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# Influence of *n*-acetylcysteine maintenance on the pharmacodynamic effects of oral ethanol

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ABSTRACT

*Rationale:* Glutamate systems play an important role in the abuse related effects of alcohol. n-Acetylcysteine, a drug that promotes glutamate homeostasis, attenuates a range of alcohol effects in preclinical models.
*Objectives:* This human laboratory study determined the influence of *n*-acetylcysteine maintenance on alcohol self-administration using a model predictive of treatment effectiveness, along with the subjective, performance and physiological effects of alcohol. We hypothesized that *n*-acetylcysteine would attenuate alcohol self-administration, as well as positive subjective effects of alcohol.
*Methods:* Nine subjects with alcohol use disorder completed this within-subjects study. Subjects were maintained on placebo, 1.2 and 2.4 g *n*-acetylcysteine in random order on an outpatient basis. After five days of maintenance on the target dose, subjects completed overnight inpatient experimental sessions in which the pharmacodynamic effects of alcohol were determined.
*Results:* Alcohol produced prototypic effects (e.g., increased breath alcohol concentration, increased ratings of Feel Drink). n-Acetylcysteine did not alter the effects of alcohol.
*Conclusions:* These results indicate that although *n*-acetylcysteine can safely be combined with alcohol, it does not attenuate the abuse related effects of alcohol and is unlikely to be an effective standalone alcohol use disorder treatment. However, considering study limitations, future work is needed to further understand whether and how *n*-acetylcysteine might be used as a treatment for alcohol use disorder (e.g., in combination with a behavioral treatment or another pharmacological agent).

## 1. Introduction

Alcohol use disorder is an unrelenting public health concern. An estimated 15 million Americans met criteria for alcohol use disorder in 2018, with 85,000 deaths directly attributable to alcohol each year, resulting in an annual economic burden of approximately $250 billion (Mokdad et al., 2004; Sacks et al., 2015; Substance Abuse and Mental Health Services Administration, 2019). Several medications have been approved for treating alcohol use disorder (i.e., disulfiram, naltrexone, acamprosate; Litten et al., 2016). These pharmacotherapies are not widely prescribed (Harris et al., 2010; Litten et al., 2016; Klein, 2016), nor are they universally effective (e.g., Kufahl et al., 2014; Mann et al., 2016) or appropriate to use in all patients with alcohol use disorder (e.g., acamprosate should only be used in detoxified patients; Kampman et al., 2009). To address the continued need to improve alcohol use disorder treatment, identifying novel pharmacotherapies is a high priority (Litten et al., 2012, 2016).

A wealth of data indicates that alcohol use produces profound changes in the brain glutamate system (Bell et al., 2016; Burnett et al., 2016; Krystal et al., 2003; Melendez et al., 2005; Rao et al., 2015a; Rao and Sari, 2012; Roberts-Wolfe and Kalivas, 2015). Preclinical studies show that chronic and binge-like alcohol dosing inhibits glutamate levels and transmission through blockade of *n*-methyl-D-aspartate (NMDA) receptors (Chen et al., 1997; Grant et al., 1990; Lovinger et al.,

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1989). Adaptations to NMDA receptors then result in increased glutamate levels during alcohol withdrawal (Dahchour and De Witte, 2003; Rossetti and Carboni, 1995; Siggins et al., 2003). Elevated glutamate levels observed during alcohol withdrawal can be reduced with further acute alcohol administration (Carboni et al., 1993; Roberto et al., 2004), driving a cycle of heavy alcohol use and withdrawal periods that characterizes alcohol use disorder.

In addition to NMDA receptor changes, disrupted glutamate homeostasis that occurs during the cycle of chronic alcohol intoxication and withdrawal has been attributed to altered glutamate transport (e.g., changes in cystine-glutamate exchanger [xCT] and glial glutamate transporter [GLT-1] expression and function; Melendez et al., 2005; Rao and Sari, 2012). A series of rigorous, placebo-controlled preclinical studies has shown that administration of ceftriaxone, which restores glutamate homeostasis by increasing xCT and GLT-1 expression and function following alcohol exposure (Alhaddad et al., 2014), robustly reduces alcohol self-administration across numerous experimental conditions (Alhaddad et al., 2014; Rao and Sari, 2014a, 2014b; Qrunfleh et al., 2013; Rao et al., 2015b; Sari et al., 2011, 2013a, 2013b) and cue-primed reinstatement of alcohol seeking (Weiland et al., 2015). Although promising in preclinical models, ceftriaxone unfortunately has a number of qualities that prevent its adoption as an alcohol use disorder pharmacotherapy. Specifically, ceftriaxone is a prescription drug that must be administered intravenously and can have serious side effects (e.g., *C. difficile* infection; Vesteinsdottir et al., 2012), and promotes antibiotic resistance, so it is *not* feasible for testing as an alcohol use disorder treatment in clinical research.

