Official Title: Characterizing the Effects of Family History of Alcoholism on Alcohol Analgesia

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Protocol

1. Project Title:

Characterizing the Effects of Family History of Alcoholism on Alcohol Analgesia

2. Investigator(s):

Michael Robinson, PhD (PI)
Sara Nixon, PhD (Co-I)
Song Lai, PhD (Co-I)
Jeff Boissoneault, PhD (Co-I)

3. Abstract:

Pain is the most common reason that patients seek medical attention. Acute pain is an essential indicator of current or impending tissue damage. However, chronic pain is a maladaptive state with strong affective, biological, and psychological components. Chronic pain is extremely costly and has strong negative effects on sufferers' quality of life. Existing treatments for chronic pain, including opioid analgesics, are relatively ineffective. Perhaps as a result of the lack of efficacious treatments, it has been recently reported that nearly 25% of individuals suffering from chronic oral and/or musculoskeletal pain self-medicate through the oral consumption of alcohol. Indeed, alcohol interacts with a wide range of relevant pharmacologic targets capable of modulating the experience of pain. However, the biological mechanisms underlying this intuitive interaction are not well established. This relationship is important to understand because alcohol analgesia may act as a potent negative reinforcer for alcohol intake, which, in turn, can have adverse health effects by increasing risk of developing an alcohol use disorder. Familial risk for alcoholism, along with sex and certain mood/personality factors, may act as critical modulators of individual sensitivity to alcohol analgesia. However, it is currently unclear whether this sensitivity is the result of neurobiological, and/or learned factors. By characterizing independent contributions of each of these factors, this proposal will improve understanding of the interplay between pain/alcohol sensitivity, sex, and family history of alcoholism as a modulator of sensitivity to alcohol analgesia. These efforts will inform further research and clinical/translational efforts regarding risk associated with self-medication of pain by consuming alcohol. Critically, the impact of these factors on the functional neural correlates of alcohol analgesia will also be determined, improving mechanistic understanding of alcohol analgesia.

4. Background:

Introduction and Clinical Relevance. Pain is a nearly ubiquitous experience. While acute pain is an essential indicator of current or impending tissue damage, chronic pain is a maladaptive state associated with biopsychosocial dysfunction. Nearly 100 million Americans suffer from chronic pain, which greatly burdens our healthcare system;

recent estimates suggest yearly expenditures reach $635B in treatment expenses and lost productivity in the United States alone (Institute of Medicine, 2011). Existing treatments for chronic pain, including opioid analgesics, are relatively ineffective (Noble et al., 2010). Meanwhile, nearly 25% of individuals suffering from chronic orofacial and/or musculoskeletal pain self-medicate through the oral consumption of alcohol (Riley and King, 2009). Alcohol interacts directly or indirectly with a wide range of neurotransmitter systems, including the serotonergic, glutamatergic, and opioidergic systems, providing a wide range of relevant pharmacologic targets capable of modulating the experience of pain (Vengeliene et al., 2008). As noted by the National Institute on Alcohol Abuse and Alcoholism (2013), this behavior is risky for several reasons: 1) interactions between alcohol and pain medications may have severe health consequences; 2) patients self- medicating pain with alcohol may exceed moderate drinking guidelines, increasing the risk of alcohol-related consequences including development of alcohol use disorders (AUDs) or painful alcohol-related small fiber neuropathies; and 3) alcohol withdrawal itself is associated with increased pain sensitivity. Indeed, the health- related consequences of alcohol use and misuse by themselves are estimated to cost over $200B per year (Spanagel, 2009). Given that 25% of treatment seeking alcoholics report past-month pain, and 25% of chronic pain patients report heavy drinking (Zale et al., 2015), it is likely that the adverse effects of self-medicating pain by consuming alcohol are responsible for a portion of the cost associated with both conditions. Mechanisms underlying alcohol analgesia, including critical modulating factors, are poorly understood. In order to better understand these mechanisms, their interaction with neural adaptations involved in the transition from acute to chronic pain, and their implications for patient education, screening, care, and management, systematic study of alcohol analgesia is needed.

Mechanisms of Pain. Acute pain is a centrally mediated sensation driven primarily by nociceptive input conducted by Aδ and C fibers responding to noxious stimulation or tissue injury in the periphery. An individual's experience of pain is modulated by the activity of a well-characterized set of neural structures involved in the contextualization and evaluation of the nociceptive stimulus, including those involved in sensory processing (primary and secondary somatosensory cortices), executive control (prefrontal and anterior cingulate cortices, anterior insula), and limbic processing (amygdala, hippocampus) (Apkarian et al., 2005; Clarke and Lawrence, 2013; Craggs et al., 2007; Staud et al., 2008). In 80-90% of cases, acute pain resolves as peripheral damage is repaired. In some cases, however, patients' pain persists even after no peripheral damage remains. In order to capitalize on the high degree of controllability and repeatability afforded by laboratory pain induction techniques, we propose the use of experimental heat pain stimulation for this project. This is a common technique used to assess pain psychophysics and neural mechanisms of pain which our research group has successfully employed many times (e.g., Craggs et al., 2012; Letzen et al., 2015; Sevel et al., 2015a; Sevel et al., 2015b).

Relief of Pain as Reward. Because pain is, by definition, an aversive sensation (International Association for the Study of Pain, 1979), the cessation of pain is associated with relief. Although conceptually distinct from typical appetitive rewards like

palatable food or drugs of abuse, substantial similarity between neurobehavioral responses to relief and appetitive rewards has been reported (Leknes et al., 2011; Seymour et al., 2005; Tanimoto et al., 2004; Ursu and Carter, 2005). For instance, Leknes and colleagues (2011) used a task designed to elicit reward associated with relief by presenting a warning signal for an intensely painful heat stimulus that was followed by a relief signal 50% of the time. In the same study, participants were tasked with imagining pleasant scenarios (e.g., “Imagine having your favorite meal”) or neutral ones (e.g., “Imagine drinking lukewarm water”). Analyses of both relief and appetitive reward were associated with activation in the ventromedial prefrontal cortex (vmPFC) and rostral anterior cingulate cortex (rACC), although they were distinguished by activations in right anterior insula, bilateral cerebellum, thalamus, and posterior cingulate. A follow-up study by the same group, distinguished primarily from the 2011 study by its contrast of neural responses to moderately painful heat stimuli presented in the context of relative relief (i.e., a less painful stimulus than expected), found that stimuli less painful than expected were associated with *pleasure* instead of unpleasantness (Leknes et al., 2013). Previously noted functional activations associated with relief reward in vmPFC and rACC were replicated, along with medial orbitofrontal cortex (mOFC). The importance of these regions in the experience of relative reward has also been demonstrated in studies of regarding hedonic aspects of monetary loss and gain in both humans and non-human primates (e.g., Padoa-Schioppa and Assad, 2008; Seymour and McClure, 2008; Tremblay and Schultz, 1999). Critically, it has been suggested that appetitive rewards are more reinforcing when simultaneously providing relief (Cabenac, 1979; Leknes et al., 2011). It seems likely this is due to the synergistic combination of positive and negative reinforcing effects and suggests self-medication of pain using alcohol may represent a “double-hit” of reinforcement, thereby increasing risk of developing an alcohol use disorder.

Acute Alcohol Effects. The effects of acute alcohol intake at legally intoxicating levels (i.e., BAC ≥ 0.08 g/dL) on neurobehavioral function are well documented. A comprehensive review of these effects is beyond the scope of this application (see Boissoneault et al., 2016; Fillmore, 2007; Oscar-Berman and Marinkovic, 2007; however, intoxicating doses of alcohol are associated with robust decrements in inhibitory control (Dougherty et al., 2008; Fillmore and Weafer, 2004; Loeber and Duka, 2009), psychomotor performance (Harrison and Fillmore, 2005), attentional function (Marczinski and Fillmore, 2006), and working memory processes (Soderlund et al., 2005; Weissenborn and Duka, 2000). Dose is a critical modulator of these effects, with subintoxicating BACs (i.e., ≤ ~0.065 g/dL) having less consistent effects on cognition and behavior. Evidence suggests, however, that these BACs can selectively disrupt critical neuropsychological processes, including sustained and/or divided attention, inhibitory control, and working memory (Boissoneault et al., 2014; Breitmeier et al., 2007; de Wit et al., 2000; Friedman et al., 2011; Gilbertson et al., 2009; Holloway, 1994; Lloyd and Rogers, 1997; Jongen et al., 2016).

There are numerous other factors besides dose that may modulate the effects of acute alcohol. Germane to this application, an individual’s family history (FH) of alcoholism may also affect their neurobehavioral response to a given dose. A substantial body of

literature indicates that FH positive (FH+) individuals have an altered profile of subjective responses to alcohol, with various conceptual models describing either consistently lower levels of response to alcohol challenge (i.e., the Low Level of Response [LLR] model), or increased stimulation on the ascending limb combined with decreased sedation on the descending limb (Morean and Corbin, 2010; Schuckit, 1994). Individuals' expectations regarding the positive and negative effects of a given alcohol dose are also critical determinants of its neurobehavioral effects. Numerous studies indicate expectation of impairment is an important predictor of behavioral compromise following alcohol administration (Field et al., 2008; Fillmore et al., 1998; Marczinski and Fillmore, 2005).

