MECHANISMS OF ALCOHOL WITHDRAWAL-INDUCED HYPERALGESIA IN YOUNG ADULTS

A Dissertation

by

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ABSTRACT

Binge drinking is one of the most dangerous types of alcohol consumption; over 38% of young adults and 24% of adults 26 years of age and older report binge drinking. Motivation to binge drink may come from comorbidity with pain conditions. Considerable animal evidence shows a biphasic relationship between alcohol and pain with intoxication-induced hypoalgesia (decreased sensitivity to painful stimuli) followed by withdrawal-induced hyperalgesia (increased sensitivity to painful stimuli). The anti-reward model of addiction suggests rewarding aspects of intoxication (e.g., analgesia) drive initial consumption. As drinking continues, anti-reward withdrawal aspects (e.g., hyperalgesia) activate brain stress axes and motivate craving and consumption. Following withdrawal, the third phase is anticipation of alcohol. Animal models of alcohol withdrawal-induced muscle mechanical hyperalgesia suggest the hyperalgesia results from changes in peripheral nociceptors mediated by increased release of stress hormones. Consistent with these findings, our laboratory recently observed withdrawal-induced muscle mechanical hyperalgesia and increased circulating levels of epinephrine in young adult binge drinkers using a between-subjects design.

The current study used a mixed between-within-subjects design to assess muscle mechanical sensitivity, cutaneous thermal sensitivity, and neurogenic inflammation, as well as a measure of central sensitization of pain — thermal temporal summation of second pain. Blood was collected to investigate moderation by epinephrine and pro-inflammatory IL-6. Individuals were prescreened for alcohol use and categorized as

moderate and binge drinkers using National Institute on Alcohol Abuse and Alcoholism (NIAAA) binge drinking criteria. Participants made two visits: one during abstinence (no alcohol within previous 48 hours) and one during withdrawal (drinking within previous 48 hours). We found binge drinkers reported more alcohol use before the withdrawal state, greater hangover symptoms, and more alcohol use disorder symptoms. Importantly, we found that participants in the withdrawal state reported mechanical hyperalgesia in skeletal muscle, partially supporting previous results in animals and our laboratory. In parallel, we found participants in withdrawal reported reduced cutaneous thermal sensitivity on multiple measures. Pain sensitivity results were predominantly driven by effects in male participants. Participants in withdrawal also reported more dominance and less anxiety and negative affect but exhibited greater psychophysiological responses. Several hypotheses for future research are presented.

DEDICATION

My dissertation is dedicated to my incredible wife, who is exceedingly supportive and understanding.

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INTRODUCTION

"Here's to alcohol: the cause of, and answer to, all of life's problems" - Matt Groening. This quote conveys both the danger and allure of alcohol consumption. It alludes to the use of alcohol as a remedy for life's problems, including a long day at work or a traumatic event, in spite of the harms associated with it.[1,73] One understudied application of this quote is pain, to which the quote may be revised to read: 'Here's to alcohol: the cause of, and answer to, one's pains'.

Problem of Binge Drinking

In particular, one of the most dangerous types of drinking is binge drinking, defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as five or more drinks for males and four or more drinks for females within 2 hours.[71] Excessive alcohol consumption, including binge drinking, has contributed to approximately 1 in 10 deaths of working-age adults in the U.S.[92] and has contributed to the prevalence of other health conditions.[109] A recent national survey reports that 24.2% of individuals aged 12 and over were estimated to binge drink during the past 30 days.[96] Binge drinking may be most prevalent in young adulthood with 38.4% of young adults (age 18-25) reporting binge drinking, which is greater than the 5% of adolescents and 24.2% of adults aged 26 and over.[96] Binge drinking during young adulthood may also have long-term consequences later in life.[31] By investigating binge withdrawal-induced increases in

pain sensitivity to already painful stimuli (hyperalgesia) in young adults, we may be able to prevent or treat hyperalgesia and chronic pain in the early stages before long-term effects, including alcohol dependence and alcohol-induced peripheral neuropathy, have been established.

Binge drinking and pain are often comorbid conditions and may be related. Studies report that between 20-25% of adults report using alcohol to self-medicate their pain with a positive dose-response relationship between greater self-medication with alcohol and greater pain frequency.[81] Similar results were found in a population of military veterans, where 24% of individuals reported using alcohol to manage pain.[39] For individuals being treated for alcohol use disorders, pain significantly predicted increased risk of heavy drinking during and after treatment for alcohol use disorders.[106] Reducing pain has also been shown to decrease the odds of relapse following treatment by 85%.[48] This relationship between pain and alcohol use may not be the case for everyone with chronic pain,[61,98] thus it is important to understand the mechanisms and individual difference variables that predict who will have comorbid pain with alcohol abuse.

Pain and Alcohol Dependence

Alcohol consumption can lead to subjective stimulation during the increase in blood alcohol content and subjective sedation during the decrease in blood alcohol content.[66] Similarly, considerable animal evidence suggests alcohol consumption leads to an initial reduction in pain sensitivity (analgesia) followed by heightened pain sensitivity (hyperalgesia).[25,27,35,37] Most research examining alcohol's effects on pain are

from animal studies that focus on the acute effects of one to three injections of alcohol.[17,33,47,50] These studies suggest that short-term use of alcohol is associated with reduced thermal pain (thermal hypoalgesia). However, other studies use chronic administration of alcohol over multiple days by including alcohol in the animal's diet.[22,27,35,37] These studies show that with chronic administration of alcohol, animals show thermal hypoalgesia during intoxication[35,37] and thermal and mechanical hyperalgesia during withdrawal.[22,27,35,37]

In humans, relatively little research has investigated alcohol's effects on pain. Most studies have examined the acute effects of alcohol on shock-induced pain.[74,95] One study inspected thermal pain sensitivity in alcohol-dependent individuals before, during, and after alcohol use disorder treatment.[49] Research from our laboratory was the first to investigate binge withdrawal-induced mechanical, thermal, and inflammatory hyperalgesia in young adults. One study used a between-subjects design, which did not account for within-subject variability, and observed mechanical muscle hyperalgesia in binge drinkers during withdrawal following a natural binge. To reduce variability and experimentally control alcohol consumption, a second study used a within-subjects design with laboratory administration of alcohol. However, the legally allowable dose of alcohol administered was insufficient to induce withdrawal symptoms comparable to a natural binge and thus no differences were observed in muscle pain.[114]

Animal models have shown that alcohol withdrawal-induced hyperalgesia may be dependent on the type of alcohol use and pain modality tested. Following a 10 day continuous diet of alcohol, cutaneous heat hyperalgesia emerged at three hours, peaked

at 6-12 hours, and disappeared at 36 hours following cessation of drinking.[35] Importantly, withdrawal from a single episode of alcohol use induced muscle mechanical hyperalgesia that strengthened over time and lasted at least 15 days following cessation.[27] Muscle mechanical hyperalgesia was exaggerated after a second episode of alcohol administration and withdrawal. These results suggest that repeated episodes of alcohol use and withdrawal more quickly induce a state of worsening hyperalgesia that lasts longer than from continuous drinking.

The bi-phasic effect of alcohol on pain may lead to self-medication and underlie the relationship between alcohol and pain.[28,38] People who have a pain condition may drink for the acute analgesic effect of alcohol.[39,81] However, when the individual is in withdrawal, the resulting hyperalgesia and increase in pain may increase their motivation to drink more. In one model of addiction linking alcohol use and dependence to pain,[28,60] people originally drink for the acute and rewarding positive mood state (euphoria) and analgesia that result from binge intoxication and are mediated by neural plasticity in specific brain regions. Indeed, greater positive stimulating effects of alcohol have been associated with greater risk for binge drinking.[56] This positive rewarding state is the "a" component of the opponent process (Fig. 1B, bottom) and is mediated by release of dopamine and opioid peptides in the ventral striatum of the basal ganglia.[60] In a human neuroimaging study, greater endorphin release in the nucleus accumbens in naïve and heavy drinkers and greater endorphin release in the orbitofrontal cortex in heavy drinkers during consumption was related to greater feelings of pleasure.[69] The rewarding euphoria and analgesia is followed by the anti-reward withdrawal state of

negative affect (dysphoria) and hyperalgesia, comprising the opposing "b" process. After the negative effects of withdrawal, the individual may become preoccupied and anticipate the next binge intoxication stage for its rewarding positive mood and analgesia. This third stage is mediated via dysregulation of the prefrontal cortex executive control circuits.[60] Over time, continued binge drinking may lead to a decrease in the brain's reward system which means the individual feels less positive, rewarding effects of the "a" process. Simultaneously, these individuals also feel an increase in the anti-reward system, or "b" process, which means the individual feels increasing negative motivational effects, including negative affect. Over the course of multiple binge withdrawal cycles, the original euphoria and analgesia they felt is slowly replaced by increasing amounts of dysphoria and hyperalgesia during withdrawal. Although the individual may continue to drink for the positive rewarding euphoria and analgesia (positive reinforcement), eventually, they will binge drink to escape the dysphoria (negative reinforcement) as they no longer feel the euphoria and analgesia. While this model suggests that alcohol withdrawal-induced negative affect and hyperalgesia contribute to the development of alcohol dependence, it remains unclear whether this model and its mechanisms translate to humans.

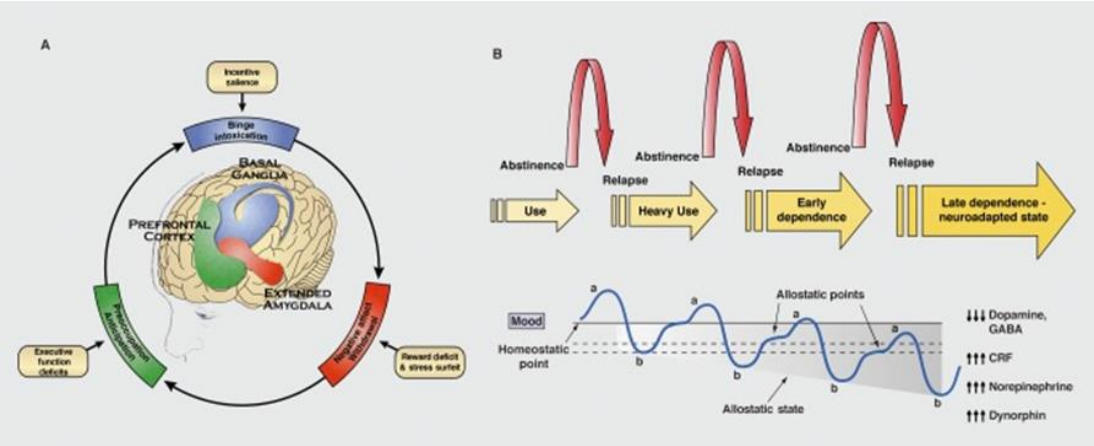

Figure 1. Model of (A) three stages of addiction and (B) the temporal development of alcohol dependence.[38] Modified from Dialogues in Clinical Neuroscience with the permission of the publisher (Institut La Conference Hippocrate, Suresnes, France), George O, Koob. Individual differences in the neuropsychopathology of addiction. Dialogues Clin Neurosci. 2017;19(3):217-229, © AICH-Servier Research Group.

Importantly, the withdrawal state is mediated by decreased activity in reward systems of the ventral striatum and an increase in the anti-reward systems of the hypothalamic-pituitary-adrenal stress (HPA)[2] axis and the brain stress systems including the nucleus accumbens, bed nucleus of the stria terminalis, central nucleus of the amygdala, among others that are collectively known as the extended amygdala.[60] This increase in stress system activity is associated with increased corticotropin-releasing factor and norepinephrine, which seem to underlie withdrawal-induced anxiety-like behaviors and self-administration of alcohol.[58,59] This increase in stress activity during withdrawal may explain the effects of withdrawal-induced mechanical hyperalgesia found in animal models[25,27] and the increased craving and self-administration of alcohol.[34] Withdrawal is also associated with increased physiological activity, including heart rate.[9]

Mechanisms

Though understudied in humans, alcohol withdrawal-induced hyperalgesia is a well-established phenomenon in animal models. This provides researchers the ability to more invasively assess mechanisms.

One such animal model of alcohol-induced mechanical muscle sensitivity is important in understanding the influence of musculoskeletal pain – one of the most common patient complaints, including in peripheral neuropathies such as alcohol-induced peripheral neuropathy.[51] This model of alcohol-induced mechanical muscle hyperalgesia has rats consume a liquid diet containing 6.5% ethanol for 4 consecutive days to mimic 0.08% blood alcohol content or levels found in binge drinking, followed by 3 consecutive days of withdrawal.[27] One 7-day cycle of this diet is sufficient to induce a state of prolonged mechanical hyperalgesia (i.e., lowered muscle pressure pain threshold) in the hindpaw that is exaggerated by a second cycle.[27] Adrenal medullectomy, thus removing the body's supply of epinephrine, blocked the induction and maintenance of alcohol-induced mechanical muscle hyperalgesia.[25] This hyperalgesia was reinstated following injection of epinephrine used to mimic stress. Likewise, local intradermal injection of the glucocorticoid receptor antagonist, RU38486, blocked the mechanical muscle hyperalgesia during both induction and maintenance.[25] These results suggest that alcohol withdrawal-induced hyperalgesia was mediated by increased levels of circulating epinephrine and corticosterone in the periphery.[25] In addition, alcohol withdrawal-induced hyperalgesia was dependent on the epsilon isoform of protein kinase C (PKCε) in the peripheral nociceptors in male and

female rats[27] and protein kinase A (PKA) in female rats.[23] These results suggest that repeated cycles of alcohol use and withdrawal tonically activate the stress axes along with a cellular mechanism in the peripheral nociceptors are needed to embed alcohol withdrawal-induced mechanical hyperalgesia. Other studies found the role of gamma-aminobutyric acid in alcohol-induced analgesia and adenosine receptors, calcium channels, and PKC in alcohol withdrawal-induced hyperalgesia.[36]

A potential mechanism of alcohol withdrawal-induced hyperalgesia may be peripheral levels of interleukin-6 (IL-6). Animal research has shown that a localized intramuscular injection of IL-6 induces mechanical muscle hyperalgesia, that is blocked by administration of an intrathecal injection of antisense to the glycoprotein 130.[24] Additionally, IL-6 was necessary for the expression of mechanical muscle hyperalgesia in a model of early life stress-induced hyperalgesia.[26] This model of stress-induced hyperalgesia is mediated by circulating epinephrine, similar to alcohol-induced mechanical muscle hyperalgesia.[25,27]

Another potential mechanism is inflammation stemming from neuronal activity (neurogenic inflammation). This erythematous flare response stems from antidromic activity in peripheral neurons that leads to a localized release of calcitonin gene related peptide and substance P.[88] These neural mediators lead to increased capillary permeability[15] and plasma extravasation and edema.[87] Topical alcohol can induce neurogenic inflammation.[99] Stress reduction techniques have been shown to protect against an increase in neurogenic inflammation[85] and be associated with a decrease in neurogenic inflammation, perceived stress, and cortisol.[86] Given the stress-inducing

nature of alcohol withdrawal,[58,59] it is possible that alcohol consumption could lead to a state of heightened neurogenic inflammatory tone that partially explains binge withdrawal-induced hyperalgesia.