Recent preclinical research has identified other promising compounds that also normalize extracellular glutamate levels by improving xCT and GLT-1 expression and function (Lebourgeois et al., 2018, 2019; Morais-Silva et al., 2016; Quintanilla et al., 2016; Schneider et al., 2015; Weiland et al., 2015). Chief among these is *n*-acetylcysteine, which ameliorates negative neuroadaptive changes produced by alcohol, as well as alcohol induced behavioral sensitization, anxiety-like behavior and alcohol withdrawal signs in preclinical models (Mocelin et al., 2019; Morais-Silva et al., 2016; Schneider et al., 2015). n-Acetylcysteine also reduces alcohol intake in rodents (Israel et al., 2019; Lebourgeois et al., 2018, 2019; Quintanilla et al., 2016). Unlike ceftriaxone, *n*-acetylcysteine is federally unscheduled, available over the counter and can be administered orally with a low incidence of side effects. Clinical data with *n*-acetylcysteine are somewhat mixed, with recent reviews indicating that *n*-acetylcysteine reduces craving in substance use disorders and may show promise as an adjunctive treatment for a range of psychiatric diagnoses (Duailibi et al., 2017; Minarini et al., 2017). However, *n*-acetylcysteine does not appear to be effective in treating autism (Minarini et al., 2017), nor did it promote cannabis abstinence or improve depressive symptoms in a cannabis use disorder treatment trial (Tomko et al., 2020). If proven effective, *n*-acetylcysteine would be widely available at low cost to treatment seekers, increasing its attractiveness and making it an ideal lead compound for evaluation as an alcohol use disorder pharmacotherapy.

The preclinical data indicate that *n*-acetylcysteine is a potential pharmacological treatment for alcohol use disorder. Although findings from secondary analyses of clinical trials in adults and adolescents with cannabis use disorder have shown that *n*-acetylcysteine maintenance reduces alcohol intake in those groups (Squeglia et al., 2016, 2018), no published clinical work has specifically examined the effects of *n*-acetylcysteine in individuals with alcohol use disorder. The purpose of this study was to fill this noted research gap by determining how *n*-acetylcysteine maintenance influenced the pharmacodynamic effects of alcohol, including alcohol drinking behavior, using rigorous human laboratory methods. The methods for this study were derived from previous human laboratory studies demonstrating the predictive validity of this approach for screening putative alcohol use disorder pharmacotherapies (Drobes et al., 2003; Hendershot et al., 2017; O'Malley et al., 2002).

## 2. Materials and methods

### 2.1. Subjects

In order to be eligible for the study, English speaking/reading adult human subjects had to be between the ages of 21–55, have a body mass index (BMI) between 19 and 35, be healthy and not report any contraindications to alcohol and *n*-acetylcysteine administration. Subjects also had to report recent use of alcohol verified through a positive ethyl glucuronide urine screen, meet diagnostic criteria for moderate to severe alcohol use disorder according to Structured Clinical Interview for DSM-5 criteria and endorse having engaged in one binge drinking episode in the past 30 days as defined by NIAAA. Screening procedures for all subjects included a medical history questionnaire, laboratory chemistries (e.g., blood chemistry screen, complete blood count and urinalysis), electrocardiogram and a brief psychiatric examination. Subjects were excluded from participation if they were seeking treatment for any drug use or if a study physician deemed the screening results to be abnormal (e.g., if the electrocardiogram was determined to be outside normal limits). Subjects with histories of serious physical disease, current physical disease or current or past histories of serious psychiatric disorder, including current or past histories of other substance use disorder, that in the opinion of a study physician would have interfered with study participation (e.g., physiologic dependence on opioids, alcohol or benzodiazepines; schizophrenia; major depression; bipolar disorder), were also excluded. Female subjects had to be using an effective form of birth control (e.g., birth control pills, IUD, condoms or abstinence) in order to participate. All subjects were paid for their participation and for compliance with the drug maintenance regimen. The Medical Institutional Review Board of the University of Kentucky approved this study, which was conducted in accordance with all relevant guidelines, including the 1964 Declaration of Helsinki.

Twenty subjects provided sober, written informed consent to participate in this randomized, placebo-controlled study. Fourteen subjects went on to receive study medication. The six consenting subjects who did not receive medication were lost to follow up (n = 3), did not fully meet inclusion/exclusion criteria (n = 1), could not make the time commitment (n = 1) or were discontinued due to the COVID-19 pandemic (n = 1). A total of nine subjects (4 women; 6 White, 2 Black, 1 biracial), all of whom met criteria for moderate alcohol use disorder, completed the study. The five subjects who did not complete withdrew due to adverse events (nausea and vomiting during placebo maintenance, n = 1; blurry vision, drowsiness and thirst during 1.2 g *n*-acetylcysteine/day maintenance, n = 1), withdrew consent due to dislike of study procedures (n = 1), withdrew in order to start a psychiatric medication prescription (n = 1) or were discharged due to cocaine use during participation (n = 1). Completing subjects were 39 ± 10 years of age on average ( ± SD), weighed 86 ± 16 kg and had BMIs of 29 ± 4. Subjects reported using alcohol 18 ± 7 days and reported binge drinking 11 ± 9 days in the month prior to screening. Alcohol Use Disorders Identification Test (Saunders et al., 1993) scores were 10 ± 3 and Michigan Alcohol Screening Test (Selzer, 1971) scores were 6 ± 4. Subjects also reported other drug use during screening. One subject was a daily cigarette smoker (8 cigarettes/day). In the month prior to screening, subjects reported cannabis use (n = 2) and amphetamine use (n = 2). Drug Abuse Screening Test (Skinner, 1982) scores were 2 ± 1.

