Neuroscience-Informed Treatment Development for Adolescent Alcohol Use
NCT03238300
November 30, 2022

Aim 1:

Hypothesis/aim	**<u>Primary:</u>** **1a. Quantify the effect of NAC versus placebo on glutamate levels in adolescent heavy drinkers.** Using MRS, we will determine the effect of NAC versus placebo on modulating anterior cingulate glutamate levels in heavy alcohol-using adolescents. *Hypothesis:* Heavy drinking youth will show decreasing levels of glutamate from baseline in the anterior cingulate while on NAC compared to placebo.
Sample size for aim	**N= 31** – all with 4 sessions (randomization visit 1, MRI visit 2 & 4, washout visit 3)
Independent variable(s)	**NAC v. Placebo**: both NAC and placebo were administered twice daily (2400 mg of NAC/day, administered as 1200 mg twice/day) over 10 days
Dependent variable(s)	dACC glutamate levels
Handling of missing data	Only participants with complete data will be included (N=31) Complete data = screener, visit 1-4
How outliers and excluded observations will be defined	Participants with all 4 sessions (visit 1-4) will be included. MRS data will be examined for quality using: 1. Linewidth (exclusion: >3 SD from mean) 2. Signal-to-noise ratio (SNR) (exclusion: >3 SD from mean) No other outliers will be removed.
Software and specific software modules or packages	Software: R Packages: Tidyverse, lmer4 OR glmer4 & lmerTest
Analytic procedure(s)	Generalized linear mixed effect models will examine the effect of NAC vs placebo on neurometabolites (Primary: glutamate) within the adolescent heavy drinking group. The primary model will contain the main effect of treatment (NAC vs. placebo), as well as day (period: scan 1 vs. scan 2) and order (sequence: NAC/placebo vs. placebo/NAC) 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 (1|id)

	Covariance structure will be determined based on best fit after trying standard options (e.g., unstructured, Toeplitz, autoregressive, etc.) Model fit will be tested using likelihood ratio tests, Akaike Information Criterion values, and interclass correlation. Parameter estimates and associated 95% confidence intervals for treatment effects on DVs.
Steps to confirm data assumptions are met	<u>Generalized linear effect mixed models</u> -Check normality of data using Shapiro-Wilks test &/or QQ plot -Divide generalized chi-square by its degrees of freedom to check for dispersion -Compare averages of outcomes to predicted values to check appropriateness of link function -Transform data (if needed) -Check for multicollinearity among predictor variables -Check for carry over effect in crossover models <u>Generalized linear models</u> -Plot deviance residuals against fitted values to evaluate linearity for GLM -Look for outliers using leverage & Cook's distance &/or a QQ plot for GLM -Transform data if needed but only on predictor variables
A priori covariates and/or means for identifying important covariates	Baseline levels of clinical and demographic characteristics will be tested for univariate associations with study outcomes; when associated, these variables will be included in the adjusted model development strategy. Primary: • Age • Sex • Alcohol use variables Exploratory: • Cannabis use variables
A priori interactions to be examined and rules for retaining interaction terms	None.
Planned subgroup or sensitivity analyses	N/A
Level of significance and whether/what corrections for multiple testing will be used	P<0.05, no correction for multiple comparisons

(if null hypothesis significance testing is used)	
Required data cleaning/formatting steps	**RedCap:** 1. Download baseline variables a. Age b. Sex c. Group (drinker v. control) d. Mental health measures (anxiety, depression) 2. Download drinking variables from TLFB a. Total drinking days (visit 1-4) b. Total drinks/drinking day (visit 1-4) c. Total binge occasions (visit 1-4) 3. Download other substance use variables from TLFB a. Total cannabis use days (visit 1-4) b. Total cannabis use/use day (visit 1-4) c. Total vaping days (visit 1-4) d. Total cigarette days (visit 1-4) **MRS:** 4. Combine excel spreadsheets for all visits and metabolites 5. Remove unnecessary variables a. Final variables: i. Metabolite levels (I.U.) ii. Linewidth iii. SNR **Combine RedCap + MRS Data:** 6. Merge RedCap and MRS data sheets 7. Remove participants without full data (final N=41) 8. Remove observations that don't meet criteria for inclusion

