Characterization of Insula to BNST Circuit Adaptations Following Chronic Ethanol Intake

By

Anne Taylor

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

In partial fulfillment of the requirements

For the degree of

DOCTOR IN PHILOSOPHY

In

Neuroscience

December 16th, 2023

Nashville, Tennessee

Approved by:

Erin S. Calipari, Ph.D. (Chair)

Max E. Joffe, Ph.D.

Carissa J. Cascio, Ph.D.

Breann L. Brown, Ph.D.

Danny G. Winder, Ph.D. (Thesis Advisor)

DEDICATION

This thesis is dedicated to those struggling
with mental illness and their families.

ACKNOWLEDGEMENTS

This work would not have been possible without funding from the National Institute on Alcohol Abuse and Alcoholism (NIAAA). A diversity supplement to Dr. Winder's parent NIAAA grant (R37AA019455-12S1) directly supported the scientific endeavors published in the journal of neuroscience and outlined in this dissertation. I would like to thank my mentor, Danny Winder, for being a great role model of how to lead by example and make science fun. I want to thank my committee members Dr. Erin Calipari, Dr. Max Joffe, Dr. Breann Brown and Dr. Carissa Cascio for their advice and support during my thesis work and job search. I am very grateful for all that you have done for my career. I would like to thank the current and previous Winder lab members who have made coming to work every day a pleasure. I especially want to thank Danielle Adank for being my partner in crime and becoming one if my closest friends. I want to thank the Vanderbilt Brain Institute and the Vanderbilt Center for Addiction Research including Dr. Bruce Carter, Roz, Pammy, Darlene, Elana and Megan for helping make my time at Vanderbilt as smooth as possible and being there whenever I needed help at each stage of my graduate career. Finally, I would like to thank my friends and family. To my best friend Alexis Jameson for constantly being my cheerleader from our first moment in the lab together. It has been a pleasure seeing you become a wife and mother and I love being a part of your little family. To my sister Elizabeth for listening to every presentation and rant and crisis I've been through these past 6 years and always being there to give advice. To my mom and dad, Aunt Patti and Uncle Bob for supporting me throughout my long schooling journey and consistently checking in to see how I'm doing and celebrate my accomplishments.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

Introduction

1.1 Homeostasis

Homeostasis is the maintenance of a stable internal environment within an organism despite fluctuations in external conditions. This process is essential for the survival and proper functioning of living organisms, as it ensures that vital physiological processes can continue within a narrow range of optimal conditions (David et al., 2022).

The maintenance of homeostasis is achieved through a range of physiological mechanisms that work together to keep the internal environment of the organism within a set range of conditions. These mechanisms include feedback loops, which are self-regulating processes that can detect changes in the internal environment and initiate responses to counteract these changes (Nose et al., 2018). For example, if the temperature in the body rises above a certain level, the hypothalamus in the brain detects this change and triggers a series of responses to lower the temperature, such as sweating and dilation of blood vessels in the skin (Figure 1) (Brengelmann et al., 1977).

Through a range of physiological mechanisms, the body can maintain a stable internal environment despite fluctuations in external conditions(Liu et al., 2023; Nose et al., 2018; Palmer & Clegg, 2016). The importance of homeostasis can be seen in many physiological processes and understanding the mechanisms of homeostasis is critical for the development of treatments for diseases and the promotion of overall health and well-being. Within the central nervous system (CNS), synaptic plasticity underlies the physiological regulation of

homoeostasis, and impairment may lead to disease (Colonna & Butovsky, 2017; Kulijewicz-Nawrot et al., 2013; Morton et al., 2006).

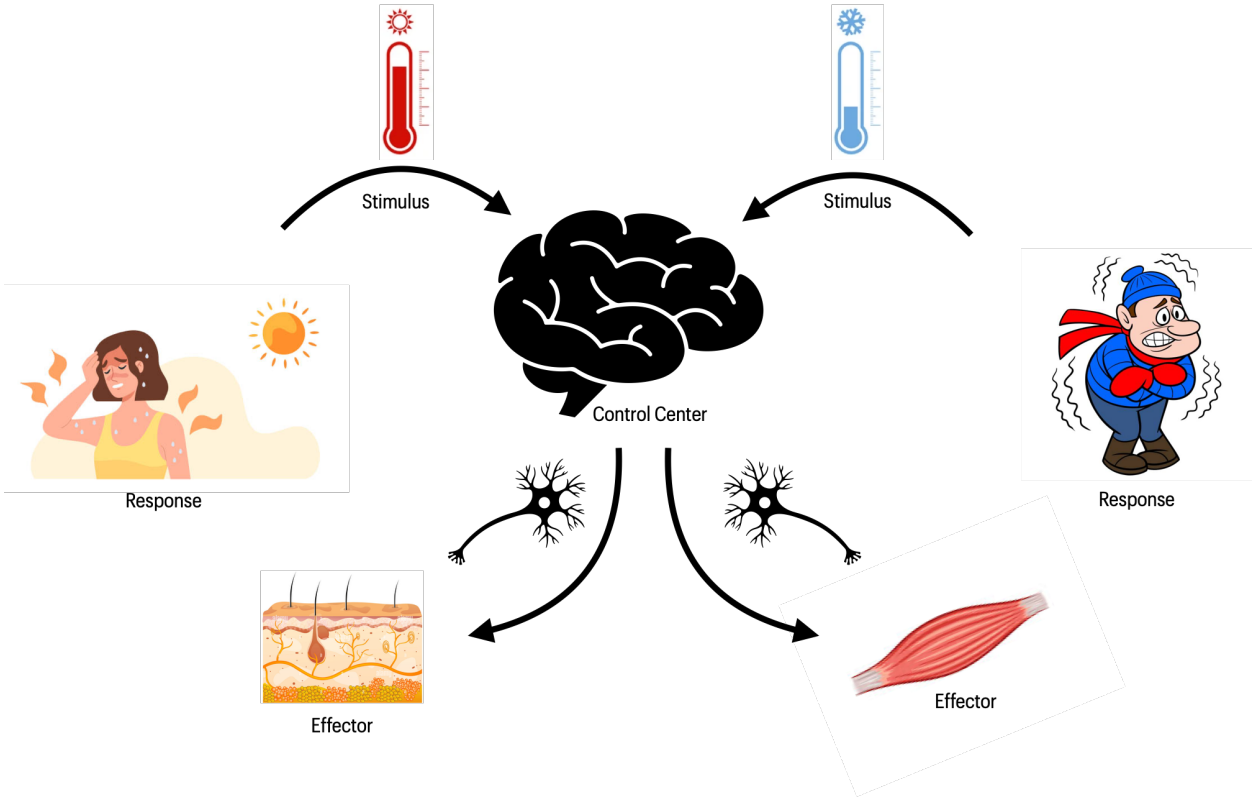

Figure 1.1 Homeostasis Example

1.2 The salience network

The human brain is a complex organ responsible for processing and integrating information, relying on a network of interconnected regions that work together to support different cognitive processes. One of these networks is the salience network, which plays a crucial role in regulating homeostasis, attention, emotions, and decision-making (Chong et al., 2017; Seeley, 2019). First characterized by Seely et al in 2007 using fMRI task activation correlations to identify a network of regions that predict individual differences in emotion and cognition (Seeley et al., 2007). Seeley coined this network the "salience network" as prior work implicating the anterior cingulate cortex (ACC) and insular cortex (IC) in response to pain,

uncertainty, and other threats to homeostasis (Craig, 2002; Grinband et al., 2006; Peyron et al., 2000).

The salience network comprises several brain regions, including the ACC, IC, and striatum (Chong et al., 2017; Schimmelpfennig et al., 2023; Sridharan et al., 2008). The ACC is a region located in the medial frontal cortex, involved in monitoring and regulating cognitive control and decision-making (Bian et al., 2019; Bliss et al., 2016; Rolls, 2019) The orbitofrontal cortex provides the ACC with information regarding rewarding and non-rewarding outcomes whereas the parietal cortical areas transmit spatial and action-related information to the posterior cingulate cortex (Rolls, 2000, 2019). This allows the cingulate cortex to engage in action-outcome learning, with the midcingulate motor area sending outputs to premotor areas (Rolls, 2019; Vogt, 2016; Vogt et al., 2003). Aberrant signaling within the ACC is associated with chronic pain, anxiety disorders and major depression (Bian et al., 2019; Bliss et al., 2016; Rolls et al., 2019).

The striatum is a region best known for its role in goal directed learning and habit formation (Graybiel & Grafton, 2015; Perez et al., 2022). This dorsal region receives dopaminergic input from the substantia nigra whereas the dorsal portion receives dopamine from the ventral tegmental area. Perturbations in striatal signaling can result in movement disorders, psychiatric disorders, compulsive behavior, affective disturbances amongst others (Gonzales & Smith, 2015; Lanz et al., 2019; Seiler et al., 2022). The insula is responsible for processing and integrating sensory information from the body and external environment. It then compiles this data and computes which downstream regions are appropriate to elicit a response that will

return the system back to a balanced state. Section 1.4 dives deeper into the structure and function of the IC.

The salience network is the intermediary between the default mode network (DMN) and the central executive network (CEN), orchestrating attentional processing in the brain. The DMN is active when the brain is at rest, while the CEN is recruited during higher level thinking and working memory. The interaction between these networks is critical for maintaining cognitive flexibility and adapting to changing environments.

The salience network plays a crucial role in regulating attention, emotions, and decision-making. It is responsible for detecting relevant stimuli in the environment and prioritizing them for further processing (Seeley, 2019). For example, if you are driving and see a pedestrian crossing the road, the salience network will detect this stimulus and activate the appropriate response, such as slowing down or stopping the car. The salience network is also involved in emotion regulation, particularly negative emotions such as fear and anxiety. It does so by monitoring the internal and external environment and modulating the response to emotional stimuli (Schimmelpfennig et al., 2023; Seeley, 2019). For example, if you are in a stressful situation, the salience network will activate the appropriate physiological response, such as increasing heart rate and respiration, to prepare you for fight or flight.

Furthermore, the salience network is involved in decision-making (Nudelman & Waltz, 2022). It does so by integrating information from different brain regions and generating a subjective value signal (Seeley, 2019). This signal reflects the expected reward or punishment associated with a particular action or choice. The salience network then modulates the activity

of other brain regions, such as the striatum and prefrontal cortex, to guide behavior (Seeley, 2019).

Dysfunction of the salience network has been implicated in several mental health disorders, including depression, anxiety, and addiction (Nudelman & Waltz, 2022; Schimmelpfennig et al., 2023; Seeley, 2019; Thomason et al., 2011). Individuals with depression show decreased activation of the ACC and insula during emotional processing tasks, suggesting impaired emotion regulation (Schimmelpfennig et al., 2023). The salience network is also implicated in the pathophysiology of schizophrenia (Palaniyappan et al., 2012). Individuals with schizophrenia show decreased connectivity between the SN and other brain networks, such as the DMN and CEN (Huang et al., 2022). This disrupted connectivity may underlie the cognitive and affective symptoms of schizophrenia, such as impaired attention and emotion regulation (Huang et al., 2022; Palaniyappan et al., 2012). Similarly, individuals with addiction show increased activation of the striatum in response to drug-related stimuli, reflecting an abnormal reward processing (Cushnie et al., 2023; Zhang & Volkow, 2019)

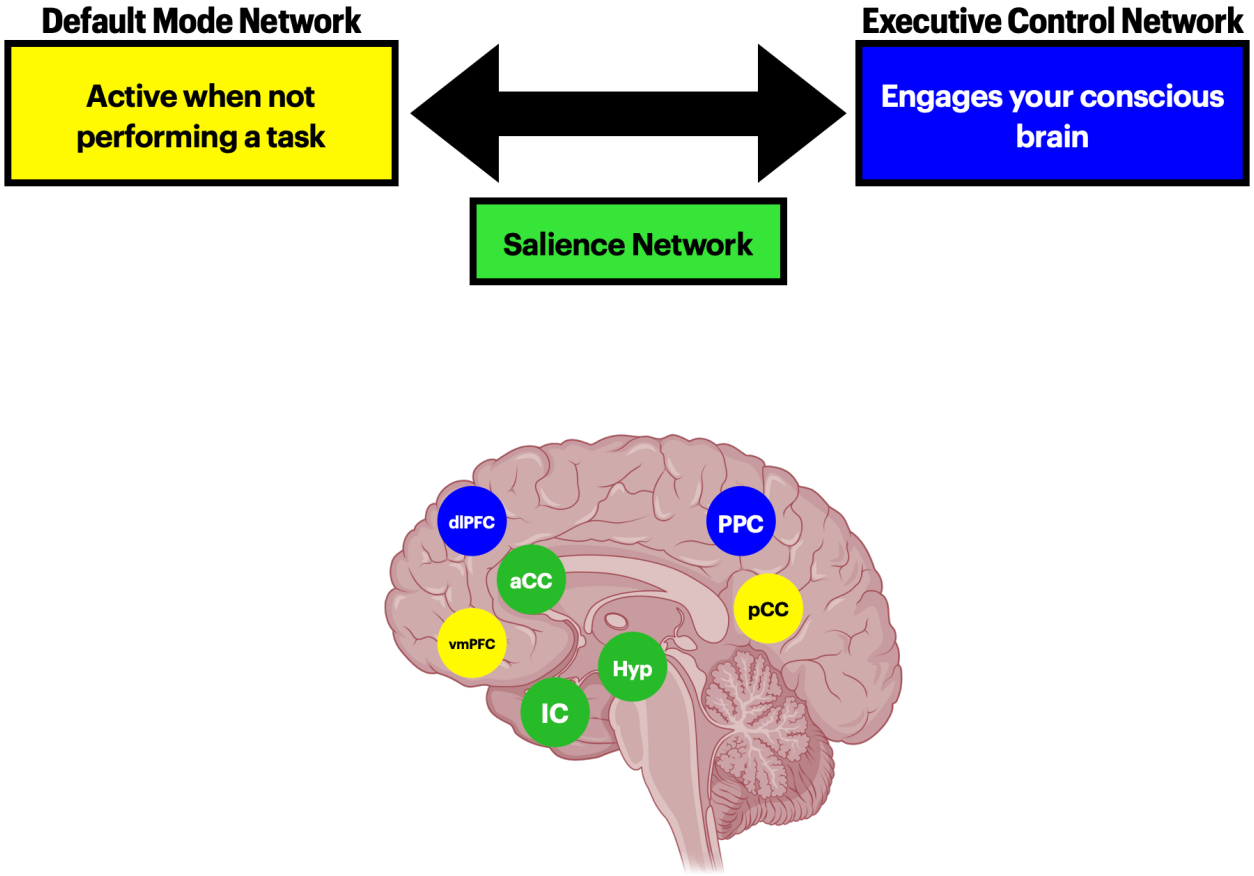

Figure 1.2 Homeostatic Neural Networks. Dorsolateral pre frontal cortex (dlPFC), ventromedial PFC (vmPFC), anterior cingulate cortex (aCC), hypothalamus (Hyp), Insular cortex (IC), posterior cingulate cortex (pCC), posterior parietal cortex (PPC)

1.3 Plasticity of the salience network: maintaining CNS homeostasis

Studies have shown that the SN can exhibit both structural and functional plasticity. Structural plasticity refers to changes in the connections between neurons or brain regions, while functional plasticity refers to changes in the activity or function of a particular brain region.

Structural plasticity of the salience network has been demonstrated in studies that have examined the effects of mindfulness meditation on the brain. Mindfulness meditation is a practice that involves paying attention to one's thoughts, feelings, and bodily sensations in a non-judgmental way. One study in humans found that long-term mindfulness meditation was associated with increased gray matter volume in the anterior insula, a key region of the salience network involved in detecting salient stimuli (Tang et al., 2015). Another study found that mindfulness meditation was associated with increased connectivity between the anterior insula and other regions of the salience network, as well as increased connectivity between the salience network and regions involved in emotion regulation and attention including the thalamus, caudate, middle frontal gyrus, and superior temporal gyrus (Jang et al., 2018; Sharp et al., 2018). These findings suggest that mindfulness meditation can lead to changes in the structure of the salience network that may enhance its ability to detect and respond to salient stimuli.

Functional plasticity of the salience network has also been demonstrated in studies that have examined the effects of stress on the brain. In patients with AHDH, chronic stress has been shown to increase activity in the amygdala, a key region of the salience involved in processing emotional stimuli, and decrease activity in the prefrontal cortex, a region involved in regulating emotion and attention (Arnsten, 2009). However, functional neuroimaging studies in healthy control patients have also shown that the salience network can adapt to chronic stress by increasing connectivity between the amygdala and regions involved in emotion regulation, such as the ventromedial prefrontal cortex (Liston et al., 2009). This increased connectivity may

help to restore homeostasis by enabling the salience network to regulate emotional responses to stress more effectively.