The neurophysiological and functional correlates of alcohol-induced neurobehavioral compromise have been explored using a number of imaging modalities, including PET (Volkow et al., 2006) and fMRI (Anderson et al., 2011; Marinkovic et al., 2012; Soderlund, et al., 2005). Taken together, these studies indicate acute alcohol- related disruption of functional activation and glucose utilization in brain areas related to affective, executive, and motor function, including anterior cingulate gyrus, prefrontal cortex, medial frontal cortex, and the basal ganglia. Notably, aberrant function of these areas and the evaluative, limbic, and executive networks they form have all been implicated as modulators of the experience of acute and chronic pain, **suggesting a common neural framework underlying both the effects of alcohol and the pain experience** (Hashmi et al., 2013). EEG/ERP studies, on the other hand, provide complementary evidence that even relatively low doses of alcohol disrupt the timing and magnitude of neurophysiological processes believed to reflect attentional and working memory processes (Bartholow et al., 2003; Kenemans et al., 2010; Lewis et al., 2013), which play an important role in the processing of nociceptive stimuli.

In summary, although a substantial body of research addresses factors that modulate the consequences of acute alcohol consumption, work to date has almost exclusively been concerned with cognitive and behavioral impairment (Fillmore, 2007; Holloway, 1994; Oscar-Berman and Marinkovic, 2007). Substantially less guidance is available regarding factors affecting analgesia resulting from alcohol use.

Alcohol Analgesia. In the past 5 years, there has been increasing interest in interactions between alcohol use/misuse and chronic pain (Apkarian et al., 2013; Egli et al., 2012; Zale et al., 2015). Recent data suggest self-medication of pain with alcohol by patients with chronic pain is widespread (Brennan et al., 2005; Riley and King, 2009). Furthermore, new evidence suggests that that a pattern of low-to-moderate alcohol use is associated with less severe pain symptomatology in fibromyalgia patients (Boissoneault et al., 2016; Kim et al., 2013). However, few experimental investigations of alcohol analgesia have been conducted. Mullin and Luckhardt (1934) were responsible for the first report on alcohol analgesia in published literature, finding that doses equivalent to ~3-5 standard drinks reduced sensitivity to punctate pain (von Frey filament). Wolff and colleagues (1941, 1942), using a radiant heat paradigm, reported elevations of up to 45% in pain threshold at doses ranging from ~1-5 standard drinks, as well as a reduction in galvanic skin response associated with onset of the painful

stimulus. A subsequent study examining differential effects of a dose of alcohol intended to produce BACs ~0.09 g/dL in alcoholics and healthy controls found that analgesic effects in a cold pressor paradigm were significantly greater in alcoholics, indicating drinking history may play a role in determining the analgesic effects of acute alcohol administration (Cutter et al., 1976). As noted by Brown and Cutter (1977), however, these studies' methodological limitations, including lack of placebo control, blinding, and accounting for sample characteristics (e.g., expectancies, age, drinking history, etc.), restrict their interpretability.

Several subsequent studies expanded and improved on initial efforts by utilizing Widmark equations (Watson et al., 1981; Widmark, 1932) to better target specific BAC levels and by accounting for aspects of participants' typical drinking pattern and history of alcoholism. Results of a double-blind, placebo controlled study using both 0.10 and 0.05 g/dL doses among healthy college students suggested analgesic responses to pressure and cold pressor stimuli depended at least partially upon participants' typical drinking pattern (Brown and Cutter, 1977). In a follow-up study, endorsement of alcohol use to increase confidence, reduce stress, and increase self-satisfaction was associated with analgesic effects following alcohol administration in nonalcoholic drinkers (Cutter et al., 1979). Importantly, these studies did not determine whether participants were at high familial risk for alcoholism, which is known to influence response to alcohol even in non-problem drinkers (Morean and Corbin, 2010). Stewart and colleagues (1995) found that, among non-alcoholic men, a legally intoxicating dose of alcohol (peak BAC ~0.09 g/dL) produced a reduction in pain ratings from electric shock, although this effect was significantly greater among those at high familial risk for alcoholism. In an attempt to minimize expectancy effects, several studies of alcohol analgesia using intravenous administration of alcohol vs. oral intake have been conducted. Although lacking ecological validity, these studies provide valuable insight into the pharmacological component of alcohol analgesia. James and colleagues (1978) found significant elevation in pressure pain thresholds over the course of infusion at both 1.5 g/kg and 0.75 g/kg. Similarly, BACs of 0.10 g/dL, but not 0.04 g/dL, produced a significant increase in pain tolerance in healthy drinkers (Perrino et al., 2008). Interestingly, this effect was not related to FH of alcoholism. A follow-up analysis by the same group revealed that a personality factor, neuroticism, interacted with FH such that those individuals with high neuroticism and a positive FH achieved a significantly greater analgesic response (i.e., reduction in pain intensity) at 0.04 g/dL than those who were FH negative or had low neuroticism, suggesting personality factors may interact with FH in determining analgesic responses to alcohol (Ralevski et al., 2010).

Despite its potential importance, sex has not been systematically examined in studies of alcohol analgesia. Only one study to date has investigated sex as a moderator of alcohol effects, finding no significant effects (Perrino, et al., 2008). However, as noted by Horn-Hofmann et al. (2015), sex differences have been noted in the literature regarding alcohol-related expectancies and the neurobehavioral consequences of alcohol consumption. Furthermore, studies examining sex differences in the efficacy of opioid analgesics have identified greater reduction of pain sensitivity and relief from pain under opioids in women than men (Fillingim and Gear, 2004; Niesters et al., 2010). As

previously noted, alcohol acts directly and indirectly on most major neurotransmitter systems, including the endogenous opioid system (Vengeliene et al., 2008). Thus, it is plausible that the analgesic effects of alcohol may also be stronger among women.

Summary. Self-administration of alcohol for pain relief is a common, yet risky (National Institute on Alcohol Abuse and Alcoholism, 2013) behavior among pain patients (Brennan, et al., 2005; Riley and King, 2009). Furthermore, alcohol use disorders are common among chronic pain patients and vice versa (Zale et al., 2015). **Although the cognitive and behavioral effects of acute alcohol intake are relatively well-documented, significant gaps in understanding remain regarding alcohol analgesia. Critically, although FH of alcoholism is currently the best-studied modulator of alcohol analgesia (i.e., Perrino et al., 2008; Ralevski et al., 2010; Stewart et al., 1995), the relative contributions of sex, alcohol-related conditioning, and expectancies have not been studied.** Furthermore, despite separate robust literatures regarding the neural correlates of acute alcohol intake and pain sensation, alterations in pain and reward- related networks associated with alcohol analgesia have never been characterized. In addition to providing an avenue for in vivo experimental evidence regarding theorized overlap between structures and networks associated with sensation and endogenous modulation of pain and alcohol intoxication (Apkarian et al., 2013; Egli et al, 2012), this project will drive the field forward by determining the relative contributions of the proposed mechanisms, allowing for a) targeted treatments to modifiable factors, including conditioning and expectancies; and b) further research on unmodifiable factors (i.e., sex) that may underlie self-medication of pain using alcohol. Healthy social drinkers provide an ideal population for initial investigations of alcohol analgesia due to their lack of complicating medical and psychosocial factors. Furthermore, understanding mechanisms underlying the analgesic effects of alcohol in healthy social drinkers who are currently pain-free is important given that these individuals may eventually develop acute and/or chronic pain and engage in self-medication behaviors. The investigative team's combined experience in mechanistic and clinical studies regarding acute, chronic, and experimental pain; the neurobehavioral consequences of both acute and chronic alcohol use; and neuroimage acquisition and analysis ensures successful completion of the proposed work.

5. Specific Aims:

<u>Aim 1</u>. Test competing hypotheses regarding the effects of family history of alcoholism on alcohol analgesia. A substantial body of evidence suggests that individuals with FH of alcoholism (FH+) have differential subjective responses to acute alcohol administration compared to FH-, including greater pain reduction (Stewart et al., 1995; Ralevski et al., 2010). **Hypothesis (H)1.** We hypothesize that alcohol administration will be associated with greater reduction in pain sensitivity in FH+ than FH- individuals. Based on our pilot data, we also predict **H2.** FH+ individuals will report lower subjective ratings of relief from pain after consuming alcohol. Confirmation would suggest FH+ individuals may self-administer greater quantities of alcohol to achieve pain relief, consistent with the Low Level of Response (LLR) model (Morean and Corbin, 2010). Disconfirmation would suggest the negative reinforcing effects of alcohol

may be especially pronounced in FH+ (i.e., sensitization). Previous studies suggest typical alcohol use, motivations to drink, and personality factors may influence alcohol analgesia, suggesting a critical role of conditioning and expectancies (Brown et al., 1977; Cutter et al., 1979; Ralevski et al., 2010). On the basis of our pilot data, we predict **H3**. FH+ individuals will have stronger positive expectancies regarding alcohol analgesia, which will predict analgesic response to alcohol administration. Results also suggest **H4.** a conditioning effect such that individuals who endorse greater and more frequent alcohol consumption will experience a greater analgesic effect of alcohol.

