns regarding coercion, adolescents and parents will be informed that they are free to choose not to participate and may withdraw at any time (this is included in the consent and

assent forms). Since this study involves minors, particular caution will be exercised in obtaining informed adolescent assent separately and independently from parental consent. To this end, an initial step in subject recruitment involves obtaining parental/legal guardian permission for participation by the adolescent. Once parental consent is secured, subjects will be asked separately and independently for informed consent (i.e., parental consent will not be used to persuade teens to participate). This approach is considered very effective in minimizing coercion to participate.

Potential risks incurred by participants include:

1. Adverse events related to study medication
2. Loss of confidentiality
3. Adverse events related to MRI

**Adverse events related to study medication.** *N*-acetylcysteine has a generally benign adverse effect profile. A meta-analysis of studies evaluating long-term oral treatment with *N*-acetylcysteine for prevention of chronic bronchitis found that *N*-acetylcysteine was well tolerated, with generally mild, most commonly gastrointestinal adverse effects that did not require treatment interruption (Grandjean et al. 2000). Our experience to date with *N*-acetylcysteine at the same dose proposed in this trial in youth with cannabis use disorder suggests a benign adverse event profile (Gray et al. 2012).

Some patients who have taken intravenous *N*-acetylcysteine for the treatment of acetaminophen overdose have had more serious reactions. Allergic reactions have occurred in about 5% of patients taking intravenous *N*-acetylcysteine (Bailey & McGuigan 1998). These reactions may be mild, consisting of flushing, rash, and itching. Less common side effects include trouble breathing, low or high blood pressure, fever, and hives. If untreated, such a reaction could lead to death. Even more rare serious side effects of intravenous *N*-acetylcysteine are irritability, confusion, and seizures. These reactions (severe allergic reaction or seizures) have never been reported when *N*-acetylcysteine is taken orally, as it will be in this study. As a precaution, we will exclude individuals with a recent history of asthma, as they are believed to possess a higher risk of allergic reaction to *N*-acetylcysteine. We will also exclude individuals with a history of seizure disorder.

**Loss of confidentiality.** Any communication of personal information carries the potential risk of breach of confidentiality.

**Adverse events related to MRI.** Risks associated with MRI (including fMRI) are minimal for individuals who do not have metal and are not claustrophobic. Some discomfort may result from lying in the scanner for up to 60 minutes.

**Procedures for protecting against potential risks**

**Adverse events related to study medication.** The informed consent process will be used to thoroughly educate participants and parents/guardians about potential medication-related risks, including adverse events. This discussion will include thorough review of adverse events associated with oral NAC treatment. Rigorous screening procedures and strict exclusion criteria are designed to exclude potential participants at elevated risk for adverse events. Drs. Squeglia and Gray will conduct serial adverse events monitoring as part of medication management. Participants and parents/guardians will have access to the study medical clinician 24 hours, 7 days a week for emergencies. Participants experiencing intolerable adverse events will have the opportunity to reduce dose or discontinue medication altogether, while remaining in the study for ongoing monitoring. Primary mentor Dr. Gray has full hospital admitting privileges in the event of an adverse event requiring hospitalization. Urine pregnancy tests will be conducted for female participants.

**Adverse events related to confidentiality.** Participants will be assured that all records will be kept confidential in research files located in a locked office and entered into a password-protected computer located behind a secure and maintained firewall. Breach of confidentiality is highly unlikely because all personally identifying information will be kept separate from data collected and will be linked only by a master subject identification list which is maintained by the PI.

**Adverse events related to MRI.** Participants will be told about the potential discomforts associated with lying in the scanner and their heads and necks will be supported for maximum comfort. Earplugs and headphones will be provided to reduce the noise of the scanner to a safe decibel range. Pregnancy testing with immediate results will ensure that no pregnant adolescent receives an MRI. Any positive pregnancy test results would be discussed with the adolescent, and counseling will be provided should the participant indicate distress concerning the result.