The ability of the salience network to maintain homeostasis may be compromised in certain conditions, such as chronic stress, anxiety, or depression. These conditions can lead to dysregulation of the salience network, resulting in increased sensitivity to salient stimuli, decreased ability to regulate emotional responses, and impaired ability to restore homeostasis after a stress.

1.3.1 Stress

While popularly seen as undesirable, stress is an adaptive response to changes in our physical or emotional environment. An appropriate stress response to negative stimuli allows the brain and body to make adjustments in physiology and behavior to constantly adjust to changing conditions and maintain homeostasis. During stress, the salience network detects and processes salient stimuli, such as threat-related cues, and initiates appropriate behavioral and physiological responses to maintain homeostasis (Nudelman & Waltz, 2022). For example, the salience network can activate the hypothalamic-pituitary-adrenal (HPA) axis via projections to amygdalar and hypothalamic circuitry, leading to the release of stress hormones, including cortisol, to prepare the organism for a fight or flight response (Packard et al., 2016; Thomason et al., 2011). Furthermore, the brainstem efferents from the salience network can regulate the autonomic nervous system, leading to changes in heart rate, blood pressure, and respiration to promote homeostasis(Macey et al., 2012; Seeley, 2019). The salience network can also modulate the activity of other brain regions involved in stress processing, including the

prefrontal cortex, extended amygdala and the hippocampus, to promote adaptive responses to stress (Luchsinger et al., 2021; Schimmelpfennig et al., 2023; Seeley, 2019; Thomason et al., 2011).

1.3.2 Affective Disorders

In healthy individuals, the salience network is responsible for maintaining homeostasis by continuously monitoring and regulating internal bodily states. However, in disease states such as chronic pain, depression, and anxiety, the salience network can become dysregulated, leading to persistent and debilitating symptoms.

Recent studies have demonstrated that the salience network is remarkably plastic, allowing it to adapt to changes in the body's environment and maintain homeostasis in the presence of disease states (Nudelman & Waltz, 2022; Schimmelpfennig et al., 2023; Seeley, 2019). For example, in chronic pain, the SALIENCE NETWORK can become hypersensitive to pain signals, leading to persistent pain perception (Bliss et al., 2016; Labrakakis, 2023; Mutschler et al., 2012). However, studies have shown that mindfulness meditation can induce plasticity in the salience network, leading to decreased pain perception and improved pain management (Grant, 2014; Wang et al., 2021).

Similarly, in depression, the salience network can become hyperactive, leading to persistent negative thoughts and emotions (Nudelman & Waltz, 2022; Schimmelpfennig et al., 2023). However, studies have shown that cognitive behavioral therapy (CBT) can induce plasticity in the SALIENCE NETWORK, leading to improved emotion regulation and decreased symptoms of depression (Bremer et al., 2022; Teng et al., 2022).

1.3.3 Addiction

Addiction is a chronic relapsing disorder characterized by compulsive drug use despite negative consequences (DSM-V). The salience network in part plays a critical role in addiction by integrating information related to drug-related cues, emotional states, and decision-making processes (Cushnie et al., 2023). Studies have demonstrated that chronic drug use can alter the function and connectivity of the salience network, leading to changes in attentional bias, emotional reactivity, and craving (Cushnie et al., 2023; Zhang & Volkow, 2019). For example, chronic drug use can lead to a hyperactive salience network, which is characterized by increased connectivity between the anterior IC and dorsal (Cushnie et al., 2023; Droutman et al., 2015; Volkow et al., 2019). This hyperactivity can lead to an increased sensitivity to drug-related cues(Gardner, 2011; Phillips & Sarter, 2020; Snelleman et al., 2014). Similarly, chronic drug use can lead to a decreased ability to regulate emotional responses, which can lead to negative affective states and increased drug use (Cushnie et al., 2023; Le Berre, 2019). Overall, the salience network's plasticity can contribute to the development and maintenance of addiction by altering the integration of sensory, emotional, and cognitive information.

Further research is needed to understand the mechanisms underlying the salience network's plasticity and how it can be targeted to treat addiction. Understanding the plasticity of the salience network is critical for developing effective treatments that involve dysregulation of attention, motivation, and affective states. One promising region of the salience network gaining recent attention is the IC, discussed below.

1.4 The Insular Cortex (IC)

The IC is a relatively enigmatic and concealed region situated within the temporal lobe. This portion of the telencephalon, comprising a mere 2% of the total cortical surface, plays a role in intricate neural networks connecting the higher cortex, limbic structures, basal ganglia, and autonomic system (Gogolla, 2017; Menon & Uddin, 2010; Nieuwenhuys, 2012). The insula is closely positioned to various vital structures, and any disorders originating in this region carry the potential for substantial neurological complications (Elder et al., 2019; Labrakakis, 2023; Nieuwenhuys, 2012).

1.4.1 Structure and Function

The most cited role of the IC may be integrating external sensory information with internal bodily sensory or state information (Chong et al., 2017). Studies have also demonstrated insular participation in emotional regulation, empathy, and physical and emotional awareness (Gogolla, 2017; Nieuwenhuys, 2012). Work in animal models has implicated insular participation in learning and memory, social behavior, malaise, drug craving, aversive state, and valence (Bernhardt & Singer, 2012; Cushnie et al., 2023; Jaramillo, Agan, et al., 2018; Klein et al., 2021; Miura et al., 2020). The IC can be divided into the anterior and posterior regions based on a gradient of granularity in which the anterior IC is agranular or lacking granule cells (neurons with small cell bodies). The mid IC is dysgranular containing some granule cells and the posterior IC is granular. This difference in granularity is due the presence of layer 4 in the posterior IC that is absent in the anterior IC. More about IC layers is discussed below in section 1.4.4.

1.4.2 Anterior IC

The anterior insular cortex (AIC) is a region of the brain that is located in the fronto-parietal operculum and plays a vital role in various functions, including emotions, decision-making, self-awareness, and social cognition (Gu et al., 2013; Nieuwenhuys, 2012). The AIC is divided into two main regions: the ventral anterior insula and the dorsal anterior insula. The ventral anterior insula is associated with emotional processing such as disgust, anger, fear, and sadness. The dorsal anterior insula is better known for its involvement in interoceptive awareness of bodily sensations such as hunger, thirst, and pain, as well as attention, and cognitive control (Evrard, 2019a).

The AIC is also involved in decision-making, particularly in situations that involve risk and uncertainty. The AIC integrates information from various brain regions, such as the prefrontal cortex and the amygdala, to guide decision-making processes (Nieuwenhuys, 2012). The AIC is also involved in social cognition, which is the ability to understand and interpret social cues and emotions. The AIC is activated when individuals perceive social cues such as facial expressions, vocal intonation, and body language.

The AIC has been implicated in various neuropsychiatric disorders such as anxiety, depression, schizophrenia, and autism spectrum disorders. The AIC is thought to play a crucial role in the pathophysiology of these disorders, particularly in emotional processing and self-awareness (Droutman et al., 2015; Mutschler et al., 2012; Shin & Liberzon, 2010a). For example, individuals with anxiety disorders have been found to have increased activation of the AIC in response to emotional stimuli such as threat and uncertainty (Hiser et al., 2021).

Similarly, individuals with depression have been found to have decreased activation of the AIC, which may contribute to the emotional dysregulation that is characteristic of this disorder (Gu et al., 2013; Rolls, 2019).

1.4.3 Posterior IC

Unlike the AIC which is involved in emotional and social processing, the posterior insular cortex (PIC) is better known for its role in somatosensory processing (Peyron & Fauchon, 2019). The PIC is involved in a variety of sensory processes, including touch, pain, and temperature sensation (Borsook et al., 2013; Gehrlach et al., 2019; Labrakakis, 2023). It receives input from the thalamus, which relays information from sensory receptors throughout the body (Nieuwenhuys, 2012; Peyron & Fauchon, 2019). This input is then processed in the posterior insula, allowing us to perceive sensations such as pressure, heat, and cold.

In addition to sensory processing, the posterior insula is also involved in interoception. This includes processes such as hunger, thirst, and fatigue. Work from Wright and colleagues have shown that the posterior insula is activated when individuals are presented with cues related to hunger or thirst, suggesting that it plays a key role in these processes (Wright et al., 2016). The posterior insula is best known for its role in pain processing. Research has shown that when individuals experience pain, the posterior insula is activated (Labrakakis, 2023; Mutschler et al., 2012). Furthermore, damage to the posterior insula has been associated with pain insensitivity (Labrakakis, 2023; Meier et al., 2012; Wang et al., 2021).

Interestingly, studies have shown that the posterior insula is activated when individuals experience emotions such as disgust and anger (Tuerke et al., 2012). Additionally, the posterior

insula is involved in empathy. Research has shown that individuals with damage to the posterior insula have difficulty with empathy tasks, suggesting that this region is crucial for social and emotional processing (Bernhardt & Singer, 2012; Novembre et al., 2015).

Overall, the posterior insular cortex is a crucial region of the brain responsible for a wide range of sensory and emotional processes. Its role in sensory processing, interoception, pain processing, and emotional processing highlights its importance in human experience and behavior. Further research into the functioning of the posterior insula may lead to a better understanding of a variety of neurological and psychiatric disorders, such as chronic pain.

1.4.4 Layers of the IC

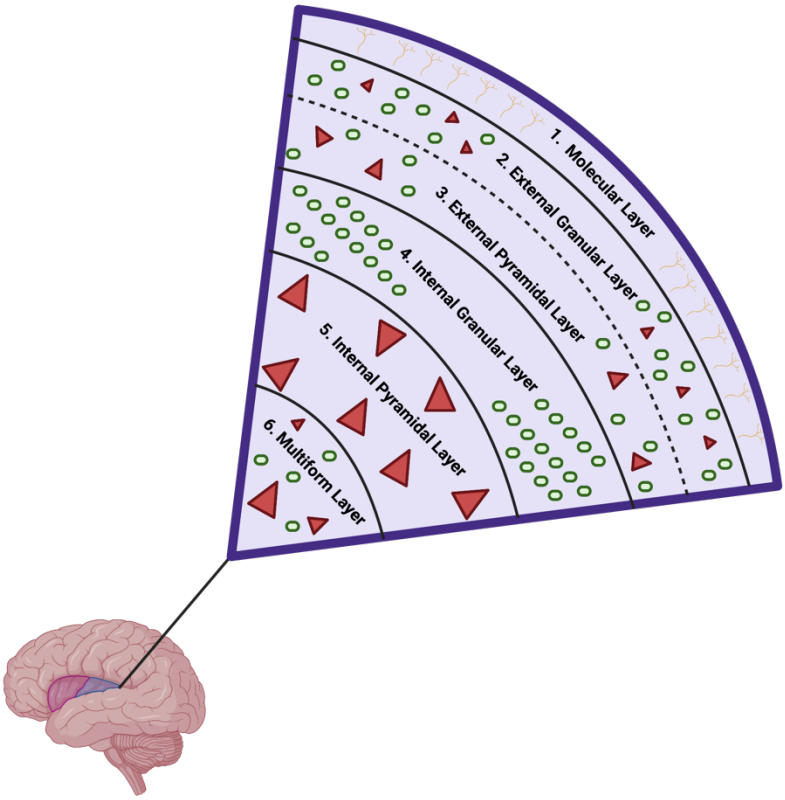

Figure 1.3 Cortical Layers. Pink= anterior IC, Purple= posterior IC. Layers 1-6 illustrating pyramidal cells (red triangles) and granule cells (green circles). Anterior IC differs in that it lacks layer 4.

The human IC is organized into several layers, each with a distinct cellular composition and functional role (Figure 3). The outermost layer of the IC is known as the molecular layer. It is composed of axons, dendrites, and synapses. It receives input from other brain regions and sends output to other cortical areas. Next there is the external granular layer which contains densely packed granule cells, which receive sensory information from the thalamus and other cortical areas. The external

pyramidal layer contains large pyramidal cells that send output to other cortical areas, as well as to the thalamus and brainstem. The internal granular layer contains smaller granule cells that receive input from the external pyramidal layer and other cortical areas. This layer is unique in that it is present in the posterior IC but absent in the anterior IC, defining the gradient of granularity along the anterior-posterior axis. It is also involved in processing somatosensory and gustatory information (Nieuwenhuys, 2012). The inner pyramidal layer contains large pyramidal cells that project to other cortical areas, as well as to subcortical structures such as the amygdala and basal ganglia (Evrard, 2019a). Finally, the innermost layer, the multiform layer contains a mixture of small and large cells with diverse functions. It is involved in the integration of sensory, emotional, and cognitive information, as well as in motor control (Evrard, 2019a).

1.4.5 Conservation Across Species

The IC is a highly evolutionarily conserved region, present in all mammalian species (Evrard, 2019a; Gehrlach et al., 2020; Nieuwenhuys, 2012). Studies have shown that the IC is particularly well-developed in primates, including humans, suggesting that it may play an important role in the unique cognitive and social abilities of these animals (Evrard, 2019a; Gu et al., 2013)In fact, some researchers have suggested that the insular cortex may be the key neural structure that distinguishes humans from other animals, as it is involved in many higher-level cognitive functions that are unique to our species, such as self-awareness, empathy, and moral reasoning (Michel, 2017)

Interestingly, despite its evolutionary conservation, there is also evidence that the insular cortex may have undergone some degree of specialization or differentiation across different species. For example, in rats and mice, the insular cortex is mainly involved in gustatory processing (taste), whereas in primates, including humans, it has a much broader range of functions (Evrard, 2019b; Gehrlach et al., 2020). This is in part due to structural differences wherein lower order species such as mice the IC is largely agranular (lacking layer 4), whereas higher order mammals like primates the IC is divided into three cytoarchitectural subdivisions that can be identified in the dorso-ventral plane: the granular (dorsal), the dysgranular (intermediate) and the agranular (ventral) subdivisions (Gallay et al., 2012; Gehrlach et al., 2020). This suggests that the insular cortex may have adapted to perform different functions in different species, while still retaining its fundamental structure and connectivity.

Overall, the evolutionary conservation of the insular cortex suggests that it is a highly important and fundamental structure, involved in a wide range of functions across many different species. Its continued study is likely to shed light on many important questions about brain evolution, cognition, and behavior.

1.4.6 IC Circuitry

Through anatomical dissection, it has been observed that the IC exhibits extensive connections both within itself and with stress-related brain regions (Shin & Liberzon, 2010b). While cortical areas have bidirectional connections with the insula, pathways to and from subcortical regions are primarily unidirectional (Bonelli & Cummings, 2007; Harris, 2011). The

insula reciprocally connects with frontal brain structures, such as the medial prefrontal cortex (mPFC), ACC, and orbitofrontal cortex. In addition to its connections with frontal and sensory regions, the insula demonstrates a strong interconnection with the limbic system (Gehrlach et al., 2020). For instance, inputs from the basolateral amygdala (BLA) project to specific parts of the insula, while one of the densest efferent pathways from the insula returns to the BLA and the central amygdala (Gehrlach et al., 2020; Luchsinger et al., 2021). Other significant outputs include the entorhinal cortices, nucleus accumbens, and the mediodorsal nucleus of the thalamus (Gehrlach et al., 2020). Furthermore, the anterior insula exhibits a distinct projection pattern compared to the posterior and mid insula, which share similar inputs and outputs (Gehrlach et al., 2020). Notably, this research confirms a one-way pathway from the insula to the bed nucleus of the stria terminalis (BNST). This innervation is primarily directed towards the lateral BNST and can modulate negative affective behaviors in mice (Luchsinger et al., 2021).

1.5 The Bed Nucleus of the Stria Terminalis (BNST)

As mentioned above, one of the major outputs from the IC is the bed nucleus of the stria terminalis (BNST). The BNST is part of the extended amygdala and plays an important role in regulating emotional and stress-related behaviors(Avery et al., 2014). The BNST sends output to areas involved in the regulation of the autonomic nervous system and receives input from regions including the amygdala, prefrontal cortex, and hippocampus. Dysregulation of the BNST has been implicated in the development of anxiety and mood disorders, as well as drug addiction(Conrad et al., 2011; Luchsinger et al., 2021; Shin & Liberzon, 2010b).

1.5.1 BNST Structure and function

Comprised predominantly of GABAergic neurons, the BNST can be subdivided into distinct regions according to its connectivity and function. It receives input from several brain regions, including the amygdala, prefrontal cortex, and hippocampus, and plays a pivotal role in governing both emotional and physiological reactions to stress and anxiety. Moreover, the BNST is involved in regulating social behaviors, fear learning and memory, as well as drug addiction.