Aim 2. Imaging functional correlates of alcohol analgesia. Empirical data regarding neural correlates alcohol analgesia are needed to inform models proposing common substrates underlying pain and acute/chronic alcohol effects (e.g., Egli et al., 2012). Unlike other analgesics (e.g., mu-opioid agonists), alcohol acts on most major neurotransmitter systems. However, no studies have examined neural mechanisms underlying alcohol analgesia. Regions of interest (ROIs) include brain structures underpinning discriminative, affective, and cognitive components of the pain experience, including anterior/posterior cingulate cortices, thalamus, insula, somatosensory cortices, hypothalamus, amygdala, and medial/dorsolateral prefrontal cortex. These regions are also implicated in addiction (Egli et al., 2012) and are vulnerable to disruption following acute alcohol administration (e.g., Anderson et al., 2011; Marinkovic et al., 2012, 2013). Thus, we predict **H5.** that alcohol administration will be associated with disruption of BOLD activation and functional connectivity in/between regions associated with the affective and cognitive aspects of pain processing (e.g., insula, prefrontal cortices, amygdala, nucleus accumbens) during application of heat pain stimuli. Based on our pilot data, we expect **H6.** FH+ individuals will demonstrate more severe alcohol-related functional disruption in these ROIs, including motivation-related circuits implicated in transition to chronic pain (Baliki et al., 2011).

Aim 3. Test sex as a moderator of alcohol and FH effects. On the basis of our pilot data, as well as studies of sex differences on the efficacy of opioid analgesics (Fillingim and Gear, 2004; Niesters et al., 2010), we expect **H7.** women will experience greater reductions in pain sensitivity as a result of alcohol consumption and report greater feelings of relief compared to men. We ask as **Empirical Question 1.** whether sex will serve as a moderator of the effect of FH of alcoholism on alcohol analgesia and its functional neural correlates. Evidence of sex by FH interactions may help to identify subgroups at particular risk for harm from self- medication of pain with alcohol.

6. Research Plan:

General Approach. For this study of healthy, community-dwelling moderating drinkers, we propose a repeated- measures, double-blind placebo-controlled factorial design with FH of alcoholism (+/-) and sex as between-subjects factors and alcohol administration (Alcohol Dose: placebo, 0.08 g/dL) as a within-subject factor. The study will include equal numbers of men and women. Study procedures will occur in the Center for Pain Research and Behavioral Health of the University of Florida under supervision of the PI (JB). The investigative team, including the PI; Drs. Robinson, Nixon, and Lai; and research assistants, will meet on a bi-weekly basis over the course of the project to

ensure successful completion. Recent progress regarding all aspects of the study will be addressed during these meetings, including ongoing review of recruitment practices and behavioral and imaging data quality. Drs. Robinson, Nixon, Lai, and Boissoneault will also work together to ensure all research assistants are fully trained to conduct the project.

Methods

Participants. Healthy volunteers (N=220; 110 women) will be recruited for the study via flyers, word of mouth, and internet and local radio advertisements with the aim of obtaining 110 completers; 55 women). Individuals aged 21-45 years will be recruited in order to avoid confounding effects of binge-drinking patterns associated with college-aged individuals and the apparent increased susceptibility of older adults to the acute neurobehavioral effects of alcohol (e.g., Boissoneault et al., 2014). We plan to enroll a representative sample of participants from our recruitment area in north central Florida (i.e., 65% white, 23% black, 7% Asian, 5% other), including ~10% identifying as Hispanic or Latino (see targeted recruitment table).

Selection Criteria. Participants will be excluded if they have a history of chronic pain (e.g., osteo- or rheumatoid arthritis, fibromyalgia, complex regional pain syndrome) or report regular (i.e., > weekly) use of analgesic medications; current major depression, or history of major depressive disorder if electroconvulsive therapy had been used; past diagnosis of any psychotic disorder; undercontrolled hypertension or diabetes (as reflected by self-report); neurological disease (e.g., multiple sclerosis, epilepsy, amyotrophic lateral sclerosis, Parkinson's disease); serious medical illness (e.g, hepatitis, HIV/AIDS); impaired cognitive function; or history of drug or alcohol dependence. Because evidence suggests chronic smoking is associated with adverse changes in brain structure and neurobehavioral function (Boissoneault et al., 2011; Durazzo et al., 2013), participants must be non-smokers. Alcohol naïve individuals will also be excluded. To increase ecological validity and improve feasibility of recruitment, use of prescription and over-the-counter (OTC) medications will be allowed provided they do not contraindicate alcohol use (Boissoneault et al., 2014; Gilbertson et al., 2009).

Screening Procedure. Individuals will complete an initial screen upon contacting the Center for Pain Research and Behavioral Health via phone or email. During the initial screen, the PI or a trained research assistant will describe the study and general entry criteria. Individuals who qualify and remain interested will be scheduled for a formal screening in the laboratory. During this screening session, additional information regarding demographics, personal and FH of drug and alcohol use (including nicotine), affective and personality measures, attitudes about pain, alcohol-related expectancies, and medical history will be collected (see Table 1). Individuals reporting at least one parent with a history of alcohol problems on the FTQ will be considered to have a family history of alcoholism (FH+; Mann et al., 1985). Notably, in addition to measures of typical consumption, the Alcohol Use Questionnaire (AUQ) includes an assessment of

the maximum quantity of alcohol they have consumed in a single setting in the last 6 months. Although not exclusionary, this maximum quantity will be considered in exploratory analyses given evidence it may modulate the acute effects of alcohol on cognitive performance (Lewis and Nixon, 2013). Expectancy of pain relief from alcohol intake will be measured using item 11 of the Alcohol Expectancy Questionnaire (Brown, et al., 1987) based on participants' responses to the item, "Alcohol can act as an anesthetic for me; that is, it can deaden the pain", expectancy will range from -10 (strong disbelief that alcohol relieves pain) to 10 (strong belief). We will also measure expectancy of pain relief from alcohol using the Expectancies for Alcohol Analgesia Inventory (Ditre et al., 2018) and a simple two item visual analog scale measure.

Screening Measure	Domain	Exclusionary Cutoff
BDI-II	Depressive Symptomology	≥ 20 (moderate depression)
STAI	State/Trait Anxiety	Not Exclusionary
AEQ	Alcohol Expectancies	Not Exclusionary
AUQ	Alcohol Use Pattern	Not Exclusionary
AUDIT	Alcohol Use Disorder Sx	≥ 8
PILL	Somatic preoccupation /neuroticism	Not Exclusionary
FTQ	FH Assessment	Not Exclusionary
PSQI	Sleep Quality	Not Exclusionary
BRS	Resilience to stressors	Not Exclusionary
DIS	Ability to tolerate physical discomfort	Not Exclusionary
EAA	Expectancies regarding pain relief from alcohol	Not Exclusionary
PCS	Pain catastrophizing	Not Exclusionary
PASS-20	Pain-related Anxiety	Not Exclusionary
IRI	Empathy	Not Exclusionary

Table 1. Screening Measures. BDI-II: Beck Depression Inventory (Beck et al., 1996); STAI: State-Trait Anxiety Inventory (Spielberger, 1983); AEQ: Alcohol Expectancies Questionnaire (Brown et al., 1987); AUQ: Alcohol Use Questionnaire (Cahalan et al., 1969); AUDIT: Alcohol Use Disorders Identification Test (Saunders et al., 1993); PILL: Pennebaker Inventory of Limbic Languidness; FTQ: Family Tree Questionnaire (Mann et al., 1985); *PSQI: Pittsburgh Sleep Quality Index (Buysse et al., 1989).* BRS: Brief Resilience Scale (Smith et al., 2008); DIS: Discomfort Intolerance Scale (Schmidt et al., 2006); EAA: Expectancies for Alcohol Analgesia Inventory (Ditre et al., 2018); PCS: Pain Catastrophizing Scale (Sullivan et al., 1995); PASS-20: Pain Anxiety Symptoms Scale (Short Version; McCracken and Dhingra, 2002); IRI: Interpersonal Reactivity Inventory (Davis, 1980).

All participants will be provided with a pamphlet containing information about local mental health services; if desired, the PI or research assistant will facilitate contact with clinical services. Any subjects endorsing suicidal or homicidal intent during screening will be withdrawn from the study. Dr. Robinson, who is a licensed clinical psychologist, will then provide appropriate referral.

Participants who continue to qualify will be given a detailed description of the two laboratory sessions, the tasks and measures to be collected, and reasons for urine samples (drug and pregnancy testing). Baseline quantitative sensory testing (QST) measures of pain sensitivity and tolerance will be taken after participants' eligibility is confirmed and they indicate their willingness to continue. Participants will provide their own transportation to screening sessions. Participants will be paid $15 for completing the screening phase of the study, which will require approximately 1-2 hours.