**Ensuring professional intervention in the event of adverse effects**: If a participant should become uncomfortable with any procedures, he/she can withdraw from the study at any time. If any medical problems arise, 911 will be phoned immediately. If psychological distress, suicidal ideation, or another problem should occur, the clinically-licensed PI or a designated backup licensed professional will be contacted immediately. Paperwork detailing the appropriate steps to handling suicidal/homicidal ideation or abuse/neglect cases is posted above every research assistant's phone, along with contact numbers of the PI and Co-Is.

**Policy on neuroimaging data:** Participants and their family (for those under age 18) are informed that this is a research study and therefore does not include clinical imaging to confirm clinical findings. Therefore, we cannot provide them with medical reports. If the research staff note a structural irregularity on a scan, a neuroradiologist will review it (fully de-identified) and note if it is of clinical significance or not. If the finding has the potential of clinical significance, the volunteer or parents (depending on age) will be notified to follow up with his or her doctor. There is a minimal risk of undue stress or concern if the finding is determined to be benign or not clinically significant. Furthermore, there may be costs associated with the recommended evaluations which are not covered by our research protocol. We include the following in the consent: "If the possibility of such a situation is too stressful for you, your child should not participate in our study."

**Potential Benefits to Subjects or Others**

**Potential benefits of the research to subjects and others**: Participation in this study involves minimal risk for participants. Benefits of participation include providing substantial long-term benefits toward understanding the extent to which NAC can affect underlying neural circuitry during adolescence and alcohol use.

**Why risks to subjects are reasonable in relation to anticipated benefits**: Given the minimal risks to participants involved, we believe the risk/benefit ratio is acceptable. Youth in the study may decrease their alcohol use which could positively affect other aspects of their lives.

**References**


Abdelmegeed, M. A., Banerjee, A., Jang, S., Yoo, S.H., Yun, J.W., Gonzalez, F. J., Keshavarzian, A., Song, B. J. (2013) CYP2E1 potentiates binge alcohol-induced gut leakiness, steatohepatitis, and apoptosis. Free Radical Biology and Medicine, 65, 1238–1245.

Achenbach TM (1991) Manual for the Child Behavior Checklist/ 4-18 and 1991 Profile. University of Vermont, Department of Psychiatry, Burlington, VT

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (5th ed.). American Psychiatric Publishing, Arlington, VA

Ames, N. J., Barb, J. J., Schuebel, K., Mudra, S., Meeks, B. K., Tuason, R. T. S., Brooks, A. T., Kazmi, N., Yang, S., Ratteree, K., Diazgranados, N., Krumlauf, M., Wallen, G. R., Goldman, D. (2020) Longitudinal gut microbiome changes in alcohol use disorder are influenced by abstinence and drinking quantity. Gut Microbes, 11(6), 1608–1631.

Bailey B, McGuigan MA (1998) Management of anaphylactoid reactions to intravenous N-acetylcysteine. Annals of Emergency Medicine 31: 710-715.

Beck AT, Steer RA, Brown GK (1996) Manual for the Beck Depression Inventory-2. Psychological Corporation, San Antonio, TX

Böttcher M, Beck O, Helander A (2008) Evaluation of a new immunoassay for urinary ethyl glucuronide testing. Alcohol and Alcoholism 43: 46-48.

Bridge JA, Iyengar S, Salary CB, Barbe RP, Birmaher B, Pincus HA, Ren L, Brent DA (2007) Clinical response and risk for reported suicidal ideation and suicide attempts in pediatric antidepressant treatment: A meta-analysis of randomized controlled trials. JAMA 297: 1683-1696.

Brown SA, Myers MG, Mott MA, Vik PW (1994) Correlates of success following treatment for adolescent substance abuse. Applied & Preventive Psychology 3: 61-73.

Chung T, Maisto SA (2006) Relapse to alcohol and other drug use in treated adolescents: Review and reconsideration of relapse as a change point in clinical course. Clinical Psychology Review 26: 149-161.