1.5.1.1 Anterior BNST

The anterior BNST has been implicated in a range of physiological and behavioral functions, including stress responses, anxiety, social behaviors, addiction, and sexual behavir (Ch'ng et al., 2018; Conrad et al., 2011). It receives inputs from a variety of sources, including the amygdala, hypothalamus, prefrontal cortex, and various sensory systems, and it sends outputs to a number of brain regions, including the hypothalamus, periaqueductal gray, and ventral tegmental area (Avery et al., 2014).

One of the key functions of the anterior BNST is its role in modulating the HPA axis, which is the primary stress response system in the body(Cole et al., 2022). The BNST receives inputs from the amygdala and other limbic regions that signal the presence of stressors, and it then sends outputs to the hypothalamus, which regulates the release of corticotropin-releasing hormone (CRH) and other stress-related hormones. Dysregulation of the anterior BNST has been implicated in the development of anxiety and other stress-related disorders (Awasthi et al., 2020; Ch'ng et al., 2018).

The anterior BNST is also involved in the regulation of social behavior, including aggression and mating behavior(Nordman et al., 2020; Zhou et al., 2023). It has been shown to play a role in the modulation of sexual behavior in both males and females, as well as in the regulation of maternal behavior in females. Dysfunction of the anterior BNST has been implicated in the development of social deficits, including autism and schizophrenia (Arakawa et al., 2023; Contestabile et al., 2023; Feola et al., 2021).

1.5.1.2 Posterior BNST

Studies have shown that the posterior BNST plays a crucial role in regulating the stress response and anxiety-related behaviors(Walker et al., 2003). It is involved in the modulation of fear-related memories and the consolidation of these memories into long-term memory(Hulsman et al., 2021; Shin & Liberzon, 2010b). It also modulates the expression of conditioned fear responses by regulating the activity of the amygdala(Davis et al., 2010; Shin & Liberzon, 2010b).

The posterior BNST is also implicated in the regulation of social behavior(Arakawa et al., 2023; Whylings et al., 2020). It is involved in the modulation of social aggression and social recognition, as well as the regulation of sexual behavior (Coria-Avila et al., 2014; Nordman et al., 2020). Dysfunction in the posterior BNST has been associated with various psychiatric disorders, including anxiety disorders, post-traumatic stress disorder (PTSD), and depression(Ch'ng et al., 2018; Feola et al., 2021; Flook et al., 2020; Rodríguez-Sierra et al., 2016). Abnormalities in the posterior BNST have also been linked to drug addiction and

alcoholism(Avery et al., 2014; Centanni, Bedse, et al., 2019; Ch'ng et al., 2018; Volkow et al., 2019; Vranjkovic et al., 2018).

1.5.2 BNST circuity

As stated earlier, the BNST exhibits a complex structure and this complexity extends to its afferent and efferent connections. Understanding this connectivity is crucial for comprehending the function of the BNST. In rodents, the dorsal bundle (stria terminalis) and the ventral bundle (ansa peduncularis) serve as pathways through which the BNST connects with other regions.

Cortical and subcortical regions throughout the brain contribute glutamatergic afferents to the BNST(Avery et al., 2014). Cortical glutamatergic inputs originate from regions such as the orbital, infralimbic, and prelimbic cortices of the prefrontal cortex, as well as the IC, entorhinal cortex, and basolateral amygdala (Alheid, 2003; Avery et al., 2014). Non-cortical regions also send glutamatergic afferents to the BNST, including the ventral subiculum of the hippocampus, paraventricular thalamus, lateral hypothalamus, and the parabrachial nucleus.

The central nucleus of the amygdala (CeA) provides the densest GABAergic input to the BNST(Francesconi et al., 2021). Other regions contributing to the GABAergic afferent network include the medial amygdala (MeA), lateral septum, shell of the nucleus accumbens (NAcc), medial preoptic area, anterior hypothalamus, paraventricular nucleus (PVN), substantia innominata, and zona inserta (Alheid, 2003).

The BNST establishes significant connections with the hypothalamus, with the connection to the paraventricular nucleus (PVN) being crucial for the stress response. These connections are mainly GABAergic, although sparse populations of glutamatergic and corticotropin-releasing

factor (CRF) cells have also been identified. The BNST also sends two distinct pathways, CRF and cholecystokinin (CCK), to the lateral hypothalamus, each responding differently to stress. Both the dorsal BNST (dBNST) and ventral BNST (vBNST) project to the ventral tegmental area (VTA), but the vBNST projection is stronger. The projections from the BNST to the VTA, which contains a significant population of dopamine neurons, are primarily GABAergic, although some glutamatergic cells are present. These two cell types appear to be activated by different stimuli and have contrasting stress-related functions. Additionally, some projections from the BNST to the VTA are also positive for CRF. The BNST also reciprocally projects to the periaqueductal gray (PAG), with this output mainly originating from CRF cells in the oval nucleus of the BNST. Projections to the parabrachial nucleus come from cells distributed throughout the BNST, particularly those containing somatostatin, neurotensin, or CRF in the dorsolateral BNST.

1.6 When use becomes misuse: the addiction cycle

An implicit tenet of the alcohol use disorder (AUD) research field is that understanding of how alcohol interacts with the brain is critical to the development of an understanding of vulnerability to AUD, and to treatment approaches. Gaining this understanding requires the mapping of brain function critical to specific components of this heterogeneous disorder. Early approaches in humans and animal models focused on determination of specific brain regions sensitive to alcohol action, and that participate in AUD-relevant behaviors. Broadly speaking, this research has focused in three domains, Binge/Intoxication, Negative Affect/Withdrawal, and Preoccupation/Anticipation, with a number of regions identified as participating in each. With the generational advances in technologies that the field of neuroscience has undergone

over the last two decades, this focus has shifted to a circuit-based analysis. A wealth of new data has sharpened the field's focus on the specific roles of the interconnectivity of multiple brain regions in AUD, and AUD-relevant behaviors, as well as demonstrating that the three major domains described above have much fuzzier edges that originally thought. In this chapter, we very briefly review brain regions previously implicated in aspects of AUD relevant behavior from animal model research, then move to a more in-depth overview of circuit-based approaches, and the utilization of these approaches in current AUD research.

1.6.1 The 3 phases of addiction model and neural circuits that underly each

One conceptualization of addiction is as a cycle typically characterized by three phases: the binge/intoxication phase, the withdrawal/negative affect phase, and the preoccupation/anticipation phase. In the binge/intoxication phase, individuals experience pleasurable effects from the substance or behavior, leading to an increase in consumption and a decrease in inhibition. During the withdrawal/negative affect phase, individuals experience physical and psychological discomfort when the substance or behavior is discontinued, leading to cravings and a desire to use again. In the preoccupation/anticipation phase, individuals become preoccupied with obtaining and using the substance or engaging in the behavior, leading to a loss of control and increased risk of relapse. These three phases can be a helpful way to conceptualize and understand the addictive process, and can inform treatment approaches to address each phase.

1.6.1.1 Binge/intoxication

The Binge/Intoxication phase refers to periods of heavy alcohol intake resulting in induction of intoxication through the pharmacological actions of alcohol. In animals, aspects of this phase can be modelled with volitional alcohol intake models, as well as alcohol conditioned place preference. A number of brain regions have been implicated in binge intake and have been heavily reviewed elsewhere (Jeanblanc et al., 2019; Koob & Volkow, 2016; Thiele & Navarro, 2014). Here we will briefly highlight key identified areas.

Ventral tegmental area (VTA)

The ventral tegmental area (VTA) has long been considered a key region for the reinforcing actions of ethanol, supported by data demonstrating that rats will self-administer intra-VTA infusions of ethanol (Gatto et al., 1994; Rodd et al., 2004). Acute exposure increases the in vivo and ex vivo firing rate of VTA dopamine (DA) neurons (Brodie et al., 1990; Gessa et al., 1985). Early work focused on and further supported VTA DA neurons as a key population of interest, as decreasing VTA DA neuron firing via intra-VTA infusion of the D2 receptor agonist quinpirole attenuates intra-VTA and oral ethanol self-administration and oral intake (Hodge et al., 1993; Nowak et al., 2000; Rodd et al., 2004). This ethanol-induced increase in DA neuron activity results in an increase in dopamine release in mesocorticolimbic targets, consistent with all drugs of abuse.

Nucleus accumbens

A major target of VTA DA neurons is the nucleus accumbens (NAc). Here, acute ethanol exposure results in increased extracellular DA levels (Di Chiara & Imperato, 1988; Robinson et al., 2009). The role for DA signaling in reinforcing ethanol behavior has been studied using NAc microinjections of DA receptor pharmacological agents. Specifically, intra-NAc infusion of D1R and D2R antagonists reduce oral ethanol operant responding in rats (Hodge et al., 1997; RASSNICK et al., 1992; Samson et al., 1993). However, while ethanol-induced increases in extracellular DA in the NAc plays a clear role in the reinforcing properties of ethanol, there is ample support for ethanol's actions through other signaling mechanisms (For review of synaptic effects of ethanol see (Lovinger & Roberto, 2013)). Ethanol is also known to bind to GABAA receptors, enhancing receptor function, (Lovinger & Roberto, 2013; Nestoros, 1980) and can act through NMDAR inhibition (Hoffman et al., 1989; Lovinger et al., 1989; Lovinger & Roberto, 2013), as intra-NAc infusion of the glutamate receptor antagonist AP-5 decreases oral ethanol self-administration (Rassnick et al., 1992).There is a large breadth of research defining ethanol's effects on specific cell types and neurotransmitters within key ethanol reward-associated regions. I have highlighted key reviews that better dive into details related to specific ethanol mechanisms and cell type-specific actions for additional information.

Focusing on this higher level of regional regulation by ethanol exposure, expression of the immediate early gene cFos is increased in the NAc following an IP injection, intraventricular (ICV), or intragastric administration of ethanol (Herring et al., 2004; Hitzemann & Hitzemann, 1997; Leriche et al., 2008; Ryabinin et al., 1997; Segovia et al., 2013a), signifying that this region has increased cell activity during exposure. Manipulation of the NAc in the context of ethanol reward is behaviorally relevant, as electrolytic lesion of the NAc decreases ethanol intake in a

drinking in the dark paradigm (Cassataro et al., 2014) and prevents the acquisition of ethanol conditioned place preference (CPP) (Gremel & Cunningham, 2008). Consistent with NAc function supporting the reinforcing properties of ethanol, pharmacological inhibition via intra-NAc infusion of the GABAA agonist muscimol decreases ethanol self-administration in rats (Hodge, Chappelle et al. 1995). Chemogenetic inhibition of the NAc using a Designer Receptors Exclusively Activated by Designer Drugs (DREADD) similarly approach decreases ethanol intake in a drinking in the dark paradigm (Cassataro et al., 2014). Together, this work supports the NAc as a region necessary in orchestrating the reinforcing properties of ethanol exposure.

Ventral pallidum

Downstream from the NAc, the ventral pallidum (VP) is also known to play a role in motivation and hedonic signaling (Kupchik et al., 2015; Lovinger & Alvarez, 2017)Intragastric infusion of ethanol decreases VP BOLD signal intensity in conscious rats (Tsurugizawa et al., 2010)and both IP and intraventricular (ICV) administration of ethanol results in increased cfos expression in the VP (Segovia et al., 2013b). Inhibition of the VP through direct infusion of muscimol (Kemppainen et al., 2012) or GABA receptor blockade using betaCCt (June et al., 2003)both decrease ethanol self-administration.

Dorsal striatum/Basal Ganglia

While many of the previous brain regions have focused on function in relation to the initial rewarding properties of ethanol, the dorsal striatum (DS, putamen) is highly implicated in habit formation (O'Tousa & Grahame, 2014). cFos expression in the dorsal striatum is increased

by an IP injection of ethanol (Hitzemann & Hitzemann, 1997; Segovia et al., 2013b). Research has also shown the behavioral relevance of DS function. Decreased dorsal striatum activity via activation of an inhibitory DREADD decreased ethanol intake (Robins et al., 2018). The DS can also be broken down into medial (DMS) and lateral segments (DLS), with control of different behavioral outcomes. For example, inactivation of the DMS via local infusion of GABA receptor agonists baclofen and muscimol decreases goal-directed responding in early training while local infusion into the DLS decreases habit-like responding after prolonged training (Corbit et al., 2012) (see for review of DS in habituation ethanol intake behaviors (O'Tousa & Grahame, 2014).

The DS projects to the dorsal pallidum, which can be divided into the globus pallidus internal (GPi) and external (GPe) segments (Lovinger and Alvarez 2017). Ex vivo bath application of ethanol to acute brain slices results in a decrease in GPe neuron firing ,(Criswell et al., 1995), which recently was further characterized as a subpopulation of GPe neurons (Abrahao et al., 2017).

Basal ganglia circuitry is thought to be critically modulated by dopamine input from the substantia nigra. In unanesthetized rats, an IV infusion of ethanol increases SNr DA firing at low doses but results in inhibition at high doses (Mereu et al., 1984). Local injection studies suggest the substantia nigra may contribute to stimulant actions of ethanol (Arizzi-LaFrance et al., 2006).

1.6.1.2 Withdrawal/negative affect

Cessation of alcohol intake following binge exposure leads to a sequalae of withdrawal symptoms dependent upon the prior pattern and history of alcohol intake. It is posited that

cycles of binge intoxication and withdrawal lead to the development of what are referred to as allostatic changes in the set point for affective behaviors and hypothalamic setpoint to result in altered stress responses (Centanni, Morris, et al., 2019; Koob, 2009; Koob & Schulkin, 2019). These changes are then viewed as contributing stimuli to drive subsequent negative reinforcement-based alcohol seeking in the Preoccupation/Anticipation phase. A number of brain regions from animal model studies have been implicated in these withdrawal-associated emergent behaviors, most notably components of the extended amygdala, as described below. Aspects of this phase can be modeled by increased alcohol intake after the development of dependence in animal models, increased conditioned place aversion, and alterations in behavior in tasks that assess affective state.

The Extended Amygdala (Central nucleus of the amygdala and Bed nucleus of the stria terminalis)

Alcohol exposure and withdrawal in rodent models induces negative affective behaviors that appear to heavily involve the central nucleus of the amygdala and the bed nucleus of the stria terminalis (CeA, BNST, for reviews see (Centanni, Bedse, et al., 2019; Roberto et al., 2021)). A variety of acute and chronic actions of ethanol on glutamatergic and GABAergic transmission have been identified, at least some of which appear to involve the neuropeptide corticotropin releasing factor (CRF). Consistently, manipulations within the CeA that decrease GABAergic transmission reduced dependence-induced increases in alcohol intake across a range of models.

In a chronic home cage drinking followed by forced abstinence model (CDFA), female mice show increased anxiety- and depressive-like behaviors after protracted withdrawal from ethanol (Holleran et al., 2016; Vranjkovic et al., 2018). At this timepoint, BNST cFos is increased in male and female mice in protracted withdrawal from chronic ethanol (+ in CRF cells) (Centanni, Morris, et al., 2019)

1.6.1.3 Preoccupation/anticipation

The preoccupation/anticipation phase of AUD represents a particularly relevant potential treatment intervention phase and is associated with increased alcohol craving. Reinstatement of alcohol seeking behavior is a common means of modeling this component. Studies have revealed a number of involved brain structures, as has been previously reviewed extensively (Domi et al., 2021; George & Hope, 2017; Mantsch et al., 2016). Here we will provide brief highlights of this research.

Prefrontal cortex (PFC)

The orbital frontal cortex (OFC) is a region heavily implicated in alcohol-related behaviors. cFos is increased in the OFC of rats during cue- and context-induced reinstatement of ethanol seeking (Bianchi et al., 2018; Jupp et al., 2011). OFC chemogenetic inhibition decreases cue-induced ethanol reinstatement in rats (Hernandez et al., 2020) and pharmacological inhibition via muscimol attenuated context-induced reinstatement (Bianchi et al., 2018) and cue-induced reinstatement via muscimol and baclofen ((Arinze & Moorman, 2020). For general review, see (Moorman, 2018).

Insular Cortex

Another cortical region we are beginning to learn more about in relation to its role in alcohol anticipation, preoccupation and craving is the insular cortex (IC). The insula participates in stress axis circuitry through its projections to the BNST, as well as with limbic, visceral and somatosensory regions (Craig, 2009). These connections position the insula well to be a central hub for regulating interoceptive states. In human imaging studies this region has been shown to be active when presented with images of drugs and drug related cues (Droutman et al., 2015; Jasinska et al., 2014; Naqvi & Bechara, 2010). Jaramillo et al have shown that inhibition of the rodent IC increases sensitivity to interoceptive effects of alcohol in an operant discrimination task (Jaramillo et al., 2016). Furthermore, chemogenetic modulation of insula activity revealed its ability to exert control over how much animals self-administration alcohol as well as their alcohol sensitivity (Jaramillo, Agan, et al., 2018; Jaramillo, Van Voorhies, et al., 2018).