Laboratory Sessions. Consistent with previous work (Boissoneault, et al., 2014; Lewis, et al., 2013), participants will be asked to fast for at least 4 hours prior to their scheduled session and abstain from consuming any alcohol in the 24 hours before laboratory sessions. Taking normal morning medications will be permitted, but no other OTC or prescribed medications (including allergy medications and analgesics) will be allowed on the day of testing. **Following provision of a urine sample, a drug screening will be performed. This screen will test for tetrahydrocannabinol, cocaine, benzodiazepines, morphine, and methamphetamine (Innovacon, Inc., San Diego, CA). Participants testing positive for any of these drugs will be discontinued. A baseline breath alcohol concentration (BrAC) measure will be taken, which must be 0 g/dL.** Women will complete a brief questionnaire regarding their menstrual cycle, including: 1) if they are currently menstruating or if they are post-menopausal; 2) the length of their menstrual cycle; 3) how many days have elapsed since their last menses began; 4) how long their menses typically lasts; and 5) if they use any hormonal preparations (e.g., birth control). **Women of child bearing potential will also be given a pregnancy test; should a woman's pregnancy test be positive, she will be excluded from the study with a recommendation to contact her physician. Women who are currently breastfeeding will also be excluded.** All testing will be conducted in private rooms within the Center for Pain Research and Behavioral Health. Participants will be provided with a light breakfast one hour before alcohol administration (~200 kcal). Following breakfast, participants will repeat affective measures (BDI-II/STAI) as well as an Irritability Questionnaire (IRQ; Craig et al., 2008). Lunch will be provided following testing, as well as another light meal should a participant be retained in the Center during dinner hours while their BrAC levels drop. For laboratory sessions, the PI or a research assistant will schedule a ride with Uber or Lyft to drive participants home after the completion of the session. Research assistants will use the GPS features of the rideshare app to ensure that participants make it home safely. If the rideshare app indicates that the participant was dropped off somewhere other than the vicinity of their home, the attending researcher will call the number left by the participant and speak with the participant. If the participant does not answer the phone, the researcher will contact the IRB to report the protocol deviation. Participants will be paid $120 for completing each laboratory session (or $15/hour up to $120 for non-completers). Laboratory sessions will be separated by at least 48 hours.

Alcohol Administration. Alcohol administration procedures are consistent with those used by Drs. Boissoneault and Nixon in their previous work, as well as NIAAA guidelines for the safe and ethical administration of alcohol in experimental settings (Brown et al., 2014). Participants will complete two laboratory sessions in which they will be administered one of two beverages: placebo (0.00 g/dL target BrAC) or active alcohol (0.08 g/dL target BrAC). Session order will be counterbalanced across participants. For laboratory sessions in which a participant will be given the active dose, the quantity of medical grade alcohol (100% or 95% ethanol) needed to achieve 0.08 g/dL will be calculated using a modification of the Widmark formula. The medical (United States Pharmacoepia) grade ethanol used in this study will be obtained from standard laboratory supply providers (e.g., Fisher Scientific). The Widmark formula

utilizes age and weight measures for men and height and weight measures for women (Watson, et al., 1981; Widmark, 1932). Only research assistants over the age of 21 will be involved in drink mixing/administration procedures, and all research assistants will be trained by the PI in drink mixing/administration procedures. In order to maintain the study's double blind, a researcher not involved in QST will be responsible for calculating alcohol doses and mixing drinks, and the dose calculation and mixing procedure confirmed by a second research assistant. Alcohol will be mixed with ice-cold sugar-free lemon-lime soda in a 1:3 ratio and split into two servings (Boissoneault, et al., 2014; Gilbertson, et al., 2009; Harrison et al., 2007). Placebo beverages will consist of only vehicle. Participants will consume both servings within 5 minutes, and both active and placebo drinks will be misted with alcohol to enhance placebo effectiveness. A small amount of alcohol will be placed on the rim of the glass and the surface of the drink in order to further mask the study condition. Participants will rinse their mouths thoroughly with water once their beverage has been consumed. To avoid influencing participants' expectations regarding alcohol analgesia, no suggestion regarding the potential pain-relieving effects of alcohol will be provided before or after beverage administration.

BrAC/BAC Assessment. Blood alcohol concentration (BAC) will be estimated using two methods. Following beverage administration, breath alcohol concentration (BrAC) measures will be obtained every 10 minutes using standard a handheld breath analysis device (e.g., Intoxylizer 400PA, CMI, Inc., Owensboro, KY) until the participant is placed in the MRI device (i.e., 2 measurements). After this point, BAC will be approximated using salivary alcohol testing strips (QED Saliva Alcohol Test, OraSure Technologies, Inc. Bethlehem, PA), with collection occurring after each scan is completed (resulting in 3 measurements occurring over the duration of scanning). Following scanning, breath measures will again be taken periodically until the participant's BrAC is ≤ 0.02, consistent with NIAAA guidelines (Brown et al., 2014). They will then be transported home.

Subjective Intoxication and Placebo Effectiveness. Participants will complete 10cm VAS measures of subjective intoxication (anchored from 'not at all intoxicated' to 'most intoxicated imaginable') (Harrison, et al., 2007). These assessments will be administered concurrently with each BrAC assessment, as well as immediately before and after QST assessments and functional image acquisitions. In order to assess the contribution of subjective stimulatory and depressant effects of alcohol to alcohol analgesia, the Subjective Effects of Alcohol Scale (SEAS; Morean et al., 2013) will also be administered immediately prior to QST assessments and the fMRI heat pain paradigm, providing measures of positive and negative aspects of stimulation and sedation (i.e., HIGH+/- and LOW+/-). At the conclusion of testing, participants will be asked whether they believe they received an alcoholic beverage; those who indicate they did not receive a beverage will be asked when they made that determination.

QST Procedure. QST testing will occur in a private room within the Center for Pain Research and Behavioral Health during screening procedures and within the scanner suite during laboratory sessions. First, during screening, all subjects will undergo a set of calibration trials to establish individualized temperatures to be used during laboratory

sessions so that degree of analgesia achieved from beverage consumption can be assessed relative to a personalized baseline. These calibration trials will consist of an ascending series of thermal pulses starting at 43ºC and increasing by 1ºC until the participant's tolerance is reached. After each pulse, participants will rate pain intensity using a visual analog scale (VAS). The lowest temperature with a VAS score of 5 will be used for thermal stimuli during laboratory sessions. Next, during both screening and laboratory sessions, we will employ a slowly ramping thermal stimulus (.5°C per second starting at 32°C) delivered to the glabrous skin of the foot. Pain ratings (using a 10 cm VAS ranging from "no pain" to "most intense pain imaginable") will be collected every 5 seconds during this process until the participant's tolerance is reached in order to determine temperatures associated with pain threshold (i.e., temperature at which the stimulus first becomes painful) and tolerance (i.e., temperature at which the stimulus becomes unbearable). This protocol also allows for the examination of pain after-sensation following removal of the thermal probe, a construct related to the clinical experience of chronic pain (Staud et al., 2007). To this end, participants will report current pain levels at 15 and 30 seconds following stimulus removal. VASs are considered the "gold standard" for laboratory and clinical pain assessment given that they have a ratio scale (Price et al., 1983) and are able to effectively separate the sensory (i.e., intensity) and affective (i.e., unpleasantness) components of the pain experience. The investigative team has extensive experience with these procedures, and is confident they can be completed safely and effectively. All thermal stimuli will be delivered using a computer-controlled Q-Sense fMRI Compatible thermal stimulator (Ramat Yishai, Israel), a Peltier-element based device. We propose to utilize a thermal stimulus paradigm because the processing of such stimuli is particularly well understood. Such paradigms have been demonstrated in the broader pain literature to be sensitive to group differences (e.g., chronic pain patients vs. healthy controls), as well as experimental manipulations like acute opioid analgesic treatment and placebo induction (e.g., Chung et al., 2007; Sevel et al., 2015; Staud et al., 2012). Furthermore, its use in this study will enhance comparability to previous work. Following each QST measure during laboratory sessions, participants will be provided with a 10 cm VAS assessing perceived relief from pain resulting from beverage consumption (anchored from "No relief at all" to "Most profound relief imaginable"). QST procedures will require approximately 10 minutes. During laboratory sessions, QST procedures will be administered prior to alcohol consumption to function as a point of reference for participants and again approximately 15 minutes after beverage consumption to allow for absorption of alcohol (Boissoneault et al., 2014).

Neuroimaging Protocol. Neuroimaging data will be acquired using a research-dedicated, whole-body scanner within the McKnight Brain Institute. Using a standard 32-channel RF head coil, we will collect both structural and functional neuroimaging data. A high-resolution, 3D structural scan will first be conducted using a T1- weighted MP-RAGE protocol. This T1-weighted structural image will predominately be used in pre-processing all functional data. Although not a primary aim, assuming sufficient time in a scanning session, we will also collect magnetic resonance spectroscopy (MRS) data using a brief sequence (~3-4 minutes) so that brain neurotransmitter and ethanol levels can be measured in regions relevant to pain processing and alcohol intoxication.