### 2.2. General procedures

Subjects were enrolled as outpatients at the University of Kentucky (UK) Laboratory of Human Behavioral Pharmacology (LHBP) and completed a total of three overnight inpatient stays at the UK Chandler Medical Center inpatient Clinical Research Unit (CRU) in order to complete their alcohol dosing experimental sessions.

#### 2.2.1. Drug maintenance periods

Subjects completed three separate drug maintenance periods, coinciding with the three *n*-acetylcysteine dose conditions, administered in random order. After completing a practice session that included all experimental procedures aside from alcohol dosing, subjects were provided with a Wisepill® dispenser loaded with the starting *n*-acetylcysteine dose (0, 1.2 or 2.4 g/day). The Wisepill® dispenser and subject self-report were used to monitor adherence to the outpatient dosing regimen whereby doses were taken orally at 0700 and 1900 h for five days. Based on this, we calculated overall adherence to be 98%. If subjects had missed more than 3 doses or took doses more than 2 h outside their scheduled window on 3 occasions within a single dose condition, they were to be coached on adherence and that dose condition repeated. However, no dose conditions were repeated due to subjects failing to meet these adherence criteria.

The *n*-acetylcysteine maintenance period was selected based on our previous work with *n*-acetylcysteine (Bolin et al., 2017), showing that a similar *n*-acetylcysteine regimen reduced cocaine self-administration in a maintenance-order dependent manner and reduced cocaine attentional bias. We also selected the maintenance period based on research published by other laboratories (Amen et al., 2011). Lastly, considering that *n*-acetylcysteine has a terminal half-life of 6.25 h (Holdiness, 1991), the selected maintenance period was substantially longer than the typical 4–5 half-lives needed to achieve steady-state blood levels. On the fifth day of maintenance on the first assigned condition, subjects completed one experimental session, as described below. Upon completion of the experimental session, subjects completed a washout period of at least seven days. The pattern of maintenance and experimental sessions was repeated two more times, with one more seven-day washout intervening. After completing a total of three experimental sessions, subjects were discharged from the study.

#### 2.2.2. Experimental sessions

On experimental session days, subjects arrived at the LHBP at approximately 1400 h. After completing a history and physical with one of the study physicians, passing a standard field sobriety test and providing urine and breath samples indicating recent drug and alcohol abstinence, as well as negative pregnancy test results in females, subjects were then transported to the CRU. Experimental sessions conducted on the CRU consisted of a Sampling Phase and two Self-Administration Phases.

*2.2.2.1. Sampling phase.* Subjects completed a sampling phase in each experimental session to acquaint them with the effects of alcohol doses that would be available later in session. Baseline subjective, physiological and cognitive-behavioral measures were completed at approximately 1530 h. At approximately 1615 h, a single dose of 95% alcohol mixed with a non-alcoholic, non-caffeinated mixer of the subject's choice (e.g., lemon lime soda, tonic water) was administered in a lidded cup with a straw. Subjects had 5 min to consume the sampling beverage. Subjective and physiological measures were completed at ten-minute intervals for 30 min after the sampling dose, with cognitive-behavioral measures completed 30 min after the dose. The sampling phase ended at approximately 1730 h.

*2.2.2.2. Self-administration phases.* Two self-administration phases were completed, one beginning at approximately 1730 h and the other beginning at approximately 1830 h. During each of these phases, subjects were given four opportunities to choose a drink containing half the sampling dose or earn $3.00 for each drink refused over a 1 h period. Thus, the maximum number of drinks that could be consumed over the two self-administration phases, the primary outcome variable, was eight. Number of drinks consumed in each phase and latency to drink in each phase were also evaluated. The second self-administration phase, and the entire experimental session, ended at approximately 1930 h. After this, subjects were allowed to recover in their individual rooms, eat dinner and engage in recreational activities until lights out at 2300 h. Subjects were then discharged from the CRU at approximately 0800 h the following day. We chose to keep subjects overnight in our inpatient unit to avoid constraints on alcohol drinking during session (e.g., subjects might drink less if they had to leave immediately after a session).

