PROTOCOL TITLE: A Randomized Controlled Trial of N-Acetylcysteine for Alcohol Use Disorder and Comorbid PTSD

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The primary objective of the proposed Phase II study is to evaluate the effects of NAC in reducing (1) AUD severity and (2) PTSD symptomatology among individuals (N=200) with current AUD and PTSD. We will also use functional magnetic resonance imaging (fMRI) and proton magnetic resonance spectroscopy (MRS) to investigate the neural circuitry and neurochemistry underlying comorbid AUD/PTSD and prognostic indicators of positive treatment response. Secondary objectives are to evaluate the effects of NAC on impairment in associated areas of functioning (e.g., depression, anxiety, sleep, risky behaviors). In order to accomplish this we will (1) employ an intent-to-treat, double-blind, placebo-controlled randomized controlled trial that will consist of 12 weeks of treatment with NAC (2400 mg per day) or placebo medication; (2) examine standardized, repeated dependent measures of clinical outcomes at baseline, week 6, week 12, and 3-, 6-, and 12-month follow-up; and (3) employ advanced neuroimaging methodologies, a laboratory cue paradigm, and collect biologic measures of alcohol consumption. The following specific aims are proposed:

Specific Aim 1: To determine the efficacy of N-acetylcysteine (NAC), as compared to placebo, in reducing alcohol use severity.

Specific Aim 2: To determine the efficacy of N-acetylcysteine (NAC), as compared to placebo, in reducing PTSD symptomatology.

Specific Aim 3: To use multimodal neuroimaging techniques to investigate the pathophysiology underlying AUD and comorbid PTSD, and prognostic indicators of treatment outcome.

Human Subjects Involvement and Characteristics: A total of 200 adults between the ages of 18 and 70 will be recruited over a 5-year period. The inclusion/exclusion criteria are as follows:

Inclusion Criteria:

1. Male or female; any race or ethnicity; age 18 to 70 years old.
2. Subjects must be able to comprehend English.
3. Meet DSM-5 criteria for current alcohol use disorder (AUD).
4. Meet DSM-5 criteria for current PTSD or subthreshold PTSD. Subjects may also meet criteria for a mood disorder (except bipolar affective disorder, see Exclusion Criteria) or other anxiety disorders (panic disorder, agoraphobia, social phobia, generalized anxiety disorder, or obsessive compulsive disorder). The inclusion of subjects with affective and other anxiety disorders is essential because of the marked frequency of the co-existence of mood and other anxiety disorders among patients with AUD and PTSD (Brady et al., 2000; Kessler et al., 2005). Subjects may meet DSM-5 criteria for another substance use disorder as long as AUD is the primary substance of choice.
5. Subjects taking psychotropic medications will be required to be maintained on a stable dose for at least four weeks before treatment initiation. This is because initiation or change of medications during the course of the trial may interfere with interpretation of results.
6. Must consent to random assignment to N-acetylcysteine (NAC) or placebo.
7. Must consent to complete all treatment and follow-up visits.
8. Must live within 50 miles (one hour) of MUSC in Charleston, SC or be willing to travel to MUSC for visits.

Exclusion Criteria:

1. Subjects meeting DSM-5 criteria for a history of or current psychotic or bipolar affective disorders, as the study protocol may be therapeutically insufficient.
2. Subjects with a current eating disorder (bulimia, anorexia nervosa) or with dissociative identity disorder, as they are likely to require specific time-intensive psychotherapy.

3. Subjects experiencing significant withdrawal symptoms, as evidence by a score of 10 or above on the Clinical Institute Withdrawal Assessment of Alcohol (CIWA). These subjects will be referred for clinical detoxification and may be re-assessed for study eligibility after medically supervised detoxification has been completed.
4. Individuals considered an immediate suicide risk or who are likely to require hospitalization during the course of the study for suicidality.
5. Women who are pregnant, nursing or not practicing an effective form of birth control.
6. Evidence of liver failure; alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels greater than 3 times the upper limit of normal; asthma or any clinically significant medical condition that in the opinion of the investigator would adversely affect safety or study participation.
7. Use of carbamazepine, phenytoin, nitrous oxide, methotrexate, 6-azauridine triacetate, or nitroglycerin within the last 14 days or any other medication felt to have a hazardous interaction if taken with NAC.
8. History of childhood or adult seizures of any cause.