1.6.2 New findings to amend the addiction cycle model

Utilizing a variety of the approaches described above in rodent models, a number of specific circuits have been investigated within the past 10 years for their modification by and/or roles in alcohol-specific behaviors. An interesting theme that has emerged from these studies is a blurring of the fidelity of specific brain regions within specific components of the AUD cycle, as will be discussed below.

Extended amygdala-VTA circuits in binge/intoxication and negative affect/withdrawal

An important source for control of the mesolimbic dopamine system in the context of alcohol bingeing and dependence are the extended amgydala structures, particularly the BNST and CeA. Avegno et al have demonstrated the VTA-projecting CeA neurons are activated during alcohol exposure and withdrawal (Avegno et al., 2021). Further, two studies demonstrate that a projection from the BNST to the VTA plays an important role in ethanol conditioned place preference (Pina & Cunningham, 2017)and binge alcohol intake (Rinker et al., 2017). While these data are not unexpected in terms of predicted roles of dopamine signaling in reinforced behaviors, the role of BNST projections here demonstrates an example of "cutting corners", in which a brain region implicated in the negative affect/withdrawal phase of the AUD cycle plays a role in alcohol intake behaviors independent of alcohol dependence.

Circuit based analysis of extended amygdala structures does provide strong support for the role of circuits involving these structures in the negative affect/withdrawal phase. For example, a circuit involving CeA CRF neurons projecting into the BNST in rat is activated during chronic alcohol withdrawal, and optogenetic inhibition of this pathway suppresses enhanced alcohol intake as well as physiological signs of withdrawal (De Guglielmo et al., 2016; de Guglielmo et al., 2019). Curiously when analogous experiments were performed utilizing chemogenetic approaches in the mouse, significantly distinct outcomes were obtained, suggesting more work needs to be done (Kreifeldt et al., 2022).

CRF neurons projecting to the VTA in the BNST have been identified as being regulated by chronic ethanol exposure (Silberman et al., 2013), and moreover, direct manipulation of the activity of these cells reveals that activity supports alcohol intake. In mice, these cells are more

excitable in females compared to males, and this difference is in part mediated by a stronger glutamatergic projection to these cells from the paraventricular nucleus of the thalamus (PVT) (Levine et al., 2021). BNST CRF neurons also receive input from the portions of the insula (Centanni, Bedse, et al., 2019; Fetterly et al., 2019). Excitatory drive in the BNST is upregulated during forced abstinence from chronic alcohol availability (Centanni, Morris, et al., 2019). Chemogenetic studies indicate that at least a portion of this upregulation occurs at insular afferents. Moreover, alcohol intake is associated with an increase of the excitability of BNST projecting insular neurons (Marino et al., 2021). Driving this circuit produces behavioral phenotypes similar to those observed with alcohol forced abstinence, and chemogenetic inhibition reduces abstinence induced negative affect-like behavioral disturbances.

Insular cortex-driven circuits participate in multiple AUD cycle components

Indeed, a variety of circuit analyses-oriented studies point to a key role insular cortex in alcohol dependent behaviors. Seif et al elegantly demonstrated that insular (and mPFC) inputs to the accumbens were uniquely regulated by GluN2C-containing NMDA receptors, and that reduction of the expression of these receptors in ventral striatum reduced aversion-independent alcohol intake (Seif et al., 2013). In dorsal striatum, insular afferents are subject to mu opiate receptor-dependent LTD, and form of synaptic plasticity disrupted by alcohol exposure (Muñoz et al., 2018). Inhibition of insular afferents to the brainstem specifically reduce aversion resistant alcohol intake (De Oliveira Sergio et al., 2021).
Canonically the IC has been posited to be a part of the anticipation/preoccupation stage of the addiction cycle. However, there is growing evidence supporting the role of the insula in the

negative affect/withdrawal phase. Data from the Winder and Sparta labs have shown that the IC is active following abstinence from ethanol in rodent models(Centanni, Morris, et al., 2019; Marino et al., 2021). Additionally, following a 2 week abstinence period from chronic ethanol intake, chemogenic inactivation of the IC blocked the subsequent increase in BNST activation previously seen during abstinence indicating the IC is a necessary component of the withdrawal phase (Centanni, Morris, et al., 2019).

Corticostriatal circuits in AUD-like behaviors

Significant circuit-based analyses have also been performed within cortico-striatal circuits in the context of alcohol intake and exposure as well. Dependence following chronic exposure to intermittent alcohol vapor is associated with a reduction in cortical control of habit formation (Renteria et al., 2018). Specifically, orbitofrontal cortex (OFC) neurons that project into the basal ganglia exhibit decreased excitability after chronic intermittent alcohol vaper exposure (Renteria et al., 2018). Further investigation revealed an endocannabinoid-dependent modulation pathway in the OFC-direct pathway circuit that is critical to this process (Renteria et al., 2021). Restoration of this cortical output via optogenetic stimulation produced an increase in goal-directed behavior in dependent mice (Renteria et al., 2018). Indirect pathway neurons also appear to play an important role in regulating alcohol-related behaviors, optogenetic (Hong et al., 2019).

Novel connections in the cycle identified by circuit-based approaches

In addition to the results described above, new strategies have been utilized to implicate interesting circuits in AUD-relevant behavior. For example, Dornellas et al, utilizing a pathway-specific chemogenetic strategy, have provided evidence that a synapse from the locus coeruleus to the rostromedial tegmental nucleus (RMTG) selectively regulates alcohol intake (Dornellas et al., 2021). Intriguingly, chemogenetic activation of this pathway reduced ethanol but not sucrose consumption (Dornellas et al., 2021). Similarly, chemogenetic inhibition of LC inputs to the lateral hypothalamus also decreased ethanol intake (Burnham et al., 2021). These data in total suggest that the LC may have a previously underappreciated role in orchestrating alcohol intake via control of lower brain centers.

Increasing emphasis is also beginning to be placed on the role of hypothalamic subnuclei containing circuits in binge ethanol intake. POMC expressing neurons are a significant cell population within the arcuate nucleus of the hypothalamus, and they project to multiple regions previously implicated in alcohol behaviors, such as the nucleus accumbens and amygdala. Leyrer-Jackson et al. recently demonstrated that POMC-containing neurons that project to the amygdala are preferentially active during the drinking-in-the-dark binge drinking model, suggesting a potential role for this pathway as well (Leyrer-Jackson et al., 2021).

Finally, one of the most enticing AUD circuit papers to date is Siciliano et al., (Science, 2019). In this paper, Siciliano and colleagues measure and modulate in vivo activity of a medial PFC to dorsal periaqueductal gray circuit (Siciliano et al., 2019). They do this within the context of a novel operant alcohol self administration model that allowed delineation of high and low alcohol drinkers, as well as “compulsive” drinkers, defined by their ability to continue high

drinking levels in the presence of the bitter tastant quinine (Siciliano et al., 2019).Remarkably, Siciliano and colleagues demonstrated that activity within this pathway was predictive of the classification scheme that the mice would fall into, suggesting the possibility for biomarker-based approaches to assess alcohol use disorder vulnerability based on MRI brain imaging approaches.

1.7 A pathway to break the cycle: the IC to BNST circuit

As stated above, both the IC and the BNST play import roles in assessing stimuli valence and coordinating an appropriate behavioral response. Work from human fMRI studies have provided evidence for functional connectivity between the IC and BNST(Flook et al., 2020, 2021). Animal models have bolstered evidence for a unilateral glutamatergic projection from the IC to the BNST (Centanni, Morris, et al., 2019; Luchsinger et al., 2021; Marino et al., 2021). Below I will discuss what is known so far about the IC to BNST circuit in the context of stress and alcohol withdrawal.

1.7.1 Role in stress

Stress related illnesses such as anxiety and depression impact almost 50 million adults in the United States. Recent published data from the Winder lab has shown that following coping bouts during an inescapable stressor, there is an increase in calcium transients in both the BNST and the insula (Luchsinger et al., 2021). Interestingly, within the insula there is a simultaneous time-locked increase in glutamate and decrease in GABA transients following acute restraint stress but only the elevation in glutamate remained after chronic restraint stress, suggesting

postsynaptic adaptations occur following repeated exposure to a homotypic stressor (Luchsinger et al., 2021).

Furthermore, IC communication with the BNST by way of the paraventricular nucleus of the thalamus (PVT) is implicated in anxiety-like behaviors in mice. Zhao et al. 2022 found that activation of IC neurons or PVT-projecting IC neurons accelerates the acute stress-induced anxiety-related behaviors (Zhao et al., 2022). Additionally, inhibition of PVT-projecting IC neurons produces an anxiolytic effect and ablation of the neurons in PVT receiving input from IC blocked the anxiety-related behaviors induced by the activated IC neurons (Zhao et al., 2022). Viral tracing revealed direct synaptic connections between the IC to the PVT to the BNST and manipulation of neuronal terminals in BNST projected from PVT altered the susceptibility to the acute stress induced anxiety-related behaviors (Zhao et al., 2022).

1.7.2 Role in ethanol withdrawal

Research suggests that the IC plays a crucial role in processing cues associated with alcohol and drugs, as well as the development of compulsive drug-seeking behaviors (Abdolahi et al., 2019; Flook et al., 2021; Joutsa et al., 2022; Nall et al., 2021; Paulus, 2007). Damage or inhibition of the IC disrupts the seeking of drugs and alcohol, as well as the normal signaling pathways linked to addiction (Centanni, Morris, et al., 2019; Naqvi et al., 2007). Functional changes in the IC and alterations in connectivity with downstream brain regions have been observed in individuals with alcohol use disorder (AUD) (Flook et al., 2020; Grodin & Momenan, 2017). Studies using rodent models of binge drinking have further supported these findings,

indicating specific alterations in IC signaling after ethanol consumption influenced by sex and neural circuitry (Haggerty et al., 2022a; Marino et al., 2021; Pina et al., 2020).

The IC and BNST play a significant role in the expression of negative emotional behaviors during alcohol abstinence, which contribute to relapse in individuals with AUD (Centanni, Morris, et al., 2019; Flook et al., 2021; Vranjkovic et al., 2018). Both the IC and BNST show activity during stress and withdrawal from repeated ethanol exposure (Centanni, Morris, et al., 2019; Luchsinger et al., 2021; Marino et al., 2021). Research conducted by the Winder lab has demonstrated the necessity of IC activity for the subsequent increase in BNST activity observed during abstinence (Centanni, Morris, et al., 2019). Moreover, short-term ethanol intake leads to a specific enhancement in the excitability of IC→BNST cells in females, which diminishes during acute withdrawal (Marino et al., 2021).

Chapter 2

BK channel Adaptations in BNST Projecting Insular Cortex Cells Following Forced Abstinence from Chronic Ethanol Intake Drive Increased Activity

2.1 Introduction

The insula is a cortical lobe that plays crucial roles in various cognitive, affective, and physiological processes, including interoception, emotion regulation, decision-making, and social cognition (Gogolla, 2017; Nagai et al., 2021; Nieuwenhuys, 2012). Growing evidence suggests that the insular cortex (IC) may be involved in the pathophysiology of several neurological and psychiatric disorders, including anxiety, depression, use disorders, and chronic pain (Campbell et al., 2019; Labrakakis, 2023; Mutschler et al., 2012; Paulus & Khalsa, 2021; Perini et al., 2020; Pushparaj et al., 2015).

In addition, studies suggest that the IC plays a critical role in processing alcohol and drug-related cues and developing compulsive drug-seeking behaviors (Abdolahi et al., 2019; Flook et al., 2021; Joutsa et al., 2022; Nall et al., 2021; Paulus, 2007). Damage or inhibition to the IC disrupts drug and alcohol seeking and canonical downstream addiction related signaling pathways (Centanni, Morris, et al., 2019; Naqvi et al., 2007). In humans with an alcohol use disorder (AUD) there are functional adaptations in the IC as well as changes in connectivity between the IC and downstream regions (Grodin et al., 2017; Flook et al., 2020). Preclinical binge drinking rodent models have bolstered these findings, indicating that there are sex specific and circuit specific alterations in IC signaling following ethanol intake (Haggerty et al., 2022a; Marino et al., 2021; Pina et al., 2020).

Both human and rodent studies have identified a unidirectional connection between the IC and the bed nucleus of the stria terminalis (BNST); a key node in regulating the hypothalamic-pituitary-adrenal axis (Conrad et al., 2011; Hulsman et al., 2021). Previous research has implicated a projection from the IC to the BNST in the emergence of negative affective behaviors during alcohol abstinence, which often contribute to relapse in those struggling with AUD (Centanni, Morris, et al., 2019; Flook et al., 2021; Vranjkovic et al., 2018). Individually these regions are active during stress as well as during withdrawal from repeated ethanol exposure (Centanni, Morris, et al., 2019; Luchsinger et al., 2021; Marino et al., 2021). Work from our lab has shown that IC activity is required for downstream elevation in BNST activity seen in abstinence (Centanni, Morris, et al., 2019). Further, short term ethanol produces a female specific increase in excitability of $IC^{\rightarrow BNST}$ cells that is diminished during acute withdrawal (Marino et al., 2021).

Given that the IC to BNST circuit is dynamically affected by ethanol, and little is known about the synaptic properties of these $IC^{\rightarrow BNST}$ cells, the current study aimed to determine (1) the impact of chronic volitional alcohol intake and subsequent forced abstinence on $IC^{\rightarrow BNST}$ excitability and (2) what mechanism may underly ethanol induced changes in neuron excitability of these cells.

Here, we assess the impact of chronic drinking forced abstinence (CDFA) on synaptic and excitable properties of insular neurons that project to the BNST ($IC^{\rightarrow BNST}$). Using whole cell patch clamp electrophysiology, we find that following forced abstinence (FA) in female C57BL/6J mice, there is an increase in action potential firing of $IC^{\rightarrow BNST}$ circuitry at 24hrs but not 2 weeks. This phenomenon is in part due to diminished BK channel expression within the region,

consistent with prior work showing perturbations of BK channel functioning in the presence of ethanol (Widmer et al., 1998). Pharmacological manipulation of BK channels in control animals was sufficient to mimic neuronal firing patterns seen in 24hrs ethanol FA mice. Acute ethanol exposure or chronic exposure to sucrose were not sufficient to induce hyperexcitability. Increased understanding of how alcohol reorganizes neural circuitry and when in abstinence alcohol induced plasticity is reversed will provide insight into not only druggable targets, but also critical time windows of treatment that may have the greatest success in preventing relapse.

2.2 Methods

Animals

Female C57BL/6J mice (Jackson Laboratories) were used in all studies. All procedures were carried out in accordance with the NIH Guide to Care and Use of Laboratory Animals and institutional guidelines and approved by the Institutional Animal Care and Use Committee at Vanderbilt University. Animals were on a 12:12 light dark cycle and all tissue collected 3-4 hours after lights turned on. All subjects were single housed at the start of the CDFA paradigm and given ad libitum access to standard rodent chow. Animals began the study at 5-6 weeks of age and were sacrificed 9 or 11 weeks later for electrophysiology and RNA scope studies.

Stereotaxic Surgery

For electrophysiology studies 5–6-week-old mice were anesthetized via isoflurane. Animals received 300nL bilateral injections of pAAV-CAG-tdTomato (Addgene, Cat no. 59462-AAVrg) into the BNST (AP 0.02mm, DV -4.18mm, ML +/- 3.76mm). Virus was infused at a rate of

40nL/min and a Hamilton syringe allowed to remain for 5 minutes after injection to increase viral uptake. Mice were treated with 2.5 mg/kg Metacam for 48-hour post-surgery and given 2 weeks to recover before undergoing CDFA.

Chronic Drinking Forced Abstinence (CDFA)

Chronic drinking followed by forced abstinence was done as described previously (Centanni et al., 2019; Holleran et at., 2016; Vranjkovic et al., 2018). Briefly, beginning at 7-8 weeks of age mice were single housed and given access to two sipper bottles filled with water. After 1 week of habituation our experimental group was given access to increasing concentrations of ethanol until reaching a maintenance dose of 10% ethanol. The control group continued to drink from bottles that both contained water for the duration of the experiment. After 6 weeks of ethanol or water, bottles were removed for either 24 hours or 2 weeks before sacrificing for electrophysiology or in situ hybridization.