### 2.3. Measures

In addition to measuring the reinforcing effects of alcohol on the choice task described above, a battery of other measures was completed during experimental sessions. Subjects reported how many standard drinks they had consumed on the Timeline Follow-back (TLFB; Sobell and Sobell, 1992), which was then used to determine number of drinking days, number of binge drinking days and total number of drinks consumed during the five-day maintenance period. Subjective measures were the: 1) Alcohol Urge Questionnaire (Bohn et al., 1995); 2) 7-item version of the Subjective High Assessment Scale (SHAS; Schuckit et al., 2000); 3) Biphasic Alcohol Effects Scale (BAES; Martin et al., 1993); 4) Profile of Mood States (POMS; Schacham, 1983) and 5) Drug Effects Questionnaire (Johanson and Uhlenhuth, 1980). Physiological measures were breath alcohol concentration, heart rate and blood pressure. Cognitive-behavioral measures were the: 1) Alcohol and Soda Purchase Tasks (Amlung et al., 2015; Bruner and Johnson, 2014; Murphy and MacKillop, 2006; Strickland and Stoops, 2017), with primary outcomes including elasticity of demand ($\alpha$) and intensity of demand ($Q_0$); 2) n-Back Task (Jaeggi et al., 2010), with the primary outcome being percentage of correct responses under two settings, the 1-back and the 2-back; 3) Cued Go/No Go Task (Marczinski and Fillmore, 2003), with the primary outcome being percentage of inhibitory failures and 4) Money and Alcohol and Money Delay Discounting Tasks (Koffarnus and Bickel, 2014) with the primary outcome being discounting rate (log10 transformed *k* values).

### 2.4. Drug administration

All drugs were administered in a double-blind fashion. Only study investigators and the Investigational Drug Service staff had access to dose orders in order to maintain the blind. These individuals did not interact with subjects during experimental sessions, nor did they collect experimental data. n-Acetylcysteine doses (0.6 and 1.2 g b.i.d.; National Vitamin Company, Casa Grande, AZ) were prepared from commercially available drug in a gelatin capsule backfilled with cornstarch. Placebo capsules contained only cornstarch. n-Acetylcysteine dose order was randomly determined for each subject.

Alcohol doses were prepared by combining the appropriate amount of 95% alcohol (Clear Spring, Beam Global Spirits and Wine, Chicago, IL) with the subject's selected non-alcoholic, non-caffeinated mixer of choice in an approximate 1:10 ratio. Alcohol doses were prepared individually for each subject based on weight, with the dose for the sampling phase being 0.244 g alcohol/kg body weight and 0.122 g alcohol/kg body weight for each of the self-administration doses. The mixer of choice could vary across subjects but was the same across sessions for each subject.

### 2.5. Data analysis

Only data from completing subjects were included in the data analysis. Self-administration data were analyzed as the total number of drinks consumed during both sampling phases, number of drinks consumed in each individual sampling phase and latency to drink in each sampling phase using a one-factor repeated measures Analysis of Variance (ANOVA) with n-Acetylcysteine Dose (0, 1.2 and 2 g/day) as the factor (Prism, GraphPad Software, San Diego, CA). The number of days drinking from the TLFB was analyzed using a one-factor repeated measures ANOVA with n-Acetylcysteine Dose (0, 1.2 and 2 g/day) as

the factor. Due to missing data from one subject for the 0 g *n*-acetylcysteine condition, the number of days binge drinking and the total number of drinks from the TLFB were analyzed using a mixed effects analysis with n-Acetylcysteine Dose (0, 1.2 and 2 g/day) as the factor. Subjective, physiological and cognitive-behavioral measures from the sampling phase were analyzed as time course using a two-factor repeated measures ANOVA with n-Acetylcysteine Dose (0, 1.2 and 2 g/day) and Time (pre, 0, 10, 20 and 30 min post dose during the sampling phase for subjective and physiological measures; pre and 30 min post dose during the sampling phase for cognitive-behavioral measures). Only data from the sampling phase were used for these measures because subjects could consume differing amounts of alcohol in the self-administration phases, making data interpretation difficult. *F* values from the ANOVAs were used to determine statistical significance with a threshold of $p \leq .05$.

Data from commodity purchase tasks were analyzed using the exponentiated demand equation (Koffarnus et al., 2015):

$$Q = Q_0 * 10^{k*(e^{(-a*Q0*c)}-1)}$$

where $Q$ = consumption; $Q_0$ = derived demand intensity; $k$ = a constant related to consumption range (a priori set to 2); $C$ = commodity price; and $\alpha$ = derived demand elasticity. Demand intensity was analyzed as the equation derived value. Analyses focused on demand intensity and demand elasticity given research showing these measures reflect a two-factor structure underlying purchase task data (e.g., Bidwell et al., 2012; Mackillop et al., 2009). The only demand curves identified as non-systematic were soda curves due to all zero consumption (15 of 54 soda demand curves). One subject also reported constant alcohol consumption across all prices on one alcohol demand curve. Consumption at the last price point for that curve was decreased by one unit to estimate a curve fit. Only subjects with non-zero soda consumption on all soda curves were analyzed for soda elasticity analyses (N = 5), but all were included for soda intensity (with intensity coded as zero). Intensity for alcohol and elasticity for alcohol and soda were log10-transformed to achieve normality.