No special classes of subjects, such as, pregnant women, prisoners, institutional individuals, or others will be recruited for this study. Both male and female participants will be recruited. There will be no exclusion based on race or ethnicity. Participants will be recruited without preference for gender, race, ethnicity or socio-economic status.

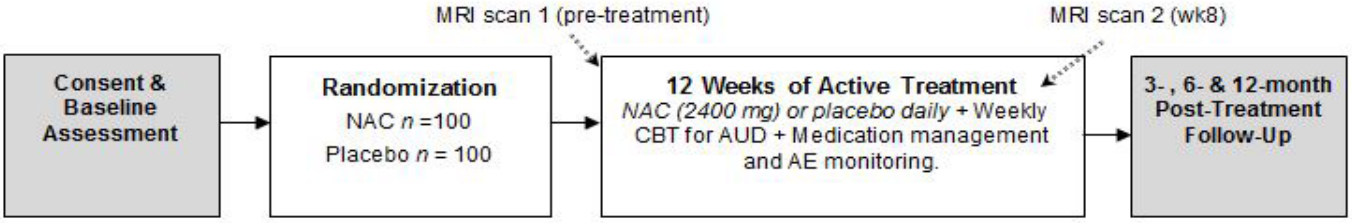
APPROACH

General Procedures. Interested individuals will be prescreened by telephone, in person, or online. Individuals who meet the basic inclusion/exclusion criteria will be invited to come into the office for a comprehensive baseline assessment. Potential participants will be given a full description of the study and asked to read and sign an IRB-approved informed consent form before any study procedures or assessments are conducted. Baseline assessment will include diagnostic interviews, history and physical examination, assessment of concomitant medications, self-report questionnaires, breathalyzer and collection of a urine sample to screen for alcohol, drug and nicotine use as well as pregnancy (females only). Ineligible participants will be referred clinically for treatment.

Following the baseline visit, eligible participants will complete a randomization visit prior to medication initiation. Eligible participants may also complete a neuroimaging session (described in the Neuroimaging Component section) prior to medication initiation. Participants will enter a 12-week, randomized, double-blind, placebo-controlled trial in which they will be seen weekly for health/safety monitoring, dosing/medication dispensation, and completion of assessments. Eligible participants may also complete a second neuroimaging session at Week 8 (Post treatment). At 3-, 6-, and 12-month post-treatment, follow-up visits will occur as specified below.

Recruitment. The primary recruitment site will be the outpatient Center for Drug and Alcohol Programs (CDAP) at MUSC. In addition, we will place IRB-approved study flyers and other approved study materials in prominent locations in MUSC and community-based clinics, and place advertisements on social networking sites (e.g., Craigslist, Facebook). Local area locations of residential hubs and community providers (e.g., restaurants, entertainment venues) will also be accessed with the appropriate permissions to have approved advertising available for public viewing. We will use the assistance of various recruitment and advertising materials such as coffee mugs, pens, etc. We will also use direct mailing methods of recruitment (through Charleston Water Systems and other as necessary) as well as other multi-media methods available through Post and Courier, including Geotargeting, Extended Email Targeting and Site re-targeting. Participants from past MUSC research studies who have consented to be contacted for future research studies will be recruited via telephone screening and/or e-mails. These individuals will be referred to us via other MUSC researchers, or they may have indicated consent to be contacted about future research studies within their MUSC medical records.

Study design overview. Participants are randomized to receive 12, 60-minute sessions of CBT for alcohol use disorder and either NAC (2400 mg qd) or placebo. Weekly visits during the treatment phase (weeks 1-12). Follow-up visits at 3, 6 and 12 months post-treatment.



Assessment Instruments. After the informed consent procedure, subjects will complete a battery of assessments. The instruments to be used (Table 1) were selected because many are standardized, have good psychometric properties, are widely used and have been used by our research group. Combined use of ethanol metabolites and traditional biomarkers (i.e., carbohydrate deficient transferrin and ethyl glucuronide) will be utilized to monitor drinking and corroborate self-report measures[108-111].