Few studies have experimentally investigated withdrawal-induced hyperalgesia in humans. One study used a between-subjects design with one group of middle-aged male participants followed through treatment at a facility for acute alcohol detoxification and a second group of males 2-3 months sober after detoxification.[49] This study found that for males admitted for acute alcohol treatment, initially increased sensitivity to thermal stimuli decreased with abstinence. However, that study used a population of middle-aged males individuals already being treated for an alcohol use disorder and therefore may not translate to a younger population of both genders in which alcohol use disorders may not yet be established. In a separate between-subjects study of young adults with histories of either abstention, moderate drinking, or binge drinking, with or without alcohol consumption in the prior 48 hours, our laboratory found a lower pressure pain threshold was found in binge drinkers that was exaggerated by withdrawal.[114] In testing potential mechanisms, binge drinkers during abstention showed greater baseline epinephrine than moderate drinkers during abstention. This was the first study to investigate binge withdrawal-induced hyperalgesia in humans and is consistent with muscle mechanical hyperalgesia that was mediated by increased epinephrine in an animal model of alcohol withdrawal.[22,25,27] Both of these studies focused on static measures of pain sensitivity, which may not predict the development of chronic pain as well as dynamic measures that reflect the mechanisms of central sensitization.[42,110]

The mechanisms underlying binge withdrawal-induced hyperalgesia likely include mechanisms of sensitization occurring in both the peripheral and central nervous systems. Pain is often a result of activation of peripheral nociceptors. These nociceptors terminate in the dorsal horn of the spinal cord and subsequent second-order neurons project up the spinal cord to the brain. Increased activity from the peripheral nociceptors can reversibly increase the excitability and efficacy of the synapses in the dorsal horn of the spinal cord in a process termed central sensitization.[7,107,108] This phenomenon may also reflect sensitization of brain areas including the thalamus and amygdala, which have been found in animal models of diabetic neuropathy[30] and arthritis,[72] respectively. In the brain, ascending spinal neurons transmit signals to brain areas associated with the sensory and affective dimensions of pain.[80] Neuroimaging and anatomical studies have implicated cortical and subcortical areas in the experience of pain, including areas that have connections to afferent neurons such as the primary and secondary somatosensory cortices, insula, prefrontal cortex, and thalamus.[4] Further research has linked patterns of brain activity in response to the painful stimulus in areas including the thalamus, posterior and anterior insulae, and periaqueductal gray, as well as a different pattern of brain activity involved in neural contributions independent of stimulus intensity.[80,101]

Animal models of alcohol withdrawal-induced hyperalgesia suggest that this form of withdrawal-induced hyperalgesia is a result of increased systemic epinephrine and corticosterone stress hormones lead to sensitization of the peripheral afferents. Alternatively, or in addition to, sensitization of the peripheral nervous system, activity from the peripheral nervous system may sensitize second-order neurons in the spinal

cord or more supraspinal areas in humans. Evidence for the possible role of central nervous system mechanisms in binge withdrawal-induced hyperalgesia may be found in the dysregulation of brain areas involved in both alcohol dependence and pain.[5,28]

As discussed earlier, one study from our laboratory used a between-subjects model to investigate binge withdrawal-induced hyperalgesia after naturally occurring alcohol use (i.e., we gave no instructions with regard to the amount that they should drink nor when to drink). We found binge drinkers in abstinence reported muscle mechanical hyperalgesia compared to moderate drinkers in abstinence and these effects were exaggerated in binge drinkers in withdrawal.[114] We also found increased epinephrine in binge drinkers during abstention when compared to moderate drinkers during abstention. These results are consistent with the pattern of results observed in an animal model of binge withdrawal-induced muscle mechanical hyperalgesia.[22,25,27] In a follow-up study, we administered alcohol to young adults until a breath alcohol content of 0.08% was achieved.[114] However, we failed to find the main effect of group on mechanical hyperalgesia and did not show an effect of group on capsaicin-induced measures of central sensitization likely due to ethical limitations that did not allow us to administer alcohol doses comparable to participants' normal levels of alcohol consumption.[114] This explanation was supported by the lower acute hangover symptoms experienced in the second study compared to the first study. These results suggest that allowing participants to choose when they binge drink based on their normal routine induces hangover symptoms needed to find mechanical hyperalgesia. Whether increased

mechanical muscle pain sensitivity is associated with enhanced central sensitization is not known.

Thesis

Building on the foregoing literature, the current dissertation was one of the only studies to investigate mechanisms of binge withdrawal-induced hyperalgesia in humans. Importantly, we used a mixed between- (Group: history of moderate drinking, history of binge drinking) and within- (State: abstain, withdrawal) subjects design to reduce individual variability in order to study two objectives. The first objective was whether young adults with a history of binge drinking showed greater pain sensitivity during withdrawal on measures of mechanical muscle pain sensitivity, neurogenic inflammation, and on a measure of central sensitization – cutaneous thermal temporal summation of second pain (between-subject comparison). Additionally, we compared binge and moderate drinkers during withdrawal from a naturally occurring drinking episode to their baseline during a period of abstinence (within-subject comparison). As part of this first objective, the present study was also the first to examine whether binge drinking altered a thermal measure of pain sensitivity that is thought to reflect the underlying process of central sensitization. Based on prior studies observing effects on thermal pain, we used measures of cutaneous thermal sensitivity, including a measure of central sensitization – cutaneous thermal temporal summation of second pain. The second objective was to determine whether this binge withdrawal-induced hyperalgesia was moderated by circulating levels of epinephrine and IL-6. Included in the second objective, this was also the first study of binge withdrawal-induced hyperalgesia in

humans to investigate the role of the pro-inflammatory cytokine, IL-6, which has been implicated in models of muscle hyperalgesia.[24] Previous studies have not measured differences in influential variables related to binge drinking. Since women may report greater experimental pain,[8] may be more sensitive to the neurological effects of alcohol,[45,102,105] and show greater alcohol withdrawal-induced hyperalgesia in an animal model,[23] we investigated gender differences in alcohol withdrawal-induced pain. This study included secondary analyses of whether pain sensitivity differs by gender. We also investigated differences in adversity, which may be related to binge drinking (e.g., those with a stressful past may be more likely to binge drink)[29,76] and has been shown to induce a state of mechanical hyperalgesia similar to the alcohol withdrawal-induced hyperalgesia[53,54] as well as including body mass index, which affects the metabolization of alcohol. Binge drinkers may also cope differently with stress than moderate drinkers, which may lead to more substance use in the early stages of alcohol use disorders. Therefore, our study included a measure of coping.[18]

This study was designed to test the following hypotheses: that individuals with a history of binge drinking will have greater pressure pain sensitivity, neurogenic inflammation, and enhanced central sensitization than individuals with a history of moderate drinking (objective 1). In addition, heightened sensitivity and inflammation will be moderated by greater baseline epinephrine and IL-6 (objective 2). These effects were hypothesized to be exaggerated by withdrawal.

METHODS

The Texas A&M University IRB approved the study and informed consent was obtained from all participants.

Recruitment

Participants were recruited from spring 2017 to spring 2018. Individuals were eligible if they were healthy, between 18 to 30 years old, and English speaking. Individuals were excluded if they endorsed having a chronic pain condition, current use of any psychoactive or prescription drugs (excluding contraceptives), a history of vasovagal syncope (i.e., fainting), a phobia that would prevent blood draws (e.g., needle or blood phobias), skin condition or injury on the lower legs and feet, or chili pepper allergy. At the beginning of each visit, participants were excluded if they had systolic blood pressure below 90 or above 160, acute illness, had any dental work within 24 hours, had 6 or fewer hours of sleep the previous night, had food within 1 hour, or brushed their teeth within 30 minutes.

Potential participants gave their informed consent for screening and completed an online screening questionnaire through Qualtrics.com assessing health status and drinking history. Two questionnaires evaluated drinking patterns to classify participants as binge or moderate drinkers. A subset of the health status questionnaire consisted of questions asking for the amount participants typically drink and the length of time they typically drink. The second questionnaire, the Daily Drinking Questionnaire (DDQ)[20]

asked participants to report the number of drinks consumed during each day of the week for their typical and heavy weeks as well as how long they typically drink during those days. Group classification from these questionnaires using the responses with the most alcohol consumed was based on National Institute of Alcohol Abuse and Alcoholism criteria with individuals reporting 4 (women) or 5 (men) standard drinks every two or fewer hour period classified as binge drinkers[71] and individuals reporting consumption less than 4 (women) or 5 (men) standard drinks per episode classified as moderate drinkers. In our previous study, there was no difference in mechanical muscle hyperalgesia or epinephrine between moderate drinkers and individuals with no history of alcohol use. Therefore, the current study did not include a control group with no history of drinking.

The mixed between-within-subjects design was devised using a recruitment procedure and naturally occurring alcohol use similar to our previous study that showed exaggerated mechanical muscle hyperalgesia in binge drinkers during withdrawal.[114] Due to ethical concerns, participants were not given directions about drinking prior to signing up for their first session, however they knew one session would be during the withdrawal state and the second visit would be during the abstinence state. The order was determined by the state the participant was in during his/her first session. Similar to our previous study there was a lower recruitment rate of binge drinkers and unexpectedly most participants in both groups were in the abstinence state during their first session. To improve recruitment of binge drinkers and to increase the proportion of participants in withdrawal during the first test session, we modified our recruitment procedures to

ensure participants would be in the withdrawal state during their first session, but this led to a marked reduction in participant recruitment (see Figure 2 for participant flowchart). Based on these recruitment and ethical concerns, my dissertation focuses on participants who were tested during the abstinence state during session 1 and during the withdrawal state during session 2. According to the power analysis below, we were sufficiently powered to test muscle mechanical pain sensitivity.

Prior to each laboratory visit, individuals were instructed to not take allergy or pain medications within 3 days, no dental work within 24 hours, no caffeine within 8 hours, no exercise that morning, no food within 1 hour, and no brushing of teeth within 30 minutes. In addition, individuals needed to have 6 hours of sleep the night before. Alcohol consumption was not mentioned to ensure individuals could naturally consume alcohol before the first visit.

Participants can be recontacted in the future to assess the occurrence of chronic pain conditions.

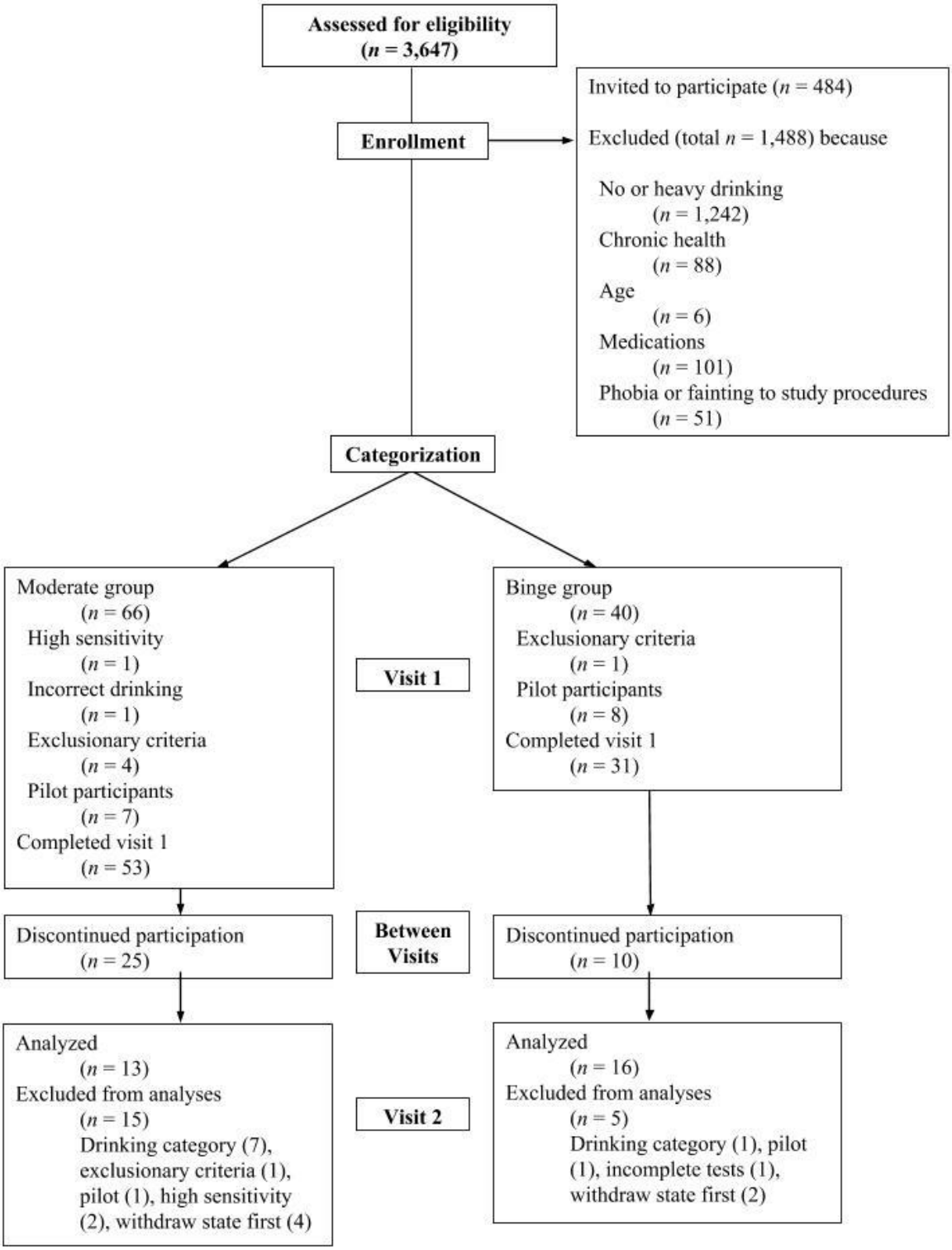

Figure 2. Flowchart for participants through study.

Measures

The following questionnaires were administered to screen individuals for study eligibility.

The pre-existing health conditions questionnaire is an in-house questionnaire with fourteen yes/no items assessing the occurrence of chronic health problems including circulatory problems, neurological disorders, numbness in extremities, fainting, and phobias.

The health status questionnaire is an in-house questionnaire consisting of 21 items assessing current health behaviors including alcohol, nicotine, and caffeine histories and use. Two questions were used to categorize individuals into binge and moderate groups, "How many drinks do you usually have on a single occasion?" with a free-response answer and "Estimate the number of hours you usually spend drinking" with a categorical response.

For individuals who self-identified as being a current drinker, categorization for drinking was also determined using the Daily Drinking Questionnaire-Revised.[20] This questionnaire asked participants to report the standard number of drinks and hours spent drinking during typical and heavy weeks in the previous 30 days.

State characteristics

Demographics regarding age, sex, and ethnicity were assessed. Socioeconomic status was measured using a battery of questions on the level of parental education and employment, family income, household size, current address, and recent parent employment.

The Early Trauma Inventory Self-Report - Short Form is a questionnaire that assesses the self-reported occurrence (*yes*, *no*) of 27 adverse events before the age of 18.[16] These events fall under general abuse (11 items; Cronbach's α = .74), physical abuse (5 items; Cronbach's α = .86), emotional abuse (5 items; Cronbach's α = .92), and sexual abuse (6 items; Cronbach's α = .92). Additional questions were added regarding the age of the first occurrence of a reported adverse event, the number of times it occurred, and the effect the events had on the individual at the time it occurred and currently. This questionnaire was added for secondary analyses to investigate a stress-induced hyperalgesia believed to occur by similar mechanisms as alcohol withdrawal-induced hyperalgesia.[53,54]

The Perceived Stress Scale consists of 10 items used to assess perceived life stress in the previous month (Cronbach's α = .84 - .86).[19] The scale ranges from 0 (*Never*) to 4 (*Very Often*). Four items were reverse coded. Scores were summed so a higher score indicates greater perceived stress.

The Center for Epidemiologic Studies Depression scale is a 20 item scale assessing depressive symptoms during the previous week (Cronbach's α = .85 - .90).[78] The scale ranges from 0 (*Rarely or none of the time [less than 1 day]*) to 3 (*Most or all of the time (5-7 days)*] with scores summed for a total range between 0 and 60. Four items were reverse coded. Higher scores indicate greater symptoms of depression.

