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Investigating the impact of chronic inflammatory pain on ethanol consumption in mouse models.

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Investigating the impact of chronic inflammatory pain on ethanol consumption in mouse models.

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List of Abbreviations and Symbols

2-BC	two-bottle choice
ANOVA	analysis of variance
AUD	Alcohol Use Disorders
BAC	Blood Alcohol Concentration
CFA	Complete Freund's Adjuvant
COMT	catechol-*O*-methyl-transferase
HPA	Hypothalamic-Pituitary-Adrenal (axis)
i.p.	Intraperitoneal
KOR	Kappa Opioid Receptor
mPFC	medial Prefrontal Cortex
NAc	Nucleus Accumbens
NIAAA	National Institute on Alcohol Abuse and Alcoholism
norBNI	norbinaltorphimine
SEM	standard error of the mean
SN	substantia nigra
VTA	Ventral Tegmental Area
WHO	World Health Organization

Abstract

Chronic pain affects nearly 20% of Americans annually and for many alcohol provides a convenient and legally acquired palliative. Population-based studies have suggested a link between increased alcohol use and reduced pain, but chronic or excessive alcohol use potentiates the risk to develop alcohol dependence and neuropathy. However, sex differences in chronic pain and alcohol abuse are still not well understood. In the present study, we investigated whether chronic inflammatory pain using the inflammatory agent Complete Freund's Adjuvant (CFA) model could alter ethanol consumption in male and female C57BL/6J mice. We observed that only CFA-treated male mice increased their ethanol intake. This increase was completely blunted by prior administration of the KOR antagonist norbinaltorphimine (norBNI) to male mice. To expand on our model of inflammatory chronic pain we repeated our experiments using paciltaxel to induce chronic neuropathy. In contrast to the CFA results, the paclitaxel study showed decreased ethanol intake in male mice without affecting female intake. Finally, we explored the impact of prior ethanol exposure on ethanol intake in our CFA paradigm. CFA-treated female but not male mice with a prior history of ethanol consumption increased their ethanol intake and preference relative to their vehicle-treated counterparts.

Chapter 1 - Introduction

1.1 Background and Significance

1.1.1. Alcohol consumption and Alcohol Use Disorders (AUD)

Alcohol has been consumed by humans for millennia for social, spiritual, and nutritional reasons. The “drunken monkey” theory proposes that hominids' first exposure to ethanol was from rotting fruit on the forest floor. In the past 6000 or so years, ethanol has been fermented intentionally by humanity[1], and many are able to consume in moderation. However, for a percentage of alcohol consumers, the drinking behavior can become harmful and is described in the literature as Alcohol Use Disorder (AUD). There are a number of social, environmental, and neurobiological conditions that ultimately affect ethanol drinking behavior. Understanding the factors that predispose an individual to developing AUD allows researchers and providers to create better treatments to manage and prevent the development of AUD and other pathologies related to excess alcohol consumption.

The National Institute on Alcohol Abuse and Alcoholism (NIAAA) describes AUD as follows, “Alcohol use disorder (AUD) is characterized by an impaired ability to stop or control alcohol use despite adverse social, occupational, or health consequences.”[2] Utilizing this definition, the prevalence of AUD in the United States is high with a 2022 SAMHSA study that 29.5 million Americans meet the criteria for an AUD diagnosis[3] (compared to a US population of 334,914,895[4]) suggesting that 11.5% of Americans are currently impacted. A study from 2017 reported that one-third of US adults could be diagnosed with AUD at some point in their life.[5] This

translates to over 88,000 deaths a year in the United States attributed to alcohol, ranking the fourth leading cause of preventable death.[6] This isn't limited to the United States; AUD is considered a leading cause of mortality and morbidity globally with over 3 million deaths annually attributed to alcohol consumption; this is approximately 5% of the global burden of disease.[7] Prior to the COVID-19 pandemic there was a gradual annual increase in AUD prevalence. In the years since, this effect has been intensified with an increase in US liquor sales increasing from 7.1 billion in 2019 to $9.5 billion in 2020.[8] This increase accounts for a 34% increase in US alcohol sales in 2020 compared to the same period in 2019. Only a 22% increase in retail alcohol sales was estimated due to the closure of bars and restaurants during the pandemic.[9] The percentage of the global population that consumes alcohol is not evenly distributed with the highest percentage of persons consuming ethanol found in the European Region and the Regions of the Americas as defined by the World Health Organization (WHO). This same WHO report estimated that some 283 million people aged over the age of 15 met the criteria for a diagnosis of AUD (representing 5.1% of adults).[10]

1.1.2 Neurobiology of Alcohol

The mesolimbic pathway consists of dopaminergic neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), amygdala, and hippocampus. This pathway is key to encoding the reward value of a stimulus. Here we need to distinguish the characteristics and relevance of phasic versus tonic dopamine (DA) release. As their name suggests, tonic dopamine releases are slower with lower overall levels of dopamine release. These releases are triggered by glutamate binding. Phasic dopamine release is characterized by a very quick release of high levels of dopamine into the synapse. D2 autoreceptors located on DA

releasing cells inhibit further dopamine release and trigger dopamine reuptake transporters to reduce the concentration of DA within the synapse. The tonic level of dopamine can be used as an estimate for the amount of reward expected from a given stimuli. Calculation of phasic dopamine levels is the difference between the received and expected value of a stimulus. In this paradigm, the phasic levels are an estimate of the net value "reward" of the given stimulus.[11] The limbic system and medial prefrontal cortex (mPFC) process the value of this reward[12] and attach it to the external context[13].

Relevant to this study is the differing ways that dopamine levels respond to alcohol in the nondependent vs the chronic user. Extracellular dopamine levels are the controlling factor in tonic levels of dopamine. Episodes of acute alcohol consumption in the nondependent user increases phasic dopamine release with little to no impact on tonic dopamine levels.[14] However in patients with chronic alcohol exposure, decreases are seen in phasic levels and increases are seen in tonic levels. Ford attributes this to complete saturation of the D2-autoreceptors located on the presynaptic cell, overloading the neuron's reuptake process.[15] Some have hypothesized that this process is the mechanism behind the tolerance in alcohol dependent users.[16]

1.1.3 Opponent Process Theory

Development of AUD can, in part, be explained by the *opponent process theory of motivation* as the equilibrium between affective states maintained by opposing systems within the Central Nervous System (CNS)[17]. Within a model of AUD this can be described as the shift in subject motivation to drink from positive to negative reinforcement.[18] This theory describes two distinct response periods to the presentation of a novel drug in a nondependent user. The "a-process" is a rewarding state characterized by a quick on and offset closely tied temporally to the

act of drug taking. This may encourage continued or repeated consumption in naive users. The "b-process" follows the a-process and is characterized as a stressful "antirewarding state". The b-process has a prolonged onset and offset compared to the a-process. In the drug naive user, there is a direct connection between drug dose and stimulus intensity. Dysregulation of the reward and stress systems is seen in users after repeated drug exposure. Now greater amounts of the drug are needed to induce the a-process and its duration and intensity will be curtailed. As dysregulation continues the b-process experiences an increase in intensity, duration and sensitivity. There is a lowering stress threshold for a given dose precipitating withdrawal.[19] The b-process becomes dominant in response to chronic drug exposure and continued consumption is incentivized through negative reinforcement i.e., temporary relief of the negative affective state. The changing of these set points for stress and reward within the CNS is known as allostasis.

1.2 Chronic Pain

Chronic pain is defined as pain lasting longer than 3 months and affects an estimated 20% of Americans[20]. The distribution of chronic pain is more consistent across the globe compared to the distribution of AUD with similar rates found in other regions globally.[21] Chronic pain impacts patients in every aspect of their life, limiting their ability to work or interact with society, and even perform activities of daily life (ADLs). The societal cost cannot be fully described monetarily, but a 2012 report estimated that $300 billion dollars was incurred as US healthcare costs due to chronic pain. When taking into account workplace productivity losses due to pain, the total was estimated at $600 billion annually for the United States.[22] It should also be noted that the 2012 study does not include military personnel, and patients living in nursing homes or persons incarcerated by the state; all three of these populations are likely to represent patients living with chronic pain and

those costs remain unaccounted for by this study. The general terminology of chronic pain encapsulates a wide range of diseases, disorders, and traumas that elicit persistent or recurrent episodes of nociceptive pain. While each chronic pain syndrome is complex and possesses its own unique taxonomy, there some shared etiologies that contribute to their pathological features[23,24,25]. There are a number of risk factors and health and lifestyle conditions that can precipitate chronic pain, some of the most common include nerve compression and injury, diabetes mellitus, autoimmune disorders, and exposure to toxic substances like chemotherapeutics and chronic alcohol.

The International Association for the Study of Pain (IASP) first published their definition of "pain" in 1979. In 2018, an IASP working group issued an updated definition, "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage,".[26] This definition of pain recognizes that while an injury usually precedes the onset of pain, it is not a prerequisite. Pain is not merely a neurological issue but also represents a psychological insult. This can present some challenges to the use of a rodent model in examining pain. If pain is partially a subjective emotional experience, how can we measure that in a mouse? Within rodent models of pain, several terms are used to more precisely describe the behavior and mechanisms experienced by the rodents. Nociception is the term for the transmission of noxious signals to the brain. Allodynia and hyperalgesia are two additional concepts we use to discuss nociception. Allodynia is defined as a pain response to stimuli not normally considered noxious. Hyperalgesia is the increased pain response to a noxious stimulus relative to their baseline. In this study, thermal (cold) allodynia is measured utilizing the acetone test, while mechanical hyperalgesia is measured using Von Frey filaments.

1.2.1 Overview of Nociception

More than a minor annoyance, pain serves an important protective function in organisms experiencing tissue insult. Pain can alert an organism to an external stimulus or internal sign motivating a change in behavior to abate the pain state. However the benefits of pain are limited and begin to take a negative toll on the physical and mental well-being of organisms in this state. Nociception is defined as the neural process of encoding a noxious stimulus when it is detected by the specialized nerve fibers within the peripheral nervous system referred to as nociceptors.[27] The cell bodies of nociceptors are located in the dorsal root ganglia (DRG) of the spinal cord and in the tissue from which they receive stimuli. Whether the initial insult is mechanical, thermal, or chemical, the stimulus is encoded into a sensory potential (voltage). Stimuli that are below the firing threshold of the neuron will not activate the nociceptive pathway. An action potential will be triggered once a large enough amplitude potential is generated at the axon hillock.[28] The action potential is conducted by primary afferent nerve fibers to the dorsal horn of the spinal cord, conveying this nociceptive signal. The transmission continues with projection neurons from the dorsal horn to the somatosensory cortex of the parietal lobe. The somatosensory cortex is then responsible for encoding the intensity and quality of the pain. The conscious perception of pain therein arises from further projections to the amygdala, thalamus, hypothalamus, and the brainstem. A process known as neurogenic inflammation is also possible; nociceptors may release peptides such as substance P or calcitonin gene-related peptide (CGRP).This second messenger release results in local vasodilation and plasma extravasation thereby attracting macrophages and encouraging the degranulation of mast cells.[29]

1.2.2 Pain Chronification

Inflammation is a common mechanism of injury and disease. Inflammatory pain conditions represent the most common cause of chronic pain and arthritis is the greatest single cause of disability in adults.[30] Adults with chronic pain also report higher frequency of alcohol consumption relative to the general population.[31]

Pain chronification is the term given to the transition from acute to chronic pain. A model proposed by Borsook defines pain chronification in terms of "reward deficiency and antireward"[32]. This model shares many features with the previously discussed model for the development of AUD. Acute pain is proposed to active neural circuits involved in stress and aversion. This is followed by activation of the corresponding reward circuit when the pain is discontinued. Much like the AUD model, repeated (chronic) exposure to the negative state can lead to dysregulation of the reward/stress system. This can result in hypersensitization of the negative affective state; which could be construed as allodynia and hyperalgesia. Conversely, anhedonia may result from the hyposensitivity to the reward system after chronic pain episodes. As this reward pathway becomes desensitized, pharmacological interventions (analgesics) may be needed to relieve pain in the absence of the endogenous pathway. This feed-forward loop disrupts the balance between reward and stress pathways.

1.2.3 Dopamine Activity during pain states

Research has shown that pain can change neuronal signaling within the Mesocorticolimbic System (MCLS). In response to pain, dopamine signaling is altered within the MCLS resulting in changes to motivation and patterns of drug use.[33] Chronic pain patients exhibit lower levels of D2-receptor binding and lower activity at the presynaptic site. This hypodopaminergic state is involved

in the reduced drive without necessarily affecting the hedonic value of a stimulus.[34] There is even evidence that there are neuroanatomical separations between these DA signaling pathways. Neurons implicated in coding salience are localized to the dorsolateral substantia nigra (SN) projecting to the NAc core while neurons that code motivational valence are more centered around the ventromedial SN and the lateral VTA.

Although most DA neurons are depressed during nociceptive events, 5% to 15% of dopaminergic neurons within the VTA fire preferentially for aversive stimuli, or for both aversive and rewarding stimuli.[35] These neurons may be responsible for the DA release after aversive stimuli such as pain. The heterogeneous nature of these DA neurons suggests that they serve a role in responding to aversive and rewarding stimuli. Within motivation theory is the concept of a stimulus' valence and salience. Salience refers to how relevant or emotionally striking a stimulus is. Valence refers to the observer's relationship to the stimulus; a stimulus with a positive valence might encourage approach behavior while a stimulus of a negative valence would support avoidance behavior. The differential activation of the DA neurons within the VTA are theorized to code a given stimulus with its salience and valence.[36] Groups of neurons activated in the presence of a reward and inhibited by punishment would be useful in coding motivational valence of a stimulus. Neurons activated by both punishing and rewarding stimuli are better adapted to coding motivational salience.

Alcohol remains an accessible means for patients to self-medicate their chronic and acute pain. Accordingly, some studies estimate 25% of chronic pain patients report consuming alcoholic beverages for pain relief.[37] Consuming alcohol even provides mild anesthetic qualities in acute settings and drinking to help with the stress of chronic pain may encourage chronic pain patients to imbibe more frequently. This despite the existence of evidence that increased frequency of

drinking can lead to addiction and significant health risks. Additionally, in higher quantities, abstinence from alcohol consumption can precipitate a withdrawal syndrome. This withdrawal syndrome has the potential to exacerbate on-going nociceptive hypersensitivity. It is possible that the recurrent urge to drink in chronic pain patients is in part to relieve withdrawal-induced or increased nociceptive hypersensitivity. Withdrawal syndromes can precipitate pain on their own. In addition, chronic alcohol consumption can produce negative effects on the central and peripheral nervous systems. One of the most common side effects with chronic AUD patients is alcohol neuropathy. This commonly presents with pain, paresthesias, and ataxia in the distal lower extremities. Several studies employing clinical and electro-diagnostic criteria have estimated that in the US the prevalence of neuropathy is present in 25–66% of defined 'chronic alcoholics'.[3839]

1.2.4 The Inflammatory Response

Inflammation is the result of the body's defense mechanisms against various injuries and pathogens. There are five hallmark physiological signs of inflammation: heat (calor), swelling (tumor), redness (rubor), pain (dolor), and loss of function (functio laesa). We divide the time course of inflammation into several stages based on duration. Acute inflammation describes inflammation immediately following the injury lasting up to a few days, subacute inflammation may last from 2 to 6 weeks, with chronic inflammation extending to months or even years.[40] Acute inflammation involves the migration of neutrophils and cytokines, mediated by cytokines, acute phase proteins, and chemokines, from the bloodstream to the affected tissue. If the inflammation remains unresolved after 6 weeks T lymphocytes and plasma cells will also migrate to the affected tissue marking the transition to chronic inflammation. Tissue damage and necrosis can result if the inflammation continues. "Inducers" are the initiating molecules in our inflammation signaling

pathway that are detected by "sensors", and acted on by effector molecules regulated by mediators. There are two general types of inducers: exogenous and endogenous with further subdivisions therein. Previous work in this lab has used various methods of inducing inflammation as their treatment, and the time course of recovery is partially dependent on the nature of the initial insult.[41]

1.3 Comorbidity of AUD and Chronic Pain

There is a strong correlation between chronic pain and alcohol consumption, but the direction of the correlation is less clear; reflecting the complex interaction between pain, alcohol use, and the brain. There is emerging evidence that both these conditions share key neural substrates. Therefore, the comorbidity of alcohol use and chronic pain likely arises from a series of neurological and psychosocial pressures.[42] The existence of a third (or more) variables that prejudice a patient to develop either one or both conditions cannot be ruled out. Previous research has largely focused on either condition in isolation. Since 2012, work by Mark Egli and others have begun to synthesize our understanding of these conditions through the shared neural pathways and genetic factors underlying the stress and reward systems.[43]

Accurately estimating the base rates of alcohol use co-occurring with chronic pain proves difficult for a few key reasons. There exist variations in how chronic pain and alcohol use or abuse is defined across various studies and state health organizations.[44] Despite this, one study from 2007 found that in a study of patients seeking treatment for a substance use disorder, 73% of patients that identified alcohol as their primary drug of choice also endorsed experiencing moderate-to-severe pain within the previous month.[45]

1.3.1 Hypothalamus-Pituitary-Adrenal (HPA) and Stress

There is considerable evidence that key neural mechanisms are shared in the development of chronic pain and AUD including the aforementioned dopaminergic release in the NAc. Adaptations in the body's primary stress management system, the hypothalamus-pituitary-adrenal axis (HPA), precipitates the development of pain chronification and AUD through the interactions of cytokines and glucocorticoids. Corticotropin-releasing factor (CRF) is produced in the paraventricular nucleus (PVN) of the hypothalamus and released in response to norepinephrine and glutamate. GABAergic inputs onto CRF releasing neurons are primarily inhibitory in nature. CRF release acts synergistically with vasopressin (AVP) and stimulates the release of adrenocorticotropic hormone (ACTH). Release of the main stress hormones corticosterone and cortisol is stimulated by ACTH release.[46] Activation of Corticotropin-releasing factor receptor 1 (CRF1 receptor) in the amygdala and hypothalamus is associated with stress and hyperalgesia during alcohol withdrawal.[47] These conditions support the development of AUD through negative reinforcement; reintroducing alcohol alleviates the anxiety and pain of withdrawal. Release of CRF1 in the amygdala has been correlated with enhanced nociception[48]; this plays a role in the development of hyperalgesia.[49] Dynorphins are the endogenous ligands for kappa opioid receptors, their activation inhibits dopamine release[50] and increases the expression of the CRF1 receptor.[51] Dopamine release is further truncated by KOR hypersensitivity in the NAc secondary to alcohol exposure.[52] Dynorphins also trigger the release of bradykinin which is correlated with inflammation and hyperalgesia.[53]

Dynophins and the KORs play a central role in moderating stress, pain, and addiction behaviors. To investigate the role of kappa Opioid Receptors (KORs) in our paradigm we used the selective and potent Kappa antagonist, norbinaltorphimine (norBNI).[54]

1.3.2 Role of sex in alcohol dependence and chronic pain

Clinical and epidemiological studies have indicated sex differences in how humans consume ethanol, and these differences are also seen in response to chronic pain. Despite a growing body of research in both clinical and preclinical models, there remain countless questions. A recent study from our lab in 2022 reported an increase in voluntary ethanol consumption in male mice following an acute surgical injury. Recent preclinical studies have also demonstrated an increase in voluntary ethanol consumption in response to chronic inflammation and neuropathy in male mice.[555657] For example, a study of patients living with chronic pain demonstrated that male patients reported alcohol consumption at a greater rate than female patients in the same study.[58] However female patients that consume alcohol on a habitual basis are more likely to develop pathological pain.[59] A study of patients with jaw/facial, joint, or arthritis reported that the use of alcohol for pain self-management was higher in younger patients, specifically non-Hispanic whites.[60] Longer-term population wide studies of alcohol consumption also support this with men demonstrating a greater frequency of drinking and high-volume drinking than women of the same age and national background.[61]

1.4 Animal Models of Ethanol consumption and chronic pain

Rodents such as rats and mice are the most widely used animal model in biomedical research worldwide.[62] Their ubiquity is due to several factors, like *drosophila m.* used in genetic research, rodents offer ease of maintenance and husbandry with relatively short gestation periods. Combined with rodent's anatomical, physiological, and genetic similarities to humans makes them a powerful tool in examining behavioral and biochemical interrelations. Rodent models of ethanol and nociception have largely focused on intravenous (i.v.) or intraperitoneal (i.p.) administration of ethanol. While these routes are effective in producing ethanol's sedative and antinociceptive

effects they may not effectively model human alcohol drinking behavior. An oral model of administration is necessary to address pharmacokinetic and pharmacodynamic factors of enteral administration. As prey creatures, mice present certain obstacles to assessing their pain through behavioral presentation. Mice attempt to conceal signs of pain from observation (i.e. abnormal posture) to seem less attractive to potential predators.[63] Despite the fact that our study is only grounded in evoked pain behaviors, further work is needed in non-evoked pain behaviors in mice.