## 3. Results

### 3.1. Reinforcing effects of alcohol

There were no statistically significant effects of n-Acetylcysteine Dose on any of the alcohol self-administration outcomes. As shown in Fig. 1, subjects consumed approximately three to four of the eight total drinks available during the self-administration phases, regardless of maintenance condition.

### 3.2. TLFB

There were no statistically significant effects of n-Acetylcysteine Dose on any of the TLFB outcomes.

### 3.3. Subjective measures

Significant main effects of Time were observed for Total Score on the Alcohol Urge Questionnaire, Total and General Scores on the SHAS, Sedative Score on the BAES and ratings of Feel Drink and Want More Drink from the Drug Effects Questionnaire ($F_{4,32}$ values > 3.35 for $p$ values ≤ .05). As shown for the representative Feel Drink item in Fig. 2, ratings on these items increased relatively rapidly after alcohol administration and slowly offset through 30 min after dosing, regardless of maintenance condition. There were no statistically significant main effects of n-Acetylcysteine Dose, nor were there any statistically significant interactions of n-Acetylcysteine Dose and Time, on these measures. No statistically significant main or interaction effects were observed for any other subjective measures.

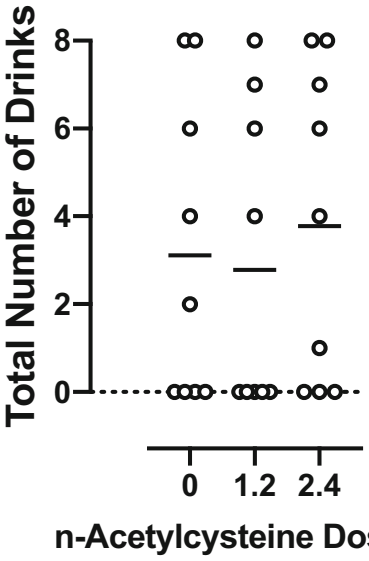


**Fig. 1.** Total number of drinks consumed following maintenance on placebo and n-Acetylcysteine (1.2 and 2.4 g/day) during self-administration phases. Circles represent individual subject data. Horizontal lines show means. X-axis: n-Acetylcysteine dose.

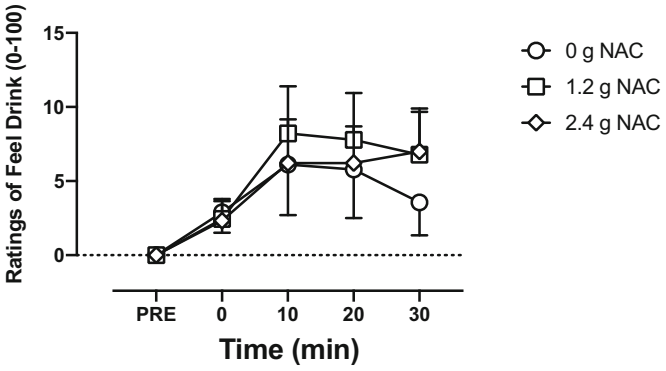


**Fig. 2.** Time-course functions for alcohol following maintenance on placebo (circles), 1.2 g (squares) and 2.4 g *n*-acetylcysteine (diamonds) for ratings of Feel Drink. X-axis: Time relative to alcohol sampling dose. Brackets indicate 1 S.E.M.

### 3.4. Physiological measures

Significant main effects of Time were observed for breath alcohol concentration and systolic blood pressure ($F_{4,32}$ values > 4.23 for $p$ values ≤ .02). As shown in Fig. 3, breath alcohol concentrations peaked at approximately 0.04% 10 min after alcohol administration and slowly offset through 30 min after administration, regardless of maintenance condition. Systolic blood pressure increased after alcohol administration, regardless of *n*-acetylcysteine dose. There were no significant main effects of n-Acetylcysteine Dose, nor were there any significant interactions of n-Acetylcysteine Dose and Time, on these measures. Significant main effects of Time ($F_{4,32}$ value = 3.14 for $p$ value = .04) and n-Acetylcysteine Dose ($F_{2,16}$ value = 4.95 for $p$ value = .04) were observed for heart rate. As shown in Fig. 3, heart rate was elevated at the pre-dose observation for both active *n*-acetylcysteine conditions. Although heart rate decreased after alcohol dosing, it was higher for both *n*-acetylcysteine conditions relative to placebo throughout the sampling phase. There were no statistically significant interactions of n-Acetylcysteine Dose and Time on heart rate. No statistically significant main or interaction effects were observed for diastolic blood pressure.

