Human Alcohol Seeking Despite Aversion

Martin Plawecki, M.D., Ph.D., 550 N University Blvd. UH5505
Indianapolis, IN 46202

Co-PI: Melissa A. Cyders, Ph.D., Associate Professor, Dept. Psychology, IUPUI
Co-I: Sean O'Connor, M.D., Professor, Dept. Psychiatry, IUSOM
Ann Kosobud, Ph.D., Assistant Professor, Dept. Neurology, IUSOM
David Kareken, Ph.D., Professor, Dept. Neurology, IUSOM
Mario Dzemidzic, Ph.D., Assistant Professor, Dept. Neurology, IUSOM
Joaquin Goñi, Ph.D., Assistant Professor, Industrial and Biomed. Engineering, Purdue

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1.0 Background

Alcohol Use Disorders (AUDs) occur in over 16.6 million adults in the United States and are the leading contributor to premature death and disability among ages 15-49. The estimated economic burden to the United States was $223.5 billion in 2006. Limiting this damage requires preventing escalation to chronic, treatment-resistant compulsive drinking. AUDs are theorized to progress through 3 overlapping and recurring stages: binge-intoxication, negative affect-withdrawal, and preoccupation-anticipation. Binge-intoxication is initially characterized by impulsive drinking, and a rewarding response to alcohol and the motivation induced by alcohol's associated cues. Repeated alcohol intoxication is thought to lead to negative affect-withdrawal, with a loss of sensitivity to, and eventually withdrawal from, alcohol. Secondary neural adaptations, including an altered stress response, occur, as well. The preoccupation-anticipation stage is thought to be marked by greater executive dysfunction, such as impaired error monitoring and compulsive consumption. By the time drinking has progressed to the more advanced stages of a diagnosed AUD, years of alcohol use have contributed to neuroadaptations in the brain which exacerbate use and make drinking behaviors more *aversion resistant* and difficult to treat. Indeed, latent trait analysis shows that drinking despite adverse interpersonal, psychological, and physical problems is a marker for the most severe stage of AUD severity. Although abstinence does result in some brain recovery, individuals at this advanced stage often have great difficulty maintaining sobriety, in part because of desensitization to adverse consequences.

Drug seeking, self-administration, and aversion resistance during self-administration are defining behaviors of preclinical models of addiction. The animals' behaviors vary according to their history of alcohol exposure. In contrast, aversion resistant drinking in humans is either established by self-report or clinical inference, without objective behavioral quantification, complicating our understanding of its relationship to lifetime drinking history. Further, the use of *quantified* lifetime drinking history as an independent variable has largely been limited to epidemiologic rather than experimental studies. Consequently, human research has failed to examine how lifetime drinking history contributes to behavioral alcohol seeking and disease progression.

There is an unfulfilled need to develop an objective measure of human ARD. Such a measure would illuminate mechanisms of disease progression and advance the translation between clinical, human laboratory, and preclinical work. "Self-report" is clearly insufficient, requiring insight, accurate perception of consequences, and willingness to report. Most individuals, even those without an AUD, minimize reporting their drinking and its consequences. Thus, many affected individuals are not identified until symptoms are severe enough to be recognized by friends, family, co-workers, or the courts. In contrast to self-report, we posit that measured alcohol "seeking despite aversion" (SDA) reflects AUD progression. We assert that SDA emerges early, progresses with lifetime drinking, presages the onset of aversion and treatment-resistant drinking and AUD diagnosis, is associated with decreased sensitivity to alcohol, and reflects AUD risk and severity.

Only a multidimensional understanding of AUD will lead to more effective treatment approaches that integrate social, behavioral, and biological aspects. This project will advance both basic research and clinical knowledge by embracing multidimensionality. Development of novel, objective measures of SDA characterizing early AUD progression is an important research tool. In basic research, it could help to understand the brain circuits involved in the transition to compulsive drinking. In clinical research, SDA would be a prime tool for testing pharmacologic and behavioral treatments. Current treatment approaches require people to self-report changes

in drinking, craving, and withdrawal symptoms, requiring both insight and objectivity. Identifying the mechanisms and neuroadaptations underlying early stages of AUD progression will lead to an integrated view of addiction across genetic, brain, behavioral, and social levels of analysis.

Our project will build on substantial evidence from the literature that supports the notion that neural adaptations from excessive and extended drinking yield aversion and treatment-resistant drinking behaviors. This project focuses on a problem with a large clinical health impact. It seeks to fill an important gap in how we assess staging of human AUDs in both basic research and clinical research. We propose a multiple level of analysis approach, consistent with the Research Domain Criteria mission and the goals of the Indiana Alcohol Research Center (IARC). Our pilot data strongly support the feasibility of this approach and viability of the proposed hypotheses.

2.0 Rationale and Specific Aims

Prolonged alcohol use results in neuroadaptations. The consequences include a decreased sensitivity to alcohol, a shift from positive to negative reinforcement-based alcohol use, increased negative affect that drives further consumption, and ultimately, aversion-resistant drinking (ARD; drinking despite resultant problems and adverse consequences). ARD is a hallmark of more severe and treatment-resistant compulsive use and is modeled well in preclinical research. There are, however, no well-validated models of human ARD and, little understanding of the neural signature underlying the transition to ARD. This project seeks to validate such a model and to examine its principal determinants in 100 subjects.