Crews FT, Qin L, Sheedy D, Vetreno RP, Zou J (2013). High mobility group box 1/Toll-like receptor danger signaling increases brain neuroimmune activation in alcohol dependence. *Biological psychiatry* 73(7): 602-612.

Deas D, Roberts JS, Randall CL, Anton RF (2002) Confirmatory analysis of the adolescent obsessive compulsive drinking scale (A-OCDS): A measure of 'craving' and problem drinking in adolescents/young adults. Journal of National Medical Association 94: 879-887.

Deas D, Thomas S (2002) Comorbid psychiatric factors contributing to adolescent alcohol and other drug use. Alcohol Research & Health 26: 116-121.

Deas DV, Riggs P, Langenbucher M, Goldman M, Brown S (2000) Adolescents are not adults: Developmental considerations in alcohol users. Alcoholism: Clinical and Experimental Research 24: 232-237.

Deas DV, Roberts JS, Randall CL, Anton RF (2001) Adolescent Obsessive-Compulsive Drinking Scale (A-OCDS): An assessment tool for problem drinking. Journal of National Medical Association 93: 92-103.

Deepmala, Slattery J, Kumar N, Delhey L, Berk M, Dean O, Spielholz C, Frye R (2015) Clinical trials of N-acetylcysteine in psychiatry and neurology: A systematic review. Neuroscience & Biobehavioral Reviews 55: 294-321.

Degenhardt L, O'Loughlin C, Swift W, Romaniuk H, Carlin J, Coffey C, Hall W, Patton G (2013) The persistence of adolescent binge drinking into adulthood: Findings from a 15-year prospective cohort study. BMJ Open 3: e003015.

Erdfelder E, Faul F, Buchner A (1996) GPOWER: A general power analysis program. Behavior Research Methods, Instruments, & Computers 28.

Eroshenko, D., Polyudova, T., Korobov, V. (2017) N-acetylcysteine inhibits growth, adhesion and biofilm formation of Gram-positive skin pathogens. Microbial Pathogenesis, 105, 145–152.

Fan, X., Peters, B. A., Jacobs, E. J., Gapstur, S. M., Purdue, M. P., Freedman, N. D., Alekseyenko, A., Wu, J., Yang, L., Pei, Z., Hayes, R., Ahn, J. (2018) Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. Microbiome, 6(1).