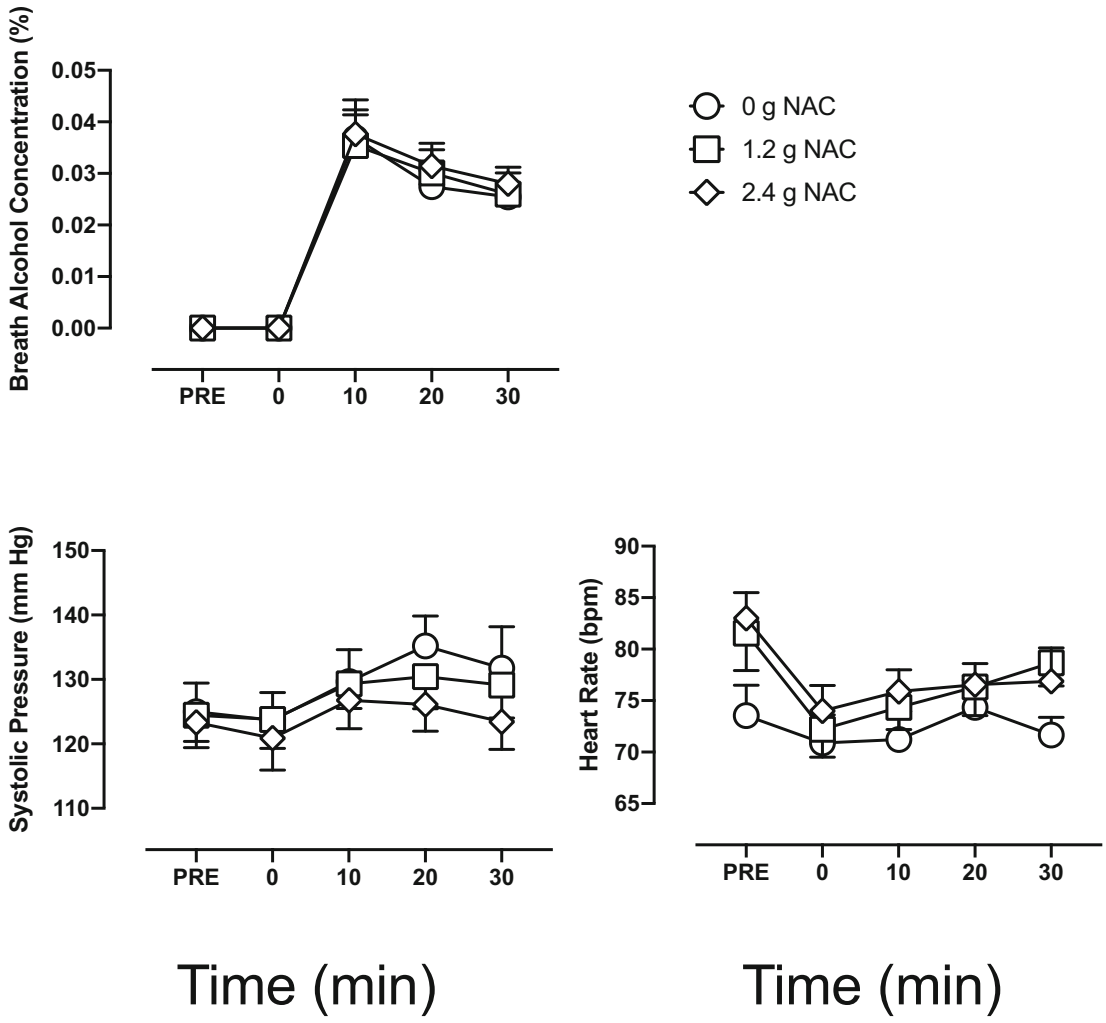


**Fig. 3.** Time-course functions for alcohol following maintenance on placebo (circles), 1.2 g (squares) and 2.4 g *n*-acetylcysteine (diamonds) for breath alcohol concentration (top left panel), systolic blood pressure (bottom left panel) and heart rate (bottom right panel). X-axis: Time relative to alcohol sampling dose. Brackets indicate 1 S.E.M.

### *3.5. Cognitive-behavioral measures*

Median alcohol demand curves and individual traces are plotted in Fig. 4. A significant main effect of Time was observed for alcohol demand intensity ($F_{1,8}$ = 7.32, $p$ = .03). This effect reflected increased alcohol demand intensity following alcohol administration, regardless of maintenance condition. No other main or interaction effects were observed for alcohol demand, nor were there any statistically significant main or interaction effects observed for soda demand intensity or elasticity. No statistically significant main or interaction effects were observed on the n-Back, Cued Go/No Go or Delay Discounting tasks.