Following these structural scans, functional imaging data acquisition will occur using two paradigms described below: 1) resting-state scanning, and 2) noxious thermal stimulation protocol. In total, participants will complete two resting-state scans and three task-based fMRI runs, amounting to a one-hour scanning session. Of particular importance is minimizing head motion during scanning when participants are intoxicated, as head motion has been shown to affect data quality (Goto et al., 2015). We will ensure that participants' heads are heavily padded inside the head coil prior to scanning, and will use the Artifact Detection Tool to remove confounds of motion during pre-processing of data. Participants will be reminded of the importance of staying awake throughout the scanning process, and we will check in with participants between each scan to ensure they did not fall asleep. Dr. Lai will work with Dr. Boissoneault to continually monitor data quality throughout the project.

Resting-State Scanning. First, participants will complete two resting-state (i.e., task-free) scans while keeping their eyes open and focused on a fixation cross. Resting-state fMRI is a powerful tool used to elucidate large- scale neural networks underlying human brain architecture (Biswal et al., 2010). Of these intrinsic connectivity networks, the subcortical limbic network is most relevant to the current project. Although previous studies have examined this resting-state network in the context of heroin (Schmidt et al., 2015) and tobacco (Janes et al., 2012) dependence, intrinsic connectivity of this network has yet to be explored in the context of acute alcohol effects. This project will use a resting-state fMRI protocol to examine differences in subcortical limbic system connectivity (as well as other pain- and reward-related resting state networks) between groups at baseline, as well as between conditions to determine whether acute alcohol consumption differentially disrupts this network in individuals with a family history of alcoholism. One resting state scan will use a conventional BOLD-sensitive T2* weighted sequence. The other will use a partial-continuous arterial spin labeling (pCASL) sequence because these scans yield estimates of cerebral blood flow (CBF) which can be used as covariates in BOLD analysis of subsequent task-based scans, accounting for the cerebrovascular effects of alcohol administration (Marinkovic et al., 2013). Each resting state scan will require approximately 9 minutes.

fMRI Heat Pain Protocol. Three task-based fMRI runs will be conducted subsequent to resting-state scanning using a noxious thermal stimulation protocol. This protocol has been previously used in an NIH-funded study in which MER is a co-investigator (R01AT006334). For this task, a long MR-compatible thermal probe attached to a Medoc Q-Sense fMRI compatible thermal stimulator will be used to deliver noxious thermal stimuli. Blocks of thermal stimuli will be delivered to the glabrous skin of the foot at the individually calibrated temperature determined during the screening visit. Following each thermal stimulation block, participants will rate levels of pain intensity and perceived relief from alcohol. Each block will be preceded and/or followed by a 30-second rest blocks. An electronic VAS will be presented following each thermal stimulation block for the purposes of rating pain intensity (anchored from "No pain at all" to "Most intense pain imaginable"). After each run, participants will rate their perceived degree of relief from pain as a result of consuming their beverage using an electronic

VAS anchored from “No relief at all” to “Most profound relief imaginable”. Runs will last for approximately 6 minutes, and will take place consecutively.

Power Analysis. Power analysis and recruitment goals are based on results of our pilot study (see attached grant document for a detailed description of results). Power analyses were performed assuming two-tailed hypothesis tests. Effect sizes are reported as Cohen’s d, r, or f^2 (Cohen, 1988).

Our pilot data suggested statistically large increases in pain threshold temperature (d = 1.16) associated with BrACs ~.052 g/dL. Effects on pain tolerance temperature and intensity ratings were smaller (d = .28); however, our targeted sample of 110 completers will provide ~ 80% power these effects, and > 99% power to detect effects of alcohol on pain threshold.

FH of alcoholism was associated with greater expectancy of pain relief from alcohol intake in our pilot work (d = 1.12), as well as lower ratings of pain relief (d = .54). Effects of sex on these measures were large (d > .88). Sex also appeared to be a potent moderator of alcohol analgesia (d > .90), with women showing stronger analgesic effects. Our sample will provide > 80% power to detect these effects. FH was associated with smaller effects on alcohol-related reductions in pain intensity (d = .42), as well as pain threshold and tolerance (d < .26), resulting in ~54% power to detect interactive effects of FH and dose on these measures.

Across participants, preliminary data suggested small-to-moderate correlations between expectancy of pain relief from alcohol, ratings of pain relief, and typical daily drinking and reductions in pain sensitivity (.26 < r < .44). We anticipate ~80-99% power to detect these effects. However, our data also suggested that sex and FH may moderate these relationships. The interaction of sex and expectancy of pain relief strongly predicted increases in pain threshold and tolerance associated with alcohol intake (f^2 > 1.11). A similar effect was detected for the interaction of FH and expectancy of pain relief (f^2 > .81). A weaker interaction of FH and ratings of relief on QST measures was also detected (f^2 > .37). Likewise, interactions of sex with typical daily drinking and ratings of relief showed some relation to QST changes (f^2 < .21). We anticipate > 94% power to detect each of these interactions. There was little evidence of Sex or FH interactions with typical drinking on alcohol analgesia (f^2 < .04). Effect sizes of FH X Sex X expectancy/relief/typical drinking interactions could not be assessed given our limited sample; however, we anticipate sensitivity to detect an interactive f^2 > .14 with 80% power given our recruitment target.

Data Analysis Strategy. Data will be analyzed using SPSS 24 (IBM Corp., Armonk, NY).

Aim 1. **Test competing hypotheses regarding the effects of family history of alcoholism on alcohol analgesia.** To assess hypotheses related to Aim 1, FH status will be included as an independent variable in repeated measures general linear models analyses (rmGLM; SPSS GLM procedure; repeated: dose), with pain threshold/tolerance and intensity ratings, associated relief ratings, expectancy of pain

relief from alcohol, and SEAS measures (i.e., HIGH+, HIGH-, LOW+, and LOW-; Morean et al., 2013, 2015) as dependent variables. Demographic, personality/affective (i.e., BDI/STAI/IRQ/NEO-derived neuroticism), and biomedical (i.e., menstrual phase for female participants, sleep quality) variables will be included as covariates should any be found to correlate significantly with magnitude of alcohol analgesia or relief ratings. A main effect of FH status such that FH+ individuals have greater magnitude of alcohol analgesia (i.e., greater increases in pain threshold/tolerance and decreases in pain intensity ratings), more positive/fewer negative subjective alcohol effects, and stronger expectancy of pain relief from alcohol consumption will be interpreted as evidence in support of H1 and H3. Lower ratings of alcohol-related relief from pain in FH+ individuals will provide support for H2.

To determine the independent contributions of expectancies and typical drinking behavior to alcohol analgesia, these measures will be included as independent variables in separate multiple regression analyses including interaction vectors with sex and FH, with magnitude of alcohol analgesia (e.g., increased pain threshold or reduction in pain intensity ratings) as the dependent variable. They will then be entered into simultaneous regression analysis to assess independence of their effects. A positive association between typical drinking and analgesic response to alcohol will provide support for H4. Risk of Type I error will be mitigated through the use of Tukey's HSD. Although not a primary aim, the relationship between individuals' rate of rise of BAC following alcohol consumption and magnitude of alcohol analgesia will be examined in exploratory analyses. For FH+ participants, potential effects of the sex of affected parents and concordance with the participants' sex (i.e., sex-matched vs. unmatched pairs) will also be assessed.

Aim 2. **Imaging functional correlates of alcohol analgesia and its modulating factors.**
H5, that alcohol administration will be associated with disruption of BOLD activation in/between regions associated with affective and cognitive aspects of pain processing during application of heat pain stimuli, and H6, that this disruption will be stronger among FH+ individuals, especially within motivation-related structures/circuits, will be evaluated using a two-part process. For all analyses described, motion will be controlled for at the individual subject level (i.e., first level) by including all six movement parameters (x/y/z translation, pitch/roll/yaw) and outlier volumes (determined using the Artifact Reduction Toolbox) as nuisance covariates. We will also co-vary for mean cerebral blood flow measured using pCASL-based resting state scans. Familywise error will be controlled for all analyses using false discovery rate correction (FDR; Benjamini and Hochberg, 1995) at $p < .05$.

Resting-state analyses: Pre-processed resting-state data will be analyzed using hybrid ICA-seed-based fcMRI analyses (Kelly et al., 2010). For this approach, independent components analysis (ICA) and seed- based correlations are used in tandem. First, ICA will be used to empirically derive a priori regions of interest (ROIs) based on resting-state networks identified from the study's participants. Networks of particular interest in this study include those associated with processing of nociceptive stimuli and those that

are disrupted in chronic pain, including default mode network (DMN), salience network (SN), executive control network (ECN), and subcortical limbic network (LN) (Martucci and Mackey, 2016). ICA will be conducted using the voxel-to- voxel processing stream in the CONN toolbox, which involves back-projection of ICs identified across subject and condition to identify spatial components, followed by back-projection of these components to individual subjects (Calhoun et al., 2001). ICs most closely matching networks of interest will be identified based on masks derived from NeuroSynth (Yarkoni et al., 2011). Second, seed-based fcMRI analyses will examine Fishers' r-to-z transformed correlations between the ICA-derived seed ROIs and all other voxels in the brain. Seed-based correlation analyses will be conducted in the Conn Toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). Hybrid ICA-seed-based fcMRI has been shown to yield accurate and reproducible results (Franco et al., 2013; Kelly et al., 2010). We will examine interactions of group and condition on connectivity of these resting-state networks using repeated measures GLM, followed by simple main effects decomposition.