Folstein MF, Luria R (1973) Reliability, validity, and clinical application of the Visual Analogue Mood Scale. Psychological Medicine 3: 479-486.
Frye MA, Thomas MA, Yue K, Binesh N, Davanzo P, Ventura J, O'Neill J, Guze B, Curran JG, Mintz J (2007) Reduced concentrations of N-acetylaspartate (NAA) and the NAA-creatine ratio in the basal ganglia in bipolar disorder: A study using 3-Tesla proton magnetic resonance spectroscopy. Psychiatry Research 154: 259-265.
Grandjean EM, Berthet P, Ruffmann R, Leuenberger P (2000) Efficacy of oral long-term N-acetylcysteine in chronic bronchopulmonary disease: a meta-analysis of published double-blind, placebo-controlled clinical trials. Clinical Therapeutics 22: 209-221.
Gray KM, Carpenter MJ, Baker NL, DeSantis SM, Kryway E, Hartwell KJ, McRae-Clark AL, Brady KT (2012) A double-blind randomized controlled trial of N-acetylcysteine in cannabis-dependent adolescents. American Journal of Psychiatry 169: 805-812.
Gray KM, Watson NL, Carpenter MJ, Larowe SD (2010) N-acetylcysteine (NAC) in young marijuana users: an open-label pilot study. American Journal of Addiction 19: 187-189.
Guerri C, Pascual M (2013). Role of Toll-Like Receptor 4 in Alcohol-Induced Neuroinflammation and Behavioral Dysfunctions. *Neural-Immune Interactions in Brain Function and Alcohol Related Disorders*. Springer, pp 279-306.
Helander A, Böttcher M, Fehr C, Dahmen N, Beck O (2009) Detection times for urinary ethyl glucuronide and ethyl sulfate in heavy drinkers during alcohol detoxification. Alcohol and Alcoholism 44: 55-61.
Helander A, Husa A, Jeppsson JO (2003) Improved HPLC method for carbohydrate-deficient transferrin in serum. Clinical Chemistry 49: 1881-1890.
Hingson RW, Heeren T, Winter MR (2006a) Age at drinking onset and alcohol dependence: Age at onset, duration, and severity. Archives of Pediatrics and Adolescent Medicine 160: 739-746.
Hingson RW, Heeren T, Winter MR (2006b) Age of alcohol-dependence onset: Associations with severity of dependence and seeking treatment. Pediatrics 118: e755-763.
Holdiness MR (1991) Clinical pharmacokinetics of N-acetylcysteine. Clinical Pharmacokinetics 20: 123-134.
Hou, Y., Wang, L., Yi, D., & Wu, G. (2015) N-acetylcysteine and intestinal health: a focus on its mechanism of action. *Frontiers in bioscience (Landmark edition)*, *20*, 872–891.
Jensen CD, Cushing CC, Aylward BS, Craig JT, Sorell DM, Steele RG (2011) Effectiveness of motivational interviewing interventions for adolescent substance use behavior change: A meta-analytic review. Journal of Consulting and Clinical Psychology 79: 433-440.
Johnston LD, O'Malley PM, Miech RA, Bachman JG, Schulenberg JE (2015) *Monitoring the Future national results on adolescent drug use: Overview of key findings, 2014*, Ann Arbor, Michigan
Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. Nature Reviews Neuroscience 10: 561-572.
Kalivas PW, Volkow ND (2011) New medications for drug addiction hiding in glutamatergic neuroplasticity. Molecular Psychiatry 16: 974-986.
Kamal, H., Tan, G. C., Ibrahim, S. F., Shaikh, M. F., Mohamed, I. N., Mohamed, R., Hamid, A. A., Ugusman, A., & Kumar, J. (2020) Alcohol Use Disorder, Neurodegeneration, Alzheimer's and Parkinson's Disease: Interplay Between Oxidative Stress, Neuroimmune Response and Excitotoxicity. *Frontiers in Cellular Neuroscience*, *14*, 282.
Kodukula, K., Faller, D. V., Harpp, D. N., Kanara, I., Pernokas, J., Pernokas, M., Powers, W. R., Soukos, N. S., Steliou, K., & Moos, W. H. (2017) Gut Microbiota and Salivary Diagnostics: The Mouth Is Salivating to Tell Us Something. *BioResearch open access*, *6*(1), 123–132.
Kristjansson AL, Sigfusdottir ID, Allegrante JP (2013) Adolescent substance use and peer use: A multilevel analysis of cross-sectional population data. Substance Abuse Treatment, Prevention, and Policy 8.
Li CS, Luo X, Yan P, Bergquist K, Sinha R (2009). Altered impulse control in alcohol dependence: neural measures of stop signal performance. *Alcohol Clin Exp Res* 33(4): 740-750.
McClure EA, Gipson CD, Malcolm RJ, Kalivas PW, Gray KM (2014) Potential role of N-acetylcysteine in the management of substance use disorders. CNS Drugs 28: 95-106.