The development of ARD marks an important transition from treatment-sensitive to treatment-resistant drinking behaviors. This creates a critical need to develop an objective measure of ARD in humans and to characterize its neural functional substrates. We propose that alcohol seeking despite aversion (SDA) is an early marker of ARD in humans, attributable to the neuroadaptations caused by excessive exposure to alcohol. An objective measure of SDA in human alcohol use will lead to better understanding of the mechanisms of disease progression in humans and advance the translation between human and animal work.

Our long-term goal is to use SDA as a platform for the laboratory testing of novel pharmacologic and behavioral interventions that can be used among those with the highest risk, but who have yet to progress to treatment-resistant drinking. The objective of this project is to test SDA across multiple levels of analysis. We consider SDA as an early marker of alcohol use disorder progression that is related to lifetime drinking history, alcohol use disorder risks, and brain physiology. Our current IARC pilot study (IRB #) demonstrates that SDA can be objectively quantified via an intravenous alcohol self-administration task, where operant work for identical incremental alcohol rewards is paired with aversive stimuli. Our preliminary data support our central hypothesis: that behavior in the SDA model is attributable to lifetime alcohol exposure, is related to alcohol use disorder (AUD) risk factors and phenotypes, and reflects alterations in neural system function. Specifically, they suggest that the degree of SDA is positively associated with recent drinking history, negative affect-based rash action (i.e. negative urgency, including action with respect to alcohol use), and self-rating of the effects of alcohol. The rationale for this work is that it would lead to the first objective, well-validated measure of SDA in humans.
Our specific aims are to:
Aim 1: Characterize SDA as a function of lifetime drinking history.
Hypothesis 1: Greater SDA will reflect greater lifetime drinking history.

Hypothesis 2: Those with a high lifetime drinking history will demonstrate less sensitivity to aversion in the preference to work for alcohol. Specifically, those with a high lifetime drinking history will work more for alcohol compared to water under both aversive and neutral experimental conditions, whereas those with a low lifetime drinking history will only work more for alcohol compared to water when alcohol seeking is paired with neutral stimuli.

Aim 2: Quantify associations between SDA and alcohol use disorder severity, and between SDA and risk phenotypes; then characterize how these risk factors classify individuals into those who will and will not work for alcohol in the context of aversive stimuli.
Hypothesis 3: Those with greater SDA will have greater AUD severity, greater negative urgency, a greater density of familial alcoholism, and reduced subjective, behavioral, and electrophysiologic sensitivity to alcohol.
Hypothesis 4: Significant factors from hypothesis 3 will identify a latent trait that classifies individuals into two types: those who have high SDA and those who have low SDA.

Aim 3: Identify signatures of neural function that underlie SDA.
Hypothesis 5: Those with greater SDA will demonstrate reduced event-related potential component amplitudes differences between negative vs. positive feedback about per-trial progress toward a reward. Those with greater SDA will demonstrate
Hypothesis 6a: greater resting state crosstalk between the default mode and fronto-parietal networks and *Hypothesis 6b*: reduced ventral striatal-ventromedial frontal connectivity.

The positive impact of this project will be to advance the translation between preclinical and clinical models and to elucidate distinct mechanisms underlying AUD progression in humans. Importantly, the proposed model uses objective behavior, rather than subjective symptom report. Our human component project is centered on the IARC theme and complements its preclinical components and methodologies. We expect that the results will facilitate the identification and evaluation of pharmacologic and behavioral interventions to minimize the risk of AUD progression risk in drinkers who have yet to manifest treatment resistant behavior.

3.0 Inclusion/Exclusion Criteria

Inclusion

- Overtly healthy men and women aged 21 – 55
- Either 25-50 kg or 250-500 kg Lifetime Drinking History (LDH) and recent drinking of at least 7 drinks/week for women and 10 drinks/week men, or any recent drinking or LDH below 500 kg at the discretion of the PI.
- Able to understand/complete questionnaires and procedures in English
- Have venous access sufficient to allow blood sampling

Exclusion

- Pregnant or breast-feeding women
- Desire to be treated for any substance use disorder or court ordered to not drink alcohol
- Medical disorders or other conditions that may influence study outcome or subject safety
- Positive urine drug screen for amphetamines/ methamphetamines, barbiturates, benzodiazapines, cocaine, opiates, or phencyclidine
- Medications (past 30 days) that could influence subject data/subject safety (e.g. antidepressants, antipsychotics, benzodiazepines, etc.) as determined by Dr. Plawecki