Task-based analyses: Alcohol-related changes in BOLD activity in pain- and reward-related brain structures during painful stimulation will be evaluated using a GLM-based approach in SPM12. Following convolution of the canonical hemodynamic response function with the heat stimulation paradigm at the single subject level, group-level effects of dose, sex, and FH on pain-related activations in ROIs and in whole brain will be assessed. To examine the changes in functional connectivity associated with alcohol analgesia, task-based data will be analyzed using the psychophysiological interaction (PPI) pipeline within the Conn Toolbox. PPI is a method of examining task-modulated increases in functional connectivity through GLM by yielding an interaction term (i.e., PPI regressor) from the product of a task's convolved time course (e.g., blocks of noxious thermal stimulation) and the time course from a seed ROI (O'Reilly et al., 2012). Relevant seed ROIs for this analysis will be generated using ICA as described above. We will examine task-dependent functional connectivity in pain- and reward-related networks, both between groups and within conditions, using rmGLM.

Aim 3. **Test sex as a moderator of alcohol and FH effects.**
To evaluate Aim 3, sex will be included as a factor of interest in rmGLM analyses described under Aims 1 and 2. A sex by dose interaction such that alcohol consumption produces greater reductions in pain sensitivity and higher VAS ratings of relief for women than men will provide support for H7. Presence or absence of a significant sex by family history by dose interactions on metrics of alcohol analgesia will inform Empirical Question 1. Sex will also be included as a factor of interest in analyses of functional neuroimaging data to determine whether sex and FH of alcoholism interact to determine the effects of acute alcohol administration on pain-related brain processes.

7. Possible Discomforts and Risks:

Psychological Discomforts and Risks. Some aspects of the questionnaires utilized in this proposal may make study participants uncomfortable, especially those dealing with affect or medical history. To ameliorate this possibility, participants will be informed they

can withdraw from the study at any time or skip individual questions that may be upsetting to them.

Acute Alcohol Intake. Alcohol intake may result in dizziness, nausea, and vomiting should a participant tolerate the active dose of alcohol poorly. The risk of these as well as more serious consequences may increase if a participant uses medications that contraindicate the use of alcohol (e.g. benzodiazepines/opioid analgesics). Should a participant experience any of these symptoms, they will be allowed to rest until symptoms subside and will be allowed to withdrawal from the study if desired. We have attempted to minimize this risk by recruiting only those who are regular moderate drinkers and excluding those regularly taking prescription medications that contraindicate the use of alcohol. Ss will be rescheduled if they report having taken prescription or over-the-counter medications that contraindicate alcohol use on the day of testing.

Pregnancy: Alcohol intake is an unacceptable risk in pregnancy. Therefore, anyone of childbearing potential will be tested for pregnancy on the morning of the laboratory session. Positive pregnancy tests will result in exclusion from study and lab staff will assist in making a referral as appropriate. Finally, any documents associated with a participant testing positive for pregnancy will be destroyed without reference to a specific cause.

Breath Analyzer Testing: Breath analyzer testing may result in dizziness or lightheadedness for some individuals. However, the PI has administered many of these tests and never observed a significant negative consequence. Staff will be trained to recognize discomfort resulting from breath analyzer testing and will assist participants in ameliorating symptoms should they occur (eg., discontinue testing, place head between knees, etc.).

MRI Scanner. The MRI scanner generates a very powerful magnetic field. To avoid all potential harm to participants as a result of exposure to this field, we will follow all UF CTSI Human Imaging Core screening procedures. By following the Human Imaging Core checklist, we will ensure no participants have metal of any kind implanted in their body, have any pacing devices (i.e., heart pacemaker), metal in their eyes, or certain types of heart valves or brain aneurysm clips. Some participants may also feel anxious about the confined space in the MRI. To attenuate this possibility, participants will be reassured that they can communicate with researchers and MRI staff through a speaker system. They will also be given the option to stop the experiment at any time during scanning. Finally, the MRI system produces very loud noises, which – in very rare cases – can produce hearing loss. To reduce this risk, participants are required to use both earplugs and headphones.

QST Discomfort and Risks. QST, by definition, will induce thermal pain in participants. However, risk of harm as a result of QST procedures is minimal for the following reasons: 1) the pain is transient in nature and generally subsides immediately after the procedure; 2) participants are instructed that they may stop any procedure at any time

with no adverse consequences; 3) the level of pain experienced by participants is below their tolerance level; and 4) although pain sensation may continue after thermode removal (at discretion of the participant), this is not expected to last longer than 90 seconds. Furthermore, because QST instrumentation cannot produce thermal stimuli above 52ºC and is equipped with an automatic shutdown system (preventing prolonged delivery of high-intensity stimuli) the risk of burning the skin is very slight. Of over 800 participants tested with QST procedures in the Center for Pain Research and Behavioral Health over several studies, less than 1.5% have suffered a first-degree burn, less than 0.5% have suffered a second-degree burn, and none have suffered a third-degree burn. Should a participant suffer a burn following thermal QST or continued pain 24 hours after pressure pain QST, an adverse event will be reported to the IRB.

Participant Confidentiality Risks. The investigative team places a high priority on protection of patient confidentiality and will use the following procedures to protect patients. Unique participant identifiers will be generated in order to collect protected health information (i.e., from questionnaires) for research purposes. Paper questionnaires and forms will be stored in a locked storage space, digital information will be stored in encrypted, password protected files on secure servers, and the data that links the participants to their unique identifiers will be stored in a separate location. When the study is completed and all raw data is entered electronically, participant identifiers will be destroyed. Despite these efforts, it is possible that participant confidentiality may be breached. If a breach occurs, it will be reported to NIH and the IRB and appropriate measures will be taken. These measures include but are not limited to informing affected participants of the breach and assisting with protective measures once the breach is detected.

Data and Safety Monitoring Plan

Because the proposed study does not comprise a clinical trial, a formal Data and Safety Monitoring Board has not been planned. The investigative team, including Drs. Boissoneault, Nixon, Robinson, and Lai, will meet quarterly to discuss data and safety monitoring issues. Any issues identified during the course of these meetings will be handled in a manner consistent with the University of Florida's policies.

8. Possible Benefits: There are no potential benefits to participants in this study.

9. Conflict of Interest: There is no conflict of interest involved with this study beyond the professional benefit from academic publication or presentation of the results.

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UF | Institutional Review Board
UNIVERSITY of FLORIDA

INFORMED CONSENT FORM
to Participate in Research, and

AUTHORIZATION
to Collect, Use, and Disclose Protected Health Information (PHI)

INTRODUCTION

Name of person seeking your consent:_____

Place of employment & position: _____

Please read this form which describes the study in some detail. A member of the research team will describe this study to you and answer all of your questions. Your participation is entirely voluntary. If you choose to participate you can change your mind at any time and withdraw from the study. You will not be penalized in any way or lose any benefits to which you would otherwise be entitled if you choose not to participate in this study or to withdraw.

We aim to create an environment of mutually respectful interactions between research staff and participants. We pledge that research staff will not engage in disrespectful behavior or use racist, sexist, or other inappropriate language, and ask the same of you. If you have questions about your rights as a research subject or have concerns regarding the conduct of our research staff during your participation, please call the University of Florida Institutional Review Board (IRB) office at (352) 273-9600.

GENERAL INFORMATION ABOUT THIS STUDY

1. Name of Participant ("Study Subject")

_____

2. What is the Title of this research study?

Characterizing Alcohol Analgesia

3. Who do you call if you have questions about this research study?

Michael Robinson, PhD, Principal Investigator (352-273-6617)

Sara Nixon, PhD, Co-Investigator (352-294-4900)

Song Lai, PhD, Co-Investigator (352-294-5597)

4. Who is paying for this research study?

The sponsor of this study is the National Institute on Alcohol Abuse and Alcoholism (NIAAA).

5. In general, what do you need to know about this Research Study?

Agreeing to become involved in any research is always voluntary. By signing this form, you are not waiving any of your legal rights. If you decide not to participate in this research, you will not be penalized in any way and you will not lose any benefits to which you are entitled. If you have questions about your rights as a research subject, please call the University of Florida Institutional Review Board (IRB) office at (352) 273-9600.