Whole Cell Patch Clamp Electrophysiology

Mice were euthanized via isoflurane anesthesia and rapidly decapitated and brains placed in ice cold preoxygenated (95% O2/5% CO2) NMDG-based slice buffer consisting of (in mM): NMDG (93), NaHCO3(30), glucose (25), HEPES (20), KCl (2.5), NaH2PO4 (1.2), MgCl2 (10), CaCl2 (0.5), Na-ascorbate (5), Na-pyruvate (3), N-acetylcysteine (5); adjusted to pH 7.3-7.4 and 300-310 mOsm. 300 µm Coronal slice slices containing the insular cortex and BNST were prepared from whole brain tissue using a Vibratome (Leica VT1200S; Leica Instruments, Nussloch, Germany). Slices were then placed into a warm bath (32-34 degrees) containing the same NMDG based

slice buffer for 10-15 minutes. Slices were then transferred to a chamber containing oxygenated artificial cerebrospinal fluid (ACSF; 119 mm NaCl, 2.5 mm KCl, 1.3 mm MgCl2-6H2O, 2.5 mm CaCl2-2H2O, 1.0 mm NaH2PO4-H2O, 26.2 mm NaHCO3, and 11 mm glucose; 287–295 mOsm) 298-302mOsm, for 1 hour at room temperature before recording.

All experiments were performed and analyzed with pClamp 11.1 (molecular devices). Recordings were made using a 10 kHz sampling rate and a 2 kHz low pass filter. Slices were transferred to an interface recording chamber and continuously-perfused with 28–32°C aCSF at 2 ml/min. BNST projecting insula neurons were visualized using a mercury lamp light source and Texas Red filter cube used to illuminate Td-tomato fluorophores.

Prior to all electrophysiology recordings, tdTomato expression was verified under the rig microscope for expression at the BNST injection site and the IC (Figures 2.2A-B). Pyramidal cells were confirmed according to morphologic (size, shape) and biophysical properties (e.g., capacitance, and membrane resistance) and patched with 3–5 MΩ recording pipettes (P-97 Micropipette Puller). Current clamp recordings were performed in a K+-based internal solution (125mM K-gluconate, 4mM NaCl, 10mM HEPES, 4mM MgATP, 0.3mM NaGTP, 10mM Tris-phosphocreatine; 285–292 mOsm). Voltage clamp recordings were performed using a cesium based internal solution (140 CsMeSO3, 5 NaCl, 10 HEPES, 0.2 EGTA, 2 MgATP, 0.2 NaGTP, 5 QX-314; 310-315mOsm).

In situ hybridization (RNAScope)

Visualization of RNA transcripts in insular coronal sections was achieved via fluorescent in situ hybridization assays using RNA-Scope Fluorescent Multiplex Reagent Kit (Advanced Cell Diagnostics). Mice were anesthetized with isoflurane prior to brain extraction. The brains were immediately flash frozen with Optimal Cutting Temperature (OCT) Solution (Fischer Scientific, CAT # 23-730-571) and dry ice and stored at -80 °C. Following this, they were sliced into 16 µm slices on a cryostat and placed on slides at -80 °C.

The RNAscope protocol was followed as previously described (Luchsinger et al., 2021). The slides were first fixed with 4% paraformaldehyde (PFA) for 15 minutes at 4 °C, then dehydrated in 200 mL 50% EtOH, 200 mL 70% EtOH, and 600 mL 100% EtOH for 5 minutes each at room temperature (RT). The slides were air-dried for 5 minutes at RT, after which a hydrophobic barrier pen (Vector Laboratories, CAT # H-4000) was used to create a barrier around the tissue. A HybEZ Humidity Control Tray with wet humidifying paper was warmed in a HybEZ oven at 40 °C for 30 minutes, after which five drops of Protease IV were added to each section for 30 minutes at RT. 3L of 1X Wash Buffer was prepared by mixing 2.94 L distilled water and 60 mL of 50X Wash Buffer. Amp 1-4-FL reagents were left at RT. The mixed probe was prepared by warming C1, C2, and C3 probes for 10 minutes at 40 °C before cooling to RT. The probe consisted of a 1:1:50 ratio of C1:C2:C3 volumes and was mixed by inverting the tube. All probes purchased from advanced cell diagnostics. PV (cat no. 421931); vGlut (Slc17a7) (cat no. 501101); BK (Kcnma1) (cat no. 476258); SST (cat no. 404631).

Four drops of the probe were added to each section, and the sealed tray containing the slide rack was put in the oven for 2 hours at 40 °C. This procedure was repeated with Amp 1-FL, Amp 2-Fl, Amp 3-Fl, and Amp 4-Fl for 30 minutes, 15 minutes, 30 minutes, and 15 minutes, respectively. The slides were washed with 1X Wash Buffer for 2 minutes at RT between each hybridization procedure. Four drops of DAPI were added to each section, and the slides were incubated for 30 seconds at RT. DAPI was removed, and 1-2 drops of fluorescent mounting (Aqua Poly/Mount, Polysciences, CAT #18606-20) medium were immediately placed on the slides, over which a coverslip was placed. The slides were then stored at 4 °C in the dark.

A Zeiss LSM 880 scanning confocal microscope was used to compile Z-stack insular images composed of mid-insula. Parameters for brightness and contrast were set using DAPI, the negative control probe, for the experimental images, while the threshold parameters for nonspecific fluorescence were set using the negative control images. ImageJ Fiji software was then used to process the images as max intensity projections, each with the combined counts of the mid-insula from each mouse. Total cell count was determined with DAPI-labeled nuclei and individual dots in a cell indicated transcripts. Cells were designated as positive (at least one dot) or negative (no dots) by two double-blinded reviewers.

BK Channel Pharmacology

The BK antagonist paxilline (Tocris Cat no. 2006) and BK channel agonist NS-19504 (Tocris Cat no. 5276) were used in slice physiology experiments. Both were diluted in DMSO to create

10mM stock solutions and subsequently diluted on recording days in aCSF for a final concentration of 10µM.

Data Analysis and Statistical Tests

Prism 9 (GraphPad Software Inc.) was used for all statistical analyses. Comparisons were made between the 24hr FA and 2 weeks FA to the control subjects. Main effects of group and week were evaluated for all data plotted across all 6 weeks of the chronic drinking period. For all analyses, a p value was set as 0.05 indicating a statistically significant difference. Statistical significance was determined by either a one-way, two-way repeated measures ANOVA or t-test as indicated in figure legends. All post hoc analysis was performed using a Bonferroni multiple comparison test, and standard error of the mean was used for all experiments. Power analyses were performed with preliminary data during the acquisition of each new data set. The sample size obtained from each power analysis calculation was then compared with sample sizes reported in the literature for similar experiments. Data represented as mean ±SEM.

2.3 Results

Acute Forced Abstinence is associated with hyperexcitability of $IC^{\rightarrow BNST}$ cells

Following stereotaxic surgery mice were randomly assigned into one of three groups (control, 24hrs FA, 2 weeks FA) and allowed ad libitum home cage access to an alcohol bottle and a water bottle (FA groups) or two water bottles (control) for 6 weeks (Figure 2.1A). There were no significant changes in body weight across groups (Figure 2.1B; ($F_{(10, 290)}$ = 1.490, p=0.1421)) but both experimental groups formed a preference for the alcohol bottle over the water bottle by week 4 (Figure 2.1C; ($F_{(2, 57)}$ = 24.31, p<0.0001)). Consumption of ethanol as

compared to water for both the 24hr FA (Figure 2.1D; p<0.0001;) and 2-week FA (Figure 2.1E; p=0.0084) groups was significantly higher for the duration of the experiment.

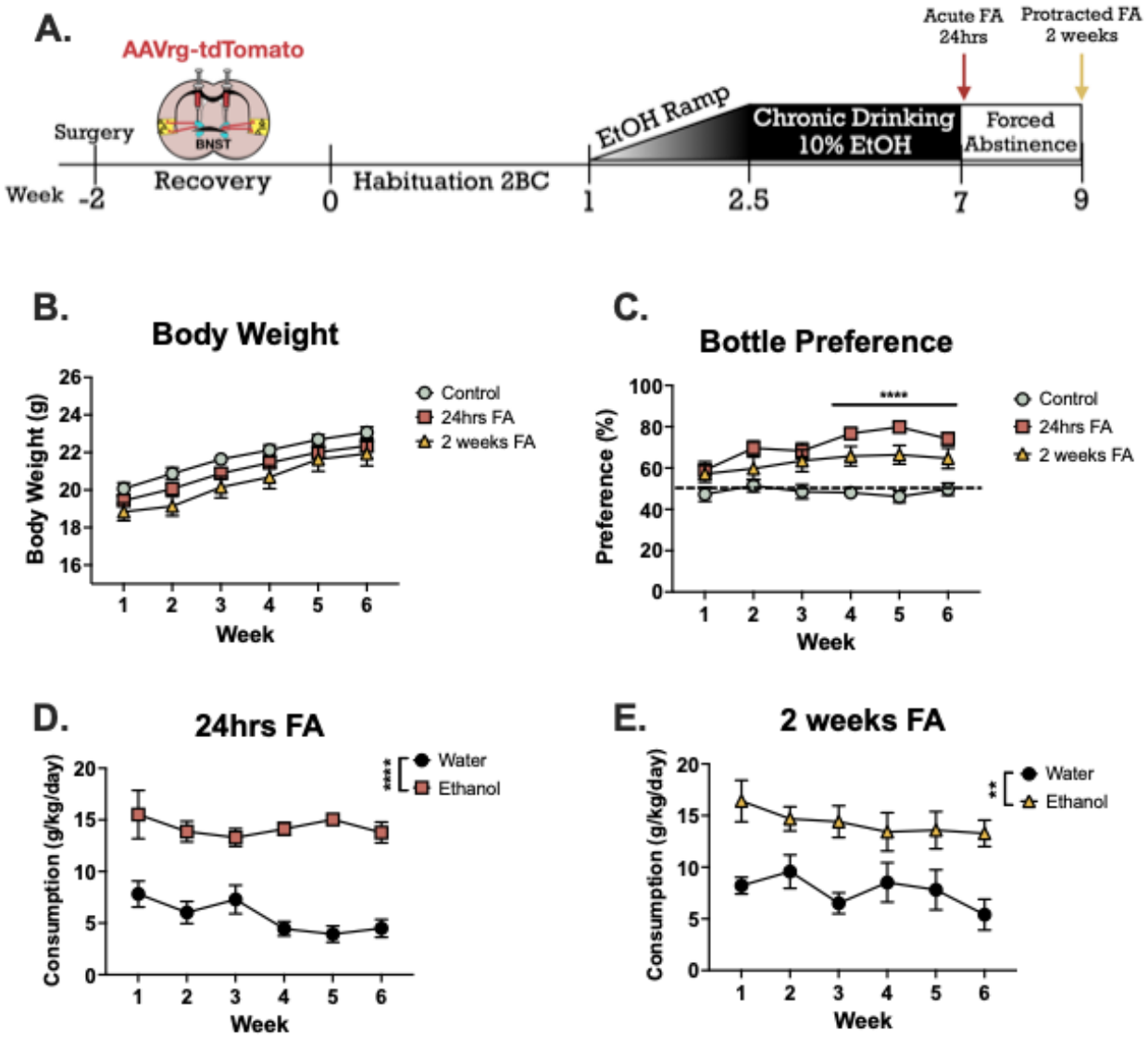

Figure 2.1: Experimental design. (A) Timeline including stereotaxic surgery, chronic drinking paradigm and data collection timepoints during forced abstinence (FA). (B) There was no main effect of group across weeks for control (N=20), 24hr FA (N=26) and 2 weeks FA (N=15) ($F_{(10, 290)}$ = 1.490, p=0.1421). (C) There was a significant main effect of group for bottle preference ($F_{(2, 57)}$ = 24.31, p<0.0001). Post hoc analysis revealed that compared to control, these differences emerged at week 4 (24hrs FA p<0.0001; 2 weeks FA p=0.0084). (D,E) There was a main effect of

group for ethanol and water consumption for both the 24hr FA group ($F_{(1, 24)}$ = 78.74, p<0.0001) and the 2 weeks FA group group ($F_{(1, 14)}$ = 9.921, p<0.0071). 2-way repeated measures ANOVA used in all analysis.

We investigated the intrinsic firing properties of $IC^{\rightarrow BNST}$ cells identified via fluorescence microscopy. Increasing injections of current into $IC^{\rightarrow BNST}$ cells produced more action potentials across current steps in the 24hrs FA group compared to control and 2 weeks FA (Figures 2.2C-D; ($F_{(2, 17)}$ = 4.488, p=0.0272)). Consistent with this finding, we observed a decrease in the afterhyperpolarization (AHP) amplitude (Figures 2.2E-F; ($F_{(2,32)}$, p=0.059)) and rheobase current (Figure 2.2G; ($F_{(2,32)}$, p=0.065)) in the 24 hrs FA but not 2 weeks FA or control groups. These changes in intrinsic excitability were not likely due to an HCN channel mechanism, as no difference was observed in the sag ratio across groups (Figure 2.2H). Membrane properties including the resting membrane potential (Rm; Figure 2.2I) and membrane voltage (Vm; Figure 2J) were also unchanged across groups. Finally, we found no correlation between individual ethanol intake over the course of the experiment and $IC^{\rightarrow BNST}$ cell firing rate (Figure 2.2K).

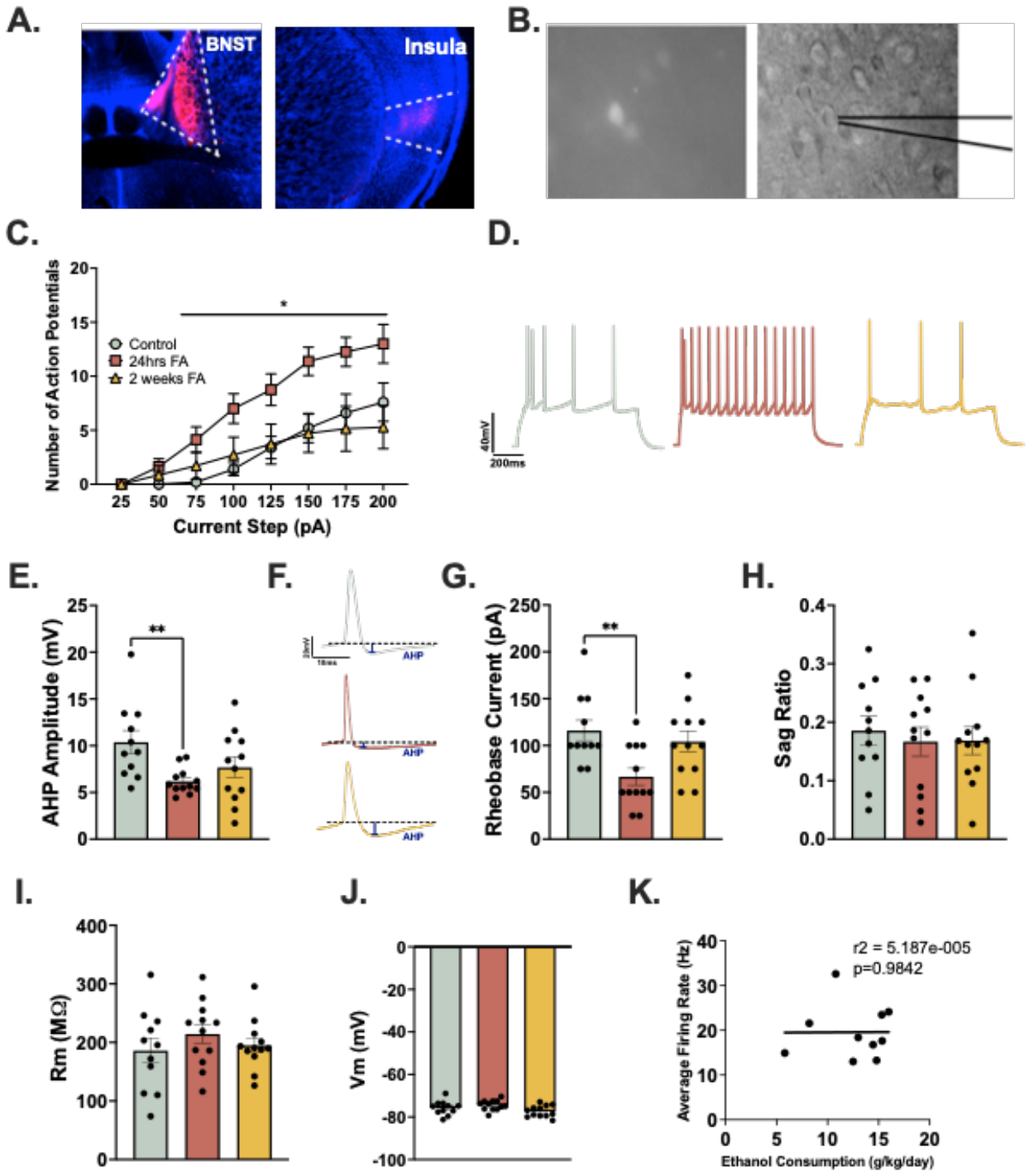

Figure 2.2: Acute abstinence increases intrinsic excitability of Insula$^{\rightarrow BNST}$ cells. N=4/group; n=11 (control), n=12 (24hrs FA, 2weeks FA). (A) AAVrg-CAG-tdTomato expression in the BNST and IC. (B) Example Insula$^{\rightarrow BNST}$ cell as viewed from electrophysiology rig. (C) Following stepwise current injections there was a main effect of group ($F_{(2, 17)}$

= 4.488, p=0.0272) and current step ($F_{(1.652, 28.09)}$ = 58.32, p<0.001) with the 24hr FA group firing more action potentials compared to control starting at the 75pA current injection (p=0.0257). (D) Representative trace at the 200pA current step. (E) After hyperpolarization amplitude ($F_{(2,32)}$ p=0.059) was significantly less in the 24hr FA group compared to control (p=0.0093). (F) Representative AHP trace. Rheobase current ($F_{(2,32)}$ p=0.065) was lower in the 24hr FA group compared to control (p=0.0052). No significant differences seen in sag ratio (H) ($F_{(2,32)}$ p=0.8465), resting membrane potential (I) ($F_{(2,32)}$ p=0.4811), or membrane voltage (J) ($F_{(2,32)}$ p=0.0612). (K) Using a simple linear regression, no correlation found between average firing rate and ethanol consumption ($F_{(1,8)}$, p=0.9842). 2-way repeated measures ANOVA used in (C). One way ANOVA used in (E, G-J).