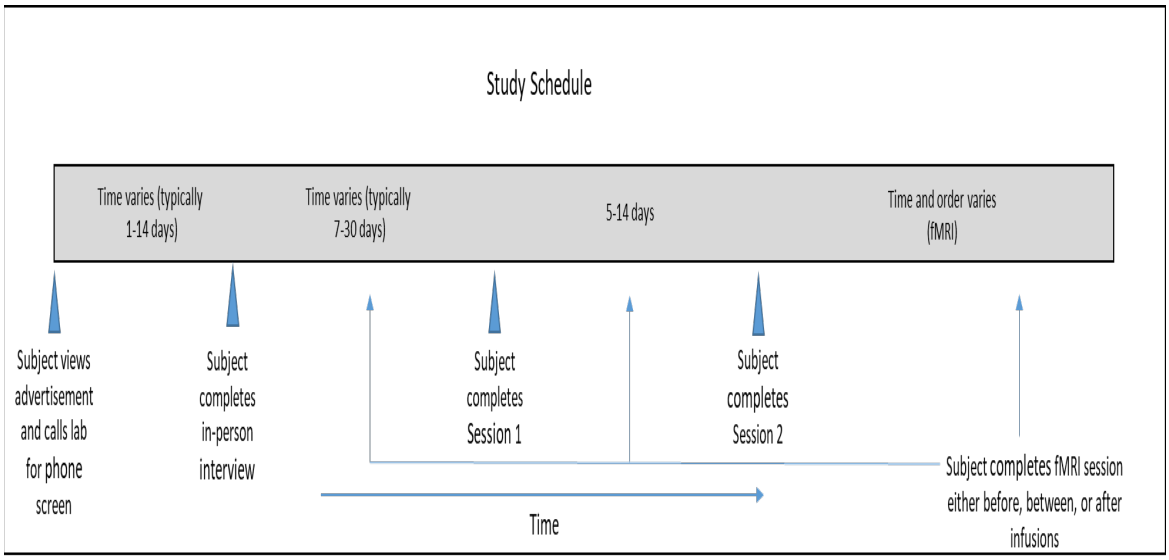
- DSM 5 Disorders (non AUD) or current/history of neurological disease of cerebral origin, or head injury with > 20 min loss of consciousness
- Positive BrAC reading on arrival at any study visit
- Actively suicidal (for example, any suicide attempts within the past year or any current suicidal intent, including a plan) or are at serious suicidal risk, by clinical evaluation by the investigator
- Any condition for which the principal investigator determines it is unsafe or not prudent to enroll a subject.

4.0 Enrollment/Randomization

The project employs a between-participants design. Each participant will undertake 3 or 4 sessions: an interview/screening session, 2 randomly counter-balanced (neutral and aversive stimuli) IV alcohol self-administration sessions, and in a subset of subjects that meet the criteria outlined in the fMRI screen, an fMRI resting state scan. All sessions will take place in the Neural Systems Laboratory at the Indiana Clinical Research Center at Indiana University Hospital or at the Neuroscience Center at Methodist Hospital.

We will consent 300 participants to enroll 150 and complete 100 participants: ***a***) 50 individuals who are current drinkers with lower lifetime drinking history, between 25-50 kg ethanol, ***b***) 50 individuals who are heavy drinkers with a higher lifetime drinking history of 250-500 kg ethanol, and **c**) individuals below 500 kg ethanol at the discretion of the principle investigator. We will target equal numbers of men and women for inclusion in the lower and higher lifetime drinking groups. Total participant payment will be $400, plus parking/travel costs.

5.0 Study Procedures



Recruiting. Participants will be recruited by local advertisements and word of mouth and they will call the study line expressing interest, OR participants will be recruited from the Neural Systems Lab research registry (IRB# 1506904956), OR from the Indiana CTSI iConnect

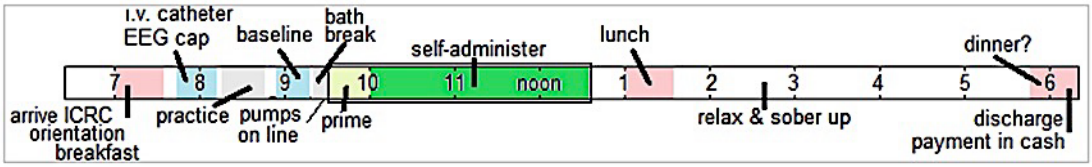
research database. An in-depth phone interview will cover all inclusion/exclusion criteria for the study that can be garnered by conversation. Those who continue to be qualified will either 1) be scheduled for a face-to-face interview conducted at the Indiana (CTSI) Clinical Research Center (ICRC) where they will consent to participate in the study, or 2) arrange for a remote interview where they will consent to participate and complete interview measures electronically and converse with study staff via IU Zoom Health or similar secure platform.

Screening. The screening interview will include informed consent, assessment of inclusion/exclusion criteria, and an introduction to study procedures to familiarize participants with infusion and fMRI methods. All or some of the following measures will be collected during the screening interview, at the discretion of the principle investigator:

- The Alcohol Use Disorders Identification Test
- The Family History of Alcoholism Module of the SSAGA
- Family History Questionnaire
- The UPPS-P Impulsive Behaviors Scale measures impulsivity traits, including urgency.
- Self-Rating of Effect of Alcohol assessing retrospective sensitivity to the subjective response to alcohol.
- Center Epidemiologic Studies – Depression screening test
- Short Inventory of Problems – Revised
- Eysenck Personality Questionnaire
- Medical History (including menstrual phase for women)
- Fagerstrom Test for Nicotine Dependence
- The Semi-structured Assessment for the Genetics of Alcoholism – II, Alcohol, and any other modules as deemed necessary by the principle investigator
- Timeline Follow-back modified to assess for one- and five-week recall of alcohol use
- Concordia Lifetime Drinking Questionnaire
- Clinical Institute Withdrawal Assessment for Alcohol Scale
- Generalized Anxiety Disorder 7 Scale
- Urine pregnancy and drug screen (~50cc)
- Breath Alcohol Concentration Measurement
- Liver function assessment (~10cc)
- Brief Trauma Questionnaire, Primary Care PTSD Screen
- Impaired Control Scale
- fMRI screening form
- Alcohol Purchase Task
- Penn Alcohol Craving Scale
- Blood sample will be taken for future polygenic risk analysis
- Penn State Electronic Cigarette Dependence Index
- Wisconsin Card Sorting Task
- Slips of Action Task