Primary Outcome Measures. The primary outcome measures are described in this section. Information regarding additional measures can be found in Table 1. The primary clinical outcomes include: (1) alcohol use severity (TLFB for alcohol consumption), and (2) PTSD symptom severity (CAPS-5 for clinician-rated and PCL-5 for self-reported symptoms). The TLFB obtains retrospective self-report of alcohol use by using a calendar and memory prompts to stimulate recall[112]. Quantity and frequency of use are obtained (e.g., total number of standard drink units, percent of days drinking, heavy drinking days as defined as 5 or more drinks for a man or 4 or more drinks for a woman) as well as abstinence (yes/no). The TLFB will assess consumption of alcohol as well as nicotine and other substances for 60 days prior to study entry, weekly during treatment, and at follow-up. The TLFB yields consistently high test-retest reliability and convergent validity with other self-reports and collateral reports[113]. The OCDS is a 14-item self-report measure of craving with high internal consistency and concurrent validity and good predictive validity of future drinking[114-115]. The CAPS for DSM-5 is a 30-item structured diagnostic interview and gold standard for assessing PTSD[116]. The CAPS assesses trauma history and has excellent psychometric properties and diagnostic efficiency[117]. The PCL for DSM-5 is a 20-item self-report measure that assesses PTSD severity and has excellent psychometric characteristics[116,118].

STUDY INTERVENTIONS AND PROCEDURES

Overview. Subjects will be randomized to NAC or placebo, and all subjects will receive cognitive-behavioral therapy (CBT) targeting AUD (12, weekly 60-minute sessions). Note that subjects will not receive any other CBT or treatments during the study. All services received (e.g., self-help groups, case management) will be carefully monitored and tracked at weekly visits.

Study Medication, Dosage, and Administration. Participants will be randomly assigned to receive NAC (2400 mg/day) or placebo for 12 weeks. The dose and length of treatment are based on previous research[92,105]. The starting dose of NAC is 1200 mg twice daily (2400 mg/day). Equivalent numbers of identical appearing placebo capsules will be dispensed. All NAC and placebo capsules will contain riboflavin 25 mg as a biomarker to assess medication compliance. Participants who wish to take a multivitamin during the treatment phase of the study will be given a multivitamin (Tri-Vi-Sol) that does not contain riboflavin. Weekly pill counts and documentation of missed doses will be carefully recorded at each visit. Study medications (USP-grade NAC and matched placebo capsules) will be compounded by Pitt Street Pharmacy in Mount Pleasant, South Carolina. Treatment assignment will follow a pre-arranged randomization scheme and will be carried out by a pharmacist not involved in clinical management of participants (to preserve the double-blind design). Research staff will administer the study medication or placebo at the twice-weekly visits, and participants will be given take-home doses to self-administer on the days in between study visits. Subjects will be maintained at the target dose for 12 weeks. Side effects and adverse events will be evaluated weekly.

Cognitive Behavioral Therapy. All subjects will receive 12, weekly 60-minute sessions of individual CBT based on NIAAAA's Project MATCH manual[119] (see Appendix for manual). Examples of session topics include: assessing high-risk situations, coping with cravings and urges to drink, managing thoughts about drinking, drink refusal skills, seemingly irrelevant decisions, and enhancing social support networks. Receipt of weekly CBT during the treatment phase will facilitate retention and medication adherence, and ensure that all participants receive adequate psychosocial support, regardless of medication arm.

Therapist Training, Supervision and Fidelity Monitoring: All therapists will be licensed Masters or Doctoral level clinicians with experience delivering CBT. During the lead-in phase of the study, therapists will receive extensive training in the CBT protocol. Study therapists will complete four phases of training: (1) didactic review of intervention-specific theory, (2) manual review, (3) a two-day training workshop, and (4) subsequent completion of two pilot study cases in which all sessions will be recorded and rated for adherence and competency. Throughout the study, therapists will receive weekly supervision focusing on manual adherence, and any clinical concerns about particular patients. If during the course of the study it is determined upon review of therapy sessions and supervisory sessions that a therapist is not competent or does not adhere sufficiently to the manual, the therapist will be replaced. To assure the therapy is delivered consistent with manual guidelines, all therapy sessions will be recorded and 25% of randomly selected sessions will be evaluated using methods developed in the NIMH Collaborative Study[121].