After examining intrinsic properties in $IC^{\rightarrow BNST}$ cells after CDFA, we next examined synaptic properties. We found no changes in either spontaneous excitatory (Figures 2.3A-C) or inhibitory (Figures 2.3D-F) transmission onto $IC^{\rightarrow BNST}$ neurons. Taken together these data demonstrate that following acute but not protracted ethanol forced abstinence increases excitability of $IC^{\rightarrow BNST}$ cells without altering spontaneous synaptic transmission.

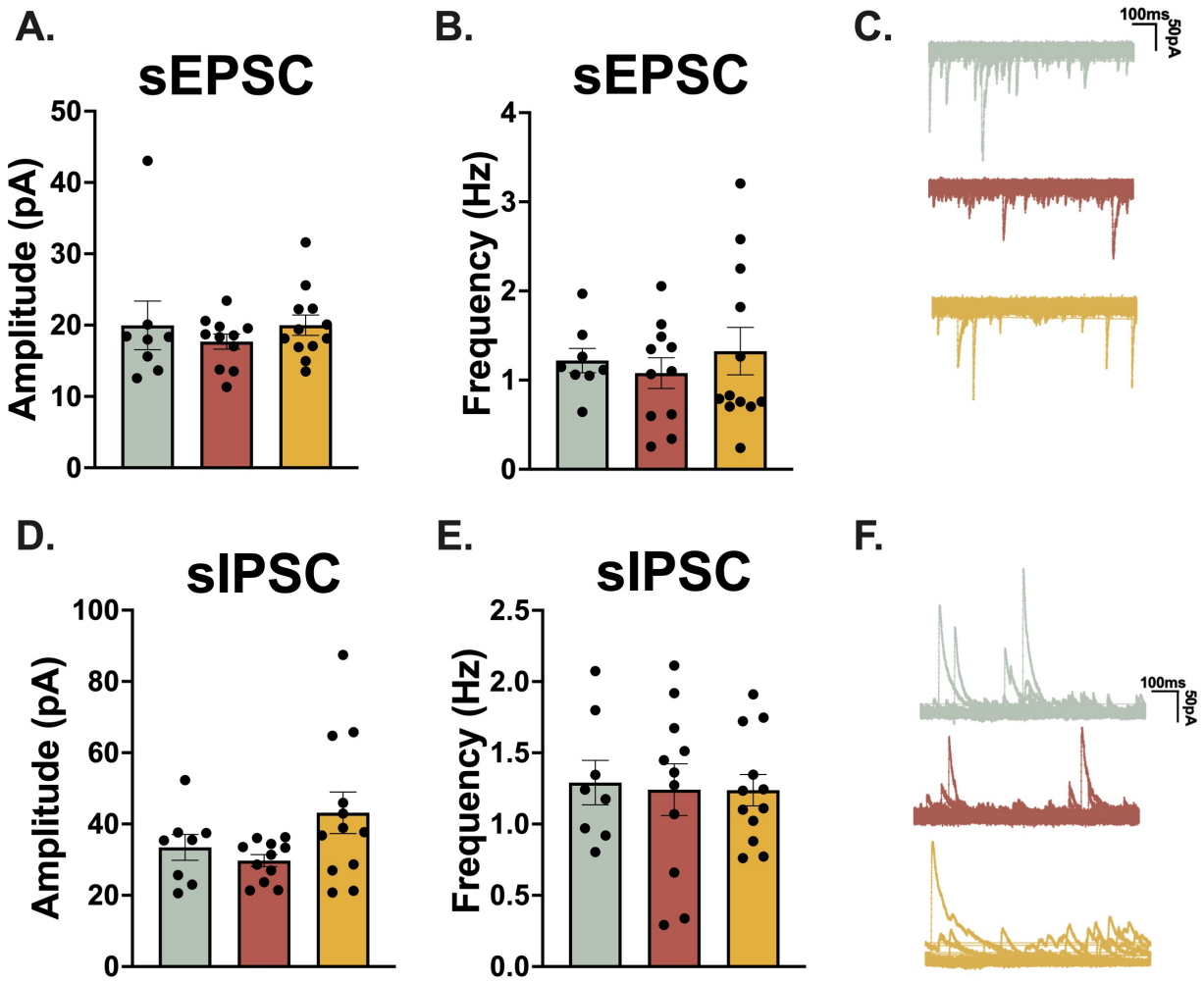

Figure 2.3: Spontaneous transmission is unaffected by forced abstinence. N=3, n= 8 control; N=4, n=11 24hrs FA; N=5, n=12 2 weeks FA. As indicated by one way ANOVA, there were no differences found in (A) sEPSC amplitude ($F_{(2, 28)} = 0.4953$, $p=0.6146$) or (B) frequency ($F_{(2, 28)} = 0.3603$, $p=0.7007$). (C) Representative sEPSC traces. There was also no change in(D) sIPSC amplitude ($F_{(2, 28)} = 2.773$, $p=0.0796$) or (E) frequency ($F_{(2, 28)} = 0.03317$, $p=0.9674$). (F) Representative sIPSC traces.

We next performed a series of experiments to assess the nature of the observed increase in $IC^{\rightarrow BNST}$ neuron excitability by examining the impact of other behavioral and pharmacological stimuli. Our first step was to examine the impact of acute ethanol *in vivo* (Figure 2.4A) by ip injecting saline or ethanol (2.5g/kg) 24hrs prior to recording from $IC^{\rightarrow BNST}$ cells. We also examined ex vivo ethanol administration by washing on ethanol (50 mM) for 20 minutes onto IC containing brain slices from naive animals (Figure 2.4B). Neither acute *in vivo* or *ex vivo* ethanol exposure produced changes in action potential firing.

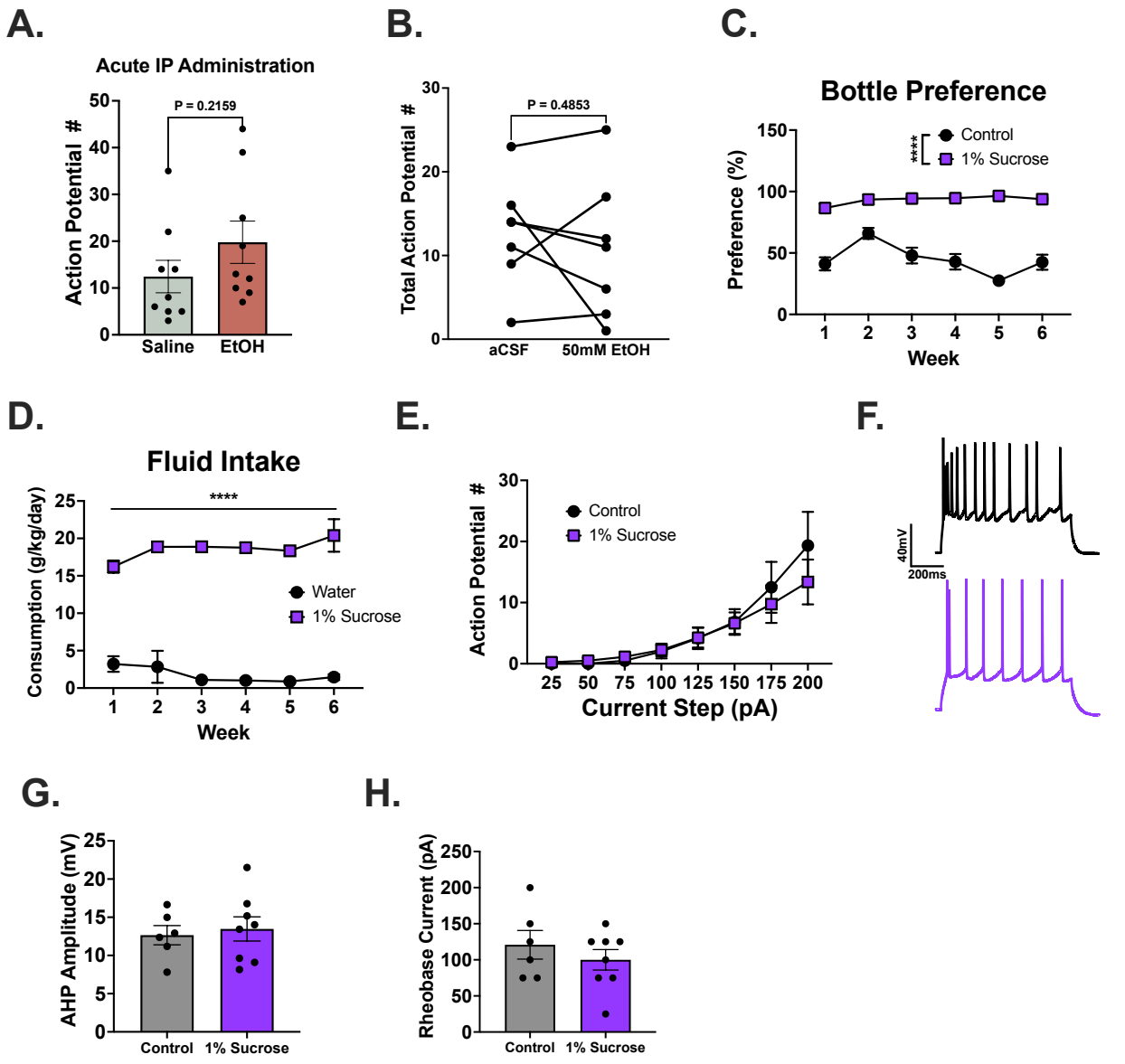

Figure 2.4. Acute ethanol exposure or chronic sucrose intake does not induce elevated excitability. (A) In vivo IP administration of 2.5g/kg ethanol followed by 24hrs wait time and then recordings from Insula$^{\rightarrow BNST}$ cells (N=3/group, n=9/group). No effect of acute ethanol administration on action potential number at the 200pA current injection (unpaired t-test; p=.2159). (B) 50mM ethanol wash on to naïve brain slices (N=3, n=7). No effect of ethanol wash on action potential number at the 200pA current injection (paired t-test; p=0.4853). (C) subjects in the sucrose group preferred the sucrose bottle (N=5/group), (main effect of group, $F_{(5, 40)} = 4.464$; p=0.0025) (control vs sucrose post hoc analysis across weeks p<.0001). (D) sucrose group animals consumed more sucrose than water (main effect of group, $F_{(1, 8)} = 821.2$, p<0.0001), (water vs sucrose post hoc analysis across weeks

p<.0001). (E) No change in action potential number across current steps (main effect of group $F_{(1, 4)}$ = 0.04649 , p=0.8398). (F) representative traces at the 200pA current step. No differences in AHP (G; p=0.5838) or rheobase current (H; p=0.4107).

To further assess the specificity of CDFA induced increases in $IC^{\rightarrow BNST}$ neuronal excitability we next ran a cohort of mice through 2 bottle choice modeled in the same way as CDFA, but with 1% sucrose instead of ethanol. As expected, sucrose animals formed a reinforcer bottle preference unlike the control water only animals (Figure 2.4C; $F_{(5, 40)}$ = 4.464; p=0.0025) and consumed more sucrose than water (Figure 2.4D; $F_{(1, 8)}$ = 821.2, p<0.0001)). Unlike with the ethanol 2 bottle choice, 24hrs FA from sucrose was not associated with any change in excitability compared to control (Figures 2.4E-H).

BK channel pharmacology recapitulates hyperexcitability phenotype seen in 24hr Forced Abstinence

The constellation of excitability changes observed in $IC^{\rightarrow BNST}$ cells in the 24hrs FA group led us to investigate potential alterations in potassium channel function and/or expression. BK potassium channels have long been identified as ethanol sensitive and have properties which enable them to regulate the aspects of cellular physiology shown to be changed here. Using the same timeline as described in figure 1A, we again used whole cell patch clamp electrophysiology to record from $IC^{\rightarrow BNST}$ neurons in control and 24hr FA animals. We began by utilizing the BK channel blocker paxilline. 1 hour incubation of control slices with paxilline (10µm), produced an increase in AP number across current steps (Figure 2.7A; ($F_{(1, 19)}$ = 8.785,

p=0.0080)), a trend towards a decrease in rheobase current (Figure 2.7B; (p=0.0572)) and significant reduction in AHP (7C; (p=0.0128)), all of which were similar in magnitude to the alterations observed in 24hr FA mice. Conversely when washing on the BK channel agonist NS19504 (10µm) for 20 minutes we find that most cells respond with diminished action potential firing (Figures 7D-E).

In the 24hr FA group, the BK ligands used above had different effects. First, paxilline produced no further increase in firing in cells recorded from these animals, consistent with endogenous FA induced occlusion of this action (Figure 7F; ($F_{(7, 56)}$ = 0.6487, p=0.7138)). Further, NS19504 wash on elicited fewer action potentials in some cells, but only in a small number of the total population (Figures 7G-H).