## 4. Discussion

The results of this experiment indicate that maintenance on a range of doses of *n*-acetylcysteine does not influence alcohol self-administration or subjective effects, nor does it change hypothetical purchases on an alcohol purchase task. It is important to note that the number of drinks administered by subjects during placebo maintenance, on average, was approximately half of the total drinks available. Thus, had *n*-acetylcysteine changed self-administration behavior, up or down, we would have been able to observe this change. n-Acetylcysteine maintenance generally did not affect physiological outcomes, with breath alcohol concentrations and blood pressure readings being similar across dose conditions. Although we observed increases in heart rate during *n*-acetylcysteine maintenance, a number of other studies have not observed this effect in populations without alcohol use disorder (e.g., Bolin et al., 2017; Leelarungrayub et al., 2011; Hirai et al., 2017), so future work is needed to confirm how *n*-acetylcysteine affects heart rate in individuals with alcohol use disorder.

Alcohol produced prototypic effects, increasing ratings of general and specific subjective effects (e.g., Feel Drink; sedative scores on the BAES), as well as breath alcohol concentration and systolic blood pressure, as a function of time. Alcohol administration also increased alcohol demand intensity on the alcohol purchase task, consistent with previous findings with oral alcohol priming (Amlung et al., 2015). The sampling alcohol dose tested was relatively low, but it was selected based on the dose tested in an initial study validating human laboratory methods for screening putative alcohol pharmacotherapies (O'Malley et al., 2002), as well as to allow subjects to familiarize themselves with the effects of the prepared drink and allow them to choose all available drinks during the self-administration phase without reaching dangerously high breath alcohol concentrations. Consequently, the subjective effects observed here were quite small in magnitude but are comparable to those observed previously with alcohol doses that produce similar alcohol concentration levels (e.g., Davidson et al., 1997). The alcohol dose also failed to impair performance on a range of measures, but this can again be attributed to testing a relatively low sampling dose administered. The peak breath alcohol concentration reached was about half of the legal limit in the United States, so it is not surprising that

