Study Protocol, including Statistical Analysis Plan

Official Title: Does endotoxin administration increase alcohol consumption in individuals with AUD?
Brief Title: Does endotoxin administration increase alcohol consumption? (Terril Verplaetse, PhD, PI)
ClinicalTrials.gov ID: NCT04527185
Protocol Date: Most recent IRB approval – 4/4/2024

Protocol and Statistical Analysis Plan for
"Does endotoxin administration increase alcohol consumption?"
NCT04527185 (Terril Verplaetse, PhD, PI)

Scientific Background

One of the principal mechanisms associated with the maintenance of and relapse to alcohol use is stress[12-17]. Neuroimmune function is a key system linked to negative reinforcement drinking and to the development of alcohol use disorder (AUD)[18,19]. Stress and chronic alcohol consumption have been found to increase microglial activity, a marker of neuroinflammation[18-20]. However, a provocation study to examine neuroinflammation-induced drinking has not been developed in humans and has direct clinical significance for the development and evaluation of pharmacotherapies targeting the neuroimmune system.

Endotoxin, also called lipopolysaccharide (LPS), is well-known to induce neuroinflammation1 and acute stress[2], and a growing literature indicates that neuroinflammatory processes are involved in alcohol-motivated behavior[3]. In rodents, systemic administration of LPS resulted in prolonged increases in levels of proinflammatory cytokines, an effect potentiated by alcohol[4]. Administration of inflammatory signaling by LPS demonstrated long-lasting increases in voluntary alcohol intake in mice5, which may be due to the effects of acute stress induced by LPS – as LPS has transient effects on neuroinflammatory responses[114]. Proinflammatory cytokines that increased in rat brain following alcohol or LPS were also increased in the brain of humans with alcohol dependence[6]. In alcohol-dependent individuals, LPS stimulated inflammatory pathways correlated with alcohol craving and short-term alcohol withdrawal was associated with the recovery of LPS-dependent receptors[7]. Regarding stress, LPS administration increased plasma ACTH in alcohol-exposed rats[8,9] and dose-dependently increased proinflammatory cytokines, cortisol, anxiety, and depressive symptoms in humans[2,10]; altogether, supporting a clear role for neuroinflammation in stress and alcohol use. To our knowledge, the effect of LPS, which directly induces neuroinflammation compared to other stress challenges, on alcohol self-administration in humans with AUD has not been studied, identifying an area of weakness in the scientific rigor of previous work. The first step in addressing this gap is to examine peripheral measures of neuroinflammation (e.g., cytokine levels) and relate that to drinking behavior in the human laboratory. By collecting subjective and objective measures of stress (e.g., mood, cortisol, ACTH), we can also determine whether LPS administration is related to stress-reactivity in individuals with AUD.

Over the past decade, rates of AUD have continued to increase more so in women (84%) than in men (35%)[50,51]. Women also experience exacerbated health risks associated with drinking, including brain atrophy, liver inflammation, cardiovascular disease, and neurotoxicity when compared to men[52-56]. Identifying effective mechanisms underlying problem alcohol use in women vs. men remains a priority. Stress and greater neuroimmune response are associated with alcohol use for both women and men, but play an especially critical role in women11. Research identifies that alcohol administration leads to higher cortical levels of proinflammatory cytokines associated with oxidative stress in female compared to male mice[57,58] and higher plasma endotoxin levels in healthy women compared to men[57,59]. Problematic drinking in women is also associated with higher plasma levels of proinflammatory cytokines compared to men[60,61].

Importantly, we plan to calculate effect sizes for the interaction between sex and treatment (endotoxin vs. placebo) on drinking behavior and associated mechanisms (e.g., peripheral cytokines, craving, cortisol, ACTH, sex hormones) during the 2-hour alcohol self-administration period, as these may be important mechanisms underlying drinking behavior in women vs. men.