Prior work by Neddendriep et al. in our lab investigated if ethanol can induce antinociceptive effects in a chronic inflammatory pain model (CFA model) in C57BL/6J male and female mice. We found that at the dose of 1.25 g/kg ethanol given orally by gavage induced significant antinociceptive effects by reversing mechanical hypersensitivity without producing a sedative effect. The study found that the blood alcohol concentration (BAC) remained stable for four hours while ethanol's antinociceptive effects rapidly declined two hours after administration. This reduction in behavioral responses despite a constant BAC suggests that the animals develop a tolerance to ethanol.[64] This comports with previous literature that describes the development of acute functional tolerance to ethanol's sedative and functional effects. Increased preference for ethanol is consistent with human experimental evidence[65] that pain can be a significant motivator for seeking addictive substances.

1.5 Thesis goals, objectives, and hypothesis

The overall goal of this thesis is to use animal models to explore the relationship between ethanol intake and chronic pain in the hope of contributing new findings that may lead to the discovery of novel therapeutic targets that may improve the treatment and management of chronic pain in relation to alcohol use. To achieve this, we characterized the time course of pain-like behaviors for C57BL/6J male and female mice after chronic inflammatory pain and neuropathic

injury by testing and monitoring their behavior through evoked pain behavioral assays. Additionally, we evaluated if these chronic pain states alter drinking behavior in mice.

Based on previous literature, we hypothesized that the presence of a chronic pain condition would exhibit a significant increase in ethanol intake and preference compared to their sham counterparts.

1.6 Aims

The first aim examined the impact of chronic inflammation (CFA model) on ethanol intake and preference using the two-bottle choice tests in naïve male and female C57BL/6J adult mice.

The second aim was to examine the impact of chronic neuropathic pain (Chemotherapy-induced peripheral neuropathy CIPN model) on ethanol intake and preference using the two-bottle choice tests in naïve male and female C57BL/6J adult mice.

The third aim examined the impact of chronic inflammation (CFA model) on ethanol intake and preference using the two-bottle choice tests in male and female C57BL/6J adult mice with a history of ethanol consumption.

1.7 General Materials and Methods

1.7.1 Animals

Male and female adult C57BL/6J mice (25-30 g; 8-10 weeks) were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were housed in a 21°C humidity-controlled Association for Assessment and Accreditation of Laboratory Animal Care (AALAC)-approved animal care facility. They were initially housed in groups of five with *ad libitum* access to food (global 18% protein chow diet; Envigo Teklad, Indianapolis, IN, USA) and water. The rooms were

on a 12-h light/dark cycle (lights on at 6:00 a.m.). Mice were 8–10 weeks of age and weighed approximately 20–25 g at the start of all the experiments. Mice were housed with Teklad corn cob bedding (#7097, Envigo Teklad, Madison, WI, United States) and cages were changed weekly. All experiments were performed during the light cycle (between 6:00 a.m. and 6:00 p.m.) and the study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. All studies were carried out in accordance with the National Institute of Health guide for the Care and Use of Laboratory Animals. Mice arrived at 6 weeks of age and were group-housed for one week to habituate to vivarium conditions. Following habituation, mice were singly housed within identical plexiglas cages and allowed to acclimate for three days prior to the beginning of the experiment.

All mice were observed daily for general well-being and their weight was measured on alternating days. C57BL/6J mice were selected for this study as they are an inbred strain known to have a high ethanol intake and preference.

1.7.2 Two-Bottle Choice (2BC)

To measure individual consumption of ethanol, mice were singly housed during the duration of the experiment with *ad libitum* access to dry food and water except during antinociceptive testing or drug administration. Water and ethanol were available through sipper tubes constructed in-house from 30ml plastic centrifugation tubes fitted with rubber stoppers and sipper tubes containing double ball bearings. Mice were housed out-of-vivarium (OOV) to minimize travel and disruption of the mice during testing sessions. Levels of water and ethanol were recorded at the same time each day with the left versus right position of the tubes being

switched every day to reduce the effect of a left/right side bias in the mouse drinking choice. An empty test cage was set up in the OOV space to measure liquid losses due solely to evaporation or mechanical disturbance of the mouse housing shelves. Ethanol for mouse consumption was prepared as a 20% (v/v) solution from 200 proof USP grade ethanol and tap water prepared freshly every 2-3 days. Due to ethical concerns with inducing pain conditions in living animals, all mice are euthanized at the end of their 15 or 21 day trial, respectively. Under a 24-hr Continuous Access Two-Bottle Choice (CA2BC) model, mice were given constant access to water, ethanol, and dry food. The only exceptions to this protocol were when mice were removed from their cages for weighing, cage changing, and nociceptive testing.

1.7.3 Von Frey testing of mechanical hypersensitivity

Mechanical withdrawal thresholds were determined by von Frey filaments as previously described by Bagdas et al.[66]. Withdrawal thresholds were measured by applying a series of calibrated von-Frey filaments. Von Frey testing was conducted prior to ethanol or CFA exposure to record baseline responses. Subsequent testing was conducted at 3, 7, 14, and 21 days post CFA injection. Mice were allowed to acclimate to this setting for 30 minutes prior to the start of testing. Five mice were under plexiglas cups placed on a steel mesh flooring. Male and female mice were tested on separate benches and using a modified up-down method, in the absence of a paw withdrawal response (paw withdrawn, licking, or shaking) to the initially selected filament, a thicker filament corresponding to a stronger stimulus was presented. Once a paw withdrawal occurred, the next weaker stimulus was chosen. Each hair was presented vertically against the paw,

with sufficient force to cause slight bending, and held for 2 to 3 seconds. A stimulation of the same intensity was applied 3 times at intervals of a few seconds.

Chapter II. Impact of Chronic Inflammation in naive animals on ethanol drinking in mice

2.1 Introduction

Rheumatoid arthritis (RA) and spondyloarthritis are two prevalent examples of degenerative inflammatory arthritic conditions which affect as much as 2% of the world population.[67] One of the most debilitating symptoms of inflammatory arthritis is the onset of severe chronic pain. Managing this chronic pain presents significant barriers to clinicians, and this condition's prevalence is likely to increase with the increase in our geriatric population in the United States. A greater understanding is needed of the mechanisms underlying the transition from peripheral inflammatory signals to alterations in nociceptors and the central nervous system that produce chronic and neuropathic pain. To support this research, a number of inducible and spontaneous models of reproducing inflammatory arthritis in animal models have been developed. This laboratory has opted to use an adjuvant-induced animal model of inflammation, utilizing local injection of Complete Freund's Adjuvant (CFA). The use of an inducible model both reduces the husbandry costs, but more importantly allows for the reproduction of our existing ethanol drinking protocols using C57 mice.[68] To study voluntary ethanol consumption in mice experiencing chronic inflammation, we needed a mouse model where the animals will drink freely without training which can complicate the use of spontaneous arthritis animal models such as K/BxN[69] and TNF-transgenic mice[70]. Section 1.3 of this thesis contains a more robust explanation of the factors underlying the comorbidity of AUD and chronic pain. The goal of these experiments was to study the impact of chronic inflammation using the CFA model on ethanol drinking behaviors and associated evoked pain behaviors in naive mice. We hypothesized that animals injected with CFA

would increase mechanical hypersensitivity (reduced paw withdrawal threshold in von Frey testing) and increased intake and preference for ethanol in sex-specific manner via kappa receptor (KOR) activation.

2.2 Materials and Methods

2.2.1 Animals

Male and female adult (10-12 weeks) C57BL/6J mice were randomly assigned to either the control or treatment group. Mechanical threshold was measured using the Von-Frey test at regular intervals both prior and after ethanol access and CFA administration, as a measure of pain-evoked behaviors.

2.2.2 Complete Freund's Adjuvant

To model inflammatory chronic pain, we used Complete Freund's Adjuvant (CFA) as its effects and time course closely mirror that of persistent injury. Studies have repeatedly used CFA as an experimental model for chronic inflammation and arthritis in rodent models.[71] CFA is an antigen emulsion consisting of heat-dried *Mycobacterium tuberculosis* bacteria.[72] The mycobacteria within CFA contain muramyl dipeptide (N-acetylmuramyl-l-alanyl-d-isoglutamine), a PAMP (pathogen-associated molecular pattern) that activates macrophages and dendritic cells.[73] Injections of 20ul of undiluted CFA (CFA; Sigma-Aldrich, MO, USA) or sterile mineral oil control were injected intraplantar (i.pl) the left or right hindpaws of test subjects 1710 TLL Hamilton microsyringe (Hamilton Company, NV, USA) and a 30½-gauge needle. Mice were restrained using Tailveiner® Restrainer for Mice (Braintree Scientific) during intraplantar injections. Inflammation induced by CFA is comparatively more severe and has greater systemic effect compared to non-antigen preparations.[74]

2.2.3. Norbinaltorphimine (norBNI)

NorBNI was administered and was dissolved in physiologic saline (0.9% sodium chloride) and injected subcutaneously (s.c.) at a total volume of 1 ml/100 g body weight in male mice, unless noted otherwise. Control animals were injected with equivalent volumes of 0.9% biologic saline. In our studies norBNI was administered 24 hours prior to the introduction of ethanol or pain condition (CFA). This was critical to ensure norBNI had sufficient time to perfuse the body. Only a single administration of norBNI is required for the entirety of the study due to its longevity of action. The half-life of NorBNI is reported as 14 days, with effective KOR blockade in mice at 28 days.[75]

2.2.4. Study Design

Male and female mice were acclimated for a week in the 2-BC paradigm using water. CFA (100%) or vehicle was then injected intraplantary (right paw) and ethanol consumption and preference were determined on a daily basis as well as the daily total fluid intake. The average ethanol intake was also determined. Mechanical sensitivity was determined at baseline (BL) and at days 3, 7 and 14 after CFA in the different groups.

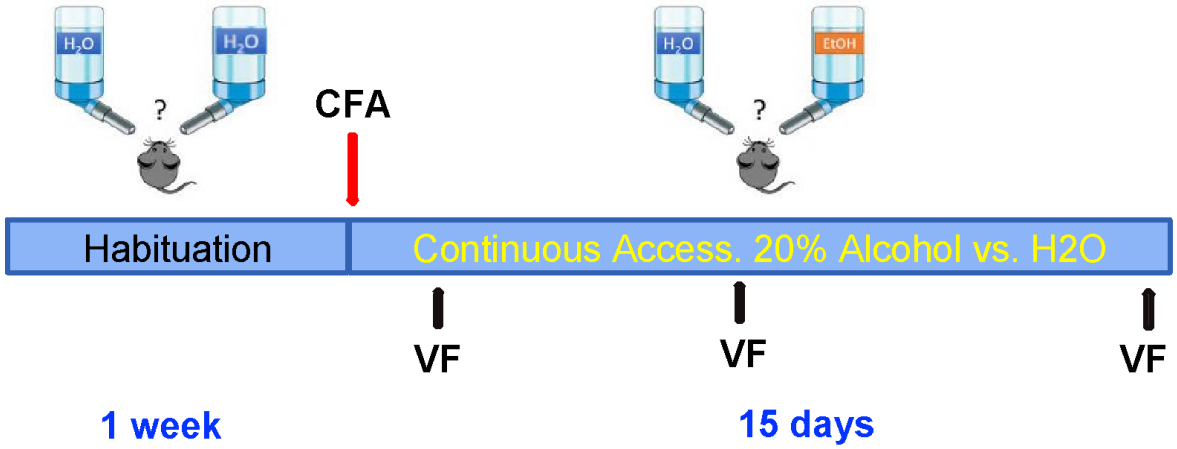


Figure 1 Study Timeline of CFA in ethanol naive mice

2.2.5 Statistical Analysis

Ethanol preference, ethanol intake, and evoked pain behaviors were analyzed using the GraphPad software, version 9.3 (GraphPad Software, Inc., La Jolla, CA), and are expressed as the mean ± S.E.M. Normality and equality of variances of all data sets were analyzed with 3- or 2-way ANOVA [post hoc analysis (Sidak test)]. Data are expressed as the mean ± S.E.M. of mice/per sex/per group for all tests. P values less than 0.05 were considered significant.

2.3 Results

2.3.1 Impact of CFA administration on ethanol intake in the 2-BC paradigm in female mice

To investigate if the increase in pain severity mediates the escalation of ethanol intake in response to chronic inflammatory pain in female mice, animals were given access to ethanol via the 2BC paradigm after the administration of CFA. ethanol-drinking behavior (20% v/v) was measured amongst C57BL/6J female mice for 14 consecutive days following intraplantar CFA or vehicle administration. As shown in Fig.2.3A, CFA administration did not produce a statistically significant change in female ethanol intake compared to the vehicle-injected group. A two-way ANOVA analysis of ethanol intake values after CFA or vehicle administration in female mice reveals no significant interaction between Time x CFA in the 2-BC assay [$F(14,238) = 1.086$, $p=0.3709$]. There was a significant effect for Time [$F(5.092,86.56) = 4.614$, $p=0.0008$], but not for CFA [$F(1,17) = 4.614$, $p=0.2112$]. Similarly, a two-way ANOVA (mixed model) was performed to compare ethanol preference in CFA-treated and Vehicle-treated mice. There was no significant effect between Time x CFA in ethanol preference within the 2-BC assay [$F(14,203) = 0.777$, $p= 0.6932$]. There was a significant effect for (Fig.2.3B). In addition, a t-test was performed to compare average ethanol intake in CFA-treated and Vehicle-treated mice. There was not a significant difference in average ethanol intake between Vehicle and CFA mice ($t=1.799(df) = 28$, $p =0.0829$) (Fig 2.1D). Total

fluid intake was calculated from the sum of water and ethanol-drinking volume (20% v/v). Results of total fluid intake were compared using two-way ANOVA with treatment and time as the factors and post-hoc Sidak's multiple comparison test (*p < 0.05 vs vehicle). A two-way ANOVA analysis of total fluid intake after CFA or vehicle administration in female mice reveals no significant effect between Time x CFA in the 2-BC assay [F(14,238) = 1.185, p=0.2875].