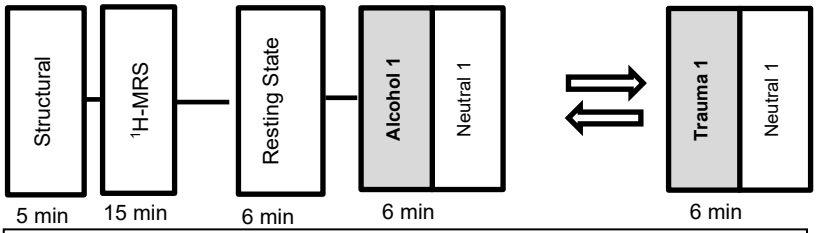
Telehealth. Participants in this research study may choose to do study visits and therapy sessions via home-based telehealth (HBT) care (i.e., service delivery to patients in their homes using consumer-friendly, video-conferencing technology) which may likely enhance retention by directly circumventing financial and transportation barriers associated with traveling to MUSC for in-person sessions. HBT sessions will be delivered via standard desk, laptop computer, tablet, or smartphone running MUSC/VA approved applications. HBT participants will be asked to come to our office for the baseline visit, week 6 visit and week 12 visit. At each visit for HBT participants, in lieu of a breathalyzer test, an alcohol saliva test will be used to measure blood alcohol concentration (BAC) on a weekly basis for telehealth visits. In front of the camera, participants will open the one-time use test strip, place the strip on tongue for 10 seconds, then hold the test strip and a color chart to the camera for a study team member to assess. Samples reading >0.01 g/dl will be considered positive.

Table 1. Assessment Instruments and Timeline

Instrument Name	Purpose/Domain	BSL	Randomization	Weekly	EOT (Wk 12)	3, 6 & 12 Months Post-Treatment
Informed Consent	Obtain informed consent	X				
Demographics Form	Characterize sample	X				
History of Head Injuries Assessment	Assess head injuries, TBI, concussion	X				
History and Physical Examination	Assess medical problems & eligibility	X				
Psychiatric Interview	Assess DSM-5 psychiatric disorders	X				
Columbia-Suicide Severity Rating Scale (CSSR)	Assess for suicidality	X		X (Wk 6)	X	
Pregnancy Test for Female Subjects+	To assess for pregnancy	X	X	X (Wk 6,)		
Clinical Institute Withdrawal Assessment of Alcohol-Revised: CIWA-Ar	Study eligibility, safety	X				
Urine Drug Screen tests: UDS	Study eligibility, assess drug use	X		X (Wk 6)	X	X
Adverse Events and Vital Signs	Monitor AEs and safety	X	X	X	X	X
Concurrent Medications Form	Monitor medications	X	X	X	X	X
Medication Adherence Log	Assess medication compliance		X	X	X	
Life Events Checklist: LEC	Assess trauma exposure	X				
Childhood Trauma Questionnaire: CTQ	Assess childhood trauma	X				

Clinician Administered PTSD Scale: CAPS-5^	PTSD symptom severity (clinician rated)	X		X (Wk 6)	X	X
PTSD Checklist: PCL-5	PTSD symptoms severity (self-report)	X		X	X	X
Time Line Follow-Back: TLFB	Alcohol use (amount and frequency)	X	X	X	X	X
Obsessive Compulsive Drinking Scale: OCDS	Craving	X	X	X	X	X
Visual Analog Craving Scale (VAS)	Measure of alcohol craving, mood, stress, amount, frequency	X	X	X	X	X
Trauma Symptoms of Discrimination Scale	Assess discrimination	X				
Everyday Discrimination Scale	Assess discrimination	X				
Race Based Trauma and Stressor Checklist	Assess race based trauma	X		X (Wk 6)	X	X
Fagerstrom Survey	Assess nicotine use	X			X	X
Satisfaction Questionnaire	Asses overall satisfaction of services				X	X (Mo. 12)
Breathalyzer test	Assess alcohol use (biological)	X	X	X	X	X
Alcohol Saliva Test (HBT care only)	Assess alcohol use (biological)	X	X	X	X	X
Alcohol Use Disorders Identification Test: AUDIT	Assess drinking behavior & problems	X				X (Mo. 12)
Urine riboflavin test	Biomarker of medication compliance		X	X (Wk 6)	X	
Ethylglucuronide (EtG)	Biomarker of alcohol use		X	X (Wk 6)	X	X
Beck Depression Inventory-II: BDI-II	Measure depression	X		X	X	X
Penetration of the Blind	Assess condition prediction				X	
Treatment Services Review	Monitor services utilization	X	X	X	X	X
Cognitive Functioning Tests	Measure cognitive functioning		X	X (Wk 6)	X	
Cognitive Behavioral Therapy: CBT	Weekly CBT for alcohol use			♦	♦	
Note. BSL = Baseline. EOT = End-of-Treatment. +at baseline, before med initiation, before imaging sessions, and at Wk 6.						