Participants that complete the interview will be compensated $25 and provided a parking pass or bus voucher. Participants that complete part of the interview remotely and appear to meet study criteria will be asked to visit the ICRC for a short visit where they will provide a blood sample, urine, and be paid the interview fee of $25. Participants who complete only the online portion of the interview and are withdrawn from the study will be compensated with $15 in the

form of an electronic or mailed gift card or mailed check. If there is a significant delay between the online and in-person portion of the interview, the subject may request compensation with $15 in the form of an electronic or mailed gift card or check. Following the interview, the information collected during the interview will be evaluated and if the participant meets all study criteria, they will be contacted and asked to schedule the alcohol self-administration sessions, while a subset of participants will be asked to participate in the fMRI sessions. The figure below depicts the flowchart for the 2 alcohol self-administration sessions:



IV alcohol self-administration protocol. Upon arrival at the Indiana Clinical Research Center, participants will be re-screened for drug use and pregnancy, and BrAC. The participant's BrAC must be at 0 by the experiment start time. We will record the subject's recent drinking. Using sterile technique, an indwelling catheter will be placed in a vein of the ante-cubital fossa of one arm. A standardized breakfast will be served and phones and car keys held for safekeeping. The participant will be fitted with a 65-lead EasyCap® and seated in one of the lab's 5'x7' Industrial Acoustic Corporation® sound-dampened chambers, or in a private room designed for ERP data collection. A closed-loop intercom system enables the participant to talk to the technician at any time without manual effort. The participant will be instructed in the use of the Draeger® BrAC meter. Baseline electrophysiologic (discussed below) and subjective response measures will be performed before the participant is prompted for a bathroom break. A single tube combining the outputs of 2 IMED PCTX infusion pumps will be joined with the indwelling catheter. Subjects will be asked to identify a comfortable sound volume from one of five choices for potential use during the A-CAT. The Computer-assisted Alcohol Infusion System (CAIS) software, developed at IU through IARC support, will be used to control the alcohol infusion rate (Figure 2). CAIS ensures an identical incremental BrAC exposure for each reward delivered within and across participants; variation in BrAC profile is determined solely by a participant's behavior. Carefully controlled infusion avoids expectancy effects and the 3-fold range of peak breath alcohol concentrations (BrACs) across individuals occurring after oral administration (Figure 3). Our IV alcohol administration techniques also achieve key features impossible to safely achieve with oral challenges. BrAC immediately descends upon termination of the infusion; after ingestion, the BrAC continues to increase until absorption is complete. CAIS continuously estimates the future time course of BrAC and prohibits infusion that would yield a BrAC exceeding the predetermined, ecologically valid safety limit (180 mg/dL for this project).

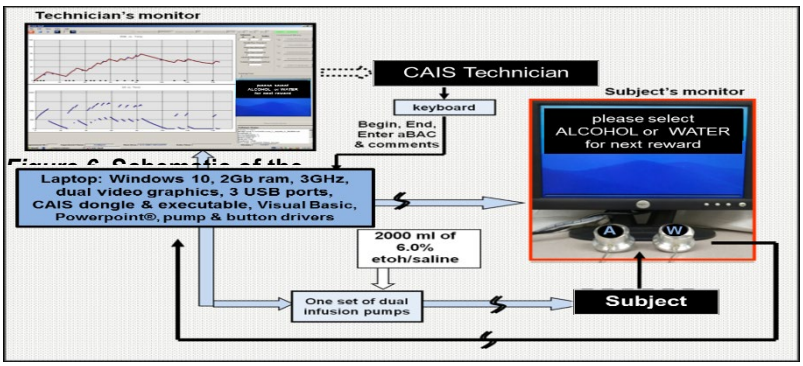


Figure 2.

Schematic of CAIS system, including computer specifications, software, and accessories.

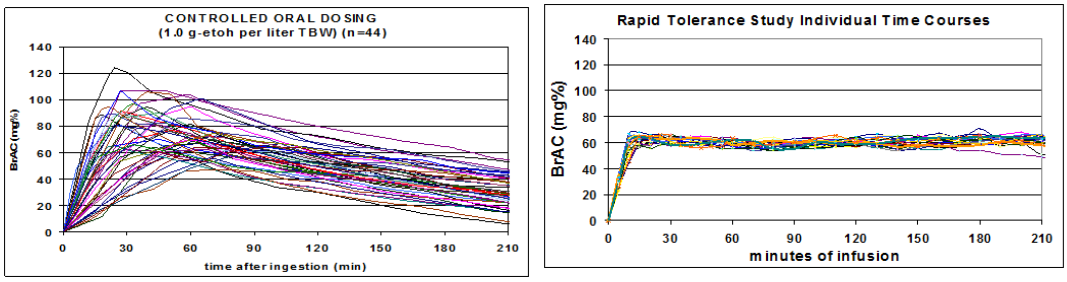


Figure 3. *Left*: variability in breath alcohol concentration exposure trajectories from a controlled oral dose of alcohol in 44 participants. Each dosage was determined for the individual based upon their total body water, and administered under identical experimental conditions. *Right:* variability in breath alcohol concentration during a breath alcohol concentration clamp experiment when participants' breath alcohol concentrations were raised to 60mg% over 15 minutes and then maintained over 3 hours.