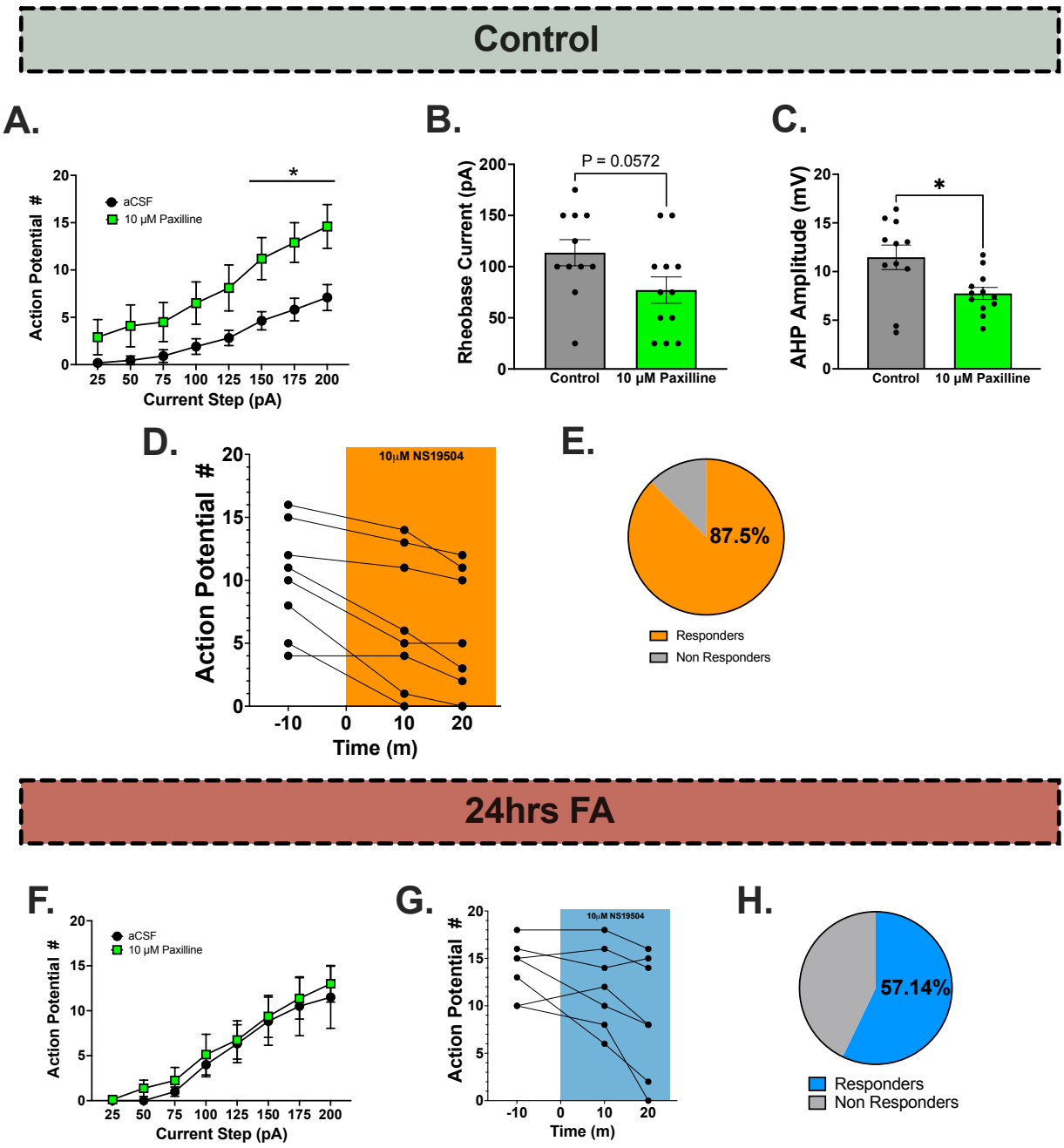

Figure 2.7. BK pharmacology can modulate firing in a similar manner to 24hrs FA from chronic ethanol. Control group slices (N=4/group, n=11 control, n=12 24hrs FA) incubated for 1 hours in BK channel antagonist paxilline mimicked the increased excitability phenotype seen in 24hrs FA mice including (A) increased action potential number across current steps (main effect of group; $F_{(1, 19)} = 8.785$, p=0.0080) starting at the 150pA current injection (p=0.03670). (B) Additionally, there is a trend towards a decrease in rheobase current and (p=0.0572) and (C) decreased AHP (p=0.0128) as indicated by unpaired t-test. (D-E) Wash on of BK agonist NS19504 reduced action

potential firing at the 200pA current injection in 87.5 of control cells sampled as indicated by a greater than 20% reduction from aCSF baseline at to 20-minute timepoint (N=3, n=8). (F) No effect of incubating 24hrs FA slices in paxilline (aCSF N=4, n=6, paxilline N=3,n=8) ($F_{(7, 56)}$ = 0.6487, p=0.7138). (G-H) Wash on of BK agonist NS19504 reduced action potential firing at the 200pA current injection in 57.14% of 24hr FA cells sampled as indicated by a greater than 20% reduction from aCSF baseline at to 20-minute timepoint (N=3, n=7).

CDFA acute abstinence is associated with decreased BK channel mRNA expression in multiple cell populations in IC.

Our experiments with BK ligands suggest the possibility that acute FA is associated with reduced BK expression and/or function. Preliminary studies utilizing fluorescent in situ hybridization approaches revealed robust expression of BK isoforms in regions of insular containing BNST-projecting neurons. Thus, we probed for BK channel (Kcnma1) mRNA expression in our 3 CDFA groups (5A). To normalize cell counts between images BK expression was reported as a percentage of total DAPI cells (5B-D). In the 24hr FA group we found IC BK mRNA expression to be significantly diminished compared to samples from control and 2 week FA (5E; ($F_{(2, 12)}$ = 23.63, p<0.0001)).

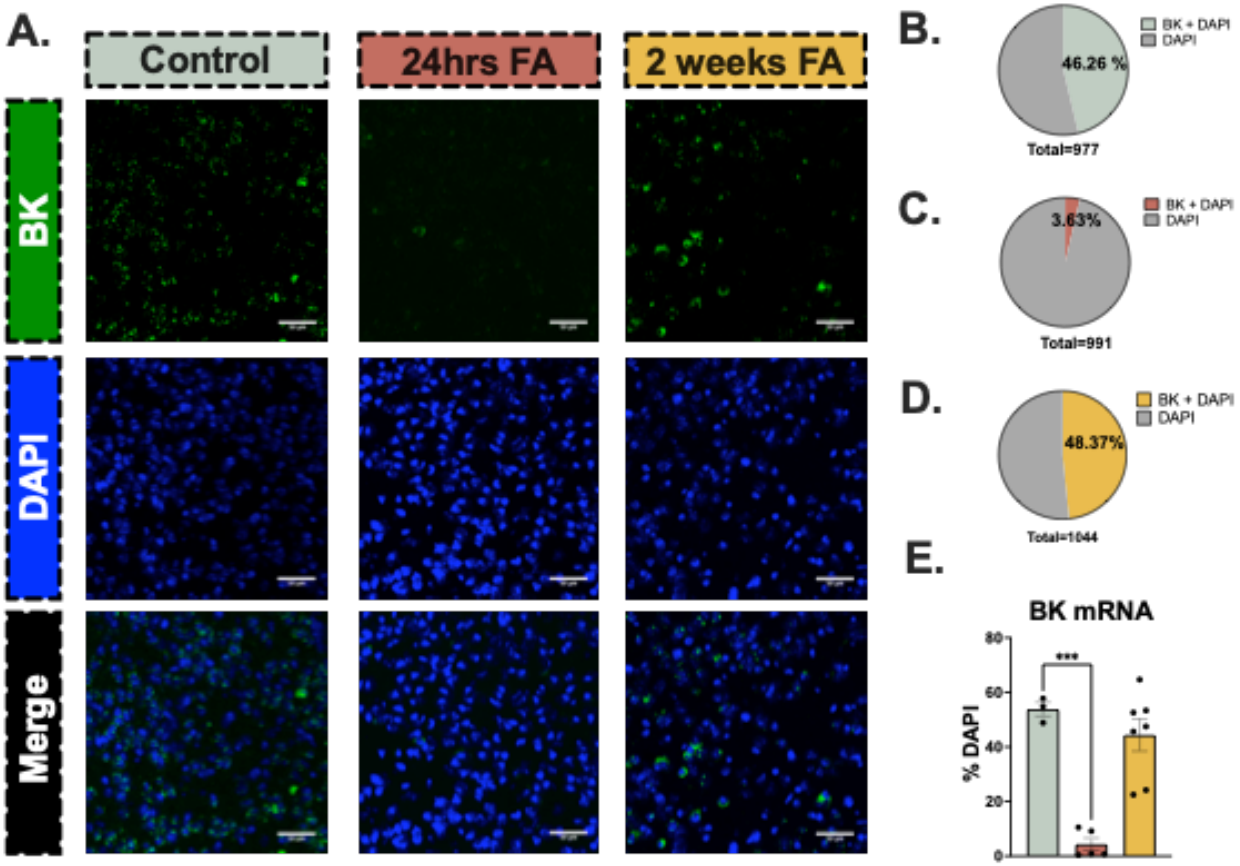

Figure 2.5: IC BK channel mRNA is decreased is decreased following 24hrs FA. (A) Representative images from control, 24hr FA and 2 weeks FA groups showing BK mRNA (green), DAPI (grey) and merged image. (B-D) Charts quantifying cell counts for BK expressing cells (BK + DAPI) and non-BK cells (DAPI only). (E) BK mRNA expression is decreased following 24hrs FA compared to control as indicated by 1 way ANOVA ($F_{(2, 12)} = 23.63$, $p<0.0001$).

To further investigate BK expression alterations in insula during CDFA, we further probed for cells co-expressing both BK and markers for 3 major cell types within the region. Principal cells marked by the glutamate vesicular transmitter vGlut1 (2.6A,B) and 2 interneuron markers: parvalbumin (PV) (2.6A,C) and somatostatin (SST) (2.6A,D). When comparing cells

containing BK mRNA with each of the forementioned markers relative to all cells, there was again a significantly decreased amount of BK mRNA colocalized with each of these cell markers. These data suggest that overall BK expression within the IC is reduced in acute- but not prolonged-abstinence.

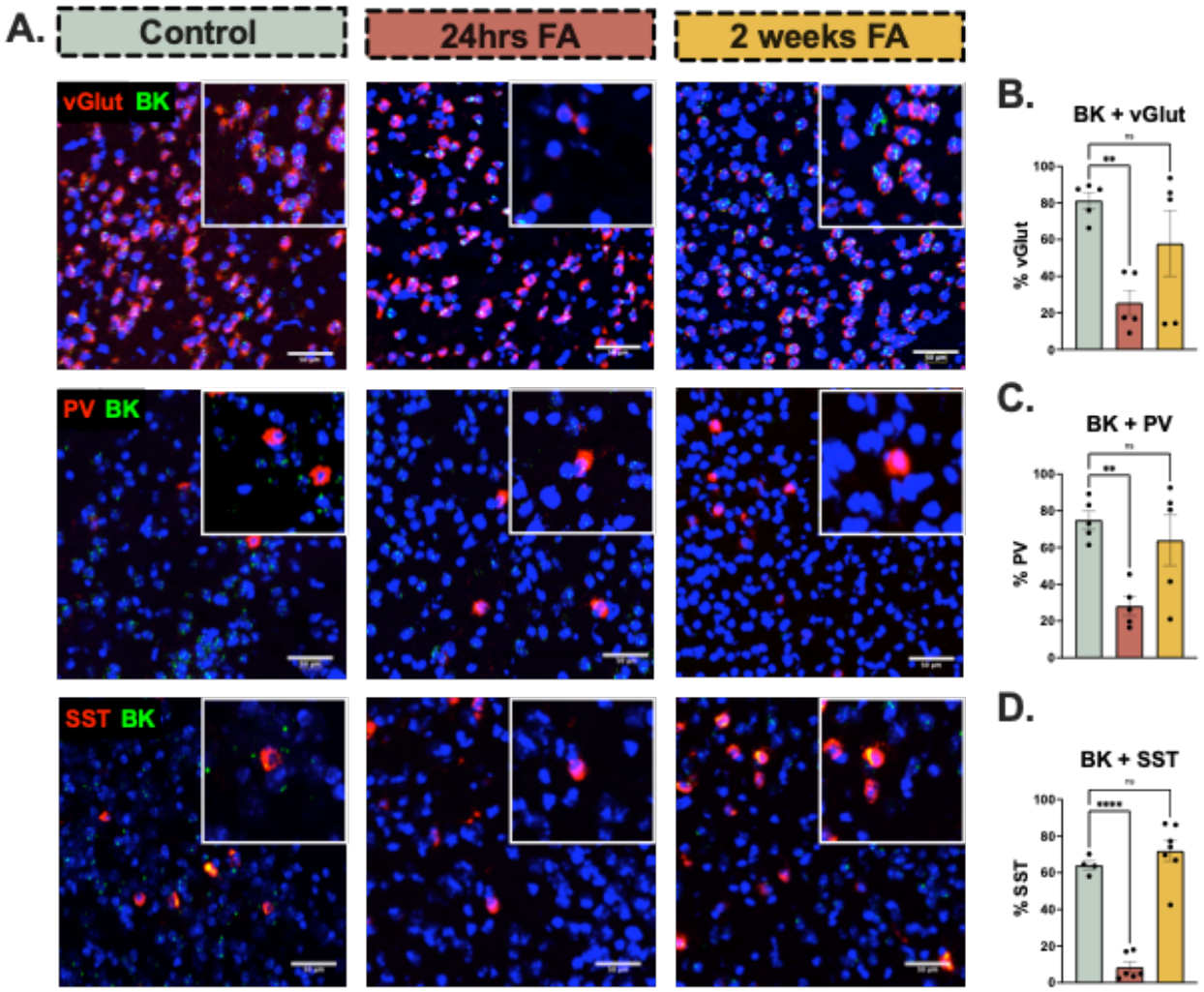

Figure 2.6: BK channel mRNA is uniformly expressed in all major cell types in the IC. (A) Representative images from control, 24hr FA and 2 weeks FA groups showing BK mRNA (green), DAPI (blue) and in red either vGlut, parvalbumin or somatostatin (red). (B-D) BK mRNA expression colocalized with either vGlut (B), PV (C), or SST (D) relative to total of each cell type. One way ANOVA revealed that compared to control, there were significantly fewer BK mRNA

puncta on cells expressing SST ($F_{(2,14)}$=60.10, p<0.0001); PV ($F_{(2, 12)}$ = 7.354, P=0.0082); and in vGlut ($F_{(2, 12)}$ = 6.043, P=0.0153).

2.4 Discussion

Here we report that during acute abstinence from chronic home cage volitional ethanol intake, BNST projecting IC cells are transiently hyperexcitable. This increased excitability was apparent 24 hrs into forced abstinence but was absent 2 weeks into forced abstinence. We found no effect of forced abstinence on either spontaneous excitatory or inhibitory transmission. The effects of CDFA were not mimicked by acute ethanol or by chronic access to 1% sucrose. *In situ* hybridization analysis revealed a significant decrease in BK channel mRNA expression in the IC of 24hr, but not 2 week FA mice. Subsequent slice pharmacology using a BK channel antagonist mimicked the 24hr FA firing phenotype in control animals, while increased firing seen in acute withdrawal animals occluded the effect of blocking BK channels on elevated action potential number. Activating BK channels decreased firing in control and 24hrs FA $IC^{\rightarrow BNST}$ cells, albeit to a lesser extent in the latter group.

BNST Projecting IC Cell Excitability is Temporally Regulated During Withdrawal

Previous studies have identified 24hrs- and 2 weeks-post forced abstinence as critical timepoints. Multiple studies have shown increased negative affective behavior in C57BL6/J mice two weeks in forced abstinence (Centanni, Bedse, et al., 2019; Holleran et al., 2016; Pang et al., 2013; Vranjkovic et al., 2018). This timepoint is also associated with increased

spontaneous excitatory transmission at IC inputs within the BNST. Chemogenetic inhibition of activity in the IC decreased CDFA induced BNST activity, while Gq-DREADD-dependent activation of this pathway mimicked forced abstinence-associated affective behaviors (Centanni, Morris, et al., 2019). Importantly, ketamine administration at the onset of abstinence, but not 2 days later, prevented the development of abstinence induced affective disturbances and increased the capacity for plasticity in the BNST (Vranjkovic et al., 2018). In the present study, we find that early- but not late-forced abstinence is associated with hyperexcitability in the BNST projecting IC neurons. This suggests the possibility that early IC hyperexcitabilty drives plasticity that results in increased excitatory transmission in the BNST later in forced abstinence.

Chronic Volitionary Moderate Ethanol Consumption Induces Novel IC Plasticity

We find that acute alcohol exposure or chronic access to a natural reward are not sufficient to induce excitability changes in this cell population. These data suggest that the effects of CDFA require the combination of volitional ethanol intake coupled with the pharmacological properties of ethanol. As previously discussed, chronic volitional ethanol intake induces changes in affective behavior in forced abstinence, which can be prevented with early intervention. Work by Marino et al. used a short-term binge drinking paradigm known as drinking in the dark to explore ethanol action on IC projections to ventral BNST neurons. Similar to our study, they found an elevation in $IC^{\rightarrow BNST}$ neuronal firing. However, they also observed an increased excitatory synaptic transmission, but not a change in rheobase. These differences

may be related to different ethanol intake conditions and/or acute versus chronic ethanol intake (Marino et al., 2021).

Pre-clinical and clinical chronic heavy drinking and binge drinking studies show aberrant IC structure and function (Chen & Lasek, 2020; Chung & Clark, 2014; Haggerty et al., 2022b, 2022a; Jaramillo et al., 2016; Jaramillo, Van Voorhies, et al., 2018; Marino et al., 2021). Unlike models of heavy/binge drinking utilizing 20% ethanol or greater that induce blood ethanol contents well above 80 mg/dL corresponding to high levels of intoxication, our model utilized a maximum dose of 10% ethanol, inducing blood ethanol contents that range from 10-40mg/dL at any given time (Crabbe et al., 2009; Hodge et al., 1997; Holleran & Winder, 2017; McBride & Li, 1998; Murphy et al., 2002). Thus these data suggest that chronic, high level ethanol intake is not necessarily required to induce abstinence-related affective disturbances. One proposal has been that harmful effects of ethanol appear in a J-shaped mortality curve where there are initial protective effects at lower doses and a reverse to negative effects with increasing levels of intake (Szmitko & Verma, 2005; Wallner & Olsen, 2008; Zakhari & Gordis, 1999). Our work suggests that low to moderate consumption can also have adverse consequences.

The transient hyperexcitability we observe highlights the importance of consideration of time in abstinence when considering treatment strategies. For example, negative affective behaviors following CDFA have been reported to emerge following 2 weeks of forced abstinence and can be prevented with ketamine administration at the 24hr FA timepoint (Vranjkovic et al., 2018). It has also been shown that the IC to BNST circuit is modulated by endocannabinoids, providing another avenue for early interventions to prevent anxio-depressive symptoms that often lead to relapse (Centanni, Morris, et al., 2019).