Neuroimaging Component. Given the severe negative outcomes associated with co-occurring AUD and PTSD and lack of effective treatments, investigation of the neural circuitry and neurochemistry underlying AUD/PTSD and involved in positive therapeutic response to NAC could be important in guiding future studies targeting this circuitry using other therapeutic agents or treatment modalities. To that end, fMRI and ^{1}H-MRS data will be acquired at baseline and end of treatment on all fMRI-eligible participants to examine (a) glutamate concentrations, (b) resting state connectivity, and (c) response to alcohol, trauma, and neutral cues. Scans will be conducted at the MUSC Center for Biomedical Imaging (see Facilities and Resources section), which houses a Siemens 3T TIM Trio MRI scanner (Siemens Medical, Germany). The neuroimaging component will involve three visits, each lasting 60-90 minutes. During visit 1 (baseline screening visit), we will develop the personalized imagery scripts for trauma and/or racial-based trauma, alcohol and neutral cues. During visit 2 (Randomization Visit, prior to medication initiation) and visit 3 (Post Treatment – 8 Visit), we will conduct the fMRI and ^{1}H-MRS scanning procedures. Women of childbearing age will receive a urine pregnancy test before each MRI. Imagery scripts will be developed according to standardized procedures[41] and employed in our ongoing research (see Preliminary Studies section). The Wk 8 scan will be conducted approximately one hour post-NAC administration to ensure findings reflect effects of NAC[122-123]. Participants will be screened for metal using a handheld metal detector. Trained staff will position subjects on the scanner bed with foam padding placed around their head to prevent motion. Participants will wear headphones to listen to the audio-recorded scripts. During initial scanner tuning, localizing, and structural



Overview of neuroimaging session. Assessment of (a) ACC glutamate, (b) resting connectivity, and (c) response to alcohol, trauma and neutral cues.

scanning, participants will be shown "relaxing" images (i.e., 20 scenic pictures, each displayed for 30 sec). For co-registration and normalization of functional images, a high resolution T1-weighted MPRAGE anatomical image will be acquired with the following parameters: TR = 2100 ms, TE = 4.18 ms, flip angle = 12°, field of view = 256 mm, slice thickness 1.0 mm. The scanning planes will be oriented parallel to the anterior commissure–posterior commissure line. The ACC voxel for ^{1}H-MRS will be placed on midsagittal T1-weighted images, anterior to the genu of the corpus callosum, with the ventral edge of the voxel aligned with the dorsal edge of the genu and a voxel size of 3 x 2.5 x 2.5 cm^3 will be selected. Following auto-shimming, single-voxel water-suppressed ^{1}H-MRS spectra will be acquired using a Point Resolved Spectroscopy (PRESS) sequence: Repetition Time (TR) = 2000ms; Echo Time (TE) = 40ms; number of averages = 128); an unsuppressed water spectrum will be co-acquired (TE = 40ms, number of averages = 16), scaled for partial volume effects and relaxation, and used as a concentration reference[123].