Prior to infusion, the participant's age, height, weight, and gender will be entered into the software, which transforms those measurements into parameters of the CAIS physiologically-based pharmacokinetic model of alcohol distribution and elimination. Computation of the infusion rate profile is based on these individualized model parameters to achieve identical incremental exposures to alcohol across participants. Each session begins with an approximately 30 min priming interval during which participants are clamped at 60 mg/dL. In the past we utilized either the investigator-prescribed exposure (BrAC clamping) or the participant-controlled Alcohol Self-Administration paradigm in our research. Here, we combine the 2 paradigms, employing our BrAC clamp as a priming exposure for an alcohol self-administration session. During the clamped priming interval, every participant will be maintained at a 60 mg/dL BrAC plateau for approximately 20 min; long enough to assess current sensitivity to alcohol in subjective and physiological domains. We will collect subjective responses to alcohol, a training run of a neutral SDA work task, and the Stop Signal Task at baseline and upon establishment of the BrAC plateau. Then, similar to our previous work, voluntary self-administration of alcohol or water begins, using the SDA innovation.

During the 2.5-hour alcohol self-administration, the participant chooses 1 of 2 rewards, either alcohol or water, at the voluntary initiation of the next work-set. The work schedules required to obtain the reward are identical, but progress is tracked separately and not communicated to the participants (Figure 4).

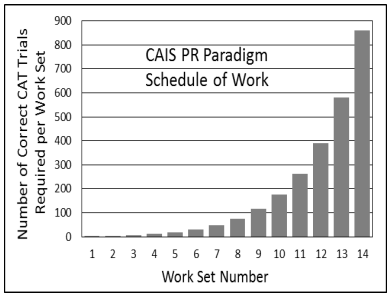
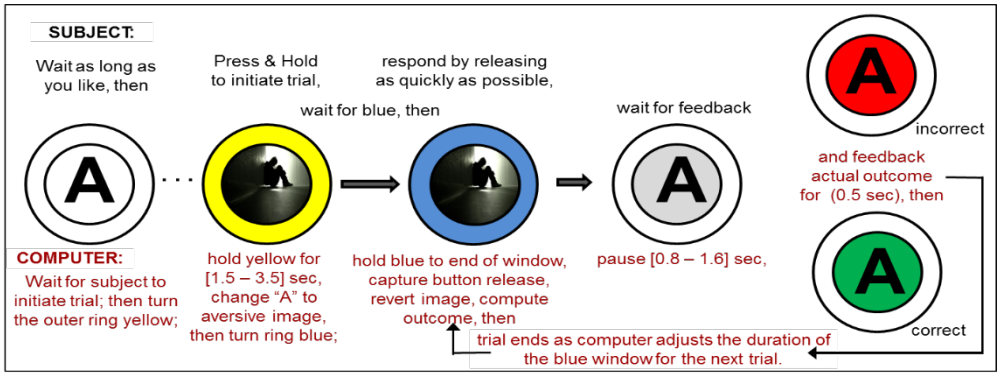


Figure 4. **Schedule of Work**. The amount of work required per reward increases geometrically. At most, 14 work-sets of a single type can be completed in the 2.5-hour voluntary self-administration interval. Thus, cumulative work will range up to 850 correct and 850 incorrect SDA trials performed. BrAC sampling or collection of subjective perceptions is performed during the 2.5 min required for reward delivery.

During a work set (below), the left-most symbol in the sequence is presented on the subject's monitor until the subject initiates a trial by pressing and holding down a 2-in diameter button (labeled here with the reward type "A" for alcohol, or "W" for the alternative reinforcer, water).



The press-and-hold causes an immediate display change: the outer ring of the symbol immediately turns from white to yellow signifying the onset of an anticipatory interval of unpredictable duration. Simultaneous with the change in outer ring color, an image will occupy the display, hiding the other visual stimuli (substituted for the reward indication for demonstration purposes). The subject may also be presented with a sound during this uncertainty interval. The nature of this image and sound will vary by session and reward – neutral for water and either neutral or aversive for alcohol dependent upon the session and selected from the International Affective Picture Scale, similar to our prior protocols (IRB # 1505788344). During this interval, the subject prepares to release the button as soon as the brief response window opens, indicated by color change of a random area of the image to blue. To count as a success, the subject must release the button within the response window; i.e. before the blue area disappears. Button release will terminate the display of the image. The SDA work task is adaptive: with success (green), the trial is counted towards the work-set requirement and the response window for the next trial is shortened; with failure (red), the response window lengthens; thus the overall success rate is specified independent of the effects of alcohol or fatigue.

Throughout any work-set, participants are free to wait, work *ad-lib*, pause, or cease working. The available rewards are a standardized increment in BrAC (alcohol, labeled "A") or an infusion of 30mL of half-normal saline (water, labeled "W"), each delivered over 2.5 min. Ethanol infusate is prepared by the Indiana University Research Pharmacy by mixing half-normal saline with 95% ethanol to create a 6.0% (v/V) solution. The Pharmacy monitors sterility and randomly checks infusate concentrations via gas chromatography. Delivery begins immediately after the last correct SDA trial required for the work-set is performed. Alcohol rewards raise the