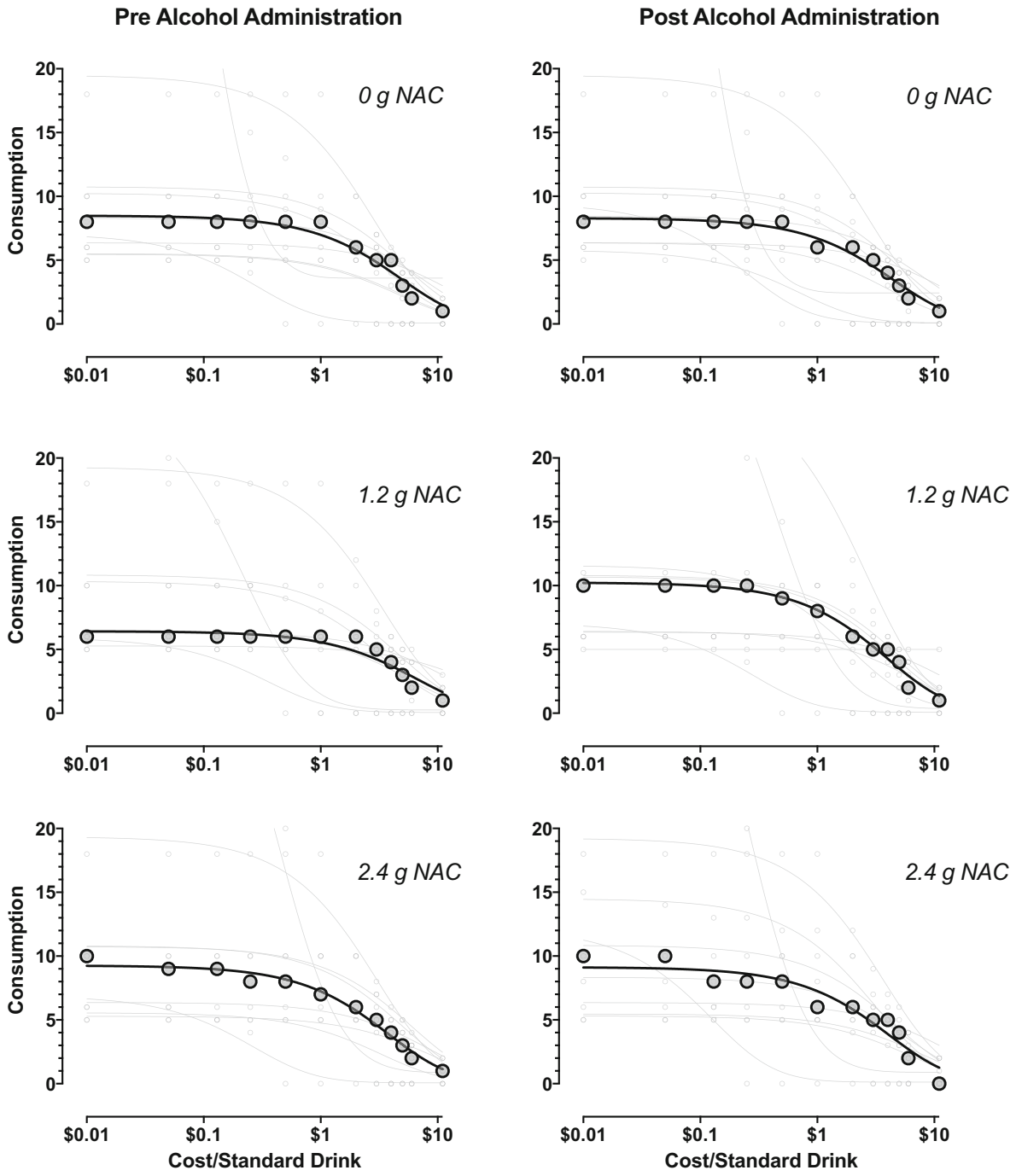


**Fig. 4.** Median economic demand curves for alcohol pre (left panels) and post (right panels) administration of the alcohol sampling dose following maintenance on placebo (top panels), 1.2 g (middle panels) and 2.4 g *n*-acetylcysteine (bottom panels). Also plotted are traces for individual subject data (light gray). X-axis: Price paid/standard drink in U.S. dollars.

impairment was not detected, especially when considering that performance impairment is typically detected following sampling doses about 2–3 fold higher than the dose administered here (e.g., Fillmore and Weafer, 2004; Weafer and Fillmore, 2012).

Several limitations to the present study should be acknowledged. First, we evaluated a relatively low alcohol dose in subjects who met criteria for alcohol use disorder, meaning that our sampling and self-administration doses resulted in breath alcohol concentrations below what this group typically achieves when drinking in the natural ecology. Second, although we tested a range of doses of *n*-acetylcysteine, including one that reduced alcohol use in individuals with cannabis use disorder (Squeglia et al., 2018), we could have tested a higher dose and perhaps observed an effect. A final limitation is the small sample size. Although we planned to enroll a greater number of subjects, slow enrollment, compounded by the advent of the COVID-19 pandemic, meant we had to close the study without reaching our enrollment target.

## 5. Conclusions

This randomized, placebo-controlled, within-subjects human laboratory experiment was designed to evaluate *n*-acetylcysteine as a putative pharmacotherapy for alcohol use disorder. The project expanded on work which showed that *n*-acetylcysteine treatment reduced

a range of alcohol related effects, including positive reinforcing effects in preclinical models (Israel et al., 2019; Lebourgeois et al., 2018, 2019; Mocelin et al., 2019; Morais-Silva et al., 2016; Quintanilla et al., 2016; Schneider et al., 2015), but the negative findings align with those of other trials which have failed to show an effect of *n*-acetylcysteine on substance use or other psychiatric disorder outcomes (e.g., Tomko et al., 2020; Yu et al., 2020). The reasons for the discrepancy between those preclinical findings and the results of this study could be due to a number of reasons. These include species differences, route of administration, dosing regimens or methodological variations. For example, in one study, 25 mg/kg intraperitoneally administered *n*-acetylcysteine reduced ethanol self-administration (Lebourgeois et al., 2019). This dose would fall between the two active doses tested here in a 70 kg human being (i.e., 1.75 g), but because it was given parenterally and did not undergo first pass metabolism, it likely produced greater *n*-acetylcysteine concentrations than the oral *n*-acetylcysteine doses administered here. Furthermore, that study tested an acute *n*-acetylcysteine dose on alcohol self-administration on a progressive ratio schedule whereas we evaluated the effects of sub-chronically administered *n*-acetylcysteine on choice between alcohol and money. Preclinical models can identify endophenotypes relevant to alcohol use disorder and spur clinical work (Perry and Lawrence, 2020), but a recent commentary has questioned their utility for developing treatments for substance use disorders because they do not fully capture the numerous factors (e.g., language, social influence) contributing to addiction (Field and Kersbegen, 2020).

The fact that *n*-acetylcysteine neither increased nor decreased alcohol self-administration or alcohol purchase behavior, taken together with the positive predictive validity of these measures in identifying alcohol pharmacotherapies (Bujarski and MacKillop, 2012; Drobes et al., 2003; Hendershot et al., 2017; O'Malley et al., 2002), suggests that *n*-acetylcysteine is likely not a strong candidate alcohol pharmacotherapy on its own. However, considering the preclinical findings and reductions in drinking shown in individuals with cannabis use disorder, as well as the safety of combining *n*-acetylcysteine with alcohol and the limitations of this study described above, further work, is needed. Previous preclinical work suggests that combining *n*-acetylcysteine with another medication (e.g., aspirin; Israel et al., 2019) enhances its ability to reduce alcohol intake. Thus, future studies, especially Phase II clinical trials, could more fully determine whether *n*-acetylcysteine is an effective adjunct treatment for alcohol use disorder in combination with another medication or with a behavioral intervention.

## Declaration of competing interest

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