The Brief COPE is a 28 item measure used to assess 14 types of situational responses to a stressor.[18] Each subscale consists of two items with each item scored on a 0 (*I haven't been doing this at all*) to 3 (*I've been doing this a lot*) range (Cronbach's α =

0.50 - 0.90). Scores were summed with higher scores indicating greater reported use of the given coping style.

Alcohol characterization

Alcohol questionnaires were used to characterize participants and the role of positive intoxication and negative withdrawal symptoms. Questionnaires assessing the state effects of alcohol were administered at the end of each visit to measure the effect of consumed alcohol compared to no alcohol consumption.

State alcohol-related questionnaires

The Acute Hangover Scale is a 9 item measure used to calculate nine current hangover symptoms (Cronbach's $\alpha = .84$).[83] Average scores for thirsty, tired, headache, dizziness, loss of appetite, stomachache, nausea, and heart racing were computed on a 0 (*None*) to 7 (*Incapacitating*) scale. Average scores ranged from 0.6 (*SD* = 0.4) the morning after placebo (nonalcoholic beer or soda and tonic water with a few drops of alcohol) and 1.4 (*SD* = 0.9) the morning after drinking alcohol to 0.10 g% breath alcohol content.[83] No items were reverse coded. A higher score indicates greater perceived withdrawal symptoms.

The Alcohol Craving Questionnaire-Short Form-Revised is a self-report measure evaluating levels of alcohol craving.[90] Scores were calculated for three subscales: emotionality (Cronbach's $\alpha = .86$), purposefulness (Cronbach's $\alpha = .77$), compulsivity (Cronbach's $\alpha = .79$), and expectancy (Cronbach's $\alpha = .77$). Each item was reported on a 1 (*strongly disagree*) to 7 (*strongly agree*) range. Eight items were reverse coded and scores were summed so higher scores indicate greater craving.

The Craving Typology Questionnaire is a 20 item questionnaire assessing three dimensions of craving[67] that more closely assess the pain and alcohol dependence model described previously.[28,38] Scores were calculated for three subscales: relief craving (5 items, Cronbach's $\alpha = 0.81$), obsessive craving (8 items, Cronbach's $\alpha = 0.88$), and reward craving (7 items, Cronbach's $\alpha = 0.83$). Each item was reported on a 1 (*completely false*) to 5 (*completely true*) range. No items were reverse coded. A higher score indicates greater craving.

The Biphasic Alcohol Effects Scale (BAES) is a 14 item self-report questionnaire to determine the subjective acute stimulant (7 items; Cronbach's $\alpha = 0.94$) and sedative (7 items; Cronbach's $\alpha = 0.87$) effects of alcohol.[66] Each item was reported on a 0 (*not at all*) to 10 (*extremely*) range. No items were reverse coded. Scores for the stimulant and sedative subscales were summed and ranged from 0 to 70 with higher scores indicating greater stimulant and sedative effects of alcohol, respectively.

Tonic alcohol-related questionnaires

The drinking quantity/frequency index is an in-house questionnaire composed of a three item questionnaire gauging typical drinking during the weekdays and weekends during the past month.

The Hangover Symptoms Scale is a 13 item measure assessing the frequency of 13 hangover symptoms in the past 12 months (Cronbach's $\alpha = 0.84$).[91] Each item was scored on a 0 (*Never*) to 4 (*Every time*) range. Responses were dichotomized to reflect the presence (1-4) or absence (0) of the symptom and then summed. Total scores ranged from 0 to 13 with a higher score indicating more hangover symptoms.

The Alcohol Use Disorders Identification Test (AUDIT) is a 10-item measure used to assess drinking behaviors and alcohol-related problems.[40] Each item is scored on a 0 to 4 range with different anchors for individual questions. No items were reverse coded. Scores were summed and range from 0 to 40 with scores of 8 or greater indicating harmful drinking.

The Urgency, Premeditation, Perseverance, and Sensation (UPPS) Seeking Impulsive Behaviour Scale is a 45 item measure used to assess four dimensions of impulsivity.[104] The current study assessed sensation seeking using the 12 items of the sensation seeking subscale (Cronbach's $\alpha = 0.85$). Each item was scored on a 1 (*Strongly Agree*) to 4 (*Disagree Strongly*). No items were reverse coded. Scores were summed with higher levels indicating more sensation seeking (i.e., more impulsive) behavior.

Responses to pain

The Spielberger State-Trait Anxiety Inventory-6 (STAI) is a 6 item short form measure used to assess levels of state anxiety (Cronbach's $\alpha = 0.82$).[65] Each item was scored on a 1 (Not at all) to 4 (Very much). Three items were reverse coded and scores were summed with higher scores indicating greater anxiety.

The Positive and Negative Affect Schedule (PANAS) is a 20 item measure used to assess current positive (Cronbach's $\alpha = .86$) and negative affective states (Cronbach's $\alpha = .87$).[103] No items were reverse coded. Each item is scored on a 0 (*Very Slightly*) to 4 (*Extremely*) range and scores were summed with higher scores indicating greater positive or negative affect.

The Self-Assessment Manikin (SAM) is a 3 item measure assessing current valence, arousal, and dominance dimensions of affect.[14] Valence is scored on a 1 (*Happy*) to 9 (*Unhappy*) range, arousal is scored on a 1 (*Calm*) to 9 (*Excited*), and dominance is scored on a 1 (*Feeling being controlled*) to 9 (*Feeling in control*). Higher scores indicate more negative affect (valence), greater arousal (arousal), and greater control (dominance).

Two visual analog scales (VAS) each consisting of a 10 centimeter line were displayed on a computer monitor and were used to measure the intensity and unpleasantness of capsaicin-induced spontaneous pain.[77] The horizontal lines were labeled with the anchors 0 (*no pain*) to 100 (*most intense pain imaginable*) for intensity and 0 (*no pain*) to 100 (*most unpleasant pain imaginable*) for unpleasantness. Higher scores indicate more intense or unpleasant aspects of pain, respectively.

Quantitative Sensory Testing

All quantitative sensory testing (QST) was conducted in a sound-attenuated and temperature controlled room (22-28°C). The dominant arm was preferred for blood draws but the non-dominant arm would be used if a venipuncture site could not be found on the dominant arm. To prevent carry-over from the blood draw, all QST, with the exception of the first three steps of the temporal summation of second pain procedure, was conducted on the side of the body contralateral to the blood draw.

Pressure pain threshold

The muscle pressure pain threshold was measured using a modified version of the German Research Network on Neuropathic Pain protocol[84] and a handheld algometer

(FPX 50, Wagner Instrument, Connecticut, USA). Three threshold tests with 15 second intervals were administered on the muscle between the toes, approximately one inch from the edge. The first stimulation was between the first (medial) and second toes, the second between the second administered third toes, and the third between the third and fourth toes. The experimenter put the $1cm^2$ diameter rubber tip on the skin at a 90° angle and increased the pressure by 50kPa/s (~$0.5kg/cm^2s$) until the participant reported pain at which point, the algometer was removed and the force recorded.

Participants listened to an audio track explaining the threshold test and were then read the following verbal script: "*Now I will put this tip on your foot and slowly increase the pressure. Please look at the black dot in front of you and say "STOP" as soon as the stimulus becomes painful. Please do not respond when you feel a lot of pressure or when you feel a lot of pain, but just when you start to feel pain. Do you have any questions?"* Prior to each test, participants were reminded to "*Please say stop as soon as you start to feel pain. Do you have any questions?*"

The method used in animal studies of alcohol withdrawal-induced muscle mechanical hyperalgesia mainly used the Randall-Selitto method to apply pressure to the muscle[3,23,25,27,79]. Similarly, we used a handheld algometer to apply pressure to the foot muscles to assess pressure pain threshold. Our procedure in humans likely stimulated a proportionally equivalent spatial area of nociceptors as the Randall-Selitto method in animal models. Due to the similar methodologies and nociceptors stimulated, we use mechanical and pressure interchangeably.

Capsaicin

Capsaicin-induced spontaneous pain was assessed over 45 minutes and flare were assessed using 0.3mL of a 0.10% topical capsaicin solution (Zostrix, Akorn Consumer Health, Ann Arbor, MI, USA).[85] First, a 16mm diameter circle was drawn on the volar aspect of the forearm contralateral to the blood draw. Care was taken to ensure visible veins or arteries were not inside the circle. Participants were then trained on how to rate the intensity and unpleasantness of their pain on a visual analog scale (VAS). After this, participants were told about the procedure after which a thin layer of Vaseline was applied around the circumference of the circle to prevent the spread of capsaicin solution. The capsaicin solution was topically applied and covered with a medical dressing (Tegaderm Film, 3M, St. Paul, Minnesota, USA). Skin temperature was measured at two opposing points around the medical dressing and at one point near the medial aspect of the wrist. Room temperature was also measured. Participants then rated the intensity and unpleasantness of their pain on the VAS and affect on the SAM every 3 minutes for 45 minutes. Ratings were prompted by a mild auditory cue. At the end of 45 minutes, skin and room temperatures were measured, the bandage quickly removed, and capsaicin wiped off. Following flare assessment, residual capsaicin was dissolved and removed using vegetable oil.

Neurogenic flare was assessed using a laser doppler imager (MoorLDI2-IR, Moor Instruments Ltd., London, UK). The imager was placed approximately 50cm from the forearm and the resulting scan (256x256 pixels; spatial resolution of 4ms/pixel) took approximately 5 minutes. Area of flare (cm^2) was quantified from the image using Moor

LDI image review 5.3 software. The size of flare was calculated as the area of hyperemia (>300pfu).[44]

Temporal summation of second pain

Cutaneous temporal summation of second pain (TSSP) was assessed using a four-step process using a 3 x 3cm^2 Peltier thermode (Medoc Advanced Medical Systems, Ramat Yishai, Israel). To reduce the potential for sensitization, during the first three steps participants switched hands for each trial, beginning with the hand ipsilateral to the blood draw. Only the four test trains occurred on the same (contralateral to blood draw) hand. First, to acclimate participants to the thermal stimuli, participants were administered three thermal pulses lasting 2 seconds each at 45°C, 46°C, and 47°C[12,13] at one minute intervals. Second, sensitivity tests using a modified staircase method were used to individualize the temperature needed to induce moderate pain ratings of 50 ± 10 NPR.[12,13,94] Pilot testing suggested using fixed step sizes of 1°C would allow for quicker discrimination between pain intensities. To determine this temperature, a maximum of 6 trains of 4 heat pulses at 0.33Hz were administered to the palmar thenar eminence. All trains of pulses began at a baseline of 38°C and, for the initial sensitivity test, increased to a peak temperature of 47°C. The target temperature for the following trials moved in 1°C increments until the participant rated 50±10 NPR. To ensure the specificity of the temperature, one more step was administered beyond the 50±10 NPR temperature followed by a 1°C step prior to the 50±10 NPR temperature. To control for differences in pain sensitivity during each drinking state, if the temperature was individualized for a participant during at least one state, the temporal summation of second pain procedure

was conducted during the other state even if the individualized temperature was outside the 50±10 NPR range during that other state. This may result in a pain rating and individualized temperature falling outside of 50±10 for some participants. Third, a 3 minute video clip created by the experimenter was shown to the participant explaining the second pain (referred to as "late sensation" so as to not bias individuals to being scared of the sensation) as opposed to the first pain (referred to as "early sensation"). Four questions were asked to the participant to identify any misunderstandings of the second pain. Then, trains of 6 stimuli at the individualized temperature were delivered in one minute intervals on alternating hands. Participants were told to recognize the late sensation and no ratings were made. For the last train, participants clicked a button on a wireless keyboard when they feel the late sensation. This, in combination with measuring the length of the arm measured at the beginning of the first visit allows for an approximate estimate of the involvement of C-fibers.[93] Fourth, during TSSP testing, four test trains of 10 heat pulses each at 0.33Hz were administered to the hand contralateral to the blood draw. Three minute breaks were administered between trains. Participants were asked to rate their pain intensity after each heat pulse and aftersensations at 15 seconds, 30 seconds, and 45 seconds, prompted by audio cues from a laptop.

Physiological Measures

Physiological responses were recorded using a BIOPAC MP150 data acquisition system (BIOPAC Systems, Inc., Goleta, CA, USA) interfacing with AcqKnowledge 4.2 software. Data was sampled at 1000Hz. Respiration was measured using a RSP100C

amplifier with a respiration transducer to measure thoracic and abdominal respiration. Heart rate was recorded using a ECG100C amplifier with two Ag-AgCl electrodes positioned in a modified lead-2 placement. Heart rate is expressed in beats per minute (BPM) as well as interbeat interval (IBI), the latter of which is linearly related to parasympathetic nervous system activity.[52] Skin conductance was recorded using a GSR100C amplifier with two Ag-AgCl electrodes attached to the volar aspect of the medial phalanx of the index and middle finger of the hand contralateral to the blood draw. Skin conductance and respiration data were analyzed offline using AcqKnowledge 4.2 software (BIOPAC Systems, Inc., Goleta, CA) and heart rate data were analyzed offline using EDF Browser v1.63 software (Teunis van Beelen). Data were filtered using band-pass finite impulse response (FIR) filters for respiration (0.05 - 1Hz),[11,111,114] heart rate (0.5 - 35Hz, 8,000 coefficients),[10,111,114] and skin conductance (1Hz FIR low pass filter).[111,114]

Enzyme-Linked Immunosorbent Assay (ELISA)

All plasma samples were thawed once prior to analyses and standards, controls, and samples were analyzed in duplicate according to the manufacturer's manual. Epinephrine kits were purchased from Abnova (Taipei, Taiwan, Catalog number KA1877) and IL-6 kits and controls were purchased from R&D Systems (Minneapolis, MN, Catalog number D6050 [ELISA kits] and QC01-1 [controls]). Plate-to-plate variability was reduced by randomly assigning all samples in duplicate for a participant to a kit with approximately equal numbers of each group assigned to each plate. All plasma samples were thawed on ice and extraction and acylation were conducted at

room temperature. Epinephrine and IL-6 analyses were conducted according to manufacturer's protocols. Absorbance values for epinephrine and optical density values for IL-6 were used to construct standard curves for determination of concentrations of epinephrine and IL-6 in plasma from participants via AssayZap software (Biosoft, Cambridge, UK). Samples from excluded participants were included when determining the standard curve in order to create a pool of sample data. During epinephrine extraction, plate 1 incubated in assay buffer and extraction buffer an additional 20 minutes and plate 2 incubated in acylation buffer and acylation reagent an additional 20 minutes. All plates were read using a Bio Rad Model 680 Microplate Reader (Bio Rad Laboratories, Inc., Hercules, California).

Procedures

Questionnaire data was collected using Qualtrics online survey software. All pain sensitivity tests were conducted by the author and both the author and trained undergraduates collected baseline blood pressure and breathalyzer data. The author was blind to the participant's group. All correspondence regarding group categorization and the visit condition was made by a trained undergraduate who did not do pain sensitivity tests.