Next, participants will be asked to relax and keep their eyes opened and fixed on a cross-hair for six-minutes while resting state data are collected. Following this, participants will be exposed to alcohol, trauma and neutral cues. We will use a block design consisting of two 6-minute runs. During the alcohol cue run, participants will hear an audio recording describing in detail the last time they consumed alcohol. The alcohol cue run will be divided into two, 3-minute blocks of alcohol and neutral cues. During the trauma cue run, participants will hear an audio recording describing in detail their traumatic event. The trauma cue run will be also divided into two, 3-minute blocks of trauma and neutral cues. During the neutral cue run, participants will hear an audio recording describing something neutral, such as their typical morning routine. To minimize potential carry-over effects, the runs will be counterbalanced so that half of the subjects in the placebo group and half of the subjects in the NAC group are exposed to the alcohol cue first and the remaining participants in each group are exposed to the trauma cue first. T2*-weighted gradient-echo planar images (EPI) will be acquired with the following parameters: TR = 2000 ms, TE = 27 ms, flip angle = 76º, matrix 64 x 64, field of view = 23 cm, slice thickness = 3.7 mm with no gap, with 36 slices to cover the entire brain.

Subject Compensation. Subjects will receive $50 for the baseline visit (plus $25 bonus for showing up on schedule), $25 for the medication initiation (randomization) visit, $40 for completing each of the subsequent weekly visits during the treatment phase, and $100 for the 3-month, $100 for the 6-month, and $100 for the 12-month post-treatment follow-up visits. Patients will also receive a $25 bonus after completing the WK 6 and a $60 bonus for completing WK 12 visit. Participants may also be eligible to participate in the optional MRI portion of the study. The participant may receive $25 for script development, $75 for the first neuroimaging visit and $125 for the second neuroimaging visit. Compensation is available in the form of cash or check. Thus, the total amount subjects may receive for study participation is $1190

STATISTICAL ANALYSES

General. Baseline clinical and demographic characteristics will be collected and contrasts performed between treatment groups. Continuous and ordinal characteristics will be compared using a Wilcoxon Rank-Sum test statistic. Categorical characteristics will be compared using a Pearson Chi-Square test statistic. Baseline characteristics found to be significantly associated with primary outcome measures will be included as covariates in the analyses. All analyses will be performed on the intent-to-treat sample consisting of all randomized subjects. All analysis will be conducted using SAS v9.3 (SAS Institute, Cary, NC, USA).

Randomization. We will stratify by (1) AUD severity (TLFB) and (2) PTSD severity (CAP-5). Stratified random block randomization will be used to balance the randomization assignment with respect to these strata. The purpose of stratification is to distribute these potential prognostic factors equally across treatment groups.

Power and Sample Size. This study is powered to estimate the efficacy of NAC on the reduction in PTSD symptoms, alcohol use, and craving at the end of the treatment phase (final 3 weeks of treatment). This study builds on a recently completed pilot trial of NAC that assessed efficacy in reduction of PTSD symptomology and craving in a cohort of Veterans with PTSD and substance use disorders. In this promising pilot trial, we observed a clinically significant decrease in CAPS total scores following 8 weeks of treatment with NAC (Δ=26.8) while the placebo response was attenuated (Δ=17.1). Assuming a similar difference in treatment effects in a larger population, we will have 80% power with a type 1 error rate of 5% to detect this difference with 75 participants randomized to each of the two treatment arms (total

N=150). In the same pilot trial, 77% of subjects completed the 8 week trial; we anticipate similar retention in the proposed study. Thus, a randomized sample size of 100 participants per treatment arm (total N=200) would maintain power to detect the clinically significant difference stated above in the presence of ~25% attrition during the study. Similarly, in the same pilot study, reductions in craving were significantly greater in the NAC vs. placebo treated group (Δ=3.0 vs. Δ=1.3). The randomized sample size of 100 per treatment arm provides >95% power with a type 1 error rate of 5% to detect this effect in the presence of 25% attrition during the study period. It is hypothesized that NAC reduces the risk of drinking as compared to placebo. With this stated sample size, a 25% decreased risk in the consumption of standard drinks can be detected with at least 80% power between the NAC and placebo treated arms (Risk Ratio=0.75).