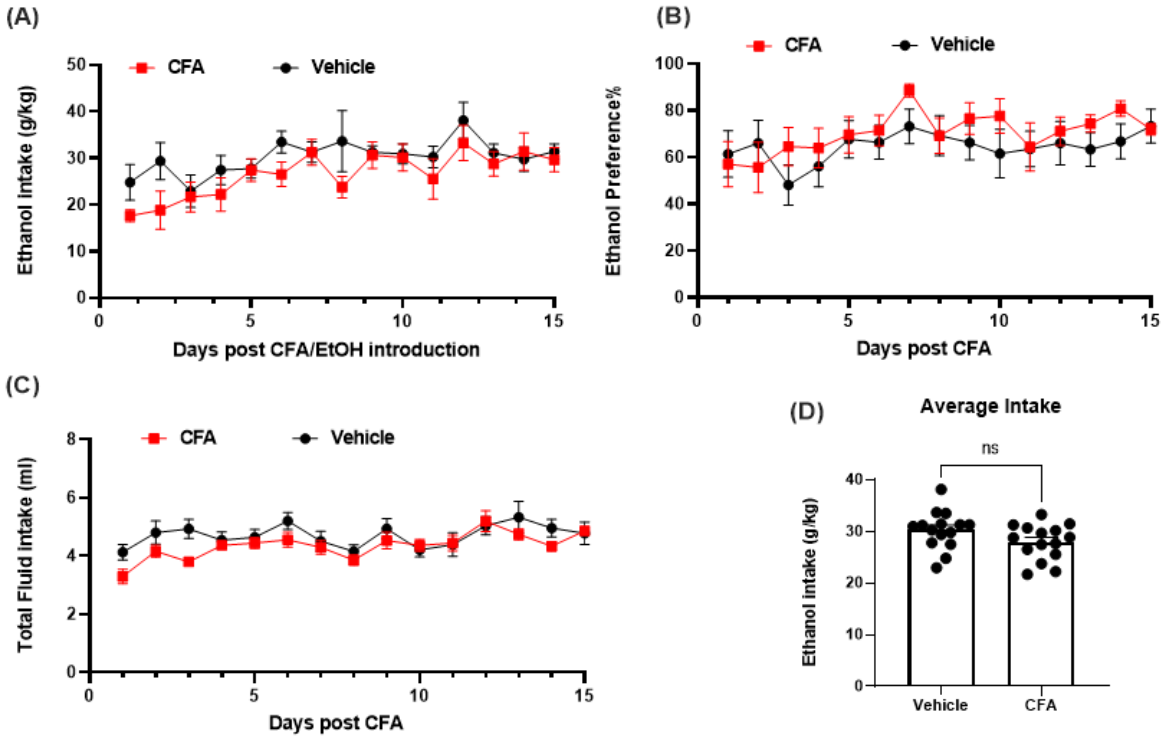


Figure 2.1 A-D Ethanol drinking in female mice after treatment with CFA. ethanol-drinking behavior (20% v/v) was measured amongst C57BL/6J female mice for 14 consecutive days following CFA or vehicle administration. **(A)** ethanol intake (g/kg), **(B)** ethanol preference (%), **(C)** total fluid intake, and **(D)** average ethanol intake. Values are expressed as mean ± SEM of n = 10/group. (*p<0.05 vs vehicle)

2.3.2 Evoked pain behaviors after administration of CFA in female mice

Changes in mechanical hypersensitivity were assessed by differences in the paw withdrawal threshold (Von Frey test) conducted on injured paws in our CFA and vehicle- treated groups in both naïve mice (ethanol free) and mice undergoing ethanol 2BC (EtOH mice). A two-way ANOVA analysis of average von Frey thresholds after CFA or vehicle administration in female mice reveals a significant interaction for Time x Treatment in the von Frey assay [$F(9,108) = 7.758$, $p<0.0001$]. Significant differences in paw withdrawal threshold were observed between the ethanol naive and vehicle injected group (Naive/Veh) and the three other treatment groups. ethanol exposure in non-CFA mice showed a progressive mechanical hypersensitivity ($p<0.05$ at days 7 and 14). CFA administration in both naïve and ethanol-drinking mice resulted in similar and significant reduction of paw withdrawal thresholds. No significant difference was noted between the CFA-treated group regardless of ethanol exposure.

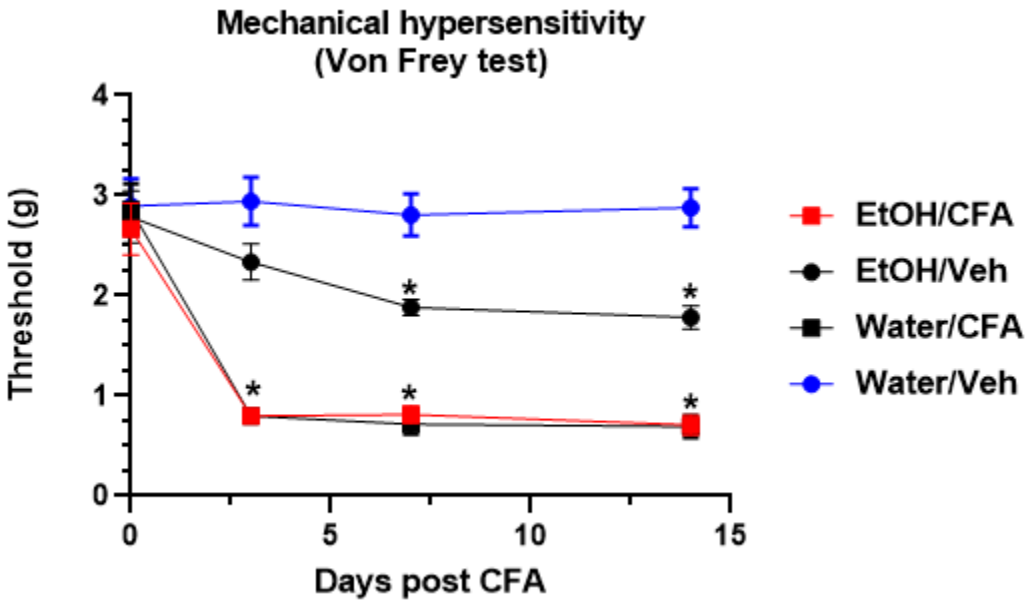


Figure 2.2 Von Frey measurements of mechanical hypersensitivity were conducted on mice at baseline, and at 3, 7, and 14 days post injection with CFA or vehicle. Results were compared using two-way ANOVA with treatment and time as the factors and post-hoc Tukey test (*$p < 0.05$ vs Naïve/vehicle group). Values are expressed as mean ± SEM of n = 10/group.

2.4.1 Impact of CFA administration on ethanol intake in male mice

To investigate if the increase in pain severity mediates the escalation of ethanol intake in response to chronic inflammatory pain in male mice, animals were given access to ethanol via the 2BC paradigm after the administration of CFA as shown in Fig. 2.4A-B CFA administration produces a statistically significant increase in male ethanol intake and ethanol preference in CFA-treated male mice as compared to their vehicle counterparts. No significant difference was found in total fluid intake between CFA-injected groups compared to the vehicle-injected group. ethanol-drinking behavior (20% v/v) was measured amongst C57BL/6J male mice for 14 consecutive days following CFA or vehicle administration. A two-way ANOVA analysis of ethanol intake after CFA or vehicle administration in male mice reveals a significant effect between Time x CFA in the 2-BC assay [$F(17,272) = 2.351$, $p=0.0022$] and significant effects for Time [$F(7.533,120.5) = 3.315$, $p=0.0023$], and CFA [$F(1,16) = 14.57$, $p=0.0015$]. In addition, a two-way ANOVA analysis of ethanol preference in male mice as shown in Fig2.4B demonstrates a significant effect between Time x CFA in the 2-BC assay [$F(18,324) = =1.705$, $p=0.0372$] and significant effects for Time [$F(7.797,140.4) = 2.867$, $p=0.0059$], CFA [$F(1,18) = 26.71$, $p<0.0001$]. Total fluid intake was measured amongst C57BL/6J male mice for 14 consecutive days following CFA or vehicle administration. This data was analyzed by fitting a mixed model, rather than by repeated measures ANOVA (which can't handle missing values). For our mixed-effects model, sphericity is not assumed, and the alpha is set to 0.05. There was not a statistically significant interaction between Time x CFA

[F(18,320)=0.9447 p=0.5246], nor was there a significant effect for CFA [F(1,18)=1.112. Finally, a two sample t-test was performed to compare average ethanol intake in CFA-treated and Vehicle-treated male mice. There was a significant difference in average ethanol intake between vehicle and CFA treated mice (t=5.410 (df) = 34, p <0.0001) with CFA-treated mice showing a higher average intake compared to vehicle-treated mice.

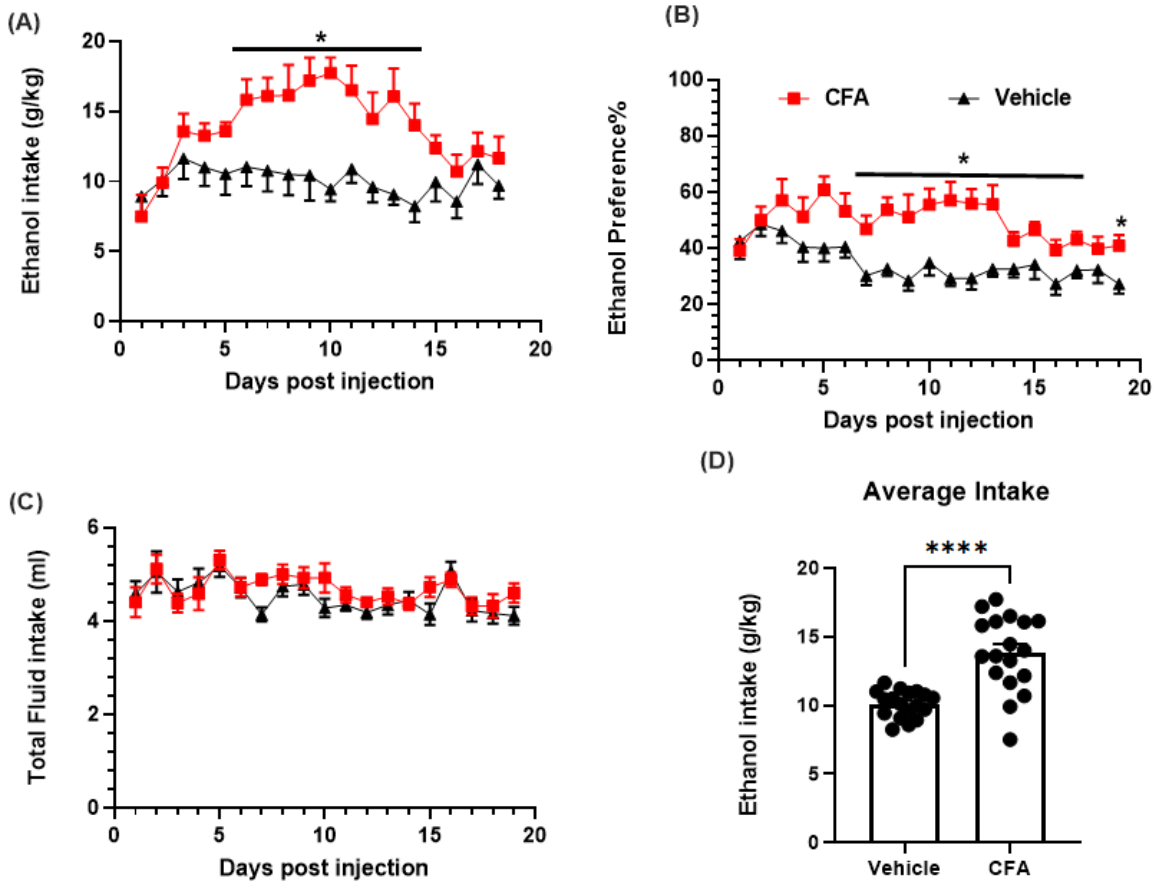


Figure 2.3A-D ethanol preference in male mice ethanol-drinking volume (20% v/v) was measured amongst C57BL/6J male mice for 14 consecutive days following CFA or vehicle administration. (A) ethanol intake (g/kg), (B) ethanol preference (%), (C) total fluid intake, and (D) average ethanol intake. Values are expressed as mean ± SEM of n = 10/group. *p<0.05 vs Vehicle group.

2.4.2 Evoked pain behaviors after administration of CFA administration in male mice

Changes in mechanical hypersensitivity were assessed by differences in the paw withdrawal threshold (Von Frey test) conducted on injured paws in our CFA and vehicle-treated groups in both naïve mice (ethanol free) and mice undergoing ethanol 2BC (EtOH mice). A two-way ANOVA analysis of von Frey thresholds after CFA or vehicle administration in male mice reveals a significant interaction for Time x Treatment in the von Frey assay [$F(9,108) = 5.857$, $p<0.0001$], Time[$F(2.831,101.9) = 30.46$, $p<0.0001$] and CFA[$F(3,36) = 32.85$, $p<0.0001$]. Significant differences in paw withdrawal threshold were observed between the naive and vehicle injected group (Naive/Veh) and the three other treatment groups. ethanol exposure or CFA administration both resulted in reduced paw withdrawal thresholds. Unlike in female mice, male mice showed after EtOH drinking a significant reduction of CFA-induced mechanical hypersensitivity compared to naïve mice.

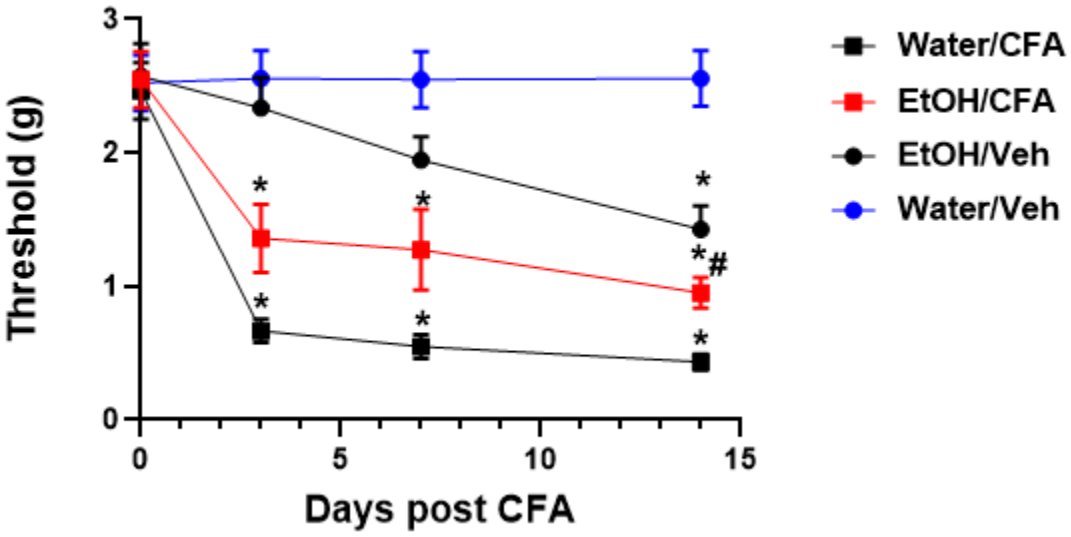


Figure 2.4 Von Frey measurements of mechanical hypersensitivity were conducted on mice at baseline, and at 4, 7, and 15 days post injection with CFA or vehicle. Average von Frey thresholds

after CFA or vehicle administration in male mice. (*p < 0.05 vs Naïve/vehicle group; #p<0.05 vs naïve/CFA group). Values are expressed as mean ± SEM of n = 10/group.

2.5.1 Kappa-Opioid activity in nociception

The Kappa opioid receptor lies at neural substrates underpinning both addiction and pain; as such, better understanding of its role is critical in treating these comorbid conditions. As opposed to a genetic or inducible KO model, we have opted for using norBNI to competitively and selectively target kappa opioid receptors.

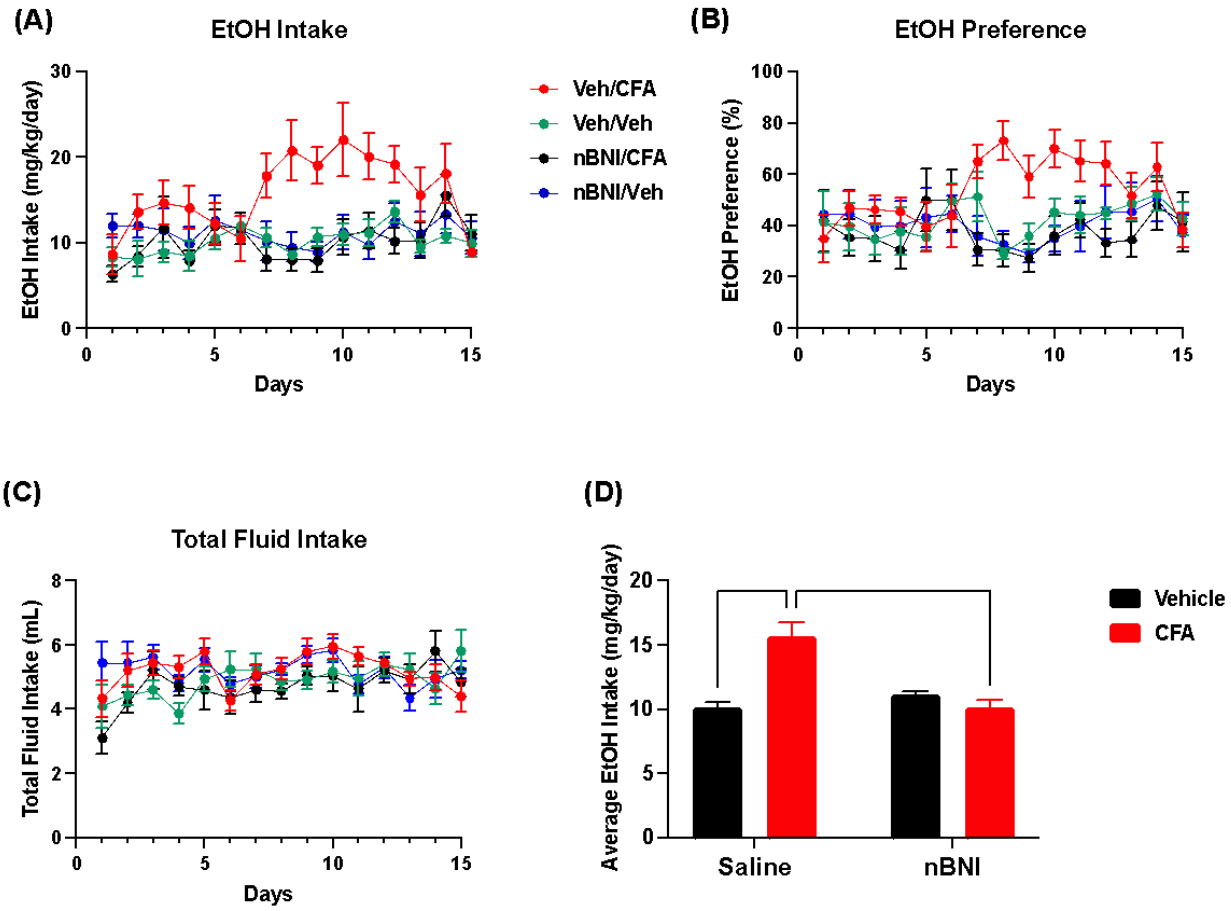
2.5.2 Effect of norBNI administration on drinking behaviors

This experiment was designed to evaluate what effect, if any, the administration of norBNI had on ethanol intake and preference in mice treated with CFA to induce inflammatory pain. Adult mice between the ages of 8 and 10 weeks were selected for this experiment. Male mice were chosen (to the exclusion of female subjects) as only male mice displayed a significant decrease in ethanol intake in response to CFA administration (See section 2.4.1). Mice were acclimated to the 2BC paradigm (water only) and baseline measurements of the mice were taken (von Frey, acetone test, and paw diameter) prior to drug or ethanol introduction. Groups I and II were injected with a nor-BNI i.p. at dose of 10mg/Kg of body weight; while groups III and IV received the mineral oil vehicle. Groups I and III were then dosed with CFA while groups II and IV received an intraplantar injection of the vehicle. All 24 mice were placed on the 20% v/v ethanol 2BC paradigm 24 hours after norBNI/vehicle administration. Ethanol and water intake were recorded at the same time each day, with weighing occurring every other day while the bottles were refilled.