Elucidation of neuroinflammatory mechanisms underlying drinking can inform on the involvement of neuroinflammation in alcohol use, as well as potential sex differences in mechanisms underlying how neuroinflammation influences acute drinking. This data will inform sex-appropriate mechanisms and potential treatment targets, such as neuroimmune modulators, for alcohol use in individuals with AUD, as well as inform future research on disentangling the effect of LPS vs. psychological stress (e.g., stress vs. neutral imagery) on subsequent drinking behavior.

Study Design and Methods

We will examine whether endotoxin, known to induce acute stress and neuroinflammation, increases acute drinking behavior in a sex-dependent manner. We will recruit 32 individuals with AUD (50% women). Subjects will undergo a single laboratory session in which we will evaluate alcohol consumption.

Subjects will be informed to not drink 24 hours prior to the laboratory session (confirmed by breath alcohol reading and self-report). Smokers will be instructed to smoke as they usually do prior to coming to the YCCI/HRU. If participants report or test positive for breath alcohol, drug use, alcohol withdrawal, or pregnancy they will not complete the laboratory session. If participants present with alcohol withdrawal (CIWA ≥ 8), the participant will be evaluated by the study MD for possible study removal and referral to detox. An IV cannulae will be inserted in the non-dominant arm to obtain blood samples throughout the session. LPS (0.4ng/kg; Escherichia coli) or placebo (saline) injections will be performed through the IV cannulae, approximately 1 hour and 45 minutes before the alcohol self-administration period. This dose has been safely administered to healthy humans previously (range 0.2 – 1.0ng/kg i.v. in healthy human subjects).

Ad-libitum drinking will occur over a 2-hour period. A video camera will be present in the room during this session to monitor drinking behavior. Subjects will be provided a pre-determined amount of their preferred beverage designed to raise BALs to 0.12 g/dL. Participants will be provided with their preferred beverage (e.g., beer, wine, liquor, mixed drink) as a bolus at the start of the session. The bolus dose is provided in a plastic pitcher (e.g., beer) or a plastic carafe (e.g., wine, mixed drink) depending on the participant's preferred beverage. The participant is also provided with a drinking vessel (e.g., plastic pint glass, plastic wine glass, plastic tumbler). The participant can pour as much or as little of their preferred beverage as they like from the pitcher or carafe into their drinking vessel, and choose to drink as much or as little as they like throughout the 2-hour alcohol self-administration session.

Prior to discharge, serum endotoxin will be drawn. Subjects will remain in the YCCI/HRU overnight, at which time their breath alcohol levels will be below 0.02% (confirmed by two BAC readings). Participants who are deemed to be belligerent by study/HRU staff will be

immediately discontinued at the discretion of the PI. Participants will be asked to remain in their room until reaching a safe BAC at which time they will be dismissed. If they continue to behave in a threatening manor, hospital security will be called to supervise the participant until they can be safely discharged from the HRU.

Eligibility Criteria

After obtaining written informed consent, potential subjects will be screened for eligibility. In other words, potential subjects will sign consent prior to beginning screening procedures. We will administer the Structured Clinical Interview for DSM-5[63] (SCID-5) to confirm AUD and exclude other primary psychiatric and substance abuse disorders (other than tobacco use disorder). We will record alcohol use over the prior 90 days with the Timeline-Follow Back Interview[64] (TLFB), and quantify lifetime patterns of alcohol use with the Lifetime Drinking History[65]. The consequences of alcohol use and severity will be measured with the Short-Inventory of Problems for Alcohol Use[66] and the Alcohol Use Disorders Test[67] (AUDIT), respectively. The Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised[68] (CIWA-R) will assess alcohol withdrawal symptoms. Patients with CIWA-R ≥ 8 will not be eligible to participate. Subjects will undergo medical screening, including medical history, medication use, allergies, a physical exam, an EKG, basic blood chemistries, urine drug toxicology screen, and a blood pregnancy test for women. The study physicians will review the subject's medical status and medical eligibility criteria. Eligible subjects will be randomized to receive endotoxin or placebo. If potential participants are found to be suicidal or are experiencing any psychiatric symptoms or distress during the screening process, they will receive short-term support from the study team (including clinical psychologists and study physicians) and will be connected to a local emergency department and their physician or therapist for ongoing care.