Michaelis T, Merboldt KD, Bruhn H, Hänicke W, Frahm J (1993) Absolute concentrations of metabolites in the adult human brain in vivo: quantification of localized proton MR spectra. Radiology 187: 219-227.
Miller JW, Naimi TS, Brewer RD, Jones SE (2007) Binge drinking and associated health risk behaviors among high school students. Pediatrics 119: 76-85.
Miller WR, Tonigan JS (1996) Assessing drinkers' motivation for change: The Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES). Psychology of Addictive Behaviors 10: 81-89.
Miranda R, Ray L, Blanchard A, Reynolds EK, Monti PM, Chun T, Justus A, Swift RM, Tidey J, Gwaltney CJ, Ramirez J (2014) Effects of naltrexone on adolescent alcohol cue reactivity and sensitivity: An initial randomized trial. Addiction Biology 19: 941-954.
Mullins PG, Chen H, Xu J, Caprihan A, Gasparovic C (2008) Comparative reliability of proton spectroscopy techniques designed to improve detection of J-coupled metabolites. Magnetic Resonance in Medicine 60: 964-969.
Olive MF, Cleva RM, Kalivas PW, Malcolm RJ (2012) Glutamatergic medications for the treatment of drug and behavioral addictions. Pharmacology Biochemistry and Behavior 100: 801-810.
Ongür D, Jensen JE, Prescot AP, Stork C, Lundy M, Cohen BM, Renshaw PF (2008) Abnormal glutamatergic neurotransmission and neuronal-glial interactions in acute mania. Biological Psychiatry 64: 718-26.
Operario D, Adler NE, Williams DR (2004) Subjective social status: Reliability and predictive utility for global health. Psychology and Health 19: 237-246.
Ozaras, R., Tahan, V., Aydin, S., Uzun, H., Kaya, S., & Senturk, H. (2003) N-acetylcysteine attenuates alcohol-induced oxidative stress in the rat. *World journal of gastroenterology*, *9*(1), 125–128.
Pascual M, Pla A, Miñarro J, Guerri C (2014). Neuroimmune activation and myelin changes in adolescent rats exposed to high-dose alcohol and associated cognitive dysfunction: a review with reference to human adolescent drinking. *Alcohol and alcoholism* 49(2): 187-192.
Petersen AC, Crockett L, Richards M, Boxer A (1988) A self-report measure of pubertal status: Reliability, validity, and initial norms. Journal of Youth and Adolescence 17: 117-133.
Provencher S (2015) LCModel1 & LCMgui User's Manual: http://www.s-provencher.com/pages/lcmodel.shtml
Rice JP, Reich T, Bucholz KK, Neuman RJ, Fishman R, Rochberg N, Hesselbrock VM, Nurnberger JIJ, Schuckit MA, Begleiter H (1995) Comparison of direct interview and family history diagnoses of alcohol dependence. Alcoholism: Clinical and Experimental Research 19: 1018–1023.
Rowe CL, Liddle HA, Greenbaum PE, Henderson CE (2004) Impact of psychiatric comorbidity on treatment of adolescent drug abusers. Journal of Substance Abuse Treatment 26: 129-140.
Schacht JP, Anton RF, Myrick H (2013a) Functional neuroimaging studies of alcohol cue reactivity: a quantitative meta-analysis and systematic review. Addiction Biology 18: 121-133.
Schacht JP, Anton RF, Randall PK, Li X, Henderson S, Myrick H (2011) Stability of fMRI striatal response to alcohol cues: a hierarchical linear modeling approach. NeuroImage 56: 61-68.
Schacht JP, Anton RF, Voronin KE, Randall PK, Li X, Henderson S, Myrick H (2013b) Interacting effects of naltrexone and OPRM1 and DAT1 variation on the neural response to alcohol cues. Neuropsychopharmacology 38: 414-422.
Schmaal L, Veltman DJ, Nederveen A, van den Brink W, Goudriaan AE (2012) N-acetylcysteine normalizes glutamate levels in cocaine-dependent patients: A randomized crossover magnetic resonance spectroscopy study. Neuropsychopharmacology 37: 2143-2152.
Schmidt LE, Dalhoff K (2001) Risk factors in the development of adverse reactions to N-acetylcysteine in patients with paracetamol poisoning. British Journal of Clinical Pharmacology 51: 87–91.
Sheehan DV, Lecrubier Y, Harnett-Sheehan K, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar G (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry 59: S22-S33.
Sheehan DV, Sheehan KH, Shytle RD, Janavs J, Bannon Y, Rogers JE, Milo KM, Stock SL, Wilkinson B (2010) Reliability and validity of the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID). Journal of Clinical Psychiatry 71: 313-326.