As seen in Fig. 2.5, CFA-induced increase in ethanol intake (Fig. 2.5. A) and preference (Fig. 2.5. B) in male mice was blocked by pretreatment with nor-BNI. A two-way ANOVA analysis of average ethanol intake after CFA or vehicle administration in male mice reveals a significant effect for norBNI in

the 2-BC assay [F (1, 56) = 11.53, p=0.0013] and significant effects for CFA [F (1, 56) = 10.91 , p=0.0017], and norBNI x CFA interaction [F(1, 56) = 22.90, P<0.0001]. Nor-BNI blocked the increase in ethanol intake without affecting the intake of vehicle-treated mice.

Figure 2.5A-D Ethanol intake and preference in male mice Ethanol-drinking volume (20% v/v) was measured amongst C57BL/6J male mice for 14 consecutive days following CFA or vehicle and norBNI or vehicle administration. (**A) ethanol intake (g/kg), (B) ethanol preference (%), (C) total fluid intake**, and **(D) average ethanol intake**. Values are expressed as mean ± SEM of n = 6/group. (*p<0.05



vs vehicle)

2.6 Discussion

Given the complex relationship between chronic pain and ethanol consumption, there is a dire need for better models and new therapeutic targets for these conditions. The C57B/6J mouse model is well

researched and valued for their ability to voluntarily consume ethanol without training or coercion. Therefore, they make a great candidate for this research into voluntary ethanol consumption and chronic pain states. In this chapter, we describe sex differences in ethanol drinking and evoked pain behaviors following CFA administration. Male mice injected with CFA consumed more ethanol and exhibited a higher preference for the drug than vehicle controls, while female mice treated with CFA consumed a similar amount of ethanol compared to the vehicle controls in the 2-BC model. Neither sex saw a significant change in total fluid intake in response to CFA administration compared to their vehicle control counterparts. Our results are similar to a 2019 study by Yu et al. that also showed that CFA-treated male mice consumed a greater amount of ethanol than their saline-treated controls. A difference that was not seen in the female mice studied. A similar increase in ethanol preference ratio was observed in male mice that was absent in female mice.[76] Their study also found that CFA-treated mice exhibited higher sensitivities to thermal nociception as measured by reduced paw withdrawal latencies (PWLs) in the Hargreaves' Test. While we did not utilize Hargreaves', mechanical allodynia was measured in our mice using the VF test. In addition, our results showed that CFA-treated male mice who consumed a higher amount of ethanol showed a reduction of mechanical hypersensitivity. This effect was not observed in CFA-treated female mice.

A 2021 study by Lorente et al.[77] of ethanol intermittent access 2-BC in Sprague-Dawley rats also utilized norBNI in investigating KOR's role in AUD and chronic pain. These researchers focused on female rats due to their findings that only female rats increased ethanol intake after an abstinence period, and further study showed that only CFA-treated female animals showed changes in KOR expression in the NAc. Induction of inflammatory pain by CFA administration induced relapse drinking behaviors. When researchers administered norBNI to these rats, it reversed these "pain-induced relapse" drinking patterns. Rather than simply reducing ethanol consumption; KOR blockade appears to prevent pain or

stress-induced increases in ethanol drinking. This suggests that norBNI administration does not make ethanol more aversive, but rather that it may prevent the rewarding effects of ethanol that serve as reinforcement to mice undergoing a pain condition.

Chapter 3 Impact of Chronic Neuropathy on drinking behavior

3.1 Introduction

Our results with the 2-BC ethanol study in CFA-treated mice showed that chronic inflammatory pain increases ethanol intake in male mice. One of the primary motivations of this experiment is to compare the responses in ethanol intake and preference using a different chronic pain model. For that, we chose to examine the effect of chronic neuropathic pain on ethanol intake using the 2BC model. We focused on chemotherapy-induced peripheral neuropathy or CIPN model. This research will allow a better understanding of the interrelations between voluntary ethanol consumption and chronic pain and could lead to new therapeutic targets to aid patients with those conditions. Would our data on ethanol intake in a CFA induced peripheral neuropathy show similar results in the CIPN model? We expect that similar to the CFA study, the induction of CIPN will increase ethanol intake and preference in male mice compared to their vehicle-treated counterparts.

3.1.1 Chemotherapy-Induced Peripheral Neuropathy

Chemotherapy Induced Peripheral Neuropathy (CIPN) describes a range of symptoms from numbness and tingling in the affected extremity to mechanical or thermal hypersensitivity after treatment with various chemotherapeutic drugs. CIPN is often a painful, dose-limiting side effect and common clinical problem; approximately 30 to 90% of patients receiving neurotoxic chemotherapy will suffer from this condition. This can seriously impact a patient's ability to continue chemotherapy. Even once a patient's cancer is in remission the side effects of certain chemotherapeutic agents leave patients with intractable neuropathic pain. This can leave patients with an extremely tough position to choose between continuing to fight their cancer and managing

the increasingly deleterious side effects of the chemotherapy. Due to CIPN's targeting of large caliber sensory neurons patients may also present with numbness and loss of vibration sense, while cold and mechanical hypersensitivity are likely due to impairment of small Aδ- and C-fibers.[78] Treating CIPN remains difficult as most analgesic therapeutics are ineffective.[79] This concern is common to several groups of classical chemotherapy agents: platinum-based drugs, taxanes, and vinca alkaloids.[80]

3.1.2 Prevalence of AUD in patients living with cancer and in remission

ethanol has been causally associated with the development of a number of different cancers (oral cavity, pharynx, esophagus, and breast cancer to name a few). ethanol consumption has also been shown to worsen patient outcomes and increase rate of recurrence in patients in remission. However, the relationship between ethanol consumption and cancer is more complex. Patients consuming ethanol while undergoing radiation or chemotherapy are at additional risks. ethanol consumption increases the risk of osteoradionecrosis in patients receiving radiation therapy for the treatment of head and neck cancers.[81] Similar risks exist for patients undergoing chemotherapy; ethanol use can worsen cognition, and increase the risk of neurotoxic, cardiotoxic and hepatotoxic effects.[82] Given these risk factors, an accurate assessment of drinking behavior in these patients and in cancer survivors is needed. Clinical data from the NIH's "All of Us Research Program" was used as the basis of the analysis with over 15,000 cancer patients included. Scoring of ethanol consumption was based on the AUDIT-C (ethanol Use Disorders Identification Test Consumption) score. 77.7% of current cancer survivors studied (11,815 patents) reported currently drinking. Of the 77.7% of current drinkers, 13% exceeded moderate drinking, 23.8% reported binge drinking and 38.3% engaged in hazardous drinking.[83]

We focused in our study on a widely used chemotherapeutic agent paclitaxel, otherwise known as taxol®. Paclitaxel is a naturally occurring compound extracted from the Pacific yew tree, Taxus brevifolia. Paclitaxel was first approved by the FDA in 1992 for use in treating refractory ovarian cancer. Since then, FDA approval has been broadened to include its use in combating breast cancer, non-small cell lung cancer (NSCLC), and Kaposi sarcoma. Several studies of paclitaxel-treated patients indicate greater frequency of impairment with some degree of neuropathy reported in 44%–66% of patients. Patients usually develop an acute pain syndrome, described in up to 60% of patients, within 1 to 3 days of paclitaxel administration and symptoms largely resolve within a week after termination of the dose. However, up to 30-35% of cancer patients treated with paclitaxel show symptoms of chronic neuropathy that could last for a long time after termination of the treatment. As an antineoplastic agent, paclitaxel, works to inhibit cell replication in a few key ways. A number of naturally occurring compounds effectively interact and disrupt microtubule organization; possibly due to their evolution as plant antifeedants or protection against predation. These compounds function as antimitotic agents by binding to microtubules and suppressing microtubule dynamics during a critical stage of mitosis. Paclitaxel and the other taxanes in its class function as "microtubule stabilizing agents" due to their mechanism of action. Paclitaxel binds to β-tubulin subunit on the inner surface of the microtubule.

Axonal transport within cells is disrupted through stabilization of their microtubule structure and altering the function and morphology of mitochondria. These changes in addition to the paclitaxel-induced inflammation result in symmetrical damage of axons and loss of nerve fiber density. Peripheral pain and neuropathy can be attributed to the accumulation of paclitaxel in the dorsal root ganglia (DRG). Development of CIPN is more likely in patients with a pre-existing neuropathic condition such as chronic ethanol consumption or diabetic peripheral neuropathy.

3.2 Materials and Methods

3.2.1 Animals

Male and female adult (10-12 weeks) C57BL/6J mice were randomly assigned to either the control or treatment group. Mechanical threshold was measured using the Von-Frey test at regular intervals both prior and after ethanol access and paclitaxel administration, as a measure of pain-evoked behaviors.

3.2.2 Paclitaxel

Paclitaxel is highly hydrophobic owing to its structure, a diterpenoid centered around a taxane ring. Due to this hydrophobicity, paclitaxel requires a vehicle for proper administration. Kolliphor EL, a 50:50 solution of dehydrated ethanol and polyethoxylated castor oil is commonly used in both the clinic and research setting for this purpose.[84] Paclitaxel (Athenex, NDC 70860-200-50, Richmond, VA, USA) was procured from VCU Health Pharmacy. Paclitaxel was dissolved in a mixture of Kolliphor (Sigma-Aldrich, St. Louis, MO, USA), ethanol (Sigma-Aldrich), and distilled water (mixture proportion 1:1:18). Intraperitoneal injections of paclitaxel were performed every other day, with four administrations in total, at a dose of 8 mg/kg. Control mice received the vehicle (1:1:18, ethanol, Kolliphor, and distilled water) at a volume of 10 mL/kg, i.p. and followed the same injection schedule.

3.2.3. Study Design

Male and female mice were acclimated for a week in the 2-BC paradigm using water. After acclimation to the 2-BC assay, intraperitoneal injections of paclitaxel were performed every other day, with four administrations in total, at a dose of 8 mg/kg, i.p. Control mice received the vehicle

(1:1:18, ethanol, Kolliphor, and distilled water) at a volume of 10 mL/kg, i.p. and followed the same injection schedule. ethanol consumption and preference were determined on a daily basis as well as the daily total fluid intake. The average ethanol intake was also determined. Mechanical sensitivity was determined at baseline (BL) and at days 3, 7 and 14 after paclitaxel (PAC) in the different groups.

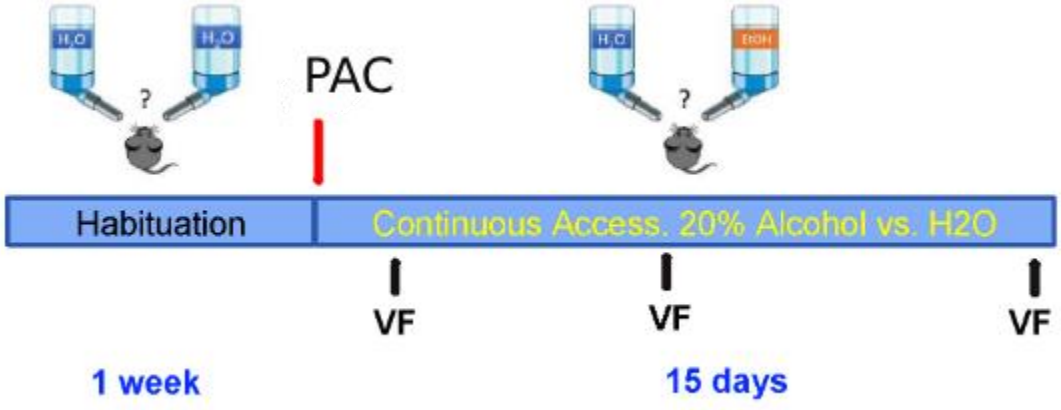


Figure 3.1 Study Timeline of Paclitaxel in ethanol naive mice

3.2.4 Statistical Analysis

Ethanol preference, ethanol intake, and evoked pain behaviors were analyzed using the GraphPad software, version 9.3 (GraphPad Software, Inc., La Jolla, CA), and are expressed as the mean ± S.E.M. Normality and equality of variances of all data sets were analyzed with 3- or 2-way ANOVA [post hoc analysis (Sidak test)]. Data are expressed as the mean ± S.E.M. of mice/per sex/per group for all tests. P values less than 0.05 were considered significant.

3.3 Results

3.3.1 Ethanol Consumption Following Paclitaxel administration in female mice.

Paclitaxel administration did not produce a statistically significant difference in ethanol intake compared to female mice treated only with vehicle. A two-way ANOVA of ethanol intake values after paclitaxel or vehicle administration in female mice reveals no significant interaction between Time x Paclitaxel in the 2-BC assay [$F(12,120) = 1.567$, $p=0.1104$]. A two-way ANOVA (mixed model) was performed to compare ethanol preference in paclitaxel-treated and Vehicle-treated mice. There was no significant effect between Time x paclitaxel in ethanol preference within the 2-BC assay [$F(12,120) = 1.621$, $p= 0.0943$. A t-test was performed to compare average ethanol intake in paclitaxel-treated and vehicle-treated female mice. There was not a significant difference in average ethanol intake between vehicle and paclitaxel treated groups ($t=1.476$ (df) $=24$, $p =0.1529$). Total fluid intake was calculated from the sum of water and ethanol-drinking volume (20% v/v). Results of total fluid intake were compared using two-way ANOVA with treatment and time as the factors and post-hoc Sidak's multiple comparison test ($*p < 0.05$ vs vehicle). A two-way ANOVA analysis of total fluid intake after paclitaxel or vehicle administration in female mice reveals no significant effect between Time x paclitaxel in the 2-BC assay [$F(12,120) = 1.059$, $p=0.4012$]. A two sample t-test was performed to compare average ethanol intake in paclitaxel-treated and vehicle-treated mice and found no significant difference between groups.

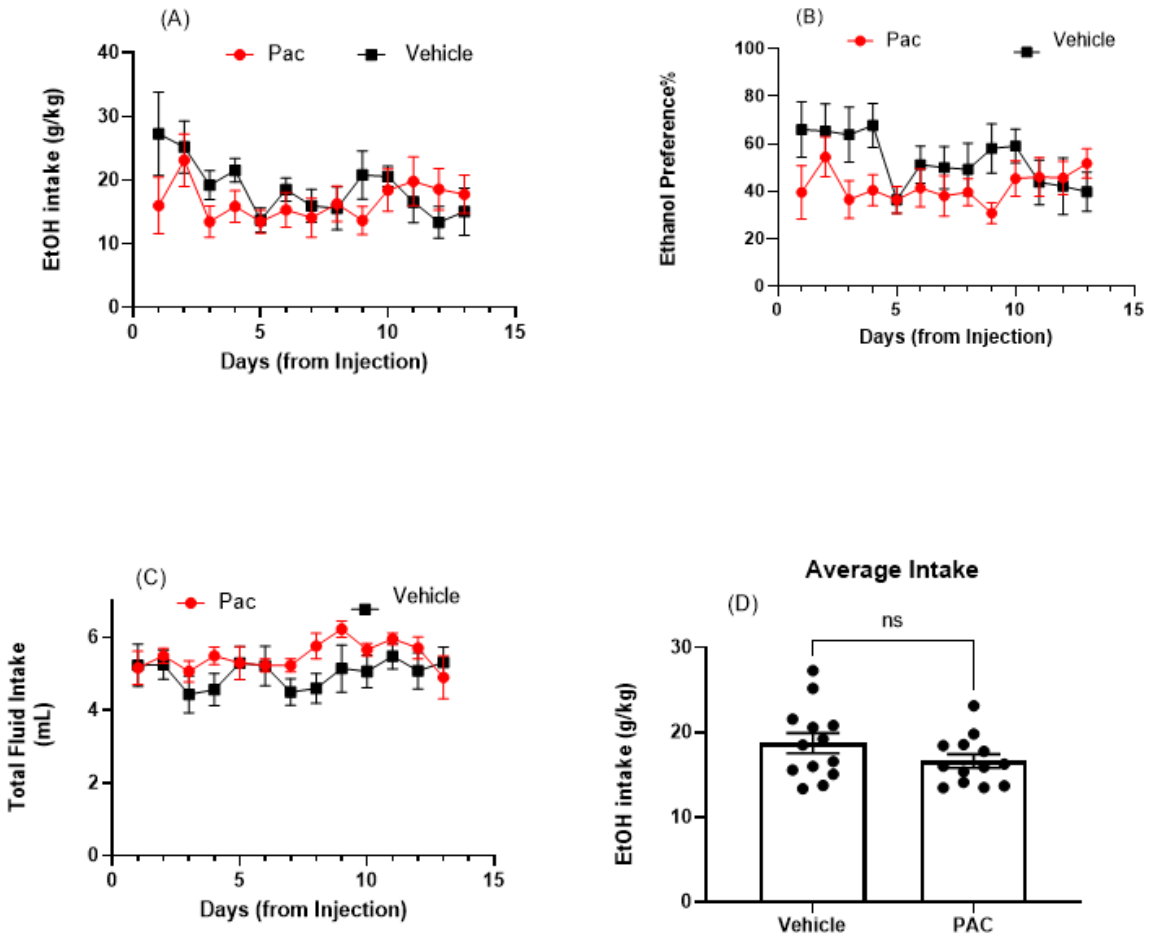


Figure 3.2.1 A-D ethanol drinking in female mice after treatment with Paclitaxel. ethanol-drinking behavior (20% v/v) was measured amongst C57BL/6J female mice for 14 consecutive days following Paclitaxel or vehicle administration. Vehicle: black squares, Paclitaxel: red circles **(A)** ethanol intake (g/kg), **(B)** ethanol preference (%), **(C)** total fluid intake, and **(D)** average ethanol intake. Values are expressed as mean ± SEM of n = 10/group. (*p<0.05 vs vehicle)

3.3.2 Ethanol consumption following paclitaxel administration in male mice.

Volume of ethanol and water consumption from 2BC was measured daily for 14 days after introduction of ethanol and paclitaxel or vehicle administration. A two-way ANOVA analysis of ethanol intake after paclitaxel or vehicle administration in male mice reveals no significant interaction between Time x Paclitaxel in the 2-BC assay [$F(12,120) = 0.6014$, $p=0.8374$] but a significant effect for Paclitaxel [$F(1,10) = 9.849$, $p=0.0105$]. A two-way ANOVA analysis of ethanol preference after paclitaxel or vehicle administration in male mice reveals a significant interaction for Time [$F(4.471,44.71) = 0.8894$, $p=0.0020$] and Paclitaxel [$F(1,10) = 7.419$, $p=0.0214$]. A two-way ANOVA analysis of ethanol preference after paclitaxel or vehicle administration in male mice reveals no significant interaction between Time x Paclitaxel in the 2-BC assay [$F(12,120) = 0.8894$, $p=0.5597$] and a significant interaction for Subject [$F(10,120) = 5.674$, $p<0.0001$], for PAC [$F(1,10) = 7.419$, $p=0.0214$] and for Time $F(4.471,44.71) = 4.758$, $p=0.0020$]). A two sample t-test was performed to compare average ethanol intake in paclitaxel and vehicle treated mice. There was a significant reduction in average ethanol intake between vehicle and paclitaxel ($t(df) = [t=7.678; df=24]$, $p = [<0.0001]$).