You are being asked to be in this research study because you are a healthy social drinker between the ages of 21 to 45. You should not be in this study if you:

- Have a medical history or condition that would conflict with study participation, including chronic pain
- Are unable to speak and read English
- Are currently participating in another research study that could interfere or influence the outcomes of this study
- Are unable to provide informed consent
- Have impaired mental function
- Have a history or presence of psychiatric, psychological, or neurological disorder(s)
- Have not consumed alcohol in the past
- Have a serious medical illness (e.g., hepatitis, HIV/AIDS)
- Have a history of drug or alcohol dependence
- Smoke cigarettes or use other tobacco/nicotine products
- Are pregnant or breastfeeding

a) In general, what is the purpose of the research, how long will you be involved?

The purpose of this research study is to improve understanding of how alcohol use affects pain and sensory function. This study has 3 total sessions. Your screening

visit will take approximately 1 to 2 hours, and the laboratory sessions will require 4 to 8 hours each.

b) What is involved with your participation, and what are the procedures to be followed in the research?

Participating in this study involves 3 different visits to our laboratory, including this screening session. During screening, you will complete a number of questionnaires and undergo thermal/sensory pain testing. During the following two laboratory sessions, you will consume a beverage that may or may not contain alcohol and undergo thermal/sensory pain testing and MRI scanning. We provide transportation to and from laboratory sessions. See Item #7 below for additional details.

c) What are the likely risks or discomforts to you?

Briefly, potential risks involved with participating in this study include alcohol intoxication, minor redness or swelling from the thermal probe, discomfort from the MRI noise, and potential uneasiness from the enclosed space in the MRI. Other potential risks and discomforts, including COVID-19 transmission, are described in detail in Item #10 below.

d) What are the likely benefits to you or to others from the research?

There is no direct benefit to you for participating in this study. However, society-at-large and the medical community may benefit by enhancing our understanding of how alcohol affects pain sensation and sensory function.

e) What are the appropriate alternative procedures or courses of treatment, if any, that might be helpful to you?

Participating in this study is entirely optional. This is not a treatment study. If you are a faculty/staff member or student at the University of Florida, your decision whether to participate will have no impact on your employment or academic status.

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT CAN YOU EXPECT IF YOU PARTICIPATE IN THIS STUDY?

6. What will be done as part of your normal clinical care (even if you did not participate in this research study)?

Your normal clinical care will not be affected by participation in this study.

7. What will be done only because you are in this research study?

The initial screening visit will include the following procedures:

a) After arriving at the Center for Pain Research and Behavioral Health (located on the ground floor of the UF Dental Tower in room DG-64), the Informed Consent form will be reviewed with you to make certain that you understand everything that is involved in the study procedures.

b) You will complete several questionnaires about your demographics (age, education, etc.), family history of drug and alcohol use (including nicotine), typical drinking behavior, affect and personality, alcohol-related expectancies, and medical history.

c) You will undergo thermal sensory/pain testing using a testing machine commonly used in clinical settings. This device has a small square piece that is used to apply heat to the skin. The amount of heat is controlled by a computer and will be applied to the bottom of your foot. You will be asked to rate the intensity of your pain experience. Although uncomfortable and/or painful, the amount of heat applied to your skin will not be sufficient to cause a burn. You can discontinue the procedures at any time so that you do not experience pain you find intolerable.

Visits 2 and 3 are laboratory sessions:

a) Laboratory sessions will occur in our laboratory (DG-64) and the McKnight Brain Institute at the University of Florida. You will be asked to fast for at least 4 hours prior to your scheduled session and abstain from alcohol consumption 24 hours before each session. You may take your normal morning medications, but you should not take any OTC medications (including allergy medications and analgesics) the morning before your session. If you are not able to meet these requirements, please let us know so your laboratory session can be rescheduled.

b) We will provide transportation to and from the laboratory sessions.

c) You will be screened via urine analysis for illicit drug use, including marijuana, cocaine, benzodiazepines, morphine, and methamphetamine.

d) You will also be initially screened for your breath alcohol concentration, which must be negative upon your arrival to the laboratory. If results of this test or the urine drug screen are positive, you will be discontinued, or your session will be rescheduled at the PI’s discretion.

e) If you are of childbearing potential, you will be given a pregnancy test. If the test is positive, the results will be destroyed, and you will be discontinued from the study.

f) You may undergo repeated thermal stimulation testing in our laboratory (DG-64). Thermal stimulation testing will be done before beverage consumption, 15 minutes after beverage consumption, and after MRI scans.

g) You will be provided with breakfast and a light lunch.

h) You will complete paper/pencil mood assessments of your mood.

i) You will be given your beverage, which will consist of alcohol (if any) mixed with sugar-free lemon-lime soda. Once finished, you will be asked to rinse your mouth thoroughly with water.

j) You will then be asked to complete assessments regarding your intoxication level and periodically breathe into a breathalyzer.

k) You will complete structural and functional MRI scans of your brain. Functional MRI scans will involve thermal stimulation testing.

l) After testing is completed and your breath alcohol concentration is at or below 0.02 g/dL, you will be transported home via Uber or Lyft. If the GPS feature of the rideshare app indicates that you were dropped off somewhere other than the vicinity of your home, the attending researcher will call to check in with you.

If you experience a physical or mental emergency during your screening or laboratory session, we will request emergency services by dialing 911.

Once this research study is completed, any information that could identify you **might** be removed from any identifiable private information or identifiable biospecimens collected and that, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or your legally authorized representative.

If you have any questions now or at any time during the study, please contact one of the research team members listed in question 3 of this form.

8. How long will you be in this research study?

This study has 3 total sessions. Your screening visit will take approximately 1 to 2 hours, and the laboratory sessions will require 4 to 8 hours each.

9. How many people are expected to take part in this research study?

Up to 220 people may participate in this study. We plan for at least 110 people to complete the study.

WHAT ARE THE RISKS AND BENEFITS OF THIS STUDY AND WHAT ARE YOUR OPTIONS?

10. What are the possible discomforts and risks from taking part in this research study?

This study might involve the following risks and discomforts to you:

- You may feel intoxicated after consuming the alcohol dose used in this study. Alcohol consumption may result in nausea and/or vomiting, as well as drowsiness, impaired memory and concentration, and decreased inhibitions. This may result in impairment to your normal function. However, the alcohol effects will have worn off by the end of the treatment day and assurance will be made that you are not intoxicated before you are transported home from the laboratory.
- There are minimal risks associated with the thermal stimulation protocol. This includes minor redness that goes away within a few minutes to hours. Burns are very rare, and you may stop the testing procedure at any time without penalty. The heat testing procedure may be uncomfortable or unpleasant. However, if the pain is greater than you wish to tolerate, you can discontinue to the procedures at any time.
- This study uses MRI. MRI is a procedure that allows researchers to look inside your body using a scanner that sends out a strong magnetic field and radio waves. The risks of MRI are:
    - The MRI scanner contains a very strong magnet. Therefore, you should not have the MRI if you have certain types of metal implanted in your body. For example, any pacing device (like a heart pacer), any metal in your eyes, or certain kinds of heart valves or brain aneurysm clips.
    - There is not much room inside the MRI scanner. You may be uncomfortable if you do not like to be in enclosed spaces (“claustrophobia”).
    - The MRI scanner produces loud noises, which have produced hearing loss in a very small number of patients. You will be given earplugs to reduce this risk.
    - If you are of childbearing potential, there may be unknown risks to the fetus. Therefore, you will be given a pregnancy test before each MRI scan.
    - MRI performed for research purposes only may not be reviewed with the same scrutiny as would be done for your specific health care needs.
- Certain medications may have harmful interactions with alcohol. For this reason, you will not be allowed to participate in the study if you use prescription medications that cause drowsiness (for example, benzodiazepines or opioids for pain).
- You may feel uncomfortable answering questions about private topics during screening and laboratory sessions. However, you may choose not to answer any questions that make you feel uncomfortable. You will not be allowed to participate in the study if you use prescription medications that cause drowsiness (for example, benzodiazepines or opioids for pain).
- You may experience dizziness or lightheadedness from breathalyzer testing.

- There is a risk of spreading or contracting COVID-19 during participation in this study. We take the following steps to limit spread of COVID-19:
    - Research personnel will wear a disposable Level 1 surgical mask during all interactions with you or other personnel during screening visits. Screening visits do not involve use of a breathalyzer device. During laboratory visits, which do involve breathalyzer use and thus production of aerosols, research staff will wear a KN95 mask. If you do not have a mask, you will be met at the door upon arrival for each session and provided a disposable Level 1 surgical mask that you are required to wear for the duration of all sessions, except when otherwise instructed (for example, while drinking a beverage or providing a breath sample).
        - If you are not willing to wear a mask, we ask that you do not participate in this study.
    - All screening material documents will be presented to you at the beginning of each study session. You will be instructed when to flip to the next page to reduce the number of times forms are passed between people. Writing utensils will be designated for participants and disinfected between study sessions.
    - All study equipment will be sanitized before and after each use. Study equipment includes but is not limited to: tabletops, door knobs, thermal stimulator for QST procedures, laptops, and breathalyzers for measuring breath alcohol concentrations. While in the MRI suites, we will follow guidelines for safety provided by the AMRIS facility.