Sobell LC, Sobell MB (1992) Timeline Follow-back: A technique for assessing self-reported ethanol consumption. In: Allen J, Litten RZ (eds) Measuring Alcohol Consumption: Psychosocial and Biological Methods. Humana Press, Totowa, NJ, pp 41-72

Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA

Squeglia LM, Dager Schweinsburg A, Pulido C, Tapert SF (2011) Adolescent binge drinking linked to abnormal spatial working memory brain activation: Differential gender effects. Alcoholism: Clinical and Experimental Research 35: 1831-1841.

Squeglia LM, Jacobus J, Tapert SF (2014) The effect of alcohol use on human adolescent brain structures and systems. Handbook of Clinical Neurology 125: 501-510.

Squeglia LM, Pulido C, Wetherill RR, Jacobus J, Brown GG, Tapert SF (2012) Brain response to working memory over three years of adolescence: Influence of initiating heavy drinking. Journal of Studies on Alcohol and Drugs 73: 749-760.

Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM (1989) Assessment of alcohol withdrawal: The revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). British Journal of Addiction 84: 1353-1357.

Swendsen J, Burstein M, Case B, Conway KP, Dierker L, He J, Merikangas KR (2012) Use and abuse of alcohol and illicit drugs in US adolescents: Results of the National Comorbidity Survey-Adolescent Supplement. Archives of General Psychiatry 69: 390-398.

Tanner-Smith EE, Wilson SJ, Lipsey MW (2013) The comparative effectiveness of outpatient treatment for adolescent substance abuse: A meta-analysis. Journal of Substance Abuse Treatment 44: 145-158.

Tripodi SJ, Bender K, Litschge C, Vaughn MG (2010) Interventions for reducing adolescent alcohol abuse: A meta-analytic review. Archives of Pediatrics and Adolescent Medicine 164: 85-91.

Vetreno RP, Crews FT (2012). Adolescent binge drinking increases expression of the danger signal receptor agonist HMGB1 and Toll-like receptors in the adult prefrontal cortex. *Neuroscience* 226: 475-488.

Vetreno RP, Qin L, Crews FT (2013). Increased receptor for advanced glycation end product expression in the human alcoholic prefrontal cortex is linked to adolescent drinking. *Neurobiology of disease* 59: 52-62.

Wellek S, Blettner M (2012) On the proper use of the crossover design in clinical trials: Part 18 of a series on evaluation of scientific publications. Deutsches Ärzteblatt International 109: 276-281.

White HR, Labouvie EW (1989) Towards the assessment of adolescent problem drinking. Journal of Studies on Alcohol 50: 30-37.

Winters KC, Botzet AM, Fahnhorst T, Koskey R (2009) Adolescent substance abuse treatment: A review of evidence-based research. Springer Academic, New York

Winters KC, Martin CS, Chung T (2011) Substance use disorders in DSM-V when applied to adolescents. Addiction 106: 882-884.

Winters KC, Stinchfield RD, Henly GA, Schwartz R (1990-91) Validity of adolescent self-report of alcohol and other drug involvement. International Journal of the Addictions 25: 1379-1395.

Xu, C. C., Yang, S. F., Zhu, L. H., Cai, X., Sheng, Y. S., Zhu, S. W., & Xu, J. X. (2014) Regulation of N-acetyl cysteine on gut redox status and major microbiota in weaned piglets1. Journal of Animal Science, 92(4), 1504–1511.

Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ*, et al* (2006). HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock* 26(2): 174-179.

Zafarullah M, Li W, Sylvester J, Ahmad M (2003). Molecular mechanisms of N-acetylcysteine actions. *Cellular and Molecular Life Sciences CMLS* 60(1): 6-20.