Power calculations for the neuroimaging component are based on previous studies of individuals with PTSD or substance use disorders. Based on our previous work and the extant literature, we conservatively estimate ~70% of participants (140/200) will be eligible and volunteer for the neuroimaging component. A recent study of 29 individuals (PTSD=14, controls=15) observed significant differences in PFC-AMY resting state connectivity[50]. The effect size for this difference was 0.8 (Cohen's d). Thus, power (1-ß) reaches .80 with a total sample size of N=26 (two-tailed, alpha=.05). Another study of 45 cocaine-dependent individuals found that individuals who relapsed (n=24) had significantly lower PFC-AMY connectivity at baseline as compared to individuals who did not relapse (n=21)[49]. The effect size for this reduction was 1.0. Thus, power (1-ß) reaches .80 with N=34 subjects (two-tailed, alpha=.05). For exploratory hypothesis 4, the only published study of NAC effects on brain glutamate found that one dose of NAC vs. placebo administered 1 hour prior to scanning, led to a significant modification in ACC glutamate with an effect size of d = 2.6 in individuals with cocaine dependence. Although the present study will be examining the effects of chronic NAC treatment in individuals with AUD and PTSD, an effect of similar magnitude would provide power > 0.99. The present study is therefore well-powered to test the proposed hypotheses.

Hypotheses. The hypotheses and statistical approaches for testing each hypothesis are listed below.

Hypothesis 1: NAC treatment will result in significantly greater reduction in AUD severity and craving, as compared to placebo. To test this hypothesis, a mixed effects modeling framework will be specified with the total number of standard drinks consumed and the OCDS total score as the primary outcomes. A Poisson distribution will be assumed with a logarithm link function to assess the effects of NAC treatment and the baseline number of total standard drinks (past 60 days) prior to treatment entry. Over-dispersion due to a wider than expected distribution in discrete count models (from heterogeneity) can have a significant impact on parameter inference, thus when detected, a negative binomial (NB) distribution will be specified. Further, data collected in substance abuse studies often contain a preponderance of zeros, and these zeros in the distribution can be considered sampling zeros in the Poisson process[125]. If this excess is present in the distribution, two-part Hurdle models (Poisson and NB) will be explored[126-127]. The hurdle part of the model will allow us to estimate the probability of abstinence during the study period (as an odds ratio) while the Poisson/NB portion will allow for the assessment of the risk of an increase in using days beyond zero during the study period (as a risk ratio). Model fit of the Poisson (Hurdle Poisson) model will be compared to the Negative Binomial (Hurdle NB) model by practical examination of the deviance and Pearson chi-square values (Likelihood Ratio) with respect to their degrees of freedom. Baseline alcohol use and craving will be included in pertinent models as covariates. Additional alcohol use outcomes will be assessed: percentage of drinking days (DD) and the number of heavy drinking day (HDD). End of study abstinence will be assessed using logistic regression models. DD and HDD will be assessed using count models.

Hypothesis 2: NAC treatment will result in significantly greater reduction in PTSD severity as compared to placebo. To test this hypothesis, generalized linear mixed effects regression models with a Gaussian distribution will be developed to assess group differences while adjusting for baseline CAPS-5 scores. Assumptions of residual normality and homoscedasticity will be checked using statistical test and graphical methods (Residual and Q-Q plots, and Shapiro-Wilk tests) and transformation will be done as necessary. Restricted maximum likelihood (REML) methods will be used to estimate the fixed effects and variance components.

Hypothesis 3A: Prefrontal cortex-amygdala connectivity at rest and in response to alcohol vs. neutral cues will predict reduction in alcohol use severity (total standard drinks).
Hypothesis 3B: Prefrontal cortex-amygdala connectivity at rest and in response to trauma vs. neutral cues will predict reduction in PTSD severity (CAPS-5).