Inclusion Criteria:
1) Age 21-65;
2) Able to read and write English;
3) Meets DSM-5 criteria for current (past 6 months) moderate to severe alcohol use disorders;
4) Drinking criteria: Males - Drinks > 14 drinks per week and exceeds 4 drinks per day at least twice per week; Females -Drinks > 7 drinks per week and exceeds 3 drinks per day at least twice per week.
5) Must meet drinking criteria during a consecutive 30-day period within the 90 days prior to baseline;
6) Laboratory sessions will be scheduled such that subjects will not have major responsibilities on the following day which might limit drinking during the self-administration session (e.g., job interview, exam);
7) Negative urine pregnancy test for women.

Exclusion Criteria:
1) Participants with any significant current medical conditions (neurological, cardiovascular [including hypertension or hypotension: sitting BP >160/100 or <90/60mmHg at baseline screening], endocrine, thyroid, renal, liver), seizures, delirium or hallucinations, or other unstable medical conditions including HIV;
2) Current DSM-5 substance use disorders, other than alcohol or nicotine;

3) A positive test result at intake appointment on urine drug screens conducted for illicit drugs, excluding cannabis;
4) Past 30 day use of psychoactive drugs including anxiolytics and antidepressants;
5) Women who are pregnant or nursing, or fail to use one of the following methods of birth control unless she or partner is surgically sterile or she is postmenopausal (hormone contraceptives [oral,
implant, injection, patch, or ring], contraceptive sponge, double barrier [diaphragm or condom plus spermicide], or IUD);
6) Suicidal, homicidal or evidence of current (past 6-month) severe mental illness such as schizophrenia, bipolar disorder or major depression, or anxiety disorders;
7) Subjects treatment-seeking or who are currently in treatment for alcohol use;
8) Subjects with medical conditions contraindicating alcohol use (e.g., liver enzymes ≥3× normal);
9) Subjects likely to exhibit clinically significant alcohol withdrawal during the study. We will exclude subjects who a) have a history of perceptual distortions, seizures, delirium, or hallucinations upon withdrawal, or b) have a score of > 8 on the Clinical Institute Withdrawal Assessment scale at intake appointments;
10) Participation within the past 8 weeks in other studies that involve additive blood sampling and/or interventional measures that would be considered excessive in combination with the current application.
11) Subjects >38 on the Alcohol Use Disorders Identification Test (AUDIT)
12) Subjects with resting pulse >100 at challenge
13) Subjects with recent (past 2 weeks) acute illness or vaccination
14) Subjects with >Grade 2 laboratory abnormalities on screening

Statistical Considerations

To determine whether endotoxin vs. placebo will increase milliliters of alcohol consumed in women and men. Linear mixed models with intervention condition (0.4ng/kg i.v. endotoxin or placebo) and sex as between subject factors will be used to evaluate milliliters consumed during ad-libitum drinking collected in the laboratory. All interactions will be considered. The best fitting variance-covariance structure will be assessed using information criteria. The mixed effects approach is advantageous in that it is unaffected by randomly missing data and allows greater flexibility in modeling the correlation structure of repeated measures data. This data will generate effect sizes for the interaction between sex and intervention (endotoxin vs. placebo) on alcohol use.

We will evaluate the safety and tolerability of endotoxin in combination with alcohol in individuals with AUD. Participants will be assessed for altered alcohol intoxication and risks associated with endotoxin (e.g., flu-like symptoms [chills, nausea]). We will take extra safety precautions when assessing adverse effects of LPS and alcohol co-administration, including drawing serum endotoxin during alcohol self-administration and discharge.