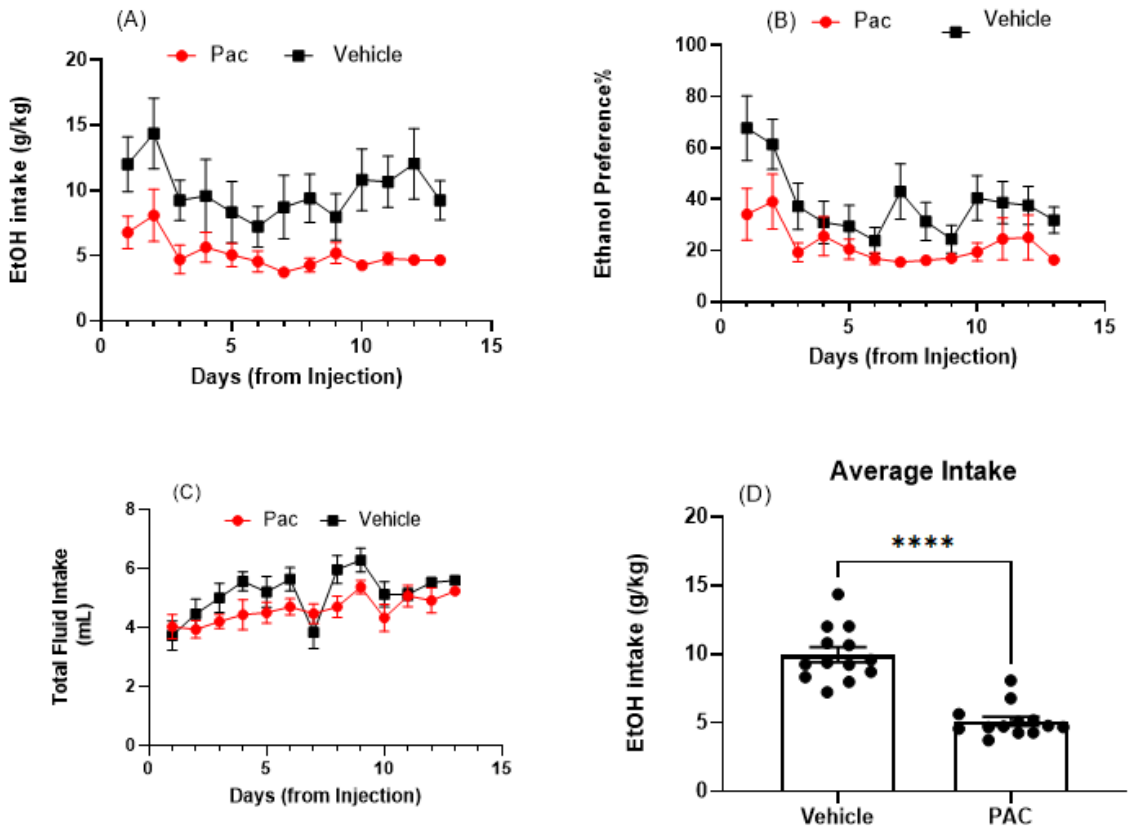


Figure 3.3.2 A-D ethanol drinking in male mice after treatment with Paclitaxel. Values are expressed as mean ± SEM. n= 10/group. Vehicle: black squares, Paclitaxel: red circles**(A) ethanol intake (g/kg)**, **(B) ethanol preference (%)**, **(C) total fluid intake,** and **(D) average ethanol intake.** Values are expressed as mean ± SEM of n = 10/group. (*p<0.05 vs vehicle)

3.4 Discussion

In this chapter we studied the effects of CIPN on ethanol intake in male and female C57BL/6J mice to compare it to our prior results on chronic inflammation's effects on ethanol intake. Our experiments with chronic inflammation showed that CFA administration had increased ethanol intake and preference in male mice compared to their vehicle-treated counterparts. This change was not seen in CFA-treated female mice. Based on this prior work, we expected to see an

increase in ethanol intake and preference in paclitaxel treated male mice without a significant difference in total fluid intake. We observed that paclitaxel administration had no significant effect on ethanol intake in female mice which was similar to our results in CFA-treated female mice. However, paclitaxel-treated male mice saw a significant decrease in ethanol intake and preference compared to their vehicle-treated mice. A review of published literature does not return any studies of paclitaxel administration on ethanol preference. Our lab, however, has done prior work measuring mechanical hypersensitivity and sucrose preference using 2BC in paclitaxel-dosed C57BL/6J mice. This study showed that paclitaxel administration in male mice resulted in changes in affective-related behaviors in mice (only male mice were tested). They observed an increase in anxiety-like behaviors (increased latency to eat in the Novel Suppressed Feeding (NSF) assay, an emotional-like deficit as measured by time spent immobile in the Forced Swim Test (FST), and an increase in anhedonia-like behavior as measured by decreased sucrose preference without a decrease in their total fluid intake as measured by 2BC[85]. It is possible that the decrease in ethanol intake seen in male mice after paclitaxel is due to the increase in aversiveness to ethanol.

Chapter 4 Impact of previous ethanol consumption on drinking and pain behaviors

4.1 Background

Our results in Chapter 2 have demonstrated that chronic inflammatory pain can induce an increase in ethanol consumption in male mice driven by KOR-mediated mechanisms. However, we used naïve mice with no history of ethanol consumption, which may not best relate to a population of patients who endure chronic inflammatory pain and would use ethanol for analgesic purposes. It will be important to examine ethanol consumption after CFA in mice with a history of ethanol exposure, as this will better reflect patients with a history of drinking who may turn to ethanol for analgesic or pain-coping effects (e.g., LaRowe et al., 2021[86]; Ferguson et al., 2022[87]). There have been comparatively few studies on drinking behavior in pain settings using rodent models. In a review of the literature by Campos-Jurado and Morón (2023) identified 13 relevant studies, and only 6 involved ethanol access prior to initiation of the pain condition. Even from this limited pool there are conflicting results on whether males or female rodents with a history of ethanol intake see changes in ethanol intake in response to chronic pain. For example, the results of a recent study show that CFA-induced inflammatory pain does not alter total ethanol intake in male or female rats with a history of ethanol exposure (intermittent access for 4 weeks) before the insult (Campos-Jurado and Morón, 2023)[88]. We therefore investigated in this chapter the impact of CFA treatment in mice with a history of ethanol consumption. We sought to design this study with male and female rodents to look for sex differences in ethanol intake and continued our use of C57BL6/J mice to compare to our previous results. Our previous work had only used a CA model of ethanol access while other labs had opted for intermittent access (IA). For better comparison between our previous studies, we used the CA 2BC model to acclimate the mice to drinking and obtain baseline values before the pain condition. A short period ethanol-free

withdrawal of 6 days was included. It is our goal to study what effect this ethanol history protocol has on mouse drinking behavior in a chronic pain paradigm.

4.2 Materials and Methods

4.2.1 Study Design

Male and female mice were acclimated to 20% ethanol 2BC for 12 days prior to the start of the ethanol withdrawal period. During the withdrawal period, both drinking bottles would contain only water. This period lasted for 6 days before the re-introduction of ethanol. Following a 10-day period of renewed ethanol access, the mice were injected with either CFA or mineral oil vehicle into their right paw (this was day 28 from the start of the initial drinking study). Following CFA administration, mice were allowed 2BC 20% ethanol access for 17 additional days with repeated measures of nociception (von Frey) at 7 day intervals.

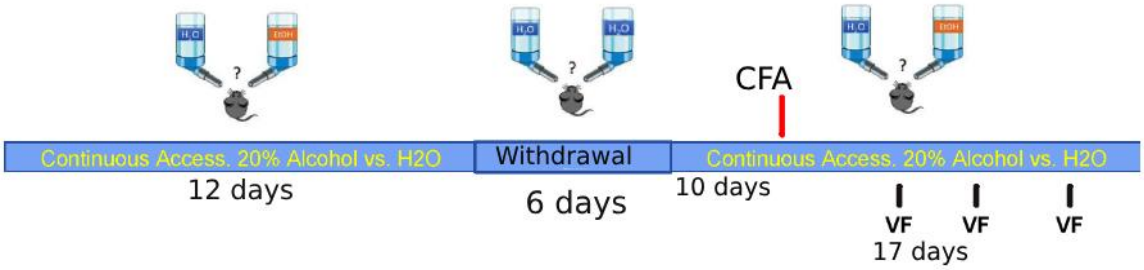


Figure 4 Study Timeline of CFA in ethanol-experienced mice

4.3 Results

4.3.1 Impact of CFA administration on ethanol intake in ethanol naïve mice

Mixed effects analysis of female ethanol intake was calculated both in total and for just the drinking periods post Day 26. A mixed effects analysis was used as ANOVA is not tolerant of missing data values. For fixed effects there was a statistically significant effect for time [(18.39, 1) ; p <0.0001], for treatment [(18.39,1) p=0.0228], and for time x treatment (p=0.0084). Mixed-effects analysis of the full time course of female ethanol intake reported similar results for Time [(17.06, 1) ; p <0.0001], and a significant effect for treatment (p=0.0484), but not for Time x Treatment (p=0.0894). A two-way ANOVA was performed on Ethanol preference in female mice post study day 26; there was no significant effect for Time x Treatment [F(9,135) = 1.591, p=0.1238], no significant effect for Treatment [F(1,15) = 2.065, p=0.1712], but a significant effect for Time [F(4.685,70.28) = 10.21, p=0.0001]. An unpaired t-test was conducted on average ethanol intake in female mice and found a significant effect for CFA administration with [(2.047,36) p=0.0480].

Mixed effects analysis of male ethanol intake was calculated both in total and for just the drinking periods post Day 26. A mixed effects analysis was used as ANOVA is not tolerant of missing data values. For fixed effects there was a statistically significant effect for time [(7.059,111.5) ; p <0.0025], but not for treatment [(1.16) p=0.3314], or time x treatment [(19,300)(p=0.0824). Mixed-effects analysis of the full time course of male ethanol intake reported similar results for Time [(7.408,117.2); p <0.0001], and treatment (p=0.0787), and a significant effect for Time x Treatment [F(17,269);p= 0.0348). A two-way ANOVA was performed on Ethanol preference in male mice post study day 26; there was no significant effect for Time x

Treatment [F(9,141) =1.200, p=0.2997], no significant effect for Treatment [F(1,16) = 0.1032, p=0.7522], and no significant effect for Time [F(1.608, 25.19) =2.979, p=0.0787]. An unpaired t-test was conducted on average ethanol intake in male mice and found no significant difference between vehicle and CFA treated groups[F(1.168,38) p=0.2499]. Mixed effects analysis of male total fluid intake was calculated for drinking periods post CFA administration and a mixed effects analysis was used as ANOVA is not tolerant of missing data values. For fixed effects there was not a statistically significant effect for time [(4.828,74.92) =1681; p <0.1517], or for treatment [(1.16) =0.8588; p=0.3678], or time x treatment [(27,419) =0.7385; p=0.8284].

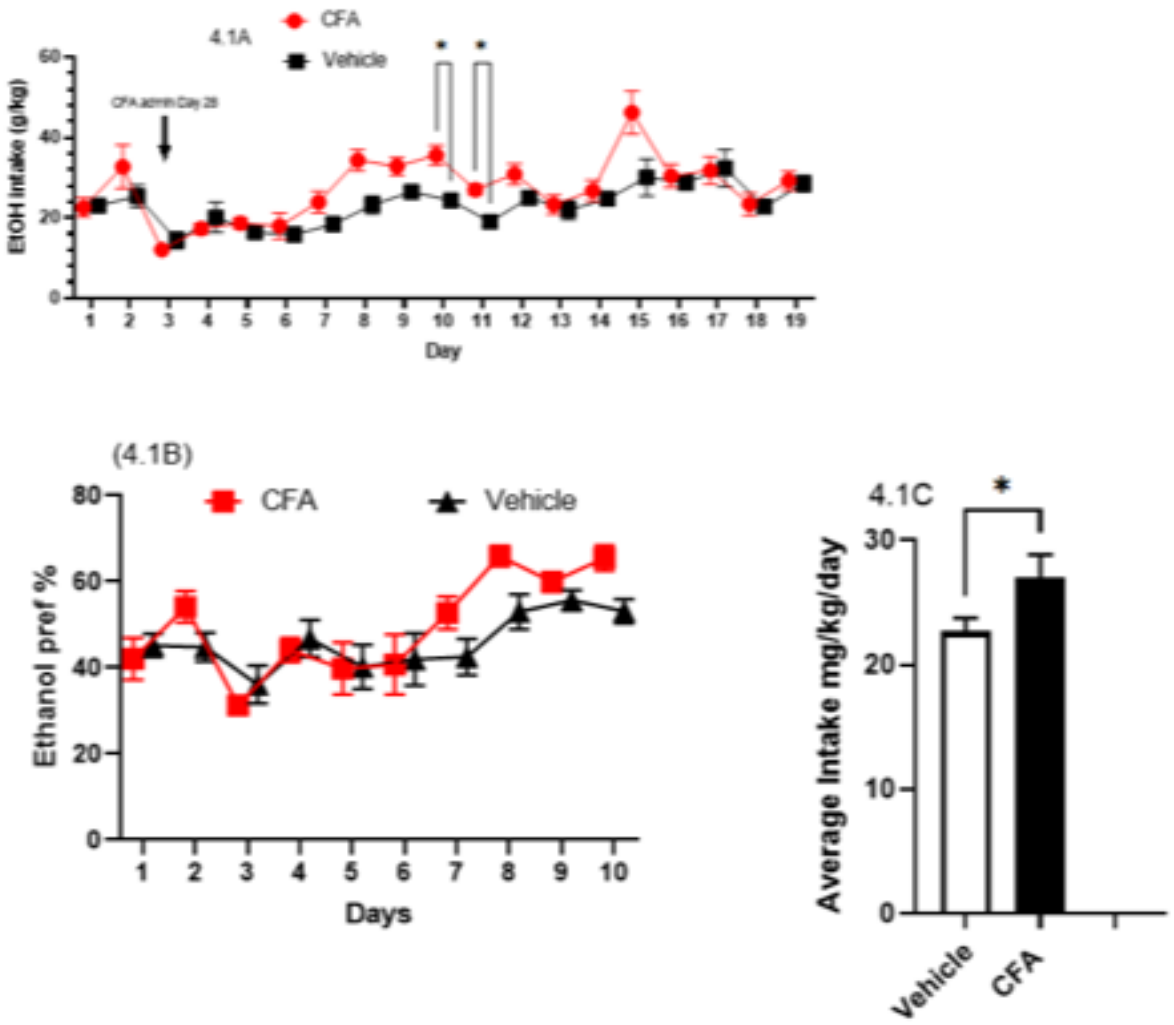


Figure 4.1 A-C Average Ethanol Intake(A) and preference (B) in CFA treated female mice; post day 26. The Figures **4.1A/B** has been cropped to focus on the period of time after the withdrawal period and the CFA/Vehicle administration. **Figure 4.1C** An unpaired t-test was performed to compare average ethanol intake in CFA-treated and Vehicle-treated mice. n = 9/group (*p<0.05 vs vehicle).