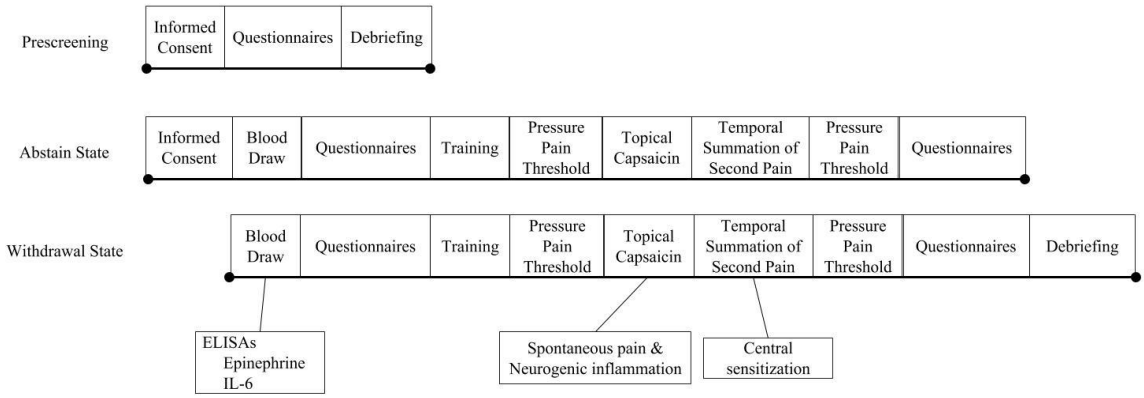

Figure 3. Timeline of study procedures. Individuals interested in participating were prescreened. Eligible participants were invited and took part in the Abstain and Withdrawal state visits.

The protocols for the two visits were largely equivalent with the exception of informed consent on the first visit and debriefing and compensation at the end of the second visit (see Figure 2 for study timeline). All state visits took place between 12:00pm and 7:00pm to reduce the effect of diurnal cortisol variability on pain and stress hormones. To improve recruitment, participants arrived at a predetermined location: either the laboratory or the local student health center for each visit and were asked questions to verify their eligibility. They were then provided with written and oral informed consent information. Following informed consent, their blood pressure was measured and 14mL of blood was drawn, with their dominant arm preferred, by a trained phlebotomist (the author or student health center phlebotomist). After the blood draw, for participants starting in the student health center, they moved to the laboratory and were asked for the intensity and unpleasantness of their pain to the blood draw. Participants already in the laboratory were asked the same questions related to the blood draw. A saliva sample was then taken. Participants moved to the experiment room and

had physiological sensors applied to measure respiration, heart rate, and skin conductance. Following this, participants filled out baseline questionnaires and took online training for the Self-Assessment Manikin. Once the questionnaires were completed, participants were asked to remove the shoe and sock contralateral to the blood draw, after which 5 minutes of physiological data were collected. After five minutes, the pressure pain threshold test was conducted, after which blood pressure was measured on their left arm and participants put their shoe and sock back on. After another 5 minutes of physiological data collection, a circle was drawn on the volar aspect of the forearm contralateral to the blood draw. The experimenter was careful to avoid visible blood vessels when possible. Participants were then trained on how to rate the intensity and unpleasantness of their pain on the visual analog scale and practiced making ratings. Once the participant was comfortable rating their pain, a thin layer of Vaseline was placed around the perimeter of the circle and 0.3mL of a mild topical capsaicin solution was applied to the skin (Zostrix) and covered with a transparent medical bandage (Tegaderm, 3M). After the capsaicin was applied, participants rated the intensity and unpleasantness of their capsaicin-induced pain on the VAS as well as their valence, affect, and dominance on the SAM every 3 minutes for 45 minutes. During this time, the participant was alone in the experiment room and was prompted to make their ratings by a mild audio cue. Following 45 minutes, the capsaicin was removed and participants moved to the next room for the flare measurement. After the flare measurement, oil was applied to the capsaicin site to dissolve and remove capsaicin any remaining capsaicin. Participants moved back to the experiment for the temporal

summation of second pain procedure. Following the temporal summation procedure, at the beginning of three minutes of physiological data collection, participants were told to remove the shoe and sock on the foot contralateral to the blood draw. After the three minutes, a second pressure pain threshold was administered followed by exit questionnaires.

Sample Size

GPower 3.1.9.2 was used to calculate a priori sample size for mixed ANOVAs. A medium effect size ($f = 0.25$) calculated using epinephrine results from a previous study,[114] for two visits per person in each group, with an alpha of .05, 80% power, a 0.5 correlation among repeated measures, and a nonsphericity correction of 1 were entered. The results indicated a minimum total sample size of 34 ($n = 17$ per group) was needed.

A second power analysis with a medium effect size ($f = 0.25$) was calculated using muscle mechanical hyperalgesia results from a previous study.[114] For two visits per person in each group, with an alpha of .05, 80% power, a 0.5 correlation among repeated measures, and a nonsphericity correction of 1 suggested a total sample size of 16 ($n = 8$ per group) was needed.

Statistical Analysis

Statistical assumptions were examined. Normality was evaluated using the Shapiro-Wilk's test. Homogeneity of variance was evaluated using Levene's test. Variables violating normality or variance were transformed according to standard conventions. Outliers were detected if they were outside the mean ± 3 SD. If outliers were detected, they were removed from analyses only if there was sufficient reason for

being an outlier. For repeated measures analyses with more than two levels for a repeated measure, the sphericity assumption was tested using Mauchly's test and deviations from sphericity were corrected using Greenhouse-Geisser if the estimated epsilon (ε) was less than 0.75 or Huynh-Feldt if the estimated epsilon was greater than 0.75. The first hypothesis regarding greater pain and neurogenic inflammation in binge drinkers and those in withdrawal was tested using Mixed ANOVAs for pressure pain threshold and flare and a Mixed RM ANOVA for temporal summation of second pain. The second hypothesis that pain and inflammation is related to greater baseline epinephrine will be tested using a Mixed ANOVA and correlations. Effects of gender were analyzed by running separate analyses on women and men.

Missing Data

No missing data values were included in pain sensitivity and psychophysiological analyses. All questionnaires were completed with the exception of one participant's CES-D during the abstain visit. To ensure the data from the same participants used for the pain sensitivity analyses were included in every analysis, individuals with missing data in the pain sensitivity tests were excluded and pairwise deletion was used for remaining analyses. Individuals with missing or unrecoverable heart rate, respiration, and skin conductance data due to equipment malfunction or missing questionnaire and affect data were analyzed using pairwise deletion.

Following data collection, participants were excluded from analyses if the participant did not follow directions regarding drinking prior to a visit, if they were a heavy drinker (i.e., consumed binge levels of alcohol at a rate below binge drinking [2

drinks/hour for women, 2.5/hour for men]), if they did not report P50 for both visits, if different pain sensitivity methods were piloted on the participant, or TSSP was not completed on both visits.

RESULTS

Participants

In total, 29 participants completed the study (see Figure 2 for participant flow chart).

Table 1 shows the demographic and coping data for the sample. Moderate and binge drinkers did not differ in age, gender, ethnicity, cigarette use, and number of adverse life event types experienced (sum of general trauma, physical abuse, emotional abuse, and sexual abuse), all *p*s > .17. Binge drinkers did endorse use of more instrumental support, $t(27) = 2.19$, $p = .038$, and behavioral disengagement, $\chi^2(1) = 5.83$, $p = .008$, when coping with stress.

Table 1. Baseline Demographic and Psychological Characteristics.

	Moderate Drinkers (*n* = 13)	Binge Drinkers (*n* = 16)		
Characteristic	*M (SD)*	*M (SD)*	*t*	*p*
Age	21.23 (2.77)	22.00 (3.50)	0.64	.53
Gender (% Female)†	56.25%	38.46%	0.91	.34
Ethnicity (% Caucasian)†	50.00%	53.85%	3.29	.51
Cigarette use (%) ‡	92.31%	75%	1.00	.34
Adversity (number of event types)	3.92 (2.50)	2.69 (2.21)	-1.41	.17
Coping				
Active Coping	6.15 (1.28)	5.63 (1.26)	-1.12	.27
Planning‡	6.00 (1.41)	6.06 (1.24)	6.00	.47
Positive Reframing	5.08 (1.61)	5.56 (1.67)	0.79	.44
Acceptance‡	5.31 (1.38)	5.50 (1.03)	6.00	.57

Table 1. Continued.

Humor	4.23 (1.48)	5.31 (1.78)	1.75	.09
Religion‡	4.15 (1.99)	3.56 (1.97)	3.00	.72
Using Emotional Support	4.23 (1.54)	4.88 (1.82)	1.02	.32
Using Instrumental Support	3.62 (1.26)[a]	4.69 (1.35)[b]	2.19	.04*
Self-Distraction	5.69 (1.11)	5.44 (1.15)	-0.60	.55
Denial‡	2.46 (1.13)	2.63 (0.96)	2.00	.45
Venting‡	4.08 (0.95)	4.40 (1.45)	4.00	.48
Substance Use‡	2.69 (1.25)	3.31 (1.49)	2.00	.26
Behavioral Disengagement‡	2.31 (1.11)[a]	3.00 (1.21)[b]	2.00	<.01**
Self-Blame‡	4.23 (1.48)	4.94 (1.53)	5.00	.09

Note. Independent samples t-tests were performed unless otherwise indicated. †chi-square test was performed with sample means and standard deviations reported. ‡Kruskal-Wallis test was performed with sample means, standard deviations, and medians reported.

*$p < .05$. **$p < .01$.

Table 2 shows alcohol use and craving characteristics. Binge drinkers reported more alcohol use disorder symptoms, $t(22.05) = 2.91$, $p = .008$, a greater number of standard drinks prior to the withdrawal state, $\chi^2(1) = 5.57$, $p = .008$, a greater number of drinks typically consumed, $\chi^2(1) = 19.45$, $p < .001$, greater typical hangover symptoms, $t(27) = 2.47$, $p = .02$, and a greater frequency of consuming 4-5 drinks for women and men, respectively, $\chi^2(1) = 14.95$, $p < .001$. Notably, despite our participants drinking more prior to withdrawal and reporting typically greater hangover symptoms, they did not differ from moderate drinkers in reported acute hangover symptoms, all *p*s > .3.

Table 2. Alcohol Use and Craving Characteristics.

	Moderate Drinkers (n = 13)		Binge Drinkers (n = 16)			
	Abstain	Withdrawal	Abstain	Withdrawal		
Characteristic	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*t*	*p*
BMI	23.03 (4.05)		24.66 (4.24)		1.05	.30
Age of first drink (years)	18.69 (1.75)		18.03 (1.97)		-0.94	.35
Years drinking	2.73 (2.31)		4.19 (2.99)		1.44	.16
Frequency (binge drink amount)	0.77 (1.17)[a]		8.81 (10.16)[b]		3.14	<.01**
Typical number of drinks	2.00 (0.91)[a]		5.13 (1.75)[b]		5.82	<.01**
Hangover Symptom Severity	4.00 (3.06)[a]		6.44 (2.25)[b]		2.47	.02*
UPPS - Sensation Seeking	20.15 (4.76)		21.06 (6.02)		0.44	.66
AUDIT	5.23 (2.39)[a]		9.44 (5.15)[b]		2.91	<.01**
Number of drinks prior to withdrawal visit‡	n/a	2.19 (0.90)[a]	n/a	4.00 (2.66)[a]	0.03	<.01**
AHS	0.70 (0.14)	0.77 (0.13)	0.94 (0.13)	0.85 (0.12)	0.77	.39
ACQ	33.85 (3.52)	30.54 (2.70)	38.50 (3.17)	37.25 (2.43)	0.34	.56
Compulsivity	5.62 (4.61)	4.54 (2.30)	5.44 (2.58)	6.06 (3.32)	2.24	.15
Expectancy	9.08 (1.26)	7.69 (1.02)	10.94 (1.14)	10.50 (0.92)	0.44	.52
Purposefulness	10.15 (1.05)	11.23 (0.93)	13.38 (0.95)	12.00 (0.84)	3.52	.07
Emotionality	9.00 (4.34)	7.08 (4.50)	8.50 (3.72)	8.69 (4.38)	2.02	.17
CTQ						
Reward	15.69 (3.07)	15.77 (2.52)	18.56 (4.59)	21.00 (4.34)	4.98	.03*
Relief	10.23 (1.09)	10.85 (1.05)	11.94 (0.99)	12.38 (0.94)	0.04	.85
Obsessive Craving	9.77 (2.74)	9.69 (3.01)	10.88 (3.83)	10.75 (3.94)	0.00	.95
BAES						
Stimulation	49.46 (14.70)[a]	40.38 (21.03)[b]	54.38 (9.85)[a]	52.19 (9.15)[b]	2.06	.16
Sedative	33.92 (3.51)	31.23 (4.33)	42.19 (3.16)	39.94 (3.91)	0.01	.91

Table 2. Continued.

Note. Independent samples t-tests and mixed ANOVAs were performed unless otherwise indicated. ‡Kruskal-Wallis test was performed with sample means, standard deviations, and medians reported. Superscript letters indicate significant differences between visits or groups. UPPS - Sensation Seeking = UPPS Impulsive Behavior Scale - Sensation Seeking subscale; AUDIT = Alcohol Use Disorder Identification Test; AHS = Acute Hangover Scale; ACQ = Alcohol Craving Questionnaire; CTQ = Craving Typology Questionnaire; BAES = Biphasic Alcohol Effects Scale.

*p < .05. **p < .01.

Table 3 shows psychological and physiological characteristics at the beginning of each drinking state visit. For perceived stress, we found a main effect of group, $F(1, 26) = 11.95$, $p = .002$, with the binge group reporting more stress at the start of each state. We also found main effects of drinking state for state anxiety, $F(1, 26) = 7.92$, $p = .009$, negative affect, $F(1, 26) = 7.46$, $p = .011$, and perceived dominance, $F(1, 24) = 5.81$, $p = .024$, indicating participants at the start of the withdrawal state felt less anxious, less negative, and more dominant. Interestingly, we also found main effects of state for heart rate in beats per minute, $F(1, 27) = 5.37$, $p = .028$, and interbeat interval, $F(1, 27) = 7.98$, $p = .009$, as well as respiration rate, $F(1, 24) = 5.46$, $p = .028$, with participants at the start of the withdrawal state having a faster heart rate and a shorter interbeat interval along with a faster respiration rate. While there was a trend for a State x Group interaction for positive affect, $F(1, 26) = 3.65$, $p = .067$, with the binge group reporting less positive affect during withdrawal, there were no differences between state, group, or interaction for depressive symptoms, positive affect, valence, arousal, and skin conductance level, all ps > .07.

Table 3. Baseline Psychological and Physiological Characteristics.

	Moderate Drinkers (n = 13)		Binge Drinkers (n = 16)			
	Abstain	Withdrawal	Abstain	Withdrawal		
Characteristic	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*t*	*p*
PSS	11.83 (1.72)[a]	10.58 (1.81)[a]	18.44 (1.49)[b]	19.19 (1.56)[b]	1.69	.21
STAI	9.83 (0.75)[a]	8.33 (0.64)[b]	9.88 (0.65)[a]	9.13 (0.55)[b]	0.88	.36
CESD	10.82 (2.48)	8.55 (2.18)	15.50 (2.06)	12.88 (1.81)	0.02	.89
PANAS						
Positive affect	28.50 (2.56)	28.42 (2.45)	27.44 (2.22)	22.81 (2.12)	3.65	.07
Negative affect	11.67 (0.69)[a]	11.17 (0.53)[b]	13.38 (0.60)[a]	11.69 (0.46)[b]	2.20	.15
SAM						
Valence	6.46 (0.31)	6.82 (0.42)	6.27 (0.27)	6.2 (0.36)	1.03	.32
Arousal	3.09 (0.48)	3.64 (0.68)	3.93 (0.41)	3.47 (0.58)	2.04	.17
Dominance	6.18 (0.52)[a]	7.09 (0.51)[b]	5.33 (0.44)[a]	6.20 (0.44)[b]	0.00	.96
Heart rate (BPM)	74.88 (3.38)[a]	76.56 (3.38)[b]	67.81 (3.05)[a]	71.55 (3.05)[b]	0.78	.39
Heart rate (IBI ms)	831.92 (164.30)[a]	804.62 (120.79)[b]	923.81 (180.91)[a]	873.38 (170.23)[b]	0.71	.41
Skin conductance level (sqrtμS)	2.13(1.04)	2.44 (0.55)	2.52 (0.56)	2.48 (0.51)	1.39	.25
Respiration rate (BrPM)	15.56 (1.79)[a]	16.00 (1.70)[b]	14.84 (3.34)[a]	16.17 (3.55)[b]	1.40	.25

Note. Mixed ANOVAs were performed. Superscript letters indicate significant differences between visits or groups. PSS = Perceived Stress Scale; STAI = State Trait Anxiety Inventory; CESD = Center for Epidemiological Studies - Depression; PANAS = Positive And Negative Affect Schedule; SAM = Self-Assessment Manikin; BPM = beats per minute; IBI ms= interbeat interval in milliseconds; sqrtμS = square root of microsiemens; BrPM = breaths per minute.