For hypotheses 3A and 3B, the following preprocessing and analytical parameters will be used. Preprocessing: Post-acquisition preprocessing and statistical analysis of imaging data will be performed using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library). Data will be preprocessed using scripting tools from FEAT. Non-brain signal will be removed using FSL's BET brain extraction. Scans will be corrected for motion using FSL's linear registration and scans will be spatially smoothed using a Gaussian kernel of 8 mm FWHM. Participants with head motion $\geq$ 0.2 mm will be excluded from the analyses[127]. Motion artifacts will be identified and scrubbed from each subject's data. Scans will be spatially co-registered with a standardized anatomical template (Montreal Neurological Institute) using a 12 parameter affine transformation. Analysis: Functional connectivity will be measured using a psychophysiologic interaction seed-based approach[129-130]. PPI analysis is used to assess the functional coupling between different brain regions. The PPI is defined as the change in connectivity of one area (i.e., the seed region) to another in relation to the experimental context (e.g., alcohol vs. neutral cues). PPI analyses for resting state, trauma and alcohol cues will be performed separately. Customized sq. wave forms representing the trauma run (1=trauma block and -1=neutral block) and the alcohol cue run (1=alcohol block and -1=neutral block) the duration of each block will be convolved with a double-gamma hemodynamic response function. A mask of the seed region will be made using a 12-mm diameter sphere located in the center of the AMY using the MNI coordinates (x, y, z = ±22, 0, -22). The transformation parameters described above will also be applied to the mask. For each subject, the mean corrected and high pass filtered time series of the BOLD signal in the AMY will be extracted and used in a single subject whole brain PPI analysis. The PPI model will include the task vector, time series of the BOLD signal in the AMY, a term representing the positive task x seed interaction, and a term representing the negative task x seed interaction. The first level analysis will generate contrast images of the parameter estimates for each of the four regressors. Voxels will be thresholded at Z>2.3 using a corrected cluster threshold of p=0.05. The contrast images of the parameter estimates of the positive and negative task x seed interactions will be combined for group-level t-tests to identify regions that exhibited altered connectivity with the AMY during the trauma and alcohol cues as compared to neutral cues. All group-level results will be thresholded at Z>2.3 using a corrected cluster threshold of p=0.05. Separate linear regression tests will be used to test for associations between PFC-AMY connectivity at baseline and improvement in AUD and PTSD symptoms during treatment. Changes in total standard drinks will be regressed against the parameter estimate obtained from the center voxel from each cluster that exhibited a significant task x seed interaction with the AMY at rest and in response to the alcohol cues (Hypothesis 3A). Changes in CAPS-5 total scores will be regressed against the parameter estimate obtained from the center voxel from each cluster that exhibited a significant task x seed interaction with the AMY at rest and in response to the trauma cue (Hypothesis 3B). Further exploratory analyses will examine change in PFC-AMY connectivity from pre- to post-treatment and associations with alcohol and PTSD outcomes, both within and between medication groups.

Exploratory Hypothesis 4: NAC treatment will be associated with a significant change in ACC glutamate concentrations, as compared to placebo. Change in ACC glutamate concentrations will be associated with alcohol and PTSD outcomes.

Analysis of PRESS data will be conducted using LC Model 6.3[131], an operator-independent curve-fitting software package that uses least-squares estimation for quantifying metabolite concentrations; the basis set for TE = 40ms is provided by the vendor and includes a number of metabolites including glutamate, creatine, glutamine, N-acetylaspartate, and phosphocholine. Only metabolites with fitting uncertainties (Cramer-Rao Lower Bound values) < 20% of SD in the LC Model output will be retained for analysis. LC Model includes standardized zero filling, Fourier transformation, and automated phase, baseline and eddy current correction. To address variability in within-voxel tissue composition we will extract and segment T_1-weighted images into partial volume maps of gray matter (GM), white matter (WM), and CSF using FSL tools, match the coordinates and size of the ^{1}H-MRS voxel with the segmented images and extract the tissue fractions within the voxel, correct the raw values obtained from the LC model (scaled

to water) for CSF and coil loading, and calculate each participant's GM to brain matter (GM/[GM+WM]) ratio for use as a covariate in the analyses. Change in glutamate levels will be analyzed as a mixed model (SAS PROC MIXED) with time as a within subject variable and medication group as between subject variables. Variables known to influence ACC glutamate levels (e.g., age, smoking status, tissue composition) will be considered as covariates. Further exploratory regression analyses will examine associations between glutamate difference scores and clinical outcomes (e.g., change in total standard drinks and CAPS-5 score) both within and between medication groups.

Summary: The proposed study will answer important questions regarding the potential of NAC as an effective pharmacotherapy for AUD and comorbid PTSD, and elucidate possible mechanisms underlying improved outcomes. The findings from this study have the potential to significantly improve the standard of patient care and accelerate research on the treatment of AUD and co-occurring PTSD

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