Aim 2:

Hypothesis/aim	Primary Aim #1: *Aim*: Quantify the effect of NAC versus placebo on alcohol cue reactivity in adolescent heavy drinkers *Hypothesis 1*: Heavy drinking youth with show decreasing alcohol cue reactivity from baseline in key reward regions (insula, striatum, and amygdala) while on NAC compared to placebo
Sample size for aim	Initial sample size: n = 31 (heavy drinkers; 17 female & 14 male) Sample size for complete data: n = 27 (14 female & 13 male)
Independent variable(s)	Medication: NAC vs. Placebo
Dependent variable(s)	Primary Aim #1: Primary contrast: BOLD response (alcohol v. non-alcohol cues) in 10 regions of interest (left and right): amygdala, insula, caudate, nucleus accumbens, and putamen
Handling of missing data	Multiple imputation will be used for missing data ('mice' package) on independent variables only.
How outliers and excluded observations will be defined	Participants with excess head motion (defined as mean relative motion >0.2 mm) and missing cue-reactivity data at baseline will be removed (n = 4). Outliers will be checked with Q-Q plots and examination of residuals.
Software and specific software modules or packages	Software: R Packages: Tidyverse, lrtest, LME, Performance
Analytic procedure(s)	Generalized linear mixed effect model will examine the effect of NAC vs placebo on alcohol cue reactivity - The primary model will contain the main effect of treatment (NAC vs. placebo), as well as day (scan 1 (visit 2) vs. scan 2 (visit 4)) and order (NAC/placebo vs. placebo/NAC) 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 - Model fit will be tested using likelihood ratio tests, Akaike Information Criterion values, and interclass correlation. - Model based parameter estimates across groups (means and standard deviations) will be used to estimate treatment effect sizes
Steps to confirm data assumptions are met	Check for normality of data with Q-Q plot Check for appropriate link function by comparing averages of outcomes versus predicted values Check for overdispersion by dividing generalized chi-square by degrees of freedom. Check for multicollinearity among predictors with VIF (cut-off of 2)
A priori covariates and/or means for identifying important covariates	Day and Order will be included in all models. Aim #1 and exploratory covariates: Baseline BOLD response (alcohol v. non-alcohol cues) in relevant region of interest (i.e. in models with ACC as the outcome, BOLD response at baseline in ACC will be the covariate), alcohol use (average standard drinks per drinking day, total quantity of standard drinks, number of drinking days, and number of binge episodes), AUD status (Yes/No defined by meeting DSM-5 criteria at screening) or AUD severity (none, mild, moderate, or severe defined by DSM-5 criteria at screening), cannabis use days, age, sex, and race.

	'Performance' package will be used to determine best fitting model with select covariates to reduce issues related to multicollinearity – only 1 alcohol covariate will be used, and will be specific to each model
A priori interactions to be examined and rules for retaining interaction terms	N/A
Planned subgroup or sensitivity analyses	Sensitivity analysis will compare results from complete data only model (n = 27) and imputed model (n = 31) to confirm the imputed data does not significantly change the results.
Level of significance and whether/what corrections for multiple testing will be used (if null hypothesis significance testing is used)	$p < 0.05$, no correction for multiple comparisons
Required data cleaning/formatting steps	1. Complete second-level cue-reactivity data processing in Freesurfer and extract relevant data for models (average percent change in BOLD signal between alcohol vs. non-alcohol cues from the ROIs) 2. Download data for covariates from Redcap and look at descriptive statistics to assess accuracy and distributions 3. Download raw TLFB data from Box 4. Calculate TLFB summary variables: average standard drinks per drinking day, total quantity of standard drinks, number of drinking days, and number of binge episodes during 10-days on treatment 5. Combine data from Redcap and output from fMRI preprocess pipeline and Freesurfer 6. Complete multiple imputation for missing data 7. Run models on imputed dataset and completers only dataset to check for any differences