*p < .05. **p < .01.

Pain Sensitivity

Muscle pressure pain

Figure 4 shows pressure pain threshold at the beginning (Fig. 4A) and end (Fig. 4B) of each state as well as a comparison of tests at the beginning and end (Fig. 4C). To

assess whether our participants with histories of binge drinking and in withdrawal report muscle mechanical hyperalgesia similar to previous studies, we measured pressure pain threshold at the beginning and end of each state.

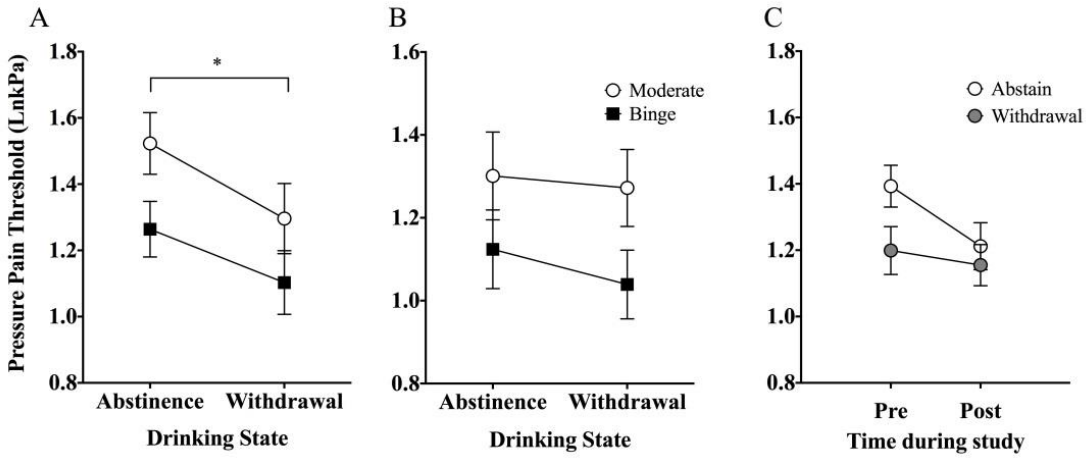

Figure 4. Pressure pain thresholds at the (A) beginning and (B) end of each drinking state as well as (C) a comparison of threshold tests. Error bars = *SEM*. * = $p < .05$.

For muscle pressure pain threshold at the beginning of the study (Fig. 4A), we found a main effect of state, $F(1, 27) = 13.38$, $p = .001$, with a lower threshold (i.e., greater pain sensitivity) during withdrawal than abstinence. This indicates withdrawal-induced mechanical hyperalgesia. While there was a trend toward a main effect of group, $F(1, 24) = 3.36$, $p = .078$, this failed to reach significance. There was no interaction, $p = .54$. Muscle pressure pain threshold at the end of each drinking state (Fig. 4B), we found no main effects nor interactions, all *p*s > .10. When we included both assessments in our analysis (Fig. 4C), we found a State x Time interaction, $F(1, 27) = 4.53$, $p = .043$. This interaction was driven by participants in withdrawal reporting

consistently lower thresholds while participants in abstinence reporting a higher threshold than during withdrawal. This reduced threshold during withdrawal is likely due to sensitization.

Capsaicin-induced neurogenic inflammation and spontaneous pain

Figure 5 shows the area (Fig. 5A) and intensity (Fig. 5B) of neurogenic flare. To determine whether a history of binge drinking or withdrawal affect neurogenic inflammation, we measured the area and intensity of capsaicin-induced neurogenic flare. Mixed between-within subjects ANOVAs on the area and intensity of flare found no main effects nor interactions, all *p*s > .25, suggesting history of alcohol use and withdrawal in our sample did not affect the area or intensity of flare.

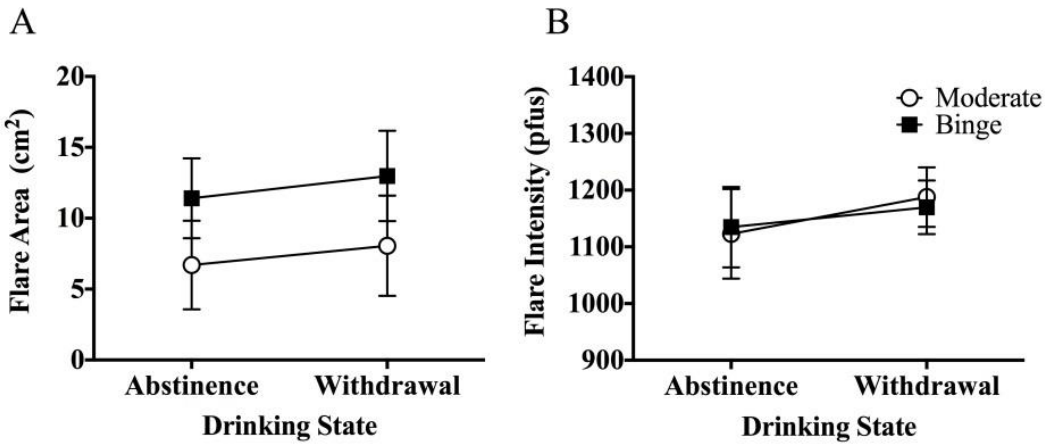

Figure 5. (A) Area and (B) intensity of capsaicin-induced neurogenic flare. Error bars = *SEM*.

Figure 6 shows the ratings of the intensity (Fig. 6A) and unpleasantness (Fig. 6B) of capsaicin-induced spontaneous pain. Mixed between-within-within subjects analyses

showed main effects of time for both the intensity, $F(1.18, 30.6) = 22.65$, $p > .001$, and unpleasantness, $F(1.26, 32.85) = 24.32$, $p < .001$, with ratings increasing over time. No other main effects nor interactions were found, all *p*s > .20. This suggests the dose of capsaicin used was sufficient to induce intense and unpleasant pain.

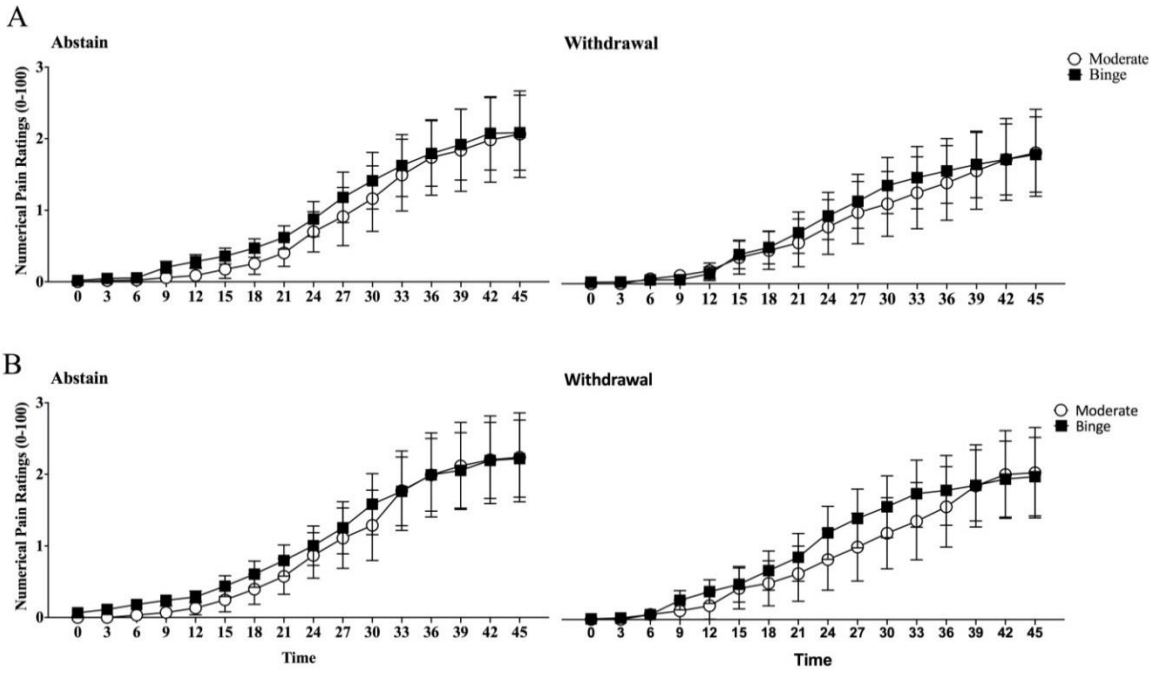

Figure 6. Capsaicin-induced (A) intensity and (B) unpleasantness of spontaneous pain during both abstain and withdrawal states. Error bars = *SEM*.

Static cutaneous thermal pain and temporal summation of second pain

We assessed thermal pain and thermal temporal summation of second pain to determine if a history of binge drinking and withdraw sensitized the ascending pain pathway.

Cutaneous thermal pain

Figure 7 shows participant ratings at 45°C (Fig. 7A), 46°C (Fig. 7B), and 47°C (Fig. 7C). To acclimate participants to the thermal device, we administered standardized thermal stimuli 2 seconds in length with each pulse at 45°C, 46°C, and 47°C with an interstimulus interval of at least 1 minute. We found a main effect of state at 45°C, $F(1, 25) = 27.45$, $p < .001$, 46°C, $F(1, 25) = 35.69$, $p < .001$, and 47°C, $F(1, 25) = 47.44$, $p < .001$. At each temperature, participants reported less pain during the withdrawal state than the abstain state. This cutaneous thermal hypoalgesia suggests that while in withdrawal, participants were less sensitive to thermal stimuli.

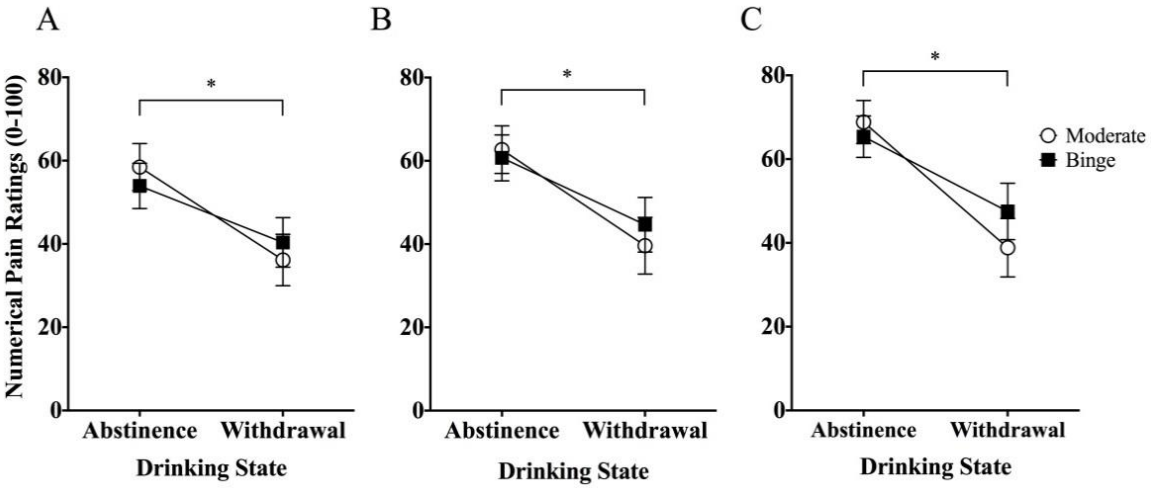

Figure 7. Pain ratings to 2 stimuli at (A) 45°C, (B) 46°C, and (C) 47°C. Error bars = *SEM*. * = $p < .05$.

Prior to cutaneous temporal summation, we individualized the temperature to be used during temporal summation to a temperature at which the participant reliably rated

their pain to be 50 out of 100 (i.e., P50). Figure 8 shows the temperature (Fig. 8A) and pain ratings (Fig. 8B) for the individualization procedure. For the temperature, we found a main effect of state, $F(1, 27) = 12.50$, $p = .001$, with individuals during the withdrawal state needing a higher temperature to reach P50 than during the abstain state. For the pain ratings, we found a State x Group interaction, $F(1, 27), = 6.61$, $p = .016$, with binge drinkers reporting similar pain levels during both states while moderate drinkers reported less pain during the withdrawal state. There was also a main effect of visit, $F(1, 27) = 12.98$, $p = .001$, for pain rating, with the participants in the withdrawal state reporting less pain than in the abstain state. This suggests that participants during the withdrawal state needed a higher temperature than during the abstain state to achieve approximately equivalent thermal pain ratings. In addition, drinkers during the withdrawal state, particularly moderate drinkers, reported lower pain to the individualized temperature than during the abstain state. These results corroborate the thermal hypoalgesia found using the standardized thermal stimuli above.

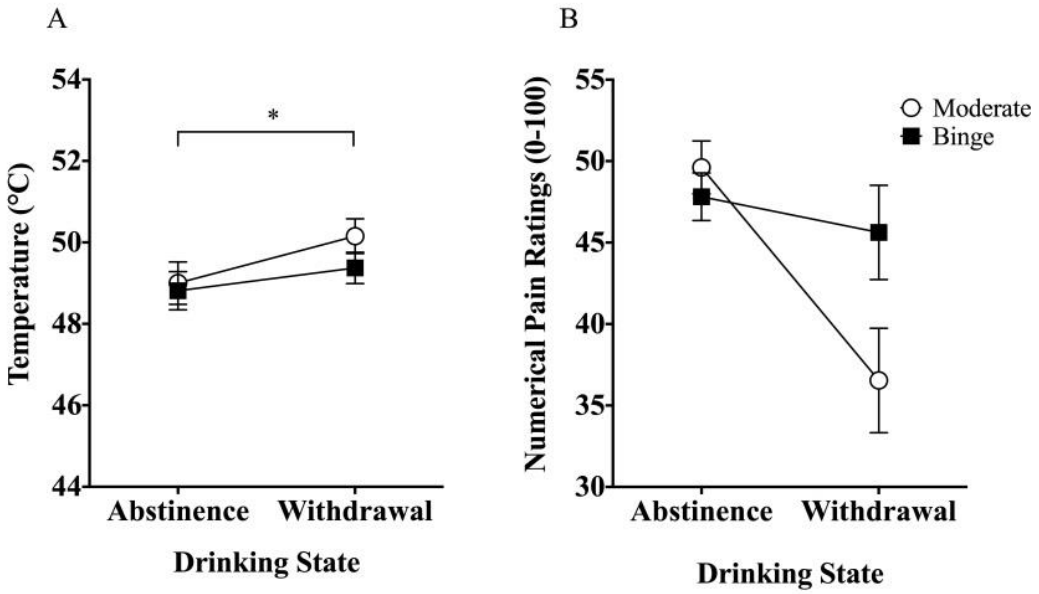

Figure 8. (A) Individualized temperature and (B) pain rating to achieve rating of 50/100 (P50). Error bars = *SEM*. * = $p < .05$.