References

1. Sandiego CM, Gallezot J-D, Pittman B, et al. Imaging robust microglial activation after

lipopolysaccharide administration in humans with PET. *Proceedings of the National Academy of Sciences.* 2015;112(40):12468-12473.
2. Grigoleit J-S, Kullmann JS, Wolf OT, et al. Dose-dependent effects of endotoxin on neurobehavioral functions in humans. *PLoS One.* 2011;6(12):e28330.
3. Hillmer A, Sandiego C, Hannestad J, et al. In vivo imaging of translocator protein, a marker of activated microglia, in alcohol dependence. *Molecular psychiatry.* 2017;22(12):1759.
4. Qin L, He J, Hanes RN, Pluzarev O, Hong J-S, Crews FT. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. *Journal of neuroinflammation.* 2008;5(1):10.
5. Blednov Y, Benavidez JM, Geil C, Perra S, Morikawa H, Harris R. Activation of inflammatory signaling by lipopolysaccharide produces a prolonged increase of voluntary alcohol intake in mice. *Brain, behavior, and immunity.* 2011;25:S92-S105.
6. He J, Crews FT. Increased MCP-1 and microglia in various regions of the human alcoholic brain. *Experimental neurology.* 2008;210(2):349-358.
7. Leclercq S, De Saeger C, Delzenne N, de Timary P, Stärkel P. Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. *Biological psychiatry.* 2014;76(9):725-733.
8. Lee S, Rivier C. Gender differences in the effect of prenatal alcohol exposure on the hypothalamic-pituitary-adrenal axis response to immune signals. *Psychoneuroendocrinology.* 1996;21(2):145-155.
9. Lee S, Schmidt D, Tilders F, Rivier C. Increased activity of the hypothalamic-pituitary-adrenal axis of rats exposed to alcohol in utero: role of altered pituitary and hypothalamic function. *Molecular and Cellular Neuroscience.* 2000;16(4):515-528.
10. Hannestad J, Subramanyam K, DellaGioia N, et al. Glucose metabolism in the insula and cingulate is affected by systemic inflammation in humans. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine.* 2012;53(4):601.
11. Koob GF, White A. Alcohol and the Female Brain. Paper presented at: 2017 National Conference on Alcohol and Opioid Use in Women & Girls: Advances in prevention, Treatment, and Recovery Research2017; Washington D.C.
12. Brady KT, Sonne SC. The role of stress in alcohol use, alcoholism treatment, and relapse. Alcohol Research & Health. 1999;23(4):263-.
13. Pohorecky LA. Stress and alcohol interaction: an update of human research. Alcoholism: Clinical and Experimental Research. 1991;15(3):438-59.
14. Sayette MA. An appraisal-disruption model of alcohol's effects on stress responses in social drinkers. Psychological bulletin. 1993;114(3):459.
15. Sher KJ, Levenson RW. Risk for alcoholism and individual differences in the stress-response dampening effect of alcohol. Journal of Abnormal Psychology. 1982;91(5):350.
16. Zywiak WH, Connors GJ, Maisto SA, Westerberg VS. Relapse research and the Reasons for Drinking Questionnaire: A factor analysis of Marlatt's relapse taxonomy. Addiction. 1996;91(12s1):121-30.
17. Marlatt GA, Donovan DM. Relapse prevention: Maintenance strategies in the treatment of addictive behaviors: Guilford Press; 2005.
18. Hillmer AT, Sandiego CM, Hannestad J, Angarita GA, Kumar A, McGovern EM, Huang Y, O'Connor KC, Carson RE, O'Malley SS, Cosgrove KP. In vivo imaging of translocator protein, a marker of activated microglia, in alcohol dependence. Mol Psychiatry. 2017;22(12):1759-1766.
19. Kalk NJ, Guo Q, Owen D, Cherian R, Erritzoe D, Gilmour A, Ribeiro AS, McGonigle J,