participant's BrAC by 10.0 mg/dL in 2.5 min before declining at a steady rate of -0.8 mg/dl/min until the next alcohol reward is delivered. About every 10-15 min, and only during an alcohol reward delivery, participants complete a brief computer-assisted assay of craving for alcohol and subjective responses. Except for *ad-libitum* bathroom breaks, participants remain in the chamber or room for the duration of the experiment; technician interaction is limited to occasional BrAC samples and dependent-measure data collection. Upon completion of the alcohol self-administration interval, participants are served a 500-calorie lunch from the ICRC kitchen and remain in an inpatient room until no concerns regarding their ability to be safely discharged exist and at least 7:00 pm or until BrAC < 35 mg/dL and with no signs of intoxication, whichever is later. Subjects with a BrAC of 35 mg/dl or below but who show signs of intoxication and/or self-report any concerns regarding their safe passage home at the time of discharge will be provided transport home via car service. Participants that have to stay past 7:00 pm for the first and/or second session will be compensated at the rate of $15/hour in 20 minute increments for the time stayed past 7:00 pm in a payment made at the second session. Any participants that stay past 7:00 pm for the first session will be informed that they have to stay until the same time for the second session (in order to reduce discharge motivated drinking behavior during the second session compared to the first). However, for the second session, participants will only actually be required to stay past 7:00 pm if their BrAC remains at or above 35 mg/dL with no signs of intoxication or a concern regarding their ability to be safely discharged exists. Participants will be offered a 1000 cal. dinner from the Indiana Clinical Research Center kitchen, *gratis*. Participants will be paid in cash upon discharge from each session, with an escalating payment schedule used to encourage retention.

Under rare circumstances, participants may express a need to leave the ICRC before protocol specified BrAC discharge criteria are met. For example, a participant may learn that a family member is seriously ill or requires care and indicates a need to leave to be with or assist the family member before the protocol stated BrAC level has been achieved. In these cases, the risk to the participant in the case of discharge must be balanced against the risks of remaining as well as their decision-making autonomy. The study staff will assess the situation and attempt to convince the subject to adhere to the protocol stated discharge criteria and discuss the situation with the PI or, if the PI is unavailable, a delegate investigator. If discharge is necessary or it is deemed the subject will simply attempt to leave without a formal discharge, study staff will implement the following procedure:

If the participant's BrAC is higher than the protocol-stated discharge criteria but less than 0.08 g% (80 mg/dL; the per se legal limit for driving under the influence) and without overt symptoms of intoxication, approval from the PI or delegate will be obtained. Participants will be strongly encouraged to allow study staff to arrange for the participant's transport via the participant's choice of a PI-approved method of transportation (for example, ride-share, taxi, pick-up by another adult, etc.) in lieu of driving themselves.

If the participant's BrAC is greater than or equal to 0.08 g% (80 mg/dL; the per se legal limit for driving under the influence) or the participant is subjectively determined to be intoxicated, the study team will implement Indiana CTSI Clinical Research Center Guidelines related to Study Participant and Visitor Behavior. Briefly, if the participant refuses a transportation option and indicates an intent to drive, they will be informed that IU Health Security may be called.

The team will attempt to follow-up with the participant following the event.

EEG. A 65-lead EasyCap® will be placed on the participant's head, with the resistance of every electrode maintained below 10K ohm. Scalp leads will be referenced to a lead taped on the bridge of the nose. The participant's EasyCap® will be connected to the input stage of Neuroscan Synamps2® EEG amplifier with output connected to a 24-bit analog-to-digital converter, sampled at 250 Hz. Three artifact leads will be calibrated for detection of eye-blink, eye-roll, frowning, swallowing, head-motion and heart-beat artifacts. Later, feedback-related ERPs will be produced using clean epochs of the continuous electrophysiological data record, defined by SDA feedback events inserted into the record.

*f*MRI *Acquisition* . Participants will be imaged in a recently installed Siemens 3T Magnetom Prisma MRI scanner equipped with a 64-channel head coil array using a multiband (MB) EPI sequence from the Center for Magnetic Resonance Research at the University of Minnesota. Prior to imaging, participants will be screened for fMRI safety, including drug, pregnancy, and BrAC screening. We will record the subject's recent drinking. They will be placed in the head coil with pads placed around their head and body for comfort. Scanning will take approximately 30 to 45 minutes and will include an MPRAGE sequence (provides high-resolution anatomical images for co-registration and normalization to the Montreal Neurological Institute (MNI) stereotactic coordinate system) and functional sequences, in which the participant will rest comfortably during the scan. Any discomfort by the participant will terminate imaging.

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Serious adverse events resulting in any physical harm associated with testing will be reported to the IRB within 24 hours; minor adverse events (e.g. nausea, infusate infiltration, discomfort associated with infusion) will be reported to the Alcohol Studies DSMB meeting at least annually.

6.0 Study Withdrawal/Discontinuation

The participant may withdraw from the study at any time for any reason, and will be paid an amount pro-rated to the duration of their participation on the testing day, including time to recover to a BrAC < 35 mg/dL. The PI may choose to terminate any subject's participation at any time if it is deemed that s/he cannot participate or cooperate in testing procedures safely, with the same pro-rated compensation to the subject.

7.0 Statistical Considerations

Aim 1: Characterize SDA across lifetime drinking history.

Aim 1 Hypothesis Testing Plan

Hypothesis 1*: Greater SDA will reflect greater lifetime drinking*. We will conduct a linear mixed-model analysis, with lifetime drinking history as the continuous independent variable and with SDA as the dependent variable. Lifetime drinking history will be defined as the kg of alcohol reported consumed via the Concordia Lifetime Drinking Questionnaire. SDA will be defined as the amount of work performed (cumulative number of SDA trials completed) for alcohol rewards in the aversive session. Age, gender, recent drinking history, and race will be included as covariates, due to their relationship with AUDs.