Cutaneous thermal temporal summation of second pain

Figure 9 shows the pain ratings of the second pain sensation following each of the 10 thermal stimulations and three aftersensations at 15 second intervals following cessation of the 10th pulse. During the 10 pulse temporal summation of second pain procedure, we found a main effect of pulse, $F(1.41, 38.18) = 6.50$, $p = .008$, with pain increasing over the course of the pulses. This suggests that the procedure did induce temporal summation. Over the 10 pulses, we found a visual trend toward binge drinkers reporting enhanced temporal summation during both abstinence and withdrawal phases compared to moderate drinkers, $F(1, 27) = 0.71$, $p = .406$. Since our data suggests sensitization over the first five pulses and based on previous studies supporting sensitization over the first 4-6 pulses,[93,113] we conducted an exploratory analysis of the

first five pulses of data. We found a main effect of pulse, $F(1.51, 40.83) = 23.00$, $p < .001$, with increasing pain over the course of the first five pulses. Additionally, we found a trend towards a State x Pulse interaction, $F(1.90, 51.17) = 3.06$, $p = .058$ with a slight linear increase over the five pulses during withdrawal while during abstinence, ratings began to plateau after the third pulse. The visual trend of enhanced temporal summation in binge drinkers compared to moderate drinkers was not supported, $F(1, 27) = 1.42$, $p = .244$.

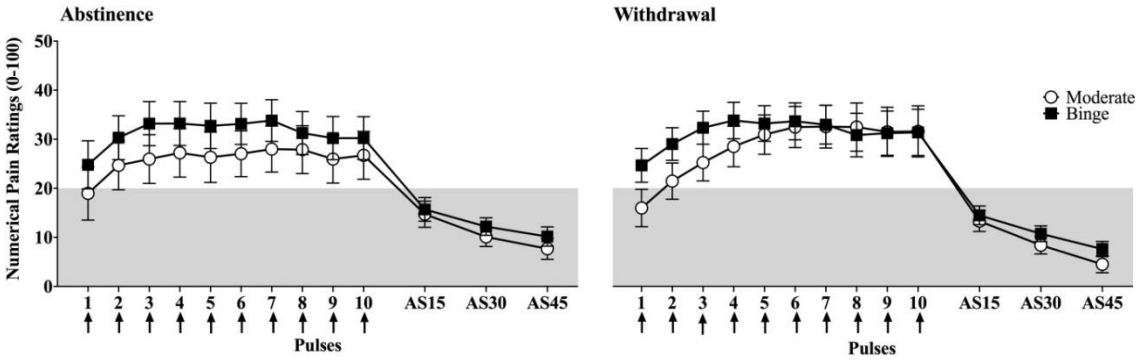

Figure 9. Second pain ratings across 10 thermal heat pulses and subsequent aftersensations. Sensitization is indicated by increasing pain over the ten pulses (pulses indicated by arrows). Pain threshold is indicated by the shaded area. Error bars = *SEM*.

Following cessation of the stimulations, we found a State x Time interaction for the aftersensations, $F(2, 54) = 3.24$, $p = .047$, (Fig. 10) with pain ratings during the withdrawal state decreasing more rapidly during the abstinence state. There was also a main effect of time, $F(1.13, 30.56) = 44.38$, $p < .001$, with pain ratings decreasing over time. This further corroborates the thermal hypoalgesia found above.

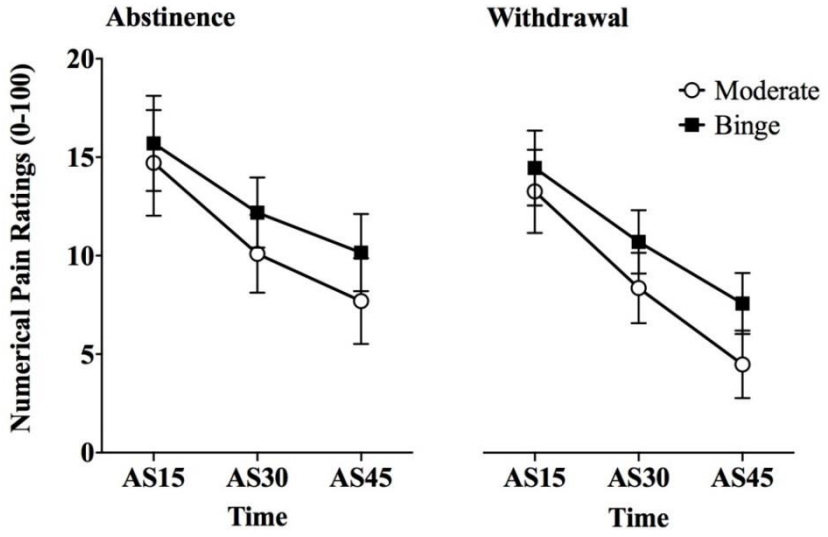

Figure 10. Aftersensations during abstinence and withdrawal states. AS15 = Aftersensation at 15 seconds; AS30 = Aftersensations at 30 seconds; AS45 = Aftersensations at 45 seconds. Error bars = *SEM*.

Role of Epinephrine and IL-6 in Pain Sensitivity

Due to the undetectable levels of epinephrine and IL-6 across participants, the epinephrine and IL-6 data are not presented.

Differences in Pain Sensitivity Between Genders

Figure 11 shows mechanical muscle sensitivity (Fig. 11A), P50 temperature (Fig. 11B), P50 pain rating (Fig. 11C), rating pain aftersensations following temporal summation of second pain (Fig. 11D), and neurogenic flare (Fig. 11E) results by gender. When analyses were conducted separately in women and men, a main effect of state was found for muscle mechanical pain threshold at the beginning of the study in men, $F(1, 13) = 6.62$, $p = .023$, while only a trend was found for women, $F(1, 12) = 4.65$, $p = .052$.

Regarding cutaneous thermal pain sensitivity, men showed main effects of state for the P50 temperature, $F(1, 13) = 14.55$, $p = .002$, and pain ratings, $F(1, 13) = 13.90$, $p = .003$. No effect was found in women, all ps > .08. In addition, for aftersensations following cutaneous thermal temporal summation of second pain, men showed a significant State x Time interaction, $F(2, 26) = 4.72$, $p = .018$ as well as a main effect of time, $F(2, 26) = 20.44$, $p < .001$. Only a main effect of time was found for women, $F(2, 24) = 24.47$, $p < .001$. This suggests that the alcohol withdrawal-induced muscle mechanical hyperalgesia and cutaneous thermal hypoalgesia were potentially driven by effects in male participants.

However, for intensity of neurogenic flare, women showed a main effect of state, $F(1, 12) = 5.75$, $p = .034$, with women during the withdrawal state showing more intense flare than when they were in the abstinence state. No effects were found in men, all ps > .34. No other main effects were found for the remaining pain tests.

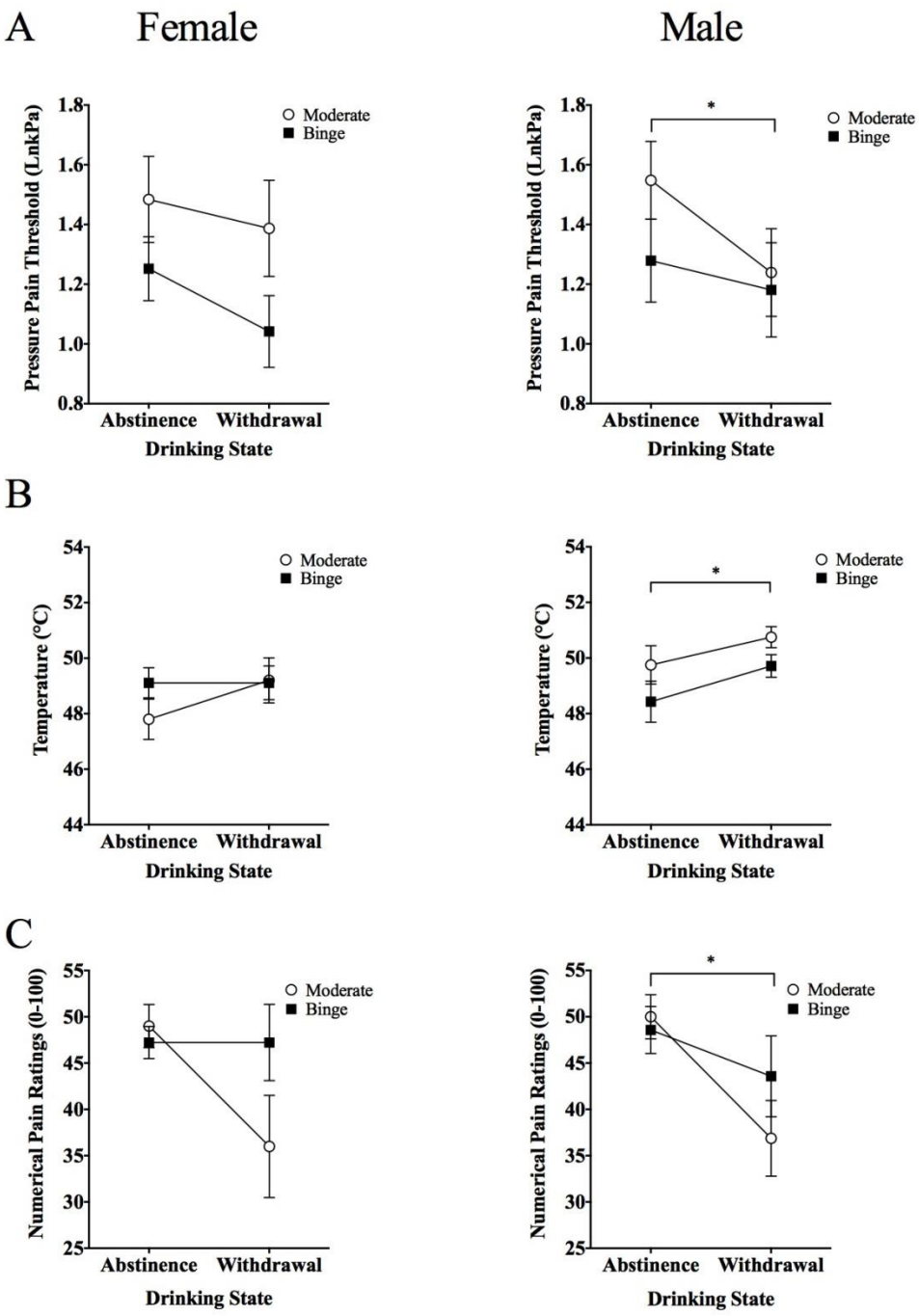

Figure 11. (A) Pressure pain thresholds, (B) individualized temperature and (C) pain ratings to achieve rating of 50/100, (P50) as well as (D) temporal summation of second pain aftersensations and (E) neurogenic flare results by gender. Error bars = *SEM*. * = $p < .05$.

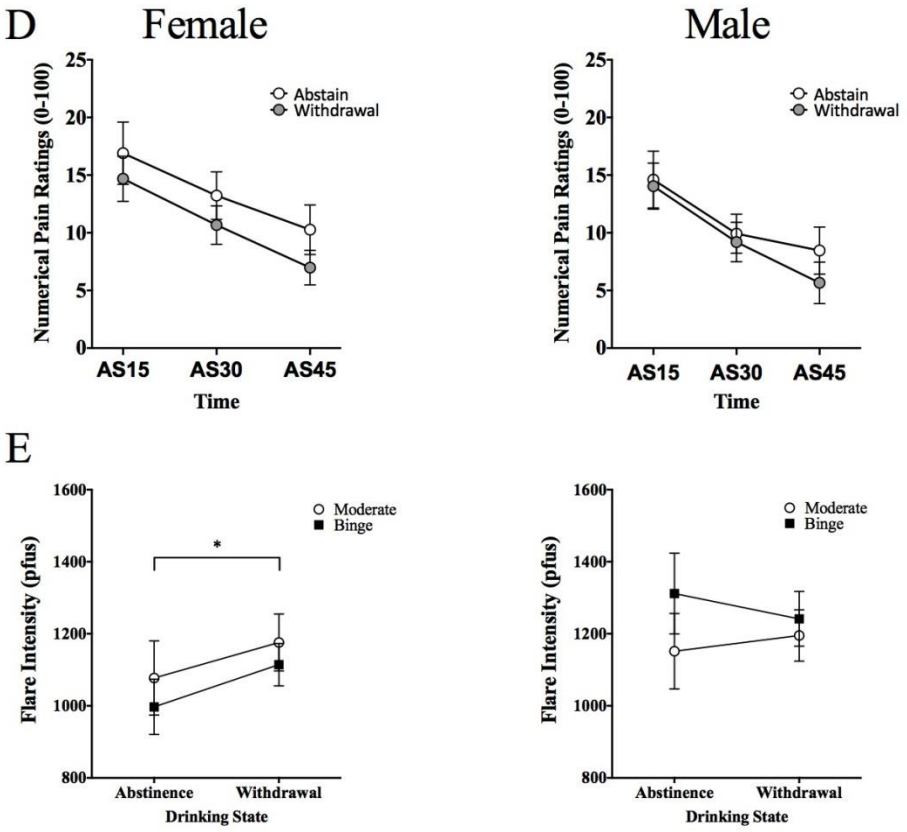

Figure 11. Continued.

Differences in Affect

Next, we wanted to assess the role of anxiety, depressive symptoms, and affect in binge- and withdrawal-induced pain sensitivity in individuals with a history of moderate or binge drinking. Figure 12 depicts state anxiety (Fig. 12A), negative affect (Fig. 12B), and positive affect (Fig. 12C) collapsed across binge and moderate drinkers. We found a main effect of state for state anxiety, $F(1, 26) = 7.92$, $p = .009$, and negative affect, $F(1, 26) = 7.61$, $p = .010$, with participants in withdrawal reporting less anxiety and less negative affect throughout the withdrawal state than when in abstinence. There was a

trend toward a main effect of group for negative affect, $F(1, 26) = 3.99$, $p = .056$, with the binge group reporting moderately more negative affect over both states. For positive affect at the beginning and end of each state, we found a main effect of time, $F(1, 26) = 10.39$, $p = .003$, with positive affect decreasing over the course of each state. No other main effects nor interactions were found including for depressive symptoms. These results suggest that when in withdrawal, participants were less anxious and had less negative affect than when they were abstaining. It also suggests that participants were less happy as each state progressed.

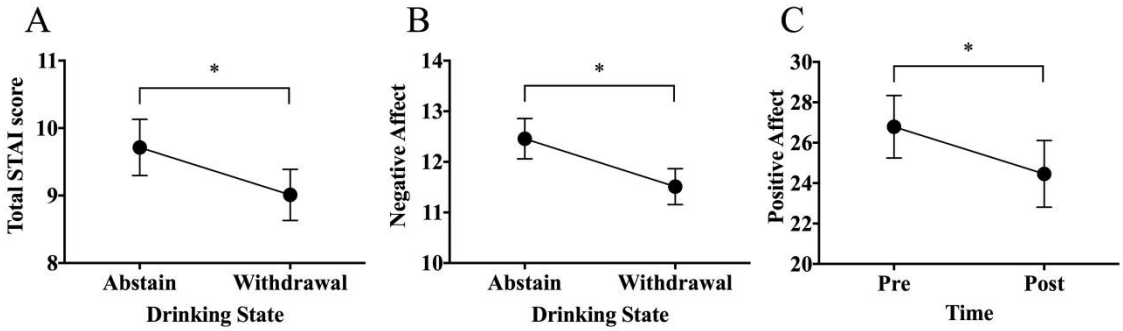

Figure 12. (A) State anxiety and (B) negative affect collapsed across the beginning and end of each drinking state as well as (C) positive affect collapsed across drinking states. Error bars = *SEM*. * = $p < .05$.