Waldman A, Matthews P, Cavanagh J, McInnes I, Dar K, Gunn R, Rabiner EA, Lingford-Hughes AR. Decreased hippocampal translocator protein (18 kDa) expression in alcohol dependence: a [(11)C]PBR28 PET study. Transl Psychiatry. 2017;7(1):e996.
20. Wohleb ES, Terwilliger R, Duman CH, Duman RS. Stress-Induced Neuronal Colony Stimulating Factor 1 Provokes Microglia-Mediated Neuronal Remodeling and Depressive-like Behavior. Biol Psychiatry. 2018;83(1):38-49.
47. Higley AE, Crane NA, Spadoni AD, Quello SB, Goodell V, Mason BJ. Craving in response to stress induction in a human laboratory paradigm predicts treatment outcome in alcohol dependent individuals. Psychopharmacology. 2011;218(1):121-9.
50. Grant B.F., Chou S.P., Saha T.D. Prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV alcohol use disorder in the United States, 2001-2002 to 2012-2013: results from the National Epidemiologic Survey on Alcohol and Related Conditions. JAMA psychiatry. 2017;74(9):911–923.
51. Dawson DA, Goldstein RB, Saha TD, Grant BF. Changes in alcohol consumption: United States, 2001-2002 to 2012-2013. Drug and Alcohol Dependence. 2015; 148: 56-61.
52. National Institute of Alcohol Abuse and Alcoholism . 2017. Women and Alcohol.
53. Agabio R., Pisanu C., Luigi Gessa G., Franconi F. Sex differences in alcohol use disorder. Curr. Med. Chem. 2017;24(24):2661–2670.
54. Szabo G. Women and alcoholic liver disease — warning of a silent danger. Nature Reviews Gastroenterology & Hepatology. 2018;15:253.
55. Agabio R., Campesi I., Pisanu C., Gessa G.L., Franconi F. Sex differences in substance use disorders: focus on side effects. Addict. Biol. 2016;21(5):1030–1042.
56. National Cancer Institute Alcohol and cancer risk. 2013.
https://www.cancer.gov/aboutcancer/causes-prevention/risk/alcohol/alcohol-fact-sheet
57. Pascual M, Montesinos J, Marcos M, Torres JL, Costa-Alba P, García-García F et al. Gender differences in the inflammatory cytokine and chemokine profiles induced by binge ethanol drinking in adolescence. Addiction biology 2016.
58. Alfonso-Loeches S, Pascual M, Guerri C. Gender differences in alcohol-induced neurotoxicity and brain damage. Toxicology 2013; 311(1-2): 27-34.
59. Bala S, Marcos M, Gattu A, Catalano D, Szabo G. Acute Binge Drinking Increases Serum Endotoxin and Bacterial DNA Levels in Healthy Individuals. PLOS ONE 2014; 9(5): e96864.
60. Kirpich IA, McClain CJ, Vatsalya V, Schwandt M, Phillips M, Falkner KC et al. Liver Injury and Endotoxemia in Male and Female Alcohol-Dependent Individuals Admitted to an Alcohol Treatment Program. Alcoholism: Clinical and Experimental Research 2017; 41(4): 747-757.
61. Wilhelm CJ, Fuller BE, Huckans M, Loftis JM. Peripheral immune factors are elevated in women with current or recent alcohol dependence and associated with altered mood and memory. Drug and alcohol dependence 2017; 176: 71-78.
62. Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, Gastfriend DR, Hosking JD, Johnson BA, LoCastro JS, Longabaugh R, Mason BJ, Mattson ME, Miller WR, Pettinati HM, Randall CL, Swift R, Weiss RD, Williams LD, Zweben A; COMBINE Study Research Group. Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. JAMA. 2006 May 3;295(17):2003-17.
63. First MB. (2015). Structured Clinical Interview for the DSM (SCID). In The Encyclopedia of Clinical Psychology (eds R. L. Cautin and S. O. Lilienfeld).
doi:10.1002/9781118625392.wbecp351

64. Sobell LC, Sobell MB. Timeline follow-back. Measuring alcohol consumption: Springer; 1992. p. 41-72.
65. Skinner HA, Sheu W-J. Reliability of alcohol use indices. The Lifetime Drinking History and the MAST. Journal of studies on alcohol. 1982;43(11):1157-70.
66. Miller WR, Tonigan JS, Longabaugh R. The Drinker Inventory of Consequences (DrInC): An instrument for assessing adverse consequences of alcohol abuse: Test manual: US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute on Alcohol Abuse and Alcoholism; 1995.
67. Saunders JB, Aasland OG, Babor TF, De la Fuente JR, Grant M. Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. Addiction. 1993;88(6):791-804.
68. Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM. Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). British journal of addiction. 1989;84(11):1353-7.