Hypothesis 2: *Those with a high lifetime drinking history will demonstrate less sensitivity to aversion in the preference to work for alcohol. Specifically, those with a high lifetime drinking history will work more for alcohol compared to water under both neutral and aversive conditions, whereas those with a low lifetime drinking history will only work more for alcohol compared to water in the neutral session*. We will conduct a mixed linear model in which lifetime drinking history is the independent variable, as in hypothesis 1, but using a different dependent measure. We will subtract work for water from work for alcohol, dividing the difference by the sum of the work done for alcohol plus water (separately under the aversive stimulus (R_a) and under the neutral stimulus (R_n)). The ratios R_a and R_n will be used as the repeated measures dependent variables, and lifetime drinking history (high vs. low), stimulus type (aversive vs. neutral) and their interaction will be independent variables. Covariates will include age, gender, recent drinking history, and race. If lifetime drinking history and the interaction with stimulus are significant predictors of the ratios of work performed, we will conduct follow-up planned contrasts to examine the ratios separately for the neutral and aversive sessions to test high and low lifetime drinking history.

<u>Aim 2 Hypothesis Testing Plan.</u>

Hypothesis 3: *Those with greater SDA will have greater AUD severity, greater LDH, greater negative urgency, greater density of familial history of alcoholism, and reduced subjective, behavioral, and electrophysiologic sensitivity to alcohol.* To minimize error inflation from multiple univariate tests, we will use structural equation modeling (R3.01 data analysis software using the lavaan statistical package; Figure 6). We will examine AUD severity as an observed independent variable of AUD symptom count. Lifetime Drinking History and density of family history of alcoholism will be observed variables, computed with information collected with Concordia Lifetime Drinking Questionnaire and the SSAGA family history module. Negative urgency will be defined as a latent independent variable, with individual item indicators from the UPPS-P negative urgency subscale. Sensitivity to alcohol will be defined as a latent independent variable with three measured variable predictors: Subjective, behavioral and electrophysiological sensitivity to 60mg/dl alcohol as compared to each individual's baseline response. The dependent measures of interest for sensitivity analysis include Subjective Response to Alcohol, Wisconsin Card Sort, and the Stop Signal Task. The dependent variable will be cumulative work performed for alcohol in the aversive session, defined as an observed variable. We will control for cumulative work performed for alcohol in the neutral session, as well as age, gender, and race, due to their relationship with alcohol use behaviors. Judgment of hypothesis viability will be assessed via **1)** fit indices (recommendations in parentheses: Root Mean Square Error of Approximation (RMSEA < 0.10); Tucker Lewis Index (TLI > 0.95); and Comparative Fit Index (CFI > 0.95) and **2)** pathway loading significance.

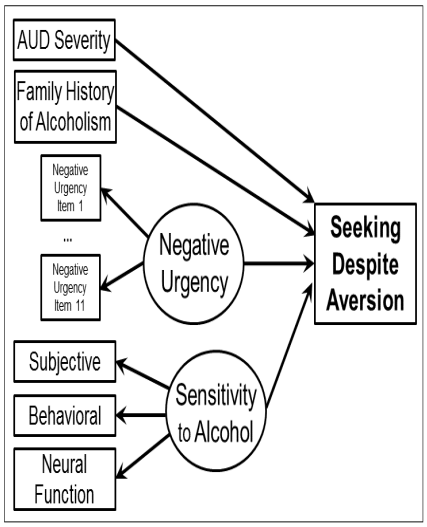


Figure 6. **Proposed path analysis**. Covariates, error terms, and correlations not shown for simplicity

Hypothesis 4: *Significant factors from hypothesis 3 can identify a latent trait that classifies individuals into at two classes of individuals: those who have high and low SDA*. The significant phenotypes from hypothesis 3 will used in a latent class analysis to identify subgroups based on

their pattern of AUD risk. The Bayesian Information Criteria and entropy will be utilized to determine the number of latent classes that best fit the data. Individuals will be assigned to the latent class with the highest probability, and discrete class membership will be characterized using *post hoc* validators such as number of AUD criteria, negative urgency, family history density and lifetime drinking history. As a final validator, we will test if SDA is different between the classes to determine if class membership reflects motivation to work for alcohol in the face of aversive stimuli.

Aim 3 Hypothesis Testing Plan

Hypothesis 5: *Those with greater SDA will demonstrate reduced event-related potential component amplitudes differences between negative vs. positive feedback about per-trial progress toward a reward*. We will employ a linear mixed model to test if SDA predicts positive and negative feedback-related N200 and P300 amplitude differences at baseline. Age, gender, biological family history of alcoholism density, and race will be used as covariates. We will explore a secondary linear mixed model analysis (co-varying for BrAC) using the ERP component differences between baseline and at **1)** maximum BrAC and **2)** terminal work for alcohol.