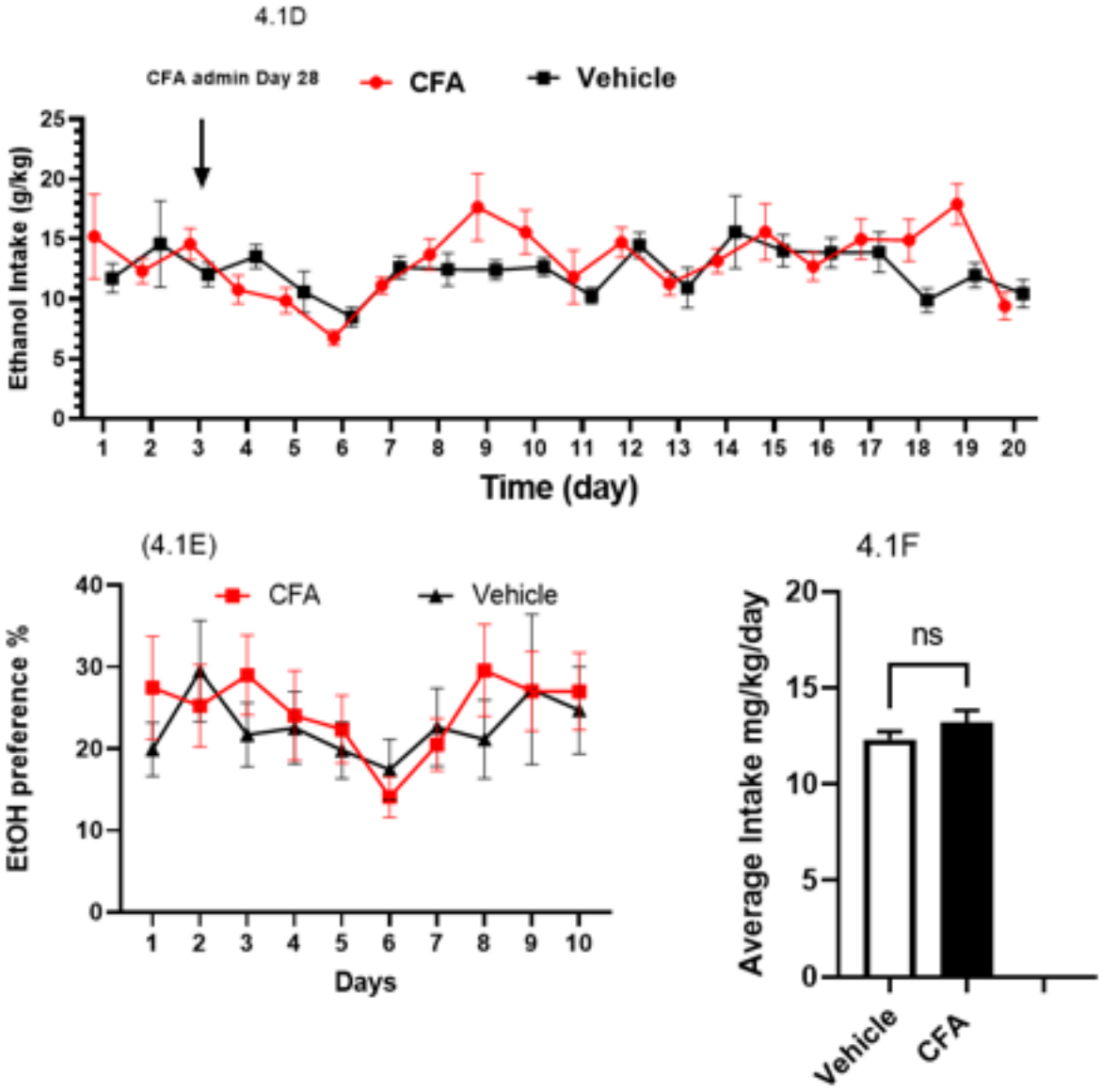


Figure 4.1D/E Average Ethanol Preference in CFA treated male mice. The Figures **4.1D/E** has been cropped to focus on the period of time after the withdrawal period and the CFA/Vehicle administration. **Figures 4.1F** An unpaired t-test was performed to compare average ethanol intake in CFA-treated and Vehicle-treated mice. n = 9/group (*p<0.05 vs vehicle)

4.4. Discussion

Previous epidemiological studies have shown that a history of AUD in chronic pain patients increases the likelihood of relapse in currently ethanol abstinent patients. A greater understanding of what factors lead to renewing or escalating ethanol consumption in these groups is critical.[89] This information can then be used to more effectively screen patients with higher risk factors based on frequency and history of ethanol consumption. Our studies showed an increase in ethanol intake and preference in CFA-treated female mice with a history of ethanol exposure. This change was not seen in male mice. As ethanol is metabolized by ADH then ALDH while paclitaxel is primarily broken down by Cytochrome P450 enzymes in the liver; there is little reason to suspect that pharmacokinetic interactions are responsible for the observed differences between paclitaxel and CFA-administered mice.[90] A study[91] comparing C57BL6/J and DBA/2J mice used capsaicin to induce a pain response after acclimation to 10% (v/v) ethanol in a single bottle drinking study. They reported that with capsaicin administration, both strains saw a decrease in ethanol intake from baseline, and that the C57BL/6J mice saw a smaller decrease in intake compared to the DBA/2J mice. This is dissimilar to our results with CFA-induced inflammation in C57BL/6J which did not show a significant difference in treatment. While this study utilizes a withdrawal period, it does not comport with previously published rodent models of ethanol deprivation or withdrawal. As such, direct comparisons between this data and other models should be performed with care. Only the recent study from Campos-Jurado and Morón of IA 2-BC with 20% ethanol utilized rodents of both sexes and had the pain condition begin after the rodents had ethanol access[92]. Although their work was conducted in Long Evans wild-type rats, it is the closest comparison to our female C57BL/6J data. In measuring male ethanol intake, their RM-ANOVA found a main effect of time, but did not detect differences in treatment or in the interaction between time and

treatment. However results from female rats differed wherein a main effect of time was detected but not for treatment or in the interaction between time and treatment.[93] A study by Lorente[94] in 2021 showed similar results in their study of an alcohol deprivation effect (ADE) in Sprague-Dawley rats. They found that only female rats under an inflammatory pain condition showed an increase in their ethanol intake after an abstinence period. While we are utilizing different rodent strains and models of IA ethanol consumption, there is the possibility that our female mice are also displaying an ADE that is absent in our male mice and our saline-treated female mice.

Chapter 5 General Discussion and Future Directions

5.1 Summary of Results

All of our work was conducted in the C57BL/6J mice inbred strain and all ethanol was presented to mice in a 2BC paradigm at a concentration of 20% (v/v). Through use of a chronic inflammation, voluntary-drinking mouse behavioral model we have observed sex-based differences in ethanol intake in response to CFA administration. This heightened intake of ethanol

in CFA-treated male mice compared to their vehicle-treated counterparts was not seen in female mice. These results are similar to the results of previous studies on 2BC in CFA-treated C57BL/6J mice (Yu et al., 2019) that also showed an increase in ethanol intake for CFA-treated male mice that was not present in their vehicle-treated counterparts or in female mice regardless of treatment group. Previous research with the C57BL/6J mouse model exposed to continuous access high concentrations of exposure has shown the inverse relationship, with female mice drinking more ethanol and exhibiting a greater preference for ethanol than their male peers. Combined with Yu's results this may suggest that the presence of chronic pain in ethanol naive mice may have a sex-dependent effect on ethanol intake.

In response to these sex-specific effects on ethanol intake in mice undergoing a chronic pain condition, we wanted to determine if these effects were moderated by dynorphins and KOR. To explore this, we used the persistent KOR antagonist norbinaltorphimine. Our work in male mice showed that norBNI administration fully negated the increased ethanol intake and preference seen in CFA-treated male mice that were not treated with norBNI. This suggests that KOR activation is required for the elevated ethanol intake and preference seen in male mice under a chronic pain condition. This may indicate that sex difference in ethanol escalation in ethanol naive mice are based on differences in the activation pathway of KOR in male and female mice.

In an effort to expand the results with our model of ethanol consumption and chronic inflammatory pain in mice, we measured ethanol drinking in another model of chronic pain such as neuropathic pain. We chose paclitaxel as our agent to induce neuropathy (CIPN) just as we had used CFA in our inflammatory response model. With the induction of CIPN we once again saw different responses in ethanol consumption based on sex. Paclitaxel-treated female mice displayed no difference in ethanol intake and preference compared to their vehicle-treated counterparts. This

time paclitaxel-treated male mice saw a decrease in ethanol intake and preference compared to their vehicle-treated counterparts.

Our final experiment examined the effect that a prior history of ethanol consumption has on ethanol intake in mice treated with CFA. We only observed an increase in ethanol intake and preference in female mice with a history of ethanol consumption. This is in contrast to our results with CFA in female C57BL/6J mice that had no history of ethanol exposure before injury. In ethanol naive females we did not see an escalation of ethanol intake and preference. Similarly, the escalation in ethanol drinking observed in male C57BL/6J mice with no history of ethanol exposure, was absent in our C57BL/6J male mice with a history of ethanol consumption. There are likely two contributing factors to this: sex-differences in escalation of drinking and the possibility that the mechanisms that support and maintain ethanol consumption may change in response to chronic or previous ethanol exposure. A similar effect has already been studied with pain (see Chapter 1.2.2 for a further discussion of pain chronification).

5.2 Significance of Results

There is limited published preclinical data on sex differences in pain as a risk factor for increasing ethanol intake. Despite this limitation, the reported studies represent a diverse set of conditions including: method of ethanol consumption, rodent strain, noxious agent used, etc. As such, our work expands needed research in this area. Given the wealth of drinking data available for the C57BL/6J strain, their use in our pain experiments may lead to easier comparison with existing data.

Why did we observe opposite responses in ethanol intake in mice treated with paclitaxel vs our results for CFA? Our research into voluntary drinking under chronic pain conditions does suggest an interesting explanation: that different mechanisms underlie pathogenesis of CIPN and

CFA induced peripheral neuropathies. If different mechanisms underlie the development of these conditions, then it is a reasonable assumption that they may not share the cellular and molecular mechanisms that promote the escalation of ethanol intake in inflammatory pain conditions. While there are not many studies published on ethanol drinking in paclitaxel; our lab's study of sucrose preference with 2BC in paclitaxel-treated mice does provide some clues. Our lab found that male mice treated with paclitaxel consumed less sucrose solution in their 2BC assay without a decrease in total fluid intake. Decreased sucrose (and ethanol) intake could be explained by a phenomenon known as dysgeusia, an alteration in taste seen in some patients treated with paclitaxel.[95] If this change in gustation occurred in our mice it could have made ethanol less palatable, driving down intake without affecting total fluid intake. One possible way to examine this would be to utilize sucrose solutions in 2BC and 3BC models to examine what effects paclitaxel administration had on ethanol preference. Another possible explanation is the decrease in ethanol intake is another component of the deficit in emotional-like behavior in paclitaxel-treated mice. Our lab had observed significant deficits in emotional-like behavior as measured by the NSF, FST and sucrose preference in 2BC after paclitaxel administration in mice.

As reported by Campos-Jurado and Morón, a review of the literature only returned 13 studies on rodent ethanol drinking behaviors in various pain settings. Of these 13 studies, only 6 included cohorts of female mice. This limits our ability to find sex-based differences in drinking behavior, a pattern already displayed in epidemiological data published on the matter. Given the sex-differences we have observed in our studies, it is imperative that more work be done in ethanol and pain research using male and female rodent models.

We have observed sex-differences in ethanol intake across varied testing paradigms, showing that only males increase their ethanol intake and preference in response to chronic pain

compared to their female counterparts that do not see this escalation in animals with no history of ethanol. This data is similar to recently published preclinical studies of ethanol and chronic pain in rodents.[96,97] These sex-based differences are also found in human studies of alcohol consumption in patients living with chronic pain.[98] This is despite both male and female mice having similar responses of mechanical hypersensitivity from CFA administration. It will be important to understand these sex differences and the mechanisms involved to develop better therapeutics for AUD and chronic pain. One possible explanation is that ethanol provides less of an analgesic effect for female mice compared to male mice, making it less reinforcing. A study in C57BL/6J has shown that inflammation in female mice was attenuated by ethanol less than males.[99] Our lab has recently observed in a test of acute thermal nociceptive pain that ethanol did not produce antinociception in females as compared to male mice.[100] A related explanation could be that female mice do not increase their ethanol drinking because they have a higher sensitivity to ethanol compared to male mice; making consumption more aversive and less likely to escalate drinking.[101] If there are sex-dependent differences in the development of a high acute functional tolerance (AFT) these might allow male rodents to increase their ethanol intake relative to female mice.[102] Exploring this concept in an acute pain model (laparotomy), our lab measured AFT to ethanol's sedative and ataxic effects using the loss-of-righting-reflex (LORR). They found that females displayed a sustained and increased sensitivity to ethanol compared to male mice. It was hypothesized that this might explain the sex-differences in ethanol consumption after laparotomy. Given that we saw an increase in ethanol consumption in female mice with a history of ethanol consumption; it is possible that prior ethanol exposure could induce tolerance in female mice against the aversive effects of future ethanol exposure and allow for escalation of drinking during painful or stressful conditions. In male mice under this paradigm, prior ethanol exposure may have

inoculated the mice against future increases in ethanol consumption under a pain condition. This may be due to differences in learning and association between ethanol naive and ethanol-exposed male mice.

5.3 Limitations

Given the complexity and distribution of opioid receptors there are potential confounding effects of norBNI's administration. Our norBNI study used global administration by i.p. Injection; other studies utilizing norBNI have used more precise dosing methods such as local injection into the posteromedial shell of the NAc.[103] While more invasive, this method of dosing would allow researchers more precision in selecting for KOR in specific regions of the nervous system rather than blocking KOR globally. With our experimental design, we cannot say with any certainty what contribution any single distribution of KORs in the CNS or PNS has to the overall observed behaviors.

The need to measure individual ethanol consumption for all studies necessitates single rodent housing. This presents its own issue as mice are naturally social animals and are subjected to relative isolation for the duration of each study. This isolation and social deprivation likely have some effect on our behavioral testing from drinking behavior to nociceptive testing. Ideally a model will emerge that allows for individualized measurement of rodent consumption in a continuous-access model while still allowing normative social housing for the studied mice. Oral gavage of ethanol would allow for individualized dosing but is incompatible with a free-drinking model.

The methods we used to measure changes in nociception also allow for some room for improvement. Mice need at least 45 minutes to acclimate in their testing conditions for Von Frey

and Acetone after being removed from their cage. This time out of their cage (and not able to access the 2BC) does not include time spent testing the mice or transferring them back to their cages. This necessary disruption in drinking occurred at minimum twice over a 7-day period. This creates two possible sources of measurement error. First, on behavioral testing days, there may be as much as 1-1.5 hours less drinking time during the light-cycle hours. Secondly, the long acclimation period prior to testing may blunt some of the ethanol's analgesic effects prior to the commencement of testing. However, this second issue is common to all use of von Frey and acetone testing, so does not represent a unique barrier to our study. One possible solution is the use of the Drinking-in-the-Dark (DID) model where water is replaced with 20% ethanol during the first four hours of the dark phase of the mouse's light/dark cycle. C57BL/6J mice exposed to this paradigm have been observed to consume high levels of ethanol.[104]

5.3.1 Future Studies

More broadly, evaluating intermittent access and escalation models of drinking in response to chronic pain will expand our results with the 2BC assay. It will be important to evaluate the effects of norBNI in the CIPN model in addition to CFA to evaluate what role KOR and dynorphins play in ethanol intake in response to neuropathic pain. Future studies could also utilize different approaches to suppressing KOR activity such as inducible genetic KOR KO mice or previously used intracranial microinjections of norBNI to selectively inhibit KOR in the NAc or other CNS targets. Global administration of norBNI does present several limitations to our studies. This administration method will affect KOR in potentially many tissues.

It will be important to evaluate in future studies the possibility of alteration of ethanol kinetics in chronic pain conditions. Measuring of BAC would allow for better comparisons of

ethanol metabolism between groups. Currently our use of only 2BC limits the ability to consider how an animal's metabolism of ethanol might affect behavior or nociception.

References

Alberti, P.; Lehmann, H.C. Chemotherapy induced peripheral neurotoxicity: Six essential articles for effective future research. Exp. Neurol. 2021, 337, 113555

Alfonso-Loeches S, Pascual M, Guerri C. Gender differences in alcohol-induced neurotoxicity and brain damage. *Toxicology (Amsterdam)*. 2013;311(1-2):27-34. doi:10.1016/j.tox.2013.03.001

Apkarian AV, Neugebauer V, Koob G, et al. Neural mechanisms of pain and alcohol dependence.*Pharmacol Biochem Behav*. 2013;112:34-41.doi:10.1016/j.pbb.2013.09.008

Bagdas, D., Alsharari, S. D., Freitas, K., Tracy, M., & Damaj, M. I. (2015). The role of alpha5 nicotinic acetylcholine receptors in mouse models of chronic inflammatory and neuropathic pain. *Biochemical Pharmacology,97*(4),590-600. doi:10.1016/j.bcp.2015.04.013

Barrett K.U.S. Population Trends Return to Pre-Pandemic Norms as More States Gain Population. United States Census Bureau. December 19th 2023. Accessed April 25th 2024.https://www.census.gov/newsroom/press-releases/2023/population-trends-return-to-pre- pandemic-norms.html

Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell.* 2009 Oct 16;139(2):267-84. doi: 10.1016/j.cell.2009.09.028. PMID: 19837031; PMCID: PMC2852643.

Boissoneault J, Lewis B, Nixon SJ. Characterizing chronic pain and alcohol use trajectory among treatment-seeking alcoholics. *Alcohol.* 2019;75:47-54

Bolon B, Stolina M, King C, et al. Rodent preclinical models for developing novel antiarthritic molecules: comparative biology and preferred methods for evaluating efficacy. *J Biomed Biotechnol*. 2011;2011:569068. doi:10.1155/2011/569068

Brennan, P. L., Schutte, K. K., Moos, B. S., & Moos, R. H. (2011). Twenty-year alcohol consumption and drinking-problem trajectories of older men and women. Journal of Studies on Alcohol and Drugs, 72, 308e321.

Brischoux F, Chakraborty S, Brierley DI, Ungless MA. Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci U S A 2009;106:4894–9.

Butler RK, Knapp DJ, Ulici V, Longobardi L, Loeser RF, Breese GR. A mouse model for chronic pain-induced increase in ethanol consumption. *Pain*. 2017;158(3):457-462. doi:10.1097/j.pain.0000000000000780

Campos-Jurado Y, Morón JA. Inflammatory pain affects alcohol intake in a dose-dependent manner in male rats in the intermittent access model. *Pain Rep*. 2023;8(4):e1082. Published 2023 Jun 27. doi:10.1097/PR9.0000000000001082

Campos-Jurado, Yolandaa,b,c; Morón, Jose A.a,b,c,d,e,*. Inflammatory pain affects alcohol intake in a dose-dependent manner in male rats in the intermittent access model. PAIN Reports 8(4):pe1082,July/August 2023. | DOI: 10.1097/PR9.0000000000001082

Chopra K, Tiwari V. Alcoholic neuropathy: possible mechanisms and future treatment possibilities. Br J Clin Pharmacol. 2012 Mar;73(3):348-62

Chronopoulos A, Zarra T, Tröltzsch M, Mahaini S, Ehrenfeld M, Otto S. Osteoradionecrosis of the mandible: a ten year single-center retrospective study.J Craniomaxillofac Surg. 2015;43(6):837-846. doi:10.1016/j.jcms. 2015.03.024

Cooper JC, Knutson B. Valence and salience contribute to nucleus accumbens activation. *Neuroimage*. 2008;39(1):538-547. doi:10.1016/j.neuroimage.2007.08.009

Dahlhamer J, Lucas J, Zelaya Carla, Nahin R, Mackey S, DeBar L, Kerns R, Von Korff M, Porter L, Helmick C (2018) Prevalence of chronic pain and high-impact chronic pain among adults — United States, 2016. MMWRMorb Mortal Wkly Rep 67:1001–1006.