We will take appropriate steps to protect any information collected about you. However, there is a slight risk that information about you could be revealed inappropriately or accidentally. Depending on the nature of the information, such a release could upset or embarrass you, or possibly affect your insurability or employability.

Questions 17-21 in this form discuss what information about you will be collected, used, protected, and shared.

This study may include risks that are unknown at this time.

Participation in more than one research study or project may further increase the risks to you. If you are already enrolled in another research study, please inform one of the research team members listed in question 3 of this form or the person reviewing this consent with you before enrolling in this or any other research study or project.

Throughout the study, the researchers will notify you of new information that may become available and might affect your decision to remain in the study.

If you wish to discuss the information above or any discomforts you may experience, please ask questions now or call one of the research team members listed in question 3 in this form.

11a. What are the potential benefits to you for taking part in this research study?

There is no direct benefit to you for participating in this study.

11b. How could others possibly benefit from this study?

Society-at-large and the medical community may benefit by enhancing our understanding of how alcohol affects pain sensation and sensory function.

11c. How could the researchers benefit from this study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator listed in question 3 of this form may benefit if the results of this study are presented at scientific meetings or in scientific journals.

12. What other choices do you have if you do not want to be in this study?

Participation in this study is entirely optional. This is not a treatment study.

13a. Can you withdraw from this study?

You are free to withdraw your consent and to stop participating in this study at any time. If you do withdraw your consent, you will not be penalized in any way and you will not lose any benefits to which you are entitled. If you decide to stop participation after you have consumed a beverage containing alcohol, we ask that you remain in the laboratory until your breath alcohol concentration is ≤ .02 g/dL for safety reasons. If you decide to withdraw your consent to participate in this study for any reason, please contact one of the research team members listed in question 3 of this form. They will tell you how to stop your participation safely.

If you have any questions regarding your rights as a research subject, please call the Institutional Review Board (IRB) office at (352) 273-9600.

13b. If you withdraw, can information about you still be used and/or collected?

If you choose to withdraw from the study, no new information will be collected. The information already collected could still be used to complete the study.

13c. Can the Principal Investigator withdraw you from this study?

You may be withdrawn from the study without your consent for the following reasons:

- The study is cancelled and/or discontinued, or other administrative reasons.
- You have an adverse reaction to the alcohol dose or pain sensitivity testing.
- You have recently used medications or drugs that are not allowed, or you are pregnant or breastfeeding
- You have not followed pre-test instructions.

WHAT ARE THE FINANCIAL ISSUES IF YOU PARTICIPATE?

14. If you choose to take part in this research study, will it cost you anything?

There will be no cost to you for participating in this research study.

15. Will you be paid for taking part in this study?

Yes. You will receive a $15 gift card for completing the screening session. You will also receive a $120 gift card for each laboratory session. The total amount you will be paid for completing all study procedures is $255. You will receive partial payment if you do not complete all study procedures.

Your payment for participation in this research study is handled through the University of Florida's Human Subject Payment (HSP) Program. Your information, which will include your name, address, and date of birth, is protected. Access to the (HSP) Program site is limited to certain staff with the assigned security role. You will be randomly assigned a specific identification (ID) number to protect your identity. The study team will provide you with an informational form called the Prepaid Card Facts document. If you have any problems regarding your payment call the HSP Office (352) 392-9057.

If you are paid more than $75 for taking part in this study, your name and social security number will be reported to the appropriate University employees for purposes of making and recording the payment as required by law. You are responsible for paying income taxes on any payments provided by the study. Payments to ***nonresident aliens*** must be processed through the University of Florida Payroll and Tax Services department. If the payments total $600 or more in a calendar year, the University must report the amount you received to the Internal Revenue Service (IRS). The IRS is not provided with the study name or its purpose. If you have questions about the collection and use of your Social Security Number, please visit: http://privacy.ufl.edu/SSNPrivacy.html.

16. What if you are injured because of the study?

If you are injured as a direct result of your participation in this study, only the professional services that you receive from any University of Florida Health Science Center healthcare provider will be provided without charge. These healthcare providers include physicians, physician assistants, nurse practitioners, dentists or psychologists. Any other expenses, including Shands hospital expenses, will be billed to you or your insurance provider.

You will be responsible for any deductible, co-insurance, or co-payments. Some insurance companies may not cover costs associated with research studies or research-related injuries. Please contact your insurance company for additional information.

The Principal Investigator will determine whether your injury is related to your participation in this study.

No additional compensation is routinely offered. The Principal Investigator and others involved in this study may be University of Florida employees.

As employees of the University, they are protected under state law, which limits financial recovery for negligence.

Please contact one of the research team members listed in question 3 of this form if you experience an injury or have questions about any discomforts that you experience while participating in this study.

17. How will your health information be collected, used and shared?

If you agree to participate in this study, the Principal Investigator will create, collect, and use private information about you and your health. This information is called protected health information or PHI. In order to do this, the Principal Investigator needs your authorization. The following section describes what PHI will be collected, used and shared, how it will be collected, used, and shared, who will collect, use or share it, who will have access to it, how it will be secured, and what your rights are to revoke this authorization.

Your protected health information may be collected, used, and shared with others to determine if you can participate in the study, and then as part of your participation in the study. This information will be gathered only through your self-report, participation in study procedures, or from your study visits or telephone calls. More specifically, the following information may be collected, used, and shared with others:

a) Your name, research record number, contact information, and dates associated with tests related to your participation.
b) Social security number (for payment purposes)
c) Date/time of negative drug and pregnancy tests (results of positive tests are destroyed)
d) Demographic and health status information
e) Responses to questionnaires
f) Results of laboratory tests
g) Results of MRI scans

This information will be stored in locked filing cabinets or on computer servers with secure passwords or encrypted electronic storage devices.

Some of the information collected could be included in a "limited data set" to be used for other research purposes. If so, the limited data set will only include information that does not directly identify you. For example, the limited data set cannot include your name, address, telephone number, social security number, photographs, or other codes that link you to the information in the limited data set. If limited data sets are created and used, agreements between the parties creating and receiving the limited data set are required in order to protect your identity and confidentiality and privacy.

18. For what study-related purposes will your protected health information be collected, used, and shared with others?

Your PHI may be collected, used, and shared with others to make sure you can participate in the research, through your participation in the research, and to evaluate the results of the research study. More specifically, your PHI may be collected, used, and shared with others to help improve understanding of mechanisms underlying the effects of alcohol on pain and sensory function.

Once this information is collected, it becomes part of the research record for this study.

19. Who will be allowed to collect, use, and share your protected health information?

Only certain people have the legal right to collect, use and share your research records, and they will protect the privacy and security of these records to the extent the law allows. These people include:

- the study Principal Investigator (listed in question 3 of this form) and research staff associated with this project.
- other professionals at the University of Florida or Shands Hospital that provide study-related treatment or procedures.
- the University of Florida Institutional Review Board (IRB; an IRB is a group of people who are responsible for looking after the rights and welfare of people taking part in research).

20. Once collected or used, who may your protected health information be shared with?

Your PHI may be shared with:

- Research staff at the University of Florida associated with this project.
- The study sponsor (listed in Question 4 of this form).
- United States governmental agencies who are responsible for overseeing research, such as the Food and Drug Administration, the Department of Health and Human Services, and the Office of Human Research Protections.

To help us protect your privacy, we have been granted a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, the researchers cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify you, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally

funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You have been informed that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information. That is, if you give written consent for the release of information, we cannot withhold that information and we cannot hold responsibility for how that person may use your information.

The Certificate of Confidentiality does not prevent the researchers from disclosing voluntarily, without your consent, information that would identify you as a participant in the research project under the following circumstances. If we learn about child abuse, elder abuse, or intent to harm yourself or others, we will report that information to appropriate authorities.

It is possible that once this information is shared with authorized persons, it could be shared by the persons or agencies who receive it and it would no longer be protected by the federal medical privacy law.

21. If you agree to take part in this research study, how long will your protected health information be used and shared with others?

Your PHI will be used and shared with others until the end of the study.

You are not required to sign this consent and authorization or allow researchers to collect, use and share your PHI. Your refusal to sign will not affect your treatment, payment, enrollment, or eligibility for any benefits outside this research study. However, you cannot participate in this research unless you allow the collection, use and sharing of your protected health information by signing this consent and authorization.

You have the right to review and copy your protected health information. However, we can make this available only after the study is finished.

You can revoke your authorization at any time before, during, or after your participation in this study. If you revoke it, no new information will be collected about you. However, information that was already collected may still be used and shared with others if the researchers have relied on it to complete the research. You can revoke your authorization by giving a written request with your signature on it to the Principal Investigator.

SIGNATURES

As an investigator or the investigator's representative, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; the alternative to being in the study; and how the participant's protected health information will be collected, used, and shared with others:

Signature of Person Obtaining Consent and Authorization _____ Date _____

You have been informed about this study's purpose, procedures, possible benefits, and risks; the alternatives to being in the study; and how your protected health information will be collected, used and shared with others. You have received a copy of this Form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your protected health information as described in sections 17-21 above. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting and Authorizing _____ Date _____