Psychological Responses to Testing

We wanted to assess the potential role of valence, arousal, and dominance during capsaicin and over the course of each state. Figure 13 depicts valence (Fig. 13A), arousal (Fig. 13B), and dominance (Fig. 13C) during the capsaicin test. We found main effects of time for valence, $F(2.09, 54.46) = 12.77$, $p < .001$, and dominance, $F(3.11,$

80.75) = 5.85, p = .001, indicating participants became less happy and less dominant over the course of each state. There were no other main effects or interactions for valence or dominance. There were also no main effects nor interactions for arousal during capsaicin, all ps > .08.

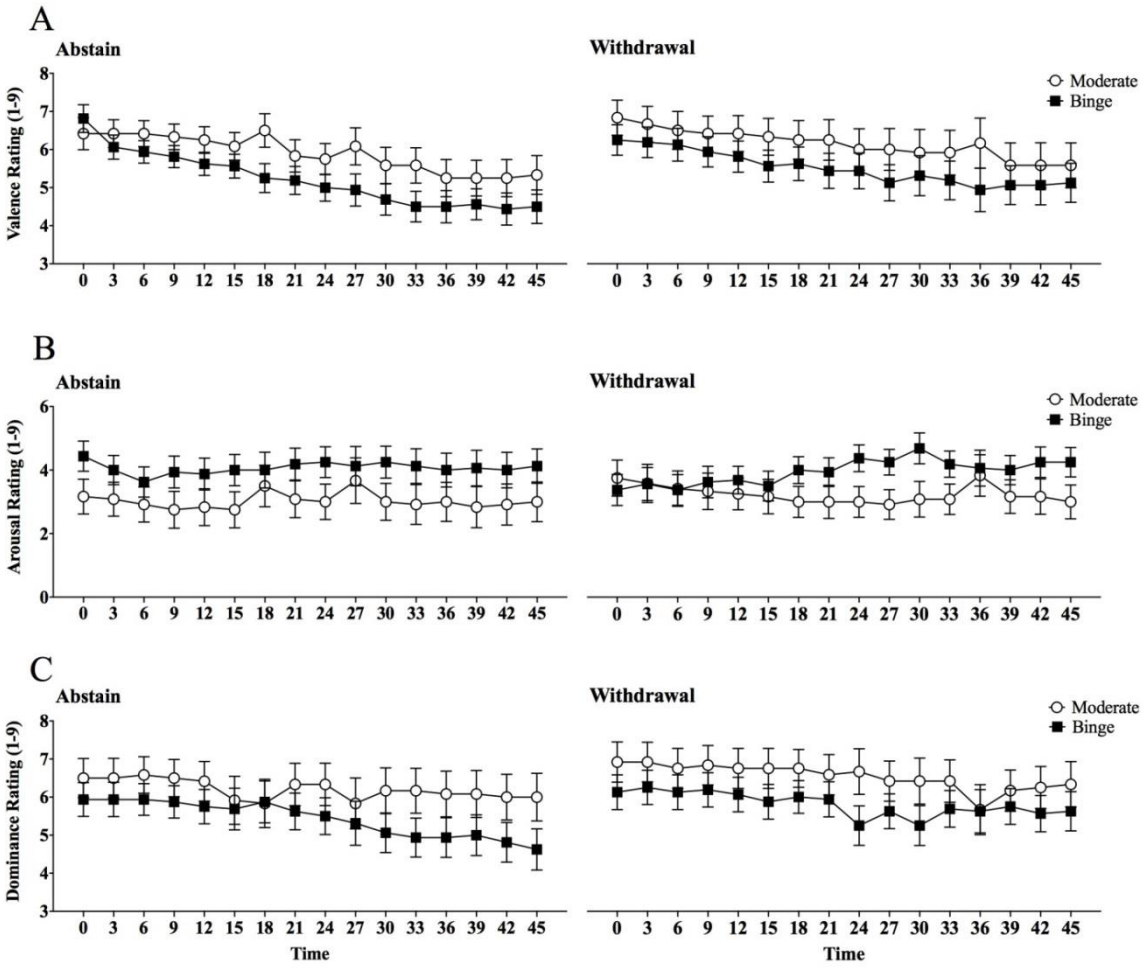

Figure 13. (A) Valence, and (B) Arousal, and (C) Dominance ratings during 45 minute capsaicin application in abstain and withdrawal states. Error bars = *SEM*.

Figure 14 depicts valence (Fig. 14A), arousal (Fig. 14B), and dominance (Fig. 14C) over the course of each drinking state. We found main effects of state for valence, $F(1, 24) = 5.42$, $p = .029$, and dominance, $F(1, 24) = 7.04$, $p = .014$, with participants during the withdrawal state reporting being happier and more dominant over the course of the study. We also found a main effect of time for valence, $F(2.74, 65.75) = 12.33$, $p < .001$, with participants generally becoming less happy over the course of each state. In addition, for arousal, there was a State x Time x Group interaction, $F(2.18, 50.21) = 3.55$, $p = .032$, with the binge group reporting increasing levels of arousal during the withdrawal state.

Physiological Responses to Testing

To determine whether participants with a history of binge drinking and withdrawal responded physiologically different to pain tests we investigated heart rate, skin conductance (a measure of perspiration rate), and respiration rate. Figure 15 shows the heart rate in beats per minute (Fig. 15A), and interbeat interval (Fig. 15B), along with skin conductance (Fig. 15C), and respiration rate (Fig. 15D) collapsed across pain tests.

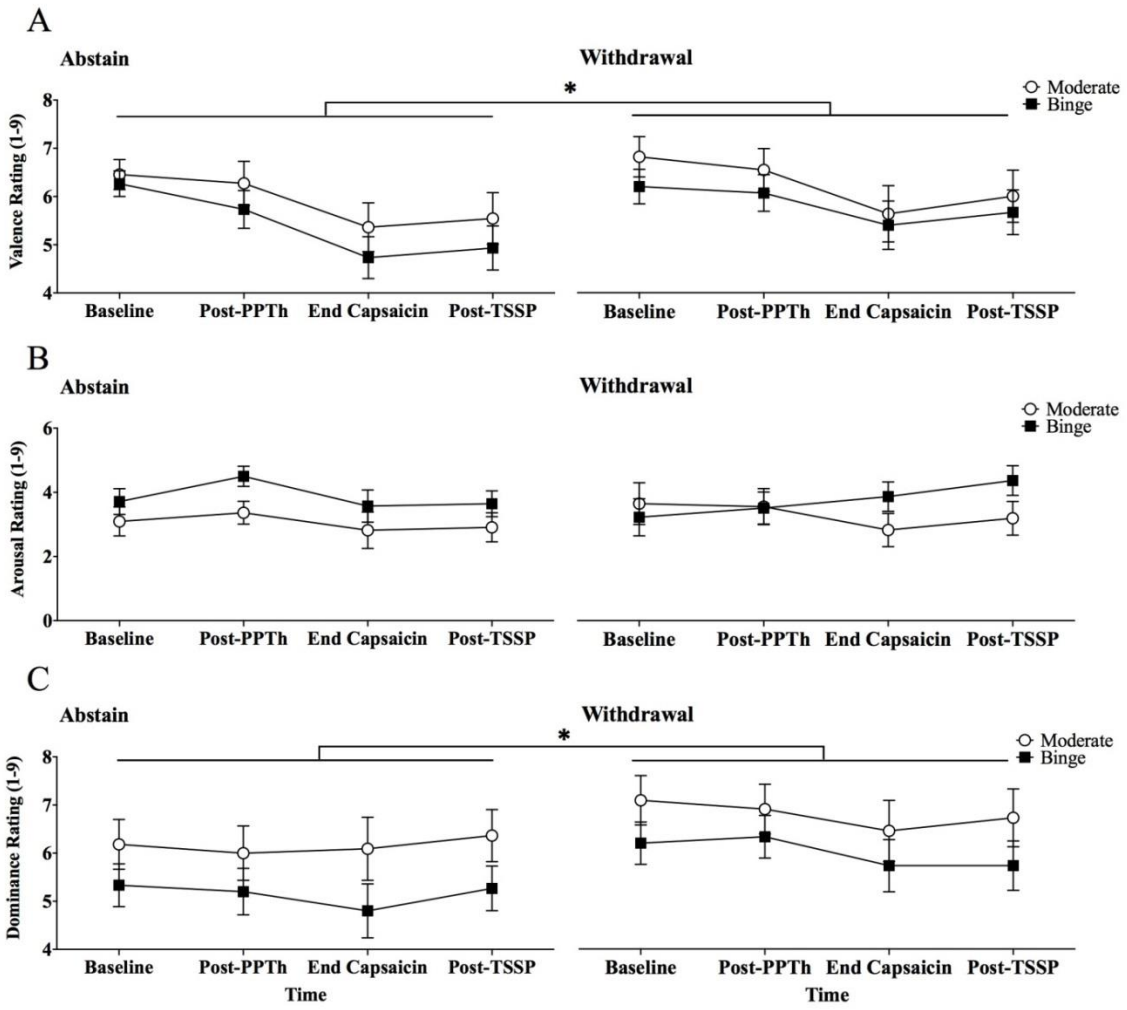

Figure 14. (A) Valence, (B) arousal, and (C) dominance ratings throughout each drinking state. Error bars = *SEM*. * = $p < .05$.

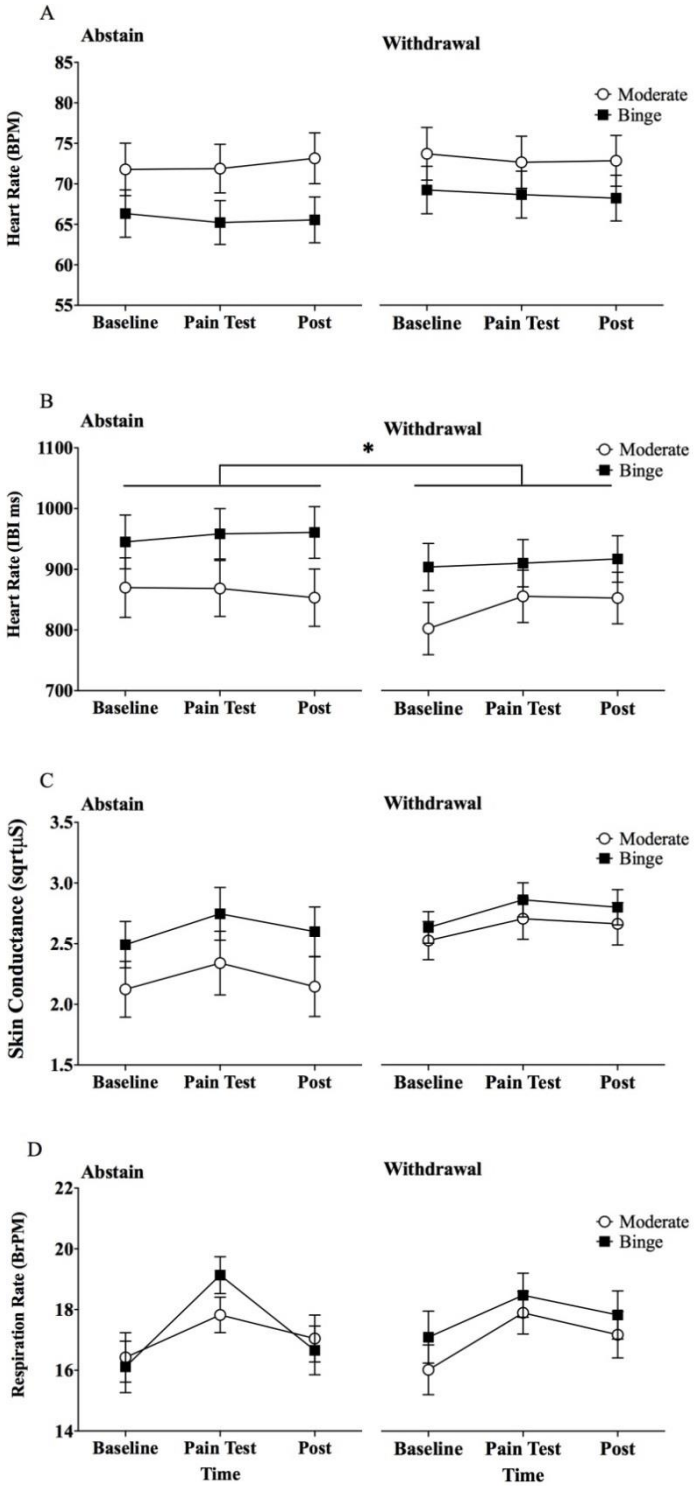

Figure 15. (A) Heart rate in (A) beats per minute and (B) interbeat interval, (B) skin conductance level, and (C) respiration rate collapsed across before (Baseline), during (Pain Test), and after (Post) pressure pain threshold, topical capsaicin, and temporal summation of second pain procedures. Error bars = *SEM*. * = $p < .05$.

For heart rate in beats per minute, we found a trend towards a State x Time interaction, $F(2, 54) = 5.85$, $p = .054$, with participants during the withdrawal state showing relatively consistent heart rate during and after the pain tests while during the abstain state, they showed a slight increase in heart rate following the pain tests. For heart rate interbeat interval, we found a main effect of state, $F(1, 27) = 5.39$, $p = .028$, suggesting participants during the withdrawal state had a shorter interbeat interval indicating a faster heart rate. We also found a State x Time interaction for skin conductance level, $F(1.79, 44.79) = 4.65$, $p = .018$, suggesting that during the withdrawal state, participants skin conductance responses were consistently higher and did not decrease following the pain tests, particularly for the moderate group. For respiration rate, we found a State x Time x Group interaction, $F(2, 46) = 3.85$, $p = .028$, suggesting that during the abstinence state, binge drinkers' respiration rates were higher during the pain tests than moderate drinkers. These results suggest that participants in withdrawal state experienced a greater physiological response throughout the study (heart rate) that did not dissipate after cessation of the test (skin conductance level).

CONCLUSION

Binge drinking is a prevalent problem, in particular for young adults, that may lead to enhanced pain sensitivity. In addition, self-medication of pain with alcohol may be a potential driver of further increases in pain and more extreme alcohol use. Animal models of alcohol withdrawal-induced hyperalgesia indicate that multiple cycles of alcohol consumption and withdrawal results in an increase in muscle mechanical pain sensitivity mediated by alterations of the peripheral nociceptors.[25,27] The current study sought to examine mechanisms of binge withdrawal-induced hyperalgesia in humans. Using a mixed between-within-subjects design with naturally occurring alcohol use, the aims of this study were to understand 1) whether young adults with a history of binge drinking showed greater pressure pain muscle sensitivity, neurogenic inflammation, and central sensitization than moderate drinkers which would be exaggerated by withdrawal and 2) whether greater pain and inflammation would be associated with baseline epinephrine levels.

Individuals tested during the withdrawal state reported reduced pressure pain threshold, indicating increased muscle pain sensitivity, when compared to testing during the abstain state. In addition, participants tested during withdraw reported reduced cutaneous thermal pain sensitivity. These effects of withdrawal were observed in both moderate and binge drinkers and the effect of withdrawal was not intensified in binge drinkers. Importantly, this pattern was observed even though binge drinkers reported typically drinking more, drinking 4 drinks for women and 5 drinks for men, greater

symptoms of withdrawal and alcohol use disorders, higher perceived stressed, and heightened preoccupation with the rewarding aspects of alcohol.

The study was unable to test the second aim that increased pain was associated with increased epinephrine and IL-6.