Deutsch-Link S, Curtis B, Singal AK. Covid-19 and alcohol associated liver disease. *Dig Liver Dis*. 2022;54(11):1459-1468. doi:10.1016/j.dld.2022.07.007

Ditre JW, Zale EL, LaRowe LR. A Reciprocal Model of Pain and Substance Use: Transdiagnostic Considerations, Clinical Implications, and Future Directions. Annu Rev Clin Psychol. 2019 May 7;15:503-528. doi: 10.1146/annurev-clinpsy-050718-095440. Epub 2018 Dec 19. PMID: 30566371.

Dudley R, Maro A. Human Evolution and Dietary Ethanol. *Nutrients*. 2021;13(7):2419. Published 2021 Jul 15. doi:10.3390/nu13072419

Dumontet, C., Jordan, M. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov* 9, 790–803 (2010). https://doi.org/10.1038/nrd3253

Egli M, Koob GF, Edwards S. Alcohol dependence as a chronic pain disorder. *Neurosci Biobehav Rev*. 2012;36(10):2179-2192. doi:10.1016/j.neubiorev.2012.07.010

Endoh T, Matsuura H, Tanaka C, Nagase H. Nor-binaltorphimine: a potent and selective kappa- opioid receptor antagonist with long-lasting activity in vivo. Arch Int Pharmacodyn Ther. 1992 Mar-Apr;316:30-42. PMID: 1326932.

Ferguson E, Vitus D, Williams M, et al. Sex Differences in Associations Between Delay Discounting and Expectancies for Alcohol Analgesia. *Experimental and clinical psychopharmacology*. 2022;30(6):862-872. doi:10.1037/pha0000517

Fischer BD, Adeyemo A, O'Leary ME, Bottaro A. Animal models of rheumatoid pain: experimental systems and insights. *Arthritis research & therapy*. 2017;19(1):146-146. doi:10.1186/s13075-017-1361-6

Fischer et al. Arthritis Research & Therapy (2017) 19:146 DOI 10.1186/s13075-017-1361-6

Ford CP (2014) The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 282:13–22.

Gaskin DJ, Richard P (2012) The economic costs of pain in the United States. J Pain 13:715–724.

George O, Le Moal M, Koob GF (2012) Allostasis and addiction: role of the dopamine and corticotropin-releasing factor systems. Physiol Behav 106:58–64.

Ghani S, Alkhlaif Y, Mann J, et al. Surgical incision pain induced an increase in alcohol consumption in mice. *Alcohol (Fayetteville, NY)*. 2024;117:1-9. doi:10.1016/j.alcohol.2024.03.005

Goldberg DS,McGee SJ (2011) Pain as a global public health priority. BMC

González-Sepúlveda M, Pozo OJ, Marcos J, Valverde O. Chronic pain causes a persistent anxiety state leading to increased ethanol intake in CD1 mice. *J Psychopharmacol*. 2016;30(2):188-203. doi:10.1177/0269881115622238

Grant BF, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Huang B, Jung J, Zhang H, Fan A, Hasin DS (2017) Prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV alcohol use disorder in the United States, 2001–2002 to 2012–2013: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. JAMA Psychiatry 74:911–923.

Hannoodee S, Nasuruddin DN. Acute Inflammatory Response. [Updated 2022 Nov 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556083/

Hargreaves K, Dubner R, Brown F, Flores C, Joris J, 1988 A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32, 77–88. 10.1016/0304-3959(88)90026-7 [PubMed: 3340425]

Hootman, J.M., Helmick, C.G., Barbour, K.E., Theis, K.A., Boring, M.A., 2016. Updated projected prevalence of self-reported doctor-diagnosed arthritis and arthritis-attributable activity limitation among US Adults, 2015–2040. Arthritis. Rheumatol. 68 (7), 1582–1587.https://doi.org/10.1002/art.39692.

Huh SY, Kim S-G, Kim H-K. Capsaicin reduces ethanol consumption in C57BL/6 but not DBA/2 mice. Clin Psychopharmacol Neurosci 2022;20: 343–9.

Ji G, Neugebauer V (2008) Pro-and anti-nociceptive effects of corticotropin releasing factor (CRF) in central amygdala neurons are mediated through different receptors. J Neurophysiol 99:1201–1212.

Jirkof P, Cesarovic N, Rettich A, Nicholls F, Seifert B, Arras M. Burrowing behavior as an indicator of post-laparotomy pain in mice. *Front Behav Neurosci.* 2010 Oct 12;4:165. doi: 10.3389/fnbeh.2010.00165. PMID: 21031028; PMCID: PMC2965018.

Julian T, Glascow N, Syeed R, Zis P. Alcohol-related peripheral neuropathy: a systematic review and meta-analysis. J Neurol. 2019 Dec;266(12):2907-2919

Kageyama K, Iwasaki Y, Daimon M. Hypothalamic Regulation of Corticotropin-Releasing Factor under Stress and Stress Resilience. *Int J Mol Sci*. 2021;22(22):12242. Published 2021 Nov 12. doi:10.3390/ijms222212242

Karkhanis AN, Al-Hasani R. Dynorphin and its role in alcohol use disorder. *Brain research.* 2020;1735:146742-146742. doi:10.1016/j.brainres.2020.146742

Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J. 1991;10(13):4025–31.

Knutson B, Cooper JC (2005) Functional magnetic resonance imaging of reward prediction. Curr Opin Neurol 18:411–417.

Koob GF (2003) Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 27:232–243.

Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. Cell. 1996; 87(5):811–22.

Lai J, LuoM-C, Chen Q,Ma S, Gardell LR, Ossipov MH, Porreca F (2006) Dynorphin A activates bradykinin receptors to maintain neuropathic pain. Nat Neurosci 9:1534–1540.

LaRowe LR, Powers JM, Maisto SA, Zvolensky MJ, Glatt SJ, Ditre JW. Brief Report: Expectancies for alcohol analgesia are associated with greater alcohol use among moderate-to-heavy drinkers without chronic pain. *The American journal on addictions*. 2022;31(1):80-84. doi:10.1111/ajad.13245

Larson MJ, Paasche-Orlow M, Cheng DM, Lloyd-Travaglini C, Saitz R, Samet JH. Persistent pain is associated with substance use after detoxification: a prospective cohort analysis. *Addiction.* 2007;102:752–760

Lee BP, Dodge JL, Leventhal A, Terrault NA. Retail Alcohol and Tobacco Sales During COVID- 19. *Ann Intern Med*. 2021;174(7):1027-1029. doi:10.7326/M20-7271

Lorente JD, Cuitavi J, Campos-Jurado Y, Montón-Molina R, González-Romero JL, Hipólito L. Kappa opioid receptor blockade in the nucleus accumbens shell prevents sex-dependent alcohol deprivation effect induced by inflammatory pain. *Pain*. 2022;163(1):e137-e147. doi:10.1097/j.pain.0000000000002332

M. Seretny, G. L. Currie, E. S. Sena et al., "Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis," Pain, vol. 155, no. 12, pp. 2461–2470, 2014.

Margolis EB, Hjelmstad GO, Bonci A, Fields HL (2003) j-Opioid agonists directly inhibit midbrain dopaminergic neurons. J Neurosci 23:9981–9986.

McCaffrey RJ, Duff K, Westervelt HJ, editors. *Practitioner's guide to evaluating change with neuropsychological assessment instruments*. New York, NY: Kluwer Academic/Plenum Publishers; 2000

Mills, S.E.E., K.P. Nicolson, and B.H. Smith, Chronic pain: a review of its epidemiology and associated factors in population-based studies. Br J Anaesth, 2019. 123(2): p. e273- e283

National Institute on Alcohol Abuse and Alcoholism (NIAAA). Health Topics: Alcohol Use Disorder. National Institute of Health (NIH). Accessed 3/4/2024.
Public Health 11:770. https://www.niaaa.nih.gov/alcohols-effects-health/alcohol- topics/health-topics-alcohol-use-disorder

Negus SS, Neddenriep B, Altarifi AA, Carroll FI, Leitl MD, Miller LL. Effects of ketoprofen, morphine, and kappa opioids on pain-related depression of nesting in mice. Pain. 2015; 156:1153– 1160. DOI: 10.1097/j.pain.0000000000000171 [PubMed: 25827812]

Neddenriep B, Bagdas D, Contreras KM, et al. Pharmacological mechanisms of alcohol analgesic-like properties in mouse models of acute and chronic pain. *Neuropharmacology*. 2019;160:107793. doi:10.1016/j.neuropharm.2019.107793

Pahwa R, Goyal A, Jialal I. Chronic Inflammation. In: *StatPearls*. Treasure Island (FL): StatPearlsPublishing; August 7, 2023.

PALMER AA, SHARPE AL, BURKHART-KASCH S, et al. Corticotropin-releasing factor overexpression decreases ethanol drinking and increases sensitivity to the sedative effects of ethanol. *Psychopharmacologia*. 2004;176(3-4):386-397. doi:10.1007/s00213-004-1896-5

Raja SN, Carr DB, Cohen M, et al. The Revised IASP definition of pain: concepts, challenges, and compromises. *Pain (Amsterdam)*. 2020;161(9):1976-1982. doi:10.1097/j.pain.0000000000001939

Riley JL 3rd, King C. Self-report of alcohol use for pain in a multi-ethnic community sample. *J Pain*. 2009 Sep;10(9):944-52. doi: 10.1016/j.jpain.2009.03.005. PMID: 19712901; PMCID: PMC2734914.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18:247–291.

Rocco A, Compare D, Angrisani D, Sanduzzi Zamparelli M, Nardone G. Alcoholic disease: liver and beyond. World J Gastroenterol. 2014;20(40):14652-14659. doi:10.3748/wjg.v20.i40.14652

Rose JH, Karkhanis AN, Chen R, Gioia D, Lopez MF, Becker HC, McCool BA, Jones SR. Supersensitive kappa opioid receptors promotes ethanol withdrawal-related behaviors and reduce dopamine signaling in the nucleus accumbens. Int J Neuropsychopharmacol 2016;19:1–10.

SAMHSA, Center for Behavioral Health Statistics and Quality. 2022 National Survey on Drug Use and Health. Table 5.9A—Alcohol use disorder in past year: among people aged 12 or older; by age group and demographic characteristics, numbers in thousands, 2021 and 2022. [cited 2023 Dec 8]. Available from: https://www.samhsa.gov/data/sites/default/files/reports/rpt42728/NSDUHDetailedTabs2022/NSDUHDetailedTabs2022/NSDUHDetTabsSect5pe2022.htm#tab5.9a

Schaible HG, Richter F. Pathophysiology of pain. *Langenbecks Arch Surg*. 2004;389(4):237-243. doi:10.1007/s00423-004-0468-9

Shi M, Luo C, Oduyale OK, Zong X, LoConte NK, Cao Y. Alcohol Consumption Among Adults With a Cancer Diagnosis in the All of Us Research Program. *JAMA Netw Open*. 2023;6(8):e2328328. doi:10.1001/jamanetworkopen.2023.28328

Solomon RL, Corbit JD (1974) An opponent-process theory of motivation: I. Temporal dynamics of affect. Psychol Rev 81:119–145.

Staff NP, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: A current review. *Annals of neurology*. 2017;81(6):772-781. doi:10.1002/ana.24951

Stahre M, Roeber J, Kanny D, Brewer RD, Zhang X (2014) Contribution of excessive alcohol consumption to deaths and years of potential life lost in the United States. Prev Chronic Dis 11:130293.

Stasiak K. L., Maul D., French E., Hellyer P. W., VandeWoude S. (2003). Species-specific assessment of pain in laboratory animals. *Contemp. Top. Lab. Anim. Sci.* 42, 13–20.

Taylor AMW, Becker S, Schweinhardt P, Cahill C. Mesolimbic dopamine signaling in acute and chronic pain: implications for motivation, analgesia, and addiction. *Pain (Amsterdam)*. 2016;157(6):1194-1198. doi:10.1097/j.pain.0000000000000494

Taylor PC, Moore A, Vasilescu R, Alvir J, Tarallo M. A structured literature review of the burden of illness and unmet needs in patients with rheumatoid arthritis: a current perspective. Rheumatol Int. 2016;36(5):685–95.

Tizard IR. Adjuvants and adjuvanticity. *Vaccines for Veterinarians*. 2021;75-86.e1. doi:10.1016/B978-0-323-68299-2.00016-2

Toma W, Kyte SL, Bagdas D, et al. Effects of paclitaxel on the development of neuropathy and affective behaviors in the mouse. *Neuropharmacology*. 2017;117:305-315. doi:10.1016/j.neuropharm.2017.02.020

Tracey I, Dunckley P (2004) Importance of anti-and pro-nociceptive mechanisms in human disease. Gut 53:1553–1555.

Tracey, I. and M.C. Bushnell, How neuroimaging studies have challenged us to rethink: is chronic pain a disease? J Pain, 2009. 10(11): p. 1113-20.

Turcott JG, Juarez-Hernandez E, De la Torre-Vallejo M, Sanchez-Lara K, Luvian-Morales J, Arrieta O. Value: Changes in the detection and recognition thresholds of three basic tastes in lung cancer patients receiving cisplatin and paclitaxel and its association with nutritional and quality of life parameters. Nutr Cancer. 2016; 68:241–249. DOI: 10.1080/01635581.2016.1144075 [PubMed: 26943275]

Turk, D.C. and R. Melzack, Handbook of pain assessment. 3rd ed. 2011, New York: Guilford Press. xvii, 542 p.

Veer AVT, Yano JM, Carroll FI, Cohen BM, Carlezon WA (2012) Corticotropin- releasing factor (CRF)-induced disruption of attention in rats is blocked by the j-opioid receptor antagonist JDTic. Neuropsychopharmacology 37:2809–2816.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Jayne M, Ma Y, Pradhan K, Wong C (2007) Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement.J Neurosci 27:12700–12706.

Vowles, K.E., Witkiewitz, K., Pielech, M., Edwards, K.A., McEntee, M.L., Bailey, R.W., Sullivan, M.D., 2018. Alcohol and opioid use in chronic pain: A cross-sectional examination of differences in functioning based on Misuse Status. J. Pain 19 (10), 1181–1188. https://doi.org/10.1016/j.jpain.2018.04.013.

Weiss F, LorangMT, Bloom FE, Koob GF (1993) Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 267:250–258.

White A, Caillaud M, Carper M, Poklis J, Miles MF, Damaj MI. Thermal antinociceptive responses to alcohol in DBA/2J and C57BL/6J inbred male and female mouse strains. *Behavioural brain research*. 2023;436:114087-114087. doi:10.1016/j.bbr.2022.114087

Wilsnack RW, Wilsnack SC, Kristjanson AF, Vogeltanz-Holm ND, Gmel G. Gender and alcohol consumption: patterns from the multinational GENACIS project. *Addiction*. 2009 Sep;104(9):1487-500. doi: 10.1111/j.1360-0443.2009.02696.x. PMID: 19686518; PMCID: PMC2844334.

World Health Organization, 2018. Global status report on alcohol and health 2018. Geneva: World Health Organization; 2018. License: CC BY-NC-SA 3.0 IGO.

Yeung EW, Craggs JG, Gizer IR. Comorbidity of Alcohol Use Disorder and Chronic Pain: Genetic Influences on Brain Reward and Stress Systems. *Alcoholism, clinical and experimental research*.2017;41(11):1831-1848. doi:10.1111/acer.13491

Yu W, Hwa LS, Makhijani VH, Besheer J, Kash TL. Chronic inflammatory pain drives alcohol drinking in a sex-dependent manner for C57BL/6J mice. *Alcohol*. 2019;77:135-145. doi:10.1016/j.alcohol.2018.10.002

Endnotes

[1] Dudley R, Maro A. Human Evolution and Dietary Ethanol. *Nutrients*. 2021;13(7):2419. Published 2021 Jul 15. doi:10.3390/nu13072419

[2] National Institute on Alcohol Abuse and Alcoholism (NIAAA). Health Topics: Alcohol Use Disorder. National Institute of Health (NIH). Accessed 3/4/2024. https://www.niaaa.nih.gov/alcohols-effects-health/alcohol-topics/health-topics-alcohol-use-disorder

[3] SAMHSA, Center for Behavioral Health Statistics and Quality. 2022 National Survey on Drug Use and Health. Table 5.9A—Alcohol use disorder in past year: among people aged 12 or older; by age group and demographic characteristics, numbers in thousands, 2021 and 2022. [cited 2023 Dec 8]. Available from: https://www.samhsa.gov/data/sites/default/files/reports/rpt42728/NSDUHDetailedTabs2022/NSDUHDetailedTabs2022/NSDUHDetTabsSect5pe2022.htm#tab5.9a

[4] Barrett K.U.S. Population Trends Return to Pre-Pandemic Norms as More States Gain Population. United States Census Bureau. December 19th 2023. Accessed April 25th 2024. https://www.census.gov/newsroom/press-releases/2023/population-trends-return-to-pre-pandemic-norms.html

[5] Grant BF, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Huang B, Jung J, Zhang H, Fan A, Hasin DS (2017) Prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV alcohol use disorder in the United States, 2001–2002 to 2012–2013: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. JAMA Psychiatry 74:911–923.

[6] Stahre M, Roeber J, Kanny D, Brewer RD, Zhang X (2014) Contribution of excessive alcohol consumption to deaths and years of potential life lost in the United States. Prev Chronic Dis 11:130293.

[7] World Health Organization, 2018.

[8] Lee BP, Dodge JL, Leventhal A, Terrault NA. Retail Alcohol and Tobacco Sales During COVID-19. *Ann Intern Med*. 2021;174(7):1027-1029. doi:10.7326/M20-7271

[9] Deutsch-Link S, Curtis B, Singal AK. Covid-19 and alcohol associated liver disease. *Dig Liver Dis*. 2022;54(11):1459-1468. doi:10.1016/j.dld.2022.07.007

[10] Global status report on alcohol and health 2018. Geneva: World Health Organization; 2018. License: CC BY-NC-SA 3.0 IGO.

[11] Yeung, 2017.

[12] Knutson B, Cooper JC (2005) Functional magnetic resonance imaging of reward prediction. Curr Opin Neurol 18:411–417.

[13] Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18:247–291.

[14] Weiss F, LorangMT, Bloom FE, Koob GF (1993) Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 267:250–258.

[15] Ford CP (2014) The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 282:13–22.

[16] Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Jayne M, Ma Y, Pradhan K, Wong C (2007) Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement.J Neurosci 27:12700–12706.