Hypothesis 6a: *Those with greater SDA will demonstrate greater resting state crosstalk (less differentiation) between the default mode and fronto-parietal networks*. Our preliminary results (Figure 5, above) suggest that advancing AUD severity increases communication between networks that are functionally independent (i.e., the default mode network is prominent at rest, while the fronto-parietal network is activated by focused mental activity). As we hypothesize that SDA marks increasing AUD severity, we similarly hypothesize that SDA is related to abnormally increased communication between the default mode and fronto-parietal networks. To test this hypothesis, whole-brain functional connectivity (FC) will be estimated for each individual participant using a data-driven, functionally based parcellation and characterized for segregation/modularity, which reflects the underlying organization into functional modules or communities. Age will be a covariate in all analyses. Furthermore, to characterize the tendency of brain regions to belong to the same module as a fine-grain continuum (as opposed to an integral membership), we use the consensus clustering approach. The pairwise correlations between module consensus and SDA of participants represent a mapping of SDA into the functional connectome domain. To control for false positives, the correlation between segregation/modularity (consensus clustering approach) and SDA will be z-normalized using 10,000 permutation tests, where the SDA vectors will be randomly shuffled and their associations with modularity consensus mapped 10,000 times, producing a distribution of expected SDA associations by chance. Based on the mean and standard deviation of this null distribution, the actual SDA associations can be z-scored and thresholded at p <0.05 (equivalent to a two tailed, i.e., $|z| > 1.96$, for significant associations). To minimize false positives, we will also incorporate the equivalent of an extent threshold for network-based associations to account for the effect size (as measured by number of edges in connected components or by their spatial pairwise clustering) of the associations in the functional connectome domain. We will also consider using the Permutation Network Framework (PNF), which makes no assumptions about the distribution of the test statistics, since they often show non-standard distributions due to the intrinsic complex nature of brain connectivity data. To evaluate the robustness and reproducibility of these results, other gray matter parcellation schemes will also be assessed, using atlases that range from coarser to finer resolutions (from anatomically-based with 84 regions to multimodal with 360 regions, respectively). Using a similar approach, we will evaluate the associations between SDA and the network properties of integration and centrality. Integration reflects communication efficiency, often reflected by distance to closure

transformations of the shortest-path-lengths between nodes. Centrality measurements rank the relevant brain regions based on how central the nodes are and cover communicability aspects such as degree (number of edges per region) or the number of shortest-paths through each region (betweenness centrality).

Hypothesis 6b: *Those with greater SDA will demonstrate reduced ventral striatal-ventromedial frontal connectivity*. We will complement the connectome approach by using a seed-based approach to test voxel-level functional connectivity between the ventral striatum and ventromedial prefrontal cortex and its relation to SDA. For each subject, the functional connectivity between the ventral striatal seed (Figure 7d, red seed region) and all other cerebral gray matter voxels will be computed and transformed to MNI standard space for each subject. Age will be a covariate in all analyses. Finally, the resulting ventral striatal seed-to-gray-matter voxel-level FC estimations will be correlated (Pearson's coefficient) with subjects' SDA, yielding a high resolution (voxel-level) mapping of SDA onto fronto-striatal connectivity patterns. To control false positive findings, a multiple comparison correction based on false discovery rate will be applied (p_{FDR}<0.05) with an extent threshold (k=50) to avoid spurious isolated voxels and small clusters.

Power Analyses

Alcohol self-administration*:* Our previous alcohol self-administration work showed effect sizes from 0.49 to 1.31 according to the phenotype of interest. Neurophysiological correlates (EEG/fMRI): Published outcome and feedback-related differences in N200 and P300 amplitudes yield effect sizes from 0.78 to 2.12 for subjects with an AUD versus controls, and 0.62 to 1.6 for the effect of alcohol. There are no standards for power analyses in whole brain connectomics. However, our pilot data showed significant results in n= 51, suggesting n=100 is sufficient to find robust connectivity patterns. We will ensure robustness using permutation tests combined with an extent threshold, or alternatively, using the Permutation Network Framework. Relationship to AUD risk variables. In our pilot data, the relationship between SDA and AUD risk correlates ranged from r=0.32 – 0.63. With a sample of n=100, we can detect an effect size of at least 0.32 and still maintain adequate *power*=0.90, α=0.01. When categorized by groups of n=50 (high/low lifetime drinking history), we can detect differences with an effect size of 0.64 and still maintain adequate *power*=0.90, α=0.01.

Supplemental and Exploratory analyses. Our careful experimental design allows for exploratory analyses in many domains including, but not limited to, comparing and contrasting the relationships between SDA, lifetime drinking history and other descriptive variables of interest including AUDIT, age of drinking onset, the DSM 5 symptom most reflective of aversion-resistance, nicotine history, stop signal task behavioral and electrophysiological response, and the PTSD screening results. We will explore the relationship between SDA and the *intensity* and *timing* of alcohol exposure – e.g. does elevated drinking during late adolescence/early adulthood have stronger relationship with SDA than either consistent low-level drinking over a longer time period or intense drinking later in life. We may also pursue structural brain alterations that may be associated with SDA or alternative connectivity analyses and interactions with smoking. We will explore differences in baseline feedback-related ERPs and stop signal task behavioral and electrophysiologic indices as a function of session type and order. We will also examine differences across men and women, including menstrual phase, as our previous data does suggest gender moderation in the effects of mood induction and post-abstinence response on alcohol self-administration. Finally, with consent, we are collecting blood samples to allow for the combination of data from this project with previous IARC human samples; enabling future analysis of polygenetic risk in this sample.

8.0 Privacy/Confidentiality Issues

Access to records that contain patient-identifiers will be kept in locked filing cabinets, inside a locked laboratory with access restricted to authorized/participating investigators and secretarial/administrative personnel who have been certified regarding methods of maintaining confidentiality. Computer records will be password protected, and subjects will be identified only by a subject number. Subjects will not be individually identified in reports.

9.0 Follow-up and Record Retention

We expect project completion in 72 months from IRB approval. The identifiable project subject files will be destroyed at the conclusion of data analysis. The records will be kept for 7 years after completion of the study. The databases will be kept on a secure server in password-protected files. The database files will be over-written with null data at the completion of the retention interval.