Mechanical Muscle Hyperalgesia During Alcohol Withdrawal

We found the general phenomenon of withdrawal-induced pressure pain muscle hyperalgesia in both binge and moderate drinkers. This is consistent with previous findings of withdrawal-induced hyperalgesia in an animal model[25,27] and in human binge drinkers.[114]

The current results differ in magnitude from a previous study that found significantly reduced muscle pressure pain threshold in binge drinkers compared to moderate drinkers that was exacerbated by withdrawal.[114] Differences in study design may explain the results. You[114] used a larger sample size (n = 23-50) with greater acute hangover symptoms (binge withdrawal group M = 1.13) than used in the current study (n = 13-16; binge withdrawal state M = 0.83). In the current study, our sample of binge drinkers was older (You et al.[114] approximate M_{age} = 19 years; current study M_{age} = 22 years) and started drinking later (You et al.[114] approximate M_{age} = 16-17 years; current study M_{age} = 18.03years) than in the previous study where You et al.[114] prescreened and recruited participants from Introductory Psychology classes who were required to participate in the subject pool. It is possible that more severe binge drinkers were harder to recruit in the present study because they are more likely to drop out of college and less likely to sign up for studies when they are not required. Moreover, the current sample

may represent higher functioning binge drinkers because they self-selected to participate in an experiment that necessitated two visits each lasting 3.5 hours. In contrast, You et al.[114] used a between-subjects design that required only a single visit lasting 1.5 hours. Despite differences in the magnitude of withdrawal symptoms and in the study design, we still found a selective withdrawal-induced mechanical hyperalgesia using a mixed between-within-subjects design. The trend toward a main effect of group is consistent with You et al.[114] and suggests that mechanical muscle hyperalgesia may be an effective early indicator of abnormalities in pain processing.

Cutaneous Thermal Hypoalgesia During Alcohol Withdrawal

Although we did not find the expected cutaneous thermal hyperalgesia and temporal summation, our results are consistent with the general phenomenon of alcohol administration and withdrawal-induced cutaneous thermal hypoalgesia found in animal models.[17,47,50] Interestingly, by including both mechanical and thermal pain assessments in the same study, we found mechanical muscle hyperalgesia and thermal cutaneous hypoalgesia. Exploratory analyses suggest these effects may be driven by the male participants. Differential effects of mechanical and thermal stimuli were found in the amygdala in an animal model of diabetic peripheral neuropathy[72] and in humans with traumatic neuropathic pain.[41] There are several testable explanations that may clarify these divergent effects of withdrawal.

One possible explanation is derived from the peripheral mechanisms found in an animal model of withdrawal-induced mechanical hyperalgesia. Using this model of alcohol-induced peripheral neuropathy, researchers found mechanical hyperalgesia was

mediated by sensitization of C-fibers.[22] In the early stages of alcohol use, the mechano-sensitive subtype of C-fibers may mediate mechanical hyperalgesia. With increasing use of alcohol, the immune system may become dysregulated resulting in a pro-inflammatory state.[21,55] In some individuals, an alcohol-induced increase in inflammation and neuropathic damage resulting from early-stages of alcohol-induced peripheral neuropathy may lead to mechano-sensitive C-fibers becoming more sensitized to thermal stimuli.[89] This would suggest mechanical hyperalgesia in early stages of alcohol use and mechanical and thermal hyperalgesia in latter stages of alcohol use. Indeed, no difference in thermal sensitivity was found in young adults with a short history of alcohol use[114] but increased thermal hyperalgesia was found in older adults undergoing treatment for alcohol use.[49] This explanation is speculative and would need electrophysiological studies in animals to determine nociceptor subtype(s) affected by alcohol use. However, this does not explain the diverging effects of mechanical and thermal sensitivity we found in the current study.

A second explanation may be the type of skin at each location. Pressure pain testing was conducted on hairy skin of the dorsal aspect of the foot while thermal temporal summation of second pain was conducted on glabrous skin (i.e., relatively absent of hair) of the palmar aspect of the hand. Previous research has shown that glabrous skin has an increased thermal pain threshold, and may therefore be less sensitive to painful stimuli, than hairy skin.[43,97] Since we tested thermal temporal summation of second pain on the glabrous palm and found reduced pain (hypoalgesia) and tested pressure pain on the hairy skin of the foot and found hyperalgesia, our

divergent results may be due to pain sensitivity differences related to glabrous and hairy skin types. Future studies should control for different skin types or parametrically study alcohol withdrawal-induced differences in pain sensitivity using different body locations.

A third explanation may be the result of different temporal dynamics of muscle and cutaneous pain during alcohol withdrawal. Peripheral neuropathies, including alcohol-induced peripheral neuropathy, are partially characterized by cutaneous pain with more recent evidence suggesting alterations in muscle pain.[3] Hand-held algometers, such as the one used in our study, are pressed into the muscle to activate nociceptors and is largely used to assess sensitivity of the muscle, while thermal devices such as the thermode the participant's hand laid on top of in our study, are used to assess more cutaneous sensitivity. Importantly, mechanical hyperalgesia of muscles and thermal hypoalgesia of cutaneous skin have been shown in trauma-induced neuropathic pain.[41,62] One animal model of alcohol withdrawal-induced peripheral neuropathy compared the time-course of mechanical cutaneous and muscle pain thresholds.[3] Mechanical cutaneous hyperalgesia was evident by day 8 of the alcohol withdrawal protocol while mechanical muscle hyperalgesia was delayed approximately one week until day 15.[3] There was also a non-significant mechanical muscle hypoalgesia at day 5, immediately following cessation of the first alcohol use cycle.[3] Evidence that neuropathies are associated with hyperalgesia and hypoalgesia along with the different temporal dynamics of muscle and cutaneous sensitivity in an animal model of alcohol withdrawal-induced peripheral neuropathy, suggest that the divergent effects we found

on mechanical (muscle) and thermal (cutaneous) pain may be due to different sensitivities of different types of pain sensitivity over the course of alcohol withdrawal.

When female and male participants were analyzed separately, males showed muscle mechanical hyperalgesia and multiple indices of cutaneous thermal hypoalgesia while women in withdrawal showed more intense neurogenic flare. Since the current study is underpowered for these exploratory analyses, these novel findings of gender need to be replicated in larger samples of women and men.

Cutaneous Thermal Central Sensitization not Affected by Alcohol

Similar to previous research in young binge drinkers with a short history of alcohol consumption that measured central sensitization using topical capsaicin-induced area of secondary hyperalgesia,[114] we did not find enhanced temporal summation which would have reflected central sensitization. However, we did find a trend towards a State x Time interaction over the first five pulses during withdrawal with sensitization of temporal summation increasing over the five pulses, while during abstinence sensitization plateaued after the third pulse. We also observed a visual trend towards binge drinkers reporting enhanced temporal summation during both abstinence and withdrawal states over all 10 cutaneous thermal pulses relative to moderate drinkers. However, these trends failed to reach significance, perhaps due to low statistical power. Previous research from our laboratory using a model of adversity-induced hyperalgesia, whose mechanisms are similar to alcohol withdrawal-induced hyperalgesia,[53,54] found enhanced temporal summation with a large sample size (n = 51-75 per group).[113] In contrast, we did not find a difference when we used a smaller sample size (n = 15 per

group),[112] though we did find the same general trend as the larger study. Given the trend we found in our data for binge drinkers and drinkers in withdrawal reporting enhanced temporal summation, a larger sample size may have increased our power to find an effect.

While our results in conjunction with previous studies suggest alcohol use may not uncover central sensitization using capsaicin or thermal modalities, the consistent finding of mechanical hyperalgesia suggests central sensitization may be modality-specific and be uncovered using mechanical temporal summation.[68] A third explanation is the possibility that mechanical hyperalgesia may be mediated by more peripheral mechanisms, while thermal hypoalgesia may be mediated by more central mechanisms. Whether this central sensitization is dependent on or independent of increased afferent barrages from peripheral sensitization, binge withdrawal may sensitize other levels of the central nervous system. Examples of sensitization in brain areas in neuropathic pain can be found in an animal model of arthritis that shows sensitization in the amygdala[72] and in an animal model of diabetic peripheral neuropathy that shows sensitization in the thalamus.[30]

Based on the progression of alcohol-induced peripheral neuropathy, it is possible we might have found significant sensitization of temporal summation of second pain in a different body location in binge drinkers. Alcohol-induced peripheral neuropathy presents in chronic alcohol users and is characterized by a distal axonopathy where axons in the distal extremities, particularly the lower extremities and to a lesser extent the upper extremities, die back.[57] Therefore, the feet may show increased sensitivity to

early signs of peripheral neuropathy than the hands. Alcohol-induced peripheral neuropathy has been shown in adults in their 20s and older, though increasing age and longer duration of alcohol abuse were associated with having neuropathic symptoms.[100,115] Based on prior work in alcohol withdrawal-induced mechanical hyperalgesia in animal models,[3,25,27] early neuropathic changes may be present in young adult binge drinkers. Therefore, in light of the progression of alcohol-induced peripheral neuropathy and animal models of withdrawal-induced hyperalgesia after short term alcohol use, it is possible we may have observed enhanced cutaneous thermal temporal summation of second pain in binge drinkers if we tested on the foot, as we did with muscle mechanical pain testing.

We did find decreased aftersensations over time in the withdrawal state compared to the abstinence state. A more rapid return to baseline during the withdrawal state corroborates the thermal hypoalgesia we found in the more static measures of thermal sensitivity.

Capsaicin-Induced Neurogenic Inflammation and Spontaneous Pain not Affected by Alcohol

Previous research showed binge drinkers had an increased area of flare during withdrawal while moderate drinkers showed a decreased area of flare during withdrawal.[114] That the current study showed no difference may be due to less acute hangover symptoms compared to the previous study. This suggests neurogenic inflammation may be related to a combination of drinking history and subjective acute withdrawal symptoms.

Issues in Translating Animal Research to Humans

Animal studies have typically used either quickly acting intraperitoneal administration of alcohol[17,32,47,50,70,75] or a 6.5% alcohol diet[27,35] with the latter resulting in a blood alcohol content approximately 3.84 times higher than the legal limit in the U.S. (307.7mg/dL in animals compared to 80mg/dL in humans). In the current study, individuals were unlikely to have achieved levels similar to the animal models and therefore the levels of drinking in the animal studies may have had stronger effects on pain sensitivity and may therefore follow a different time course of intoxication and withdrawal that does not directly translate to humans.

Furthermore, animal studies have found effects without typically following the definition of binge drinking used by the NIAAA (0.08% BAC or 4 or 5 drinks [i.e., 4/5] in approximately 2 hours for women and men, respectively) and other levels of drinking. That effects of withdrawal on pain sensitivity can be found regardless of NIAAA levels of binge drinking is supported by the rationale behind the binge drinking definition. The 0.08% BAC was determined by the resulting cognitive and motor impairments that hinder driving ability.[82] Even this distinction of 0.08% BAC is flexible as a recent publication from the U.S. National Transportation Safety Board recommended lowering the legal BAC limit in the US to 0.05%.[46] Indeed, previous research in humans using experimental administration of alcohol to reach 0.08% BAC was insufficient to uncover hyperalgesia or enhanced central sensitization.[114] While the current 0.08% or 4/5 drink definition of binge drinking represents important cognitive and motor impairments and is effective for establishing categories for laws and research, it may not inherently

represent a threshold for pain sensitivity. Future studies would need to determine such a threshold or if continuous measures of alcohol are sufficient.

Clinical Implications

The current study found mechanical hyperalgesia and thermal hypoalgesia in the withdrawal state of alcohol consumption regardless of drinking category. This suggests that clinicians may want to focus on withdrawal from a continuum of alcohol consumption. This pain phenotype may also be a relatively quick way for doctors to have a quantitative measure for individuals at risk for alcohol withdrawal-induced hyperalgesia and further increases in substance use by potentially comparing pain ratings to normative values (e.g., Magerl et al.[64]).

Limitations

While we found novel mechanical hyperalgesia and thermal hypoalgesia in humans using a mixed between-within-subject design, the time-course of withdrawal experienced by our participants may have limited our ability to see the hypothesized effects.

The binge drinkers in our study showed greater symptoms of alcohol use (higher AUDIT scores, more drinking, and more typical hangover symptoms), however the lack of difference in acute hangover symptoms in binge drinkers compared to moderate drinkers may have limited our statistical power in finding group differences or Group x State interactions in mechanical hyperalgesia, neurogenic flare, epinephrine, and IL-6.

In our study, participants arrived first in the control state of abstinence and in a separate visit were in the experimental state of withdrawal. This may have resulted in

participants being more anxious during the first state (abstinence) resulting in stress-induced hyperalgesia and more relaxed during the second state (withdrawal). Future studies should run a counter-balanced design to determine if order of visits has an effect on alcohol withdrawal-induced pain sensitivity.

Future Directions

While our study helps advance the literature on alcohol and binge withdrawal-induced hyperalgesia in humans, it also suggests future studies. The divergent effects of muscle mechanical hyperalgesia and cutaneous thermal hypoalgesia as well as self-report and psychophysiological measures of affect and arousal may be the result of different time courses for alcohol withdrawal's effects. As previously discussed, muscle mechanical sensitivity and cutaneous thermal sensitivity may be dependent on the time course of withdrawal. We also found positive self-report potentially resulting from a decreasing "a" process and increased psychophysiological arousal potentially from an increasing "b" process. It is possible that self-report negative affect may emerge at a later time point in withdrawal than we assessed. Therefore, future studies should parametrically investigate the time course of alcohol use and withdrawal on different measures of pain, affect, and arousal.

Future studies should also systematically vary the level of severity of alcohol use and age participants began drinking along with their drinking history. In younger individuals or those with a short drinking history, a longitudinal study should be conducted to determine how their alcohol use and drinking history predict their risk of developing increased pain sensitivity and alcohol use disorders. One particular subset of

individuals that should be included in separate analyses is people with clinical alcohol use disorders showing withdrawal-related symptoms.

Differences in opioidergic function, particularly of the mu opioid subtype, should be investigated. Previous research in humans showed greater binding of endogenous endorphins to mu opioid receptors in the nucleus accumbens and orbitofrontal cortex was related to greater subjective pleasure.[69] More pleasure during intoxication may help drive the initial stages of alcohol use. In addition, research has shown that the presence of a single nucleotide polymorphism in the opioid receptor mu 1 (OPRM1) gene associated with a decreased response to opioids predicted decreased pain in women following sexual assault[6] or a motor vehicle accident in women with peritraumatic distress.[63] This effect is sexually dimorphic as men with the same polymorphism showed increased pain following a motor vehicle accident.[63] Future studies should include measures of opioid response in individuals with varieties of drinking history as this may not only affect the initial stages of alcohol use but the development of alcohol withdrawal-induced hyperalgesia.

Summary

The current study used a mixed between- and within-subjects design comparing moderate drinkers and binge drinkers during the abstinence and withdrawal states of alcohol use. Binge drinkers reported more typical alcohol use and more alcohol use prior to the withdrawal state as well as reporting more typical hangover symptoms and alcohol use disorder symptoms. We found participants in the withdrawal state reported muscle mechanical hyperalgesia and cutaneous thermal hypoalgesia when compared to

the abstain visit, regardless of drinking group. Participants in the withdrawal state also reported more dominance, less anxiety, and less negative affect but greater cardiovascular and respiratory activity than when they were in the abstinence state. Several hypotheses for future research were presented along with recommendations for future studies. This research suggests the divergent effects of alcohol on pain sensitivity may depend on the specific modality being tested. This research suggests that the revised quote, 'Here's to alcohol: the cause of, and answer to, one's pain' is more complicated.

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