[17] Solomon RL, Corbit JD (1974) An opponent-process theory of motivation: I. Temporal dynamics of affect. Psychol Rev 81:119–145.

[18] Koob GF (2003) Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 27:232–243.

[19] George O, Le Moal M, Koob GF (2012) Allostasis and addiction: role of the dopamine and corticotropin-releasing factor systems. Physiol Behav 106:58–64.

[20] Dahlhamer J, Lucas J, Zelaya Carla, Nahin R, Mackey S, DeBar L, Kerns R, Von Korff M, Porter L, Helmick C (2018) Prevalence of chronic pain and high-impact chronic pain among adults — United States, 2016. MMWRMorb Mortal Wkly Rep 67:1001–1006.

[21] Goldberg DS,McGee SJ (2011) Pain as a global public health priority. BMC Public Health 11:770.

[22] Gaskin DJ, Richard P (2012) The economic costs of pain in the United States. J Pain 13:715–724.

[23] Turk, D.C. and R. Melzack, Handbook of pain assessment. 3rd ed. 2011, New York: Guilford Press. xvii, 542 p.

[24] Tracey, I. and M.C. Bushnell, How neuroimaging studies have challenged us to rethink: is chronic pain a disease? J Pain, 2009. 10(11): p. 1113-20.

[25] Mills, S.E.E., K.P. Nicolson, and B.H. Smith, Chronic pain: a review of its epidemiology and associated factors in population-based studies. Br J Anaesth, 2019. 123(2): p. e273-e283

[26] Raja SN, Carr DB, Cohen M, et al. The Revised IASP definition of pain: concepts, challenges, and compromises. *Pain (Amsterdam)*. 2020;161(9):1976-1982. doi:10.1097/j.pain.0000000000001939

[27] Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell.* 2009 Oct 16;139(2):267-84. doi: 10.1016/j.cell.2009.09.028. PMID: 19837031; PMCID: PMC2852643.

[28] Basbaum, 2009.

[29] Schaible HG, Richter F. Pathophysiology of pain. *Langenbecks Arch Surg*. 2004;389(4):237-243. doi:10.1007/s00423-004-0468-9

[30] Hootman, J.M., Helmick, C.G., Barbour, K.E., Theis, K.A., Boring, M.A., 2016. Updated projected prevalence of self-reported doctor-diagnosed arthritis and arthritis-attributable activity limitation among US Adults, 2015–2040. Arthritis. Rheumatol. 68 (7), 1582–1587. https://doi.org/10.1002/art.39692.

[31] Vowles, K.E., Witkiewitz, K., Pielech, M., Edwards, K.A., McEntee, M.L., Bailey, R.W., Sullivan, M.D., 2018. Alcohol and opioid use in chronic pain: A cross-sectional examination of differences in functioning based on Misuse Status. J. Pain 19 (10), 1181–1188. https://doi.org/10.1016/j.jpain.2018.04.013.

[32] Yeung, 2017.

[33] Karkhanis AN, Al-Hasani R. Dynorphin and its role in alcohol use disorder. *Brain research*. 2020;1735:146742-146742. doi:10.1016/j.brainres.2020.146742

[34] Taylor AMW, Becker S, Schweinhardt P, Cahill C. Mesolimbic dopamine signaling in acute and chronic pain: implications for motivation, analgesia, and addiction. *Pain (Amsterdam)*. 2016;157(6):1194-1198. doi:10.1097/j.pain.0000000000000494

[35] Brischoux F, Chakraborty S, Brierley DI, Ungless MA. Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci U S A 2009;106:4894–9.

[36] Cooper JC, Knutson B. Valence and salience contribute to nucleus accumbens activation. *Neuroimage*. 2008;39(1):538-547. doi:10.1016/j.neuroimage.2007.08.009

[37]Riley JL, King C, 2009 Self-report of alcohol use for pain in a multi-ethnic community sample. J. Pain Off. J. Am. Pain Soc 10, 944–952. 10.1016/j.jpain.2009.03.005

[38] Alcoholic neuropathy: possible mechanisms and future treatment possibilities. Chopra K, Tiwari V. Br J Clin Pharmacol. 2012 Mar;73(3):348-62

[39] Alcohol-related peripheral neuropathy: a systematic review and meta-analysis. Julian T, Glascow N, Syeed R, Zis P. J Neurol. 2019 Dec;266(12):2907-2919

[40] Pahwa R, Goyal A, Jialal I. Chronic Inflammation. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; August 7, 2023.

[41] Hannoodee S, Nasuruddin DN. Acute Inflammatory Response. [Updated 2022 Nov 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556083/

[42] Yeung EW, Craggs JG, Gizer IR. Comorbidity of Alcohol Use Disorder and Chronic Pain: Genetic Influences on Brain Reward and Stress Systems. *Alcoholism, clinical and experimental research*. 2017;41(11):1831-1848. doi:10.1111/acer.13491

[43] Egli M, Koob GF, Edwards S. Alcohol dependence as a chronic pain disorder. *Neurosci Biobehav Rev*. 2012;36(10):2179-2192. doi:10.1016/j.neubiorev.2012.07.010

[44] McCaffrey RJ, Duff K, Westervelt HJ, editors. *Practitioner's guide to evaluating change with neurophsychological assessment instruments.* New York, NY: Kluwer Academic/Plenum Publishers; 2000

[45] Larson MJ, Paasche-Orlow M, Cheng DM, Lloyd-Travaglini C, Saitz R, Samet JH. Persistent pain is associated with substance use after detoxification: a prospective cohort analysis. *Addiction.* 2007;102:752–760

[46] Kageyama K, Iwasaki Y, Daimon M. Hypothalamic Regulation of Corticotropin-Releasing Factor under Stress and Stress Resilience. *Int J Mol Sci*. 2021;22(22):12242. Published 2021 Nov 12. doi:10.3390/ijms222212242

[47] Apkarian AV, Neugebauer V, Koob G, et al. Neural mechanisms of pain and alcohol dependence. *Pharmacol Biochem Behav*. 2013;112:34-41. doi:10.1016/j.pbb.2013.09.008

[48] Ji G, Neugebauer V (2008) Pro-and anti-nociceptive effects of corticotropin releasing factor (CRF) in central amygdala neurons are mediated through different receptors. J Neurophysiol 99:1201–1212.

[49] Tracey I, Dunckley P (2004) Importance of anti-and pro-nociceptive mechanisms in human disease. Gut 53:1553–1555.

[50] Margolis EB, Hjelmstad GO, Bonci A, Fields HL (2003) j-Opioid agonists directly inhibit midbrain dopaminergic neurons. J Neurosci 23:9981–9986.

[51] Veer AVT, Yano JM, Carroll FI, Cohen BM, Carlezon WA (2012) Corticotropin- releasing factor (CRF)-induced disruption of attention in rats is blocked by the j-opioid receptor antagonist JDTic. Neuropsychopharmacology 37:2809–2816.

[52] Rose JH, Karkhanis AN, Chen R, Gioia D, Lopez MF, Becker HC, McCool BA, Jones SR. Supersensitive kappa opioid receptors promotes ethanol withdrawal-related behaviors and reduce dopamine signaling in the nucleus accumbens. Int J Neuropsychopharmacol 2016;19:1–10.

[53] Lai J, LuoM-C, Chen Q,Ma S, Gardell LR, Ossipov MH, Porreca F (2006) Dynorphin A activates bradykinin receptors to maintain neuropathic pain. Nat Neurosci 9:1534–1540.

[54] Endoh T, Matsuura H, Tanaka C, Nagase H. Nor-binaltorphimine: a potent and selective kappa-opioid receptor antagonist with long-lasting activity in vivo. Arch Int Pharmacodyn Ther. 1992 Mar-Apr;316:30-42. PMID: 1326932.

[55] Yu W, Hwa LS, Makhijani VH, Besheer J, Kash TL. Chronic inflammatory pain drives alcohol drinking in a sex-dependent manner for C57BL/6J mice. *Alcohol*. 2019;77:135-145. doi:10.1016/j.alcohol.2018.10.002

[56] González-Sepúlveda M, Pozo OJ, Marcos J, Valverde O. Chronic pain causes a persistent anxiety state leading to increased ethanol intake in CD1 mice. *J Psychopharmacol*. 2016;30(2):188-203. doi:10.1177/0269881115622238

[57] Butler RK, Knapp DJ, Ulici V, Longobardi L, Loeser RF, Breese GR. A mouse model for chronic pain-induced increase in ethanol consumption. *Pain*. 2017;158(3):457-462. doi:10.1097/j.pain.0000000000000780

[58] Brennan, P. L., Schutte, K. K., Moos, B. S., & Moos, R. H. (2011). Twenty-year alcohol consumption and drinking-problem trajectories of older men and women.Journal of Studies on Alcohol and Drugs, 72, 308e321.

[59] Boissoneault J, Lewis B, Nixon SJ. Characterizing chronic pain and alcohol use trajectory among treatment-seeking alcoholics. *Alcohol.* 2019;75:47-54

[60] Riley JL 3rd, King C. Self-report of alcohol use for pain in a multi-ethnic community sample. *J Pain*. 2009 Sep;10(9):944-52. doi: 10.1016/j.jpain.2009.03.005. PMID: 19712901; PMCID: PMC2734914.

[61] Wilsnack RW, Wilsnack SC, Kristjanson AF, Vogeltanz-Holm ND, Gmel G. Gender and alcohol consumption: patterns from the multinational GENACIS project. *Addiction*. 2009 Sep;104(9):1487-500. doi: 10.1111/j.1360-0443.2009.02696.x. PMID: 19686518; PMCID: PMC2844334.

[62] Jirkof P, Cesarovic N, Rettich A, Nicholls F, Seifert B, Arras M. Burrowing behavior as an indicator of post-laparotomy pain in mice. *Front Behav Neurosci.* 2010 Oct 12;4:165. doi: 10.3389/fnbeh.2010.00165. PMID: 21031028; PMCID: PMC2965018.

[63] Stasiak K. L., Maul D., French E., Hellyer P. W., VandeWoude S. (2003). Species-specific assessment of pain in laboratory animals. *Contemp. Top. Lab. Anim. Sci.* 42, 13–20.

[64] Neddenriep B, Bagdas D, Contreras KM, et al. Pharmacological mechanisms of alcohol analgesic-like properties in mouse models of acute and chronic pain. *Neuropharmacology*. 2019;160:107793. doi:10.1016/j.neuropharm.2019.107793

[65] Ditre JW, Zale EL, LaRowe LR. A Reciprocal Model of Pain and Substance Use: Transdiagnostic Considerations, Clinical Implications, and Future Directions. Annu Rev Clin Psychol. 2019 May 7;15:503-528. doi: 10.1146/annurev-clinpsy-050718-095440. Epub 2018 Dec 19. PMID: 30566371.

[66] Bagdas, D., Alsharari, S. D., Freitas, K., Tracy, M., & Damaj, M. I. (2015). The role of alpha5 nicotinic acetylcholine receptors in mouse models of chronic inflammatory and neuropathic pain. *Biochemical Pharmacology*,*97*(4), 590-600. doi:10.1016/j.bcp.2015.04.013

[67] Taylor PC, Moore A, Vasilescu R, Alvir J, Tarallo M. A structured literature review of the burden of illness and unmet needs in patients with rheumatoid arthritis: a current perspective. Rheumatol Int. 2016;36(5):685–95.

[68] Fischer et al. Arthritis Research & Therapy (2017) 19:146 DOI 10.1186/s13075-017-1361-6

[69] Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. Cell. 1996; 87(5):811–22.

[70] Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J. 1991;10(13):4025–31.

[71] Fischer BD, Adeyemo A, O'Leary ME, Bottaro A. Animal models of rheumatoid pain: experimental systems and insights. *Arthritis research & therapy*. 2017;19(1):146-146. doi:10.1186/s13075-017-1361-6

[72] Hargreaves K, Dubner R, Brown F, Flores C, Joris J, 1988 A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32, 77–88. 10.1016/0304-3959(88)90026-7 [PubMed: 3340425]

[73] Tizard IR. Adjuvants and adjuvanticity. *Vaccines for Veterinarians*. 2021;75-86.e1. doi:10.1016/B978-0-323-68299-2.00016-2

[74] Bolon B, Stolina M, King C, et al. Rodent preclinical models for developing novel antiarthritic molecules: comparative biology and preferred methods for evaluating efficacy. *J Biomed Biotechnol*. 2011;2011:569068. doi:10.1155/2011/569068

[75] Bruchas M. R., Yang T., Schreiber S., Defino M., Kwan S. C., Li S., et al. (2007). Long-acting kappa opioid antagonists disrupt receptor signaling and produce noncompetitive effects by activating c-Jun N-terminal kinase. *J. Biol. Chem.* 282 29803–29811. 10.1074/jbc.M705540200

[76] Yu, 2019.

[77] Lorente JD, Cuitavi J, Campos-Jurado Y, Montón-Molina R, González-Romero JL, Hipólito L. Kappa opioid receptor blockade in the nucleus accumbens shell prevents sex-dependent alcohol deprivation effect induced by inflammatory pain. *Pain*. 2022;163(1):e137-e147. doi:10.1097/j.pain.0000000000002332

[78] Alberti, P.; Lehmann, H.C. Chemotherapy induced peripheral neurotoxicity: Six essential articles for effective future research. Exp. Neurol. 2021, 337, 113555

[79] M. Seretny, G. L. Currie, E. S. Sena et al., "Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis," Pain, vol. 155, no. 12, pp. 2461–2470, 2014.

[80] Staff NP, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: A current review. *Annals of neurology*. 2017;81(6):772-781. doi:10.1002/ana.24951

[81] Chronopoulos A, Zarra T, Tröltzsch M, Mahaini S, Ehrenfeld M, Otto S. Osteoradionecrosis of the mandible: a ten year single-center retrospective study.J Craniomaxillofac Surg. 2015;43(6):837-846. doi:10.1016/j.jcms.2015.03.024

[82] Rocco A, Compare D, Angrisani D, Sanduzzi Zamparelli M, Nardone G. Alcoholic disease: liver and beyond. World J Gastroenterol. 2014;20(40):14652-14659. doi:10.3748/wjg.v20.i40.14652

[83] Shi M, Luo C, Oduyale OK, Zong X, LoConte NK, Cao Y. Alcohol Consumption Among Adults With a Cancer Diagnosis in the All of Us Research Program. *JAMA Netw Open*. 2023;6(8):e2328328. doi:10.1001/jamanetworkopen.2023.28328

[84]Dumontet C,2010.

[85] Toma W, Kyte SL, Bagdas D, et al. Effects of paclitaxel on the development of neuropathy and affective behaviors in the mouse. *Neuropharmacology*. 2017;117:305-315. doi:10.1016/j.neuropharm.2017.02.020

[86] LaRowe LR, Powers JM, Maisto SA, Zvolensky MJ, Glatt SJ, Ditre JW. Brief Report: Expectancies for alcohol analgesia are associated with greater alcohol use among moderate-to-heavy drinkers without chronic pain. *The American journal on addictions*. 2022;31(1):80-84. doi:10.1111/ajad.13245

[87] Ferguson E, Vitus D, Williams M, et al. Sex Differences in Associations Between Delay Discounting and Expectancies for Alcohol Analgesia. *Experimental and clinical psychopharmacology*. 2022;30(6):862-872. doi:10.1037/pha0000517

[88] Campos-Jurado, Yolandaa,b,c; Morón, Jose A.a,b,c,d,e,*. Inflammatory pain affects alcohol intake in a dose-dependent manner in male rats in the intermittent access model. PAIN Reports 8(4):p e1082, July/August 2023. | DOI: 10.1097/PR9.0000000000001082

[89] Boissoneault J (2019)

[90] Spratlin J, Sawyer MB. Pharmacogenetics of paclitaxel metabolism. *Crit Rev Oncol Hematol*. 2007;61(3):222-229. doi:10.1016/j.critrevonc.2006.09.006

[91] Huh SY, Kim S-G, Kim H-K. Capsaicin reduces ethanol consumption in C57BL/6 but not DBA/2 mice. Clin Psychopharmacol Neurosci 2022;20: 343–9.

[92] Campos-Jurado, 2023.

[93] Campos-Jurado Y, Morón JA. Inflammatory pain affects alcohol intake in a dose-dependent manner in male rats in the intermittent access model. *Pain Rep*. 2023;8(4):e1082. Published 2023 Jun 27. doi:10.1097/PR9.0000000000001082

[94] Lorente,2021

[95] Turcott JG, Juarez-Hernandez E, De la Torre-Vallejo M, Sanchez-Lara K, Luvian-Morales J, Arrieta O. Value: Changes in the detection and recognition thresholds of three basic tastes in lung cancer patients receiving cisplatin and paclitaxel and its association with nutritional and quality of life parameters. Nutr Cancer. 2016; 68:241–249. DOI: 10.1080/01635581.2016.1144075 [PubMed: 26943275]

[96] González-Sepúlveda, 2016

[97] Yu et al., 2019

[98] Riley & King, 2009.

[99] Alfonso-Loeches S, Pascual M, Guerri C. Gender differences in alcohol-induced neurotoxicity and brain damage. *Toxicology (Amsterdam)*. 2013;311(1-2):27-34. doi:10.1016/j.tox.2013.03.001

[100] White A, Caillaud M, Carper M, Poklis J, Miles MF, Damaj MI. Thermal antinociceptive responses to alcohol in DBA/2J and C57BL/6J inbred male and female mouse strains. *Behavioural brain research*. 2023;436:114087-114087. doi:10.1016/j.bbr.2022.114087

[101] Palmer AA, Sharpe AL, Burkhart-Kasch S, et al. Corticotropin-releasing factor overexpression decreases ethanol drinking and increases sensitivity to the sedative effects of ethanol. *Psychopharmacologia*. 2004;176(3-4):386-397. doi:10.1007/s00213-004-1896-5

[102] Ghani S, Alkhlaif Y, Mann J, et al. Surgical incision pain induced an increase in alcohol consumption in mice. *Alcohol (Fayetteville, NY)*. 2024;117:1-9. doi:10.1016/j.alcohol.2024.03.005

[103] Lorente, 2022.

[104] Rhodes JS, Ford MM, Yu CH, Brown LL, Finn DA, Garland T Jr. *et al* (2007). Mouse inbred strain differences in ethanol drinking to intoxication. *Genes Brain Behav* 6: 1–18.