Ethanol Induced BK Channel Downregulation and IC Hyperexcitability

Ethanol induces a wide array of effects on neuronal function via modulation of a variety of proteins (Abrahao et al., 2017; Corbit & Janak, 2016; Vena et al., 2020). BK channels have been repeatedly implicated in ethanol action in various systems (Bettinger & Davies, 2014; Bukiya et al., 2014; Dopico et al., 2016; Palacio et al., 2015). Prior work indicates that ethanol reduces excitability, and that this reduction can be blocked by the BK channel antagonist iberiotoxin (Gruß et al., 2001). We assessed both expression and functional level changes in BK channels using *in situ* hybridization and brain slice electrophysiology. We found that in the 24hrs FA but not the 2weeks FA group, there was significantly less IC BK channel mRNA compared to control animals. Flourescent *in situ* hybridization is advantageous for its specificity and relative ease in tagging mRNA, however it does not assess channel expressed at the membrane. Thus we also utilized pharmacological approaches as in Figure 7, and found that in control animals, blocking BK channels with the antagonist paxilline elevates neuronal firing to levels similar to those seen in 24hrs FA mice. However, when repeating this experiment with tissue from 24hrs FA subjects, there was no effect of paxilline on action potential number across current steps. Pharmacological activation of BK channels blunted the number of potentials fired at the 200pA current step in both control and 24hrs FA animals but to a lesser extent in the latter group. These data suggest that in our acute forced abstinence animals there is a blunted or absent response to *ex vivo* BK channel agonism/antagonism, however we cannot parse apart if that is due solely to differential expression of these channel or if their ability to bind calcium and properly flux ion is also compromised.

Prior studies have shown that in adult drinkers and in animal models of AUD that still have ethanol in their system, there is decreased IC activity (Haggerty et al., 2022a; Le et al., 2022). In context, our IC hyperexcitability phenotype in early abstinence is likely not due to residual ethanol intoxication effects but rather this could be a compensatory mechanism that emerged to counteract ethanol's depressive effects on synaptic transmission. Without ethanol present at the 24hr FA timepoint, these animals have increased excitability in this BNST projecting cell population. The BNST is canonically known for its role in mediating anxiety and fear responses via its downstream connectivity with the hypothalamic pituitary axis (Cole et al., 2022; Davis et al., 2010; Walker et al., 2003). IC interoceptive cues signaling a deviation from homeostasis, trigger a response in the BNST which in part elucidates an underlying mechanism for negative affect in early withdrawal (Centanni, Morris, et al., 2019; Flook et al., 2021; Luchsinger et al., 2021).

The ability of IC cells to compensate to the absence of alcohol in more protracted abstinence shown in our study at the 2-week timepoint highlights the critical importance of early abstinence for 2 reasons. First, the risk of relapse is greatest as withdrawal symptoms are most severe and the central nervous system is still wired to counterbalance for the presence of ethanol (Becker, 2008; Fox et al., 2007; Sinha et al., 2009). Second, early intervention is critical to future long-term abstinence (Haber et al., 2021; Holzhauer et al., 2017; Ledda et al., 2019). Using a personalized medicine approach to determine the timeline of neural plasticity and withdrawal to pinpoint which targetable circuits can be manipulated to hasten the return to homeostasis will inevitably increase success rates of those recovering from AUD.

Conclusions

The IC plays a critical role in the development and maintenance of AUD. Chronic alcohol consumption can cause structural and functional changes in the insular cortex, leading to altered emotional and cognitive processing, which may contribute to AUD-relevant behaviors. Furthermore, targeted interventions aimed at modulating IC, such as non-invasive brain stimulation or pharmacological treatments, may be effective in reducing alcohol craving and promoting abstinence. Understanding the role of the IC in AUD may therefore provide important insights for the development of novel treatments for this widespread and devastating disorder.

Chapter 3

Discussion and Future Directions

3.1 Contributions to the field

In this work I used whole cell patch clamp electrophysiology and fluorescent *in situ* hybridization to explore adaptations of the $IC^{\rightarrow BNST}$ circuit following abstinence from chronic volitional ethanol intake. Previous work has implicated this circuitry in the development of negative affective symptoms present during withdrawal (Centanni, Morris, et al., 2019; Flook et al., 2021; Marino et al., 2021). The present study is the first to describe $IC^{\rightarrow BNST}$ circuit hyperexcitability early in forced abstinence due to a downregulation in BK channel expression.

In the broader context of homeostasis in AUD, it can be extrapolated from my work that during chronic drinking there is an upregulation of BK channels (Figure 3.1). Human and animal work showing increased IC activity in chronic drinkers supports this hypothesis (Haggerty et al., 2022a; Le et al., 2022). This could be a result of direct ethanol binding to BK (Brodie et al., 1990; Martin et al., 2008). When ethanol is abruptly removed from the system, there is an acute response of BK channel downregulation resulting in neuronal hyperexcitability (Figure 2.2). This could be the catalyst for downstream hyperactivity in downstream regions like the BNST, previously reported by the Winder lab (Centanni, Morris, et al., 2019; Vranjkovic et al., 2018). Notably, elevated neuronal firing of BNST projecting insula cells is a specific response to volitional chronic ethanol intake. Neither acute ethanol administration nor chronic volitional exposure to sucrose could recapitulate

these results. These data taken together fit into a growing narrative in the AUD field and the broader media landscape that contrary to prior thinking, frequent moderate alcohol intake can be similarly detrimental to human health as binge drinking or heavy drinking (The New York Times, 2023.).

The mouse model used in my studies aims to mimic an individual who may not necessarily meet AUD criteria, but who does consume alcohol on a daily basis – something that 30 percent of Americans report to do (NIAAA, 2023). I hope that my work inspires further research using models of chronic moderate volitional ethanol intake to better understand the effects of a drug that is so engrained in our culture.

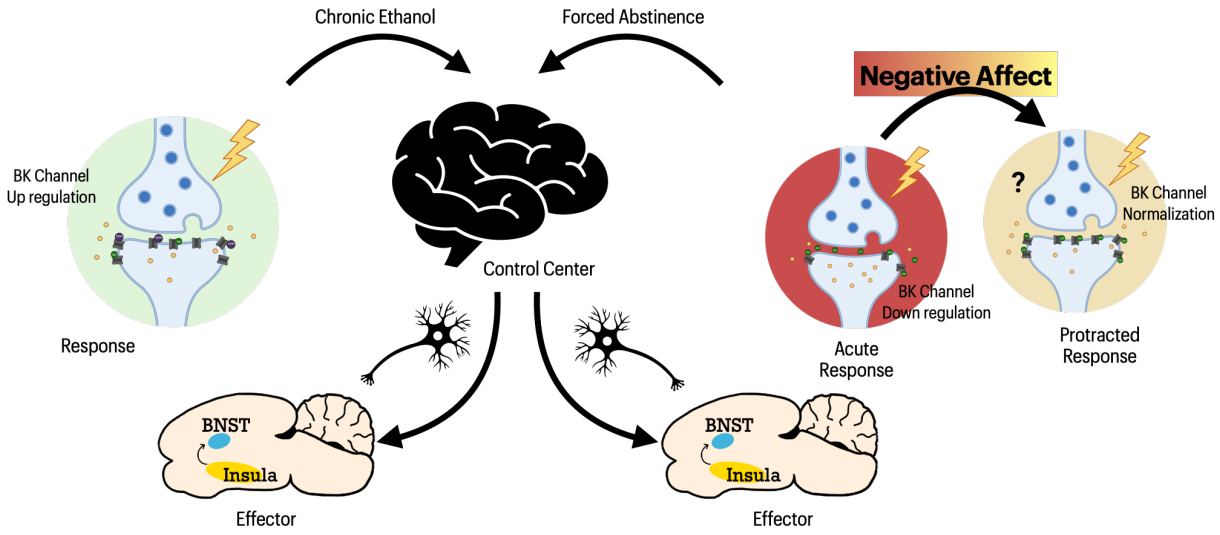

Figure 3.1 The Homeostatic response of IC to BNST Circuit in AUD

3.2 New areas of exploration

Prior studies have explored the actions of ethanol and the GABAergic system in detail. Alcohol enhances the inhibitory effects of GABA on neurons by binding of GABA to GABA-A receptors. This leads to an overall increase in inhibitory neurotransmission, resulting in the

dampening of neuronal activity (Handforth et al., 2023; Weight et al., 1992). The increased inhibitory effects of alcohol on GABAergic neurons contribute to its sedative and anxiolytic properties (Weight et al., 1992). Chronic alcohol consumption can lead to adaptations in GABAergic neurotransmission, resulting in the development of tolerance and dependence (Elvig et al., 2021; Liang & Olsen, 2014). Similarly, ethanol acts on the glutamatergic system which I will discuss further in the next section.

3.2.1 Alcohol's impact on glutamatergic neurons

My work focuses on the consequences of ethanol on the lesser studied glutamatergic population. I show that BK channels on glutamatergic neurons can be modulated by ethanol. However, there is evidence that ethanol can also act on excitatory neurons by reducing glutamate release and NMDA receptor activity, dampening excitatory neurotransmission (Abrahao et al., 2017; Allgaier, 2002; McGinnis et al., 2020; Wirkner et al., 1999). Alcohol's inhibitory effects on NMDA receptors can lead to impaired synaptic plasticity and synaptic transmission contributing to difficulties with memory, learning, and cognitive function (Cain, 1997; Wirkner et al., 1999).

Chronic and heavy alcohol use can lead to excitotoxicity, a process in which excessive glutamate release can cause damage to neurons (Peng et al., 2020; Yang et al., 2020). Additionally, alcohol withdrawal is associated with hyperexcitability of glutamatergic neurons(Airagnes et al., 2019; Gupta et al., 2021; Hillmer et al., 2015). When alcohol is abruptly discontinued after chronic use, the compensatory adaptations that occurred in the brain to counteract alcohol's inhibitory effects are unmasked. This can result in increased glutamate

release and heightened excitability, leading to withdrawal symptoms such as seizures, tremors, and hyperarousal (Airagnes et al., 2019; Becker, 2008; Gupta et al., 2021).

3.2.2 Determining the role of social isolation on the IC and BNST

Due to the nature of the rodent model of AUD used in my studies, animals are single housed which may introduce another variable of social isolation stress. However, this is not necessarily a confounding variable as many in the clinical population struggling with AUD are often isolated. Regardless, the effects of social isolation can have significant effects on the IC and the BNST that I will outline below.

The IC is involved in the perception and understanding of social cues, including facial expressions, body language, and vocal intonations (Lamm & Singer, 2010; Miura et al., 2020). Social isolation can limit exposure to these cues, leading to reduced interpersonal connections and potentially impacting the IC's ability to accurately interpret and respond to social signals. Social isolation can also heighten sensitivity to potential social threats, leading to increased vigilance, anxiety, and hypervigilance within the IC (Kavaliers et al., 2022). This heightened sensitivity to social threats can contribute to a cycle of further withdrawal and isolation. Social isolation can disrupt the formation and maintenance of a healthy self-identity, as the absence of social feedback and interactions can limit the development of a coherent sense of self (Brandt et al., 2022; Holt-Lunstad et al., 2015). This can lead to feelings of loneliness, reduced self-esteem, and an altered perception of one's social identity (Brandt et al., 2022; Gardiner et al., 2018; Holt-Lunstad et al., 2015). Furthermore, the IC is involved in the regulation of autonomic functions, including cardiovascular activity and immune responses (Klein et al.,

2021; Macey et al., 2012; Nieuwenhuys, 2012). Social isolation and loneliness have been linked to negative health outcomes, including increased inflammation, elevated blood pressure, and compromised immune system functioning (Cacioppo et al., 2011; Du Preez et al., 2021; Leigh-Hunt et al., 2017).

Social isolation can also lead to heightened activation of the BNST. When individuals experience social isolation, the brain's perception of social threats may be heightened, leading to increased BNST activation and therefore increased anxiety and vigilance (Campagne, 2019; Conrad & Winder, 2011; Hajek & König, 2022). Social isolation can disrupt the normal connectivity patterns of the BNST with other brain regions. A study in humans using a fMRI cue anticipation task found that in socially anxious individuals there was a decrease in functional connectivity between the BNST and downstream regions involved in emotional regulation, such as the PFC and amygdala (Clauss et al., 2019). Decreased BNST connectivity may lead to difficulties in regulating emotions and increased vulnerability to stress and anxiety.

The BNST plays a crucial role in the stress response system. Social isolation can dysregulate the stress response, leading to an overactive or dysregulated BNST response to stressors. This dysregulation can contribute to increased anxiety and difficulties in coping with stress. Social isolation is a known risk factor for the development of anxiety and depression. The BNST's involvement in anxiety and fear processing suggests that dysregulation in this region may contribute to the increased risk of developing these mental health conditions in socially isolated individuals. The BNST is also involved in social behavior and the processing of social information. Social isolation can disrupt the normal functioning of the BNST, potentially leading

to alterations in social behavior, reduced social motivation, and difficulties in forming and maintaining social relationships.

It is important to note that while social isolation can have negative effects on the BNST, the specific impacts can vary among individuals. The duration and intensity of social isolation, as well as an individual's pre-existing vulnerability to stress and anxiety can influence the extent of IC and BNST alterations.

3.2.3 Better defining the abstinence timeline

Animal models of AUD have demonstrated that negative symptoms associated with alcohol withdrawal vary based on how long a subject has been abstinent. For example, in acute withdrawal there is evidence from rodent studies indicating disruptions in sleep homeostasis (Liang & Olsen, 2014; Thakkar et al., 2015), enhanced autonomic function and sensory hyperactivity (Becker, 1999). At protracted timepoints, greater than 2 weeks, researchers have observed negative affective anxiety and depressive like behaviors in mice (Centanni et al., 2022; Centanni, Morris, et al., 2019; Holleran et al., 2016). Notably, 2 hours after abstinence there is a reported overall decrease in activation of cortical regions, in addition to striatal, hypothalamic and regions of the extended amygdala (Smith et al., 2020). At 7 days these changes are no longer observed in these regions (Smith et al., 2020), consistent with my data indicating differential excitability of neurons in early and late abstinence timepoints.

In the human population, the timeline of alcohol withdrawal can vary depending on several factors, including the individual's level of alcohol dependence, the duration and amount of alcohol consumed, and their overall health. However, here is a general timeline that outlines

the common stages and symptoms of alcohol withdrawal (Airagnes et al., 2019; Gupta et al., 2021; Maldonado et al., 2014):

6-12 Hours after the Last Drink: mild withdrawal symptoms may begin to appear, such as tremors (shakes), anxiety, insomnia, nausea, and restlessness. Some individuals may also experience increased heart rate and blood pressure.

24-72 Hours: symptoms typically peak during this period. Delirium Tremens (DTs), a severe and potentially life-threatening form of withdrawal, may occur in some cases. Symptoms of DTs may include confusion, hallucinations, seizures, severe agitation, and high fever.
Other symptoms can include increased tremors, sweating, rapid breathing, elevated heart rate, irritability, headache, gastrointestinal distress, and fatigue.

2-5 Days: the intensity of withdrawal symptoms usually starts to decrease, although some symptoms may persist. Symptoms such as anxiety, depression, insomnia, and irritability may continue.

5-7 Days: many physical symptoms of withdrawal tend to resolve by this point.
Psychological symptoms, such as anxiety, depression, and cravings for alcohol, may persist.
Fatigue and difficulty concentrating may still be present.

Beyond 7 Days: Most physical symptoms should have resolved by this stage. Psychological symptoms and cravings may continue, requiring ongoing support and treatment. Some individuals may experience a condition called "protracted withdrawal" or "post-acute withdrawal syndrome" (PAWS), where symptoms such as mood swings, sleep disturbances, and difficulty with memory and concentration can persist for several weeks or months.
It's crucial to note that alcohol withdrawal can be unpredictable, and the severity and duration of symptoms can vary widely among individuals. Severe withdrawal symptoms, including delirium tremens, require immediate medical attention, as they can be life-threatening. If you or someone you know is experiencing alcohol withdrawal, it is important to seek medical help to ensure safe and appropriate management of the withdrawal process.

The current standard of treatment for alcohol use disorder (AUD) is a combination of psychosocial interventions, medications, and support services. It is important to note that treatment for AUD should be tailored to the individual's specific needs, and a comprehensive assessment by qualified healthcare professionals is essential to determine the most appropriate treatment plan. Ongoing support and monitoring are crucial for maintaining long-term recovery from AUD.

My research along with data published by Vranjkovic et al. suggests there is a short window of optimal therapeutic intervention occurring very early in abstinence (Vranjkovic et al., 2018). In the mouse we have found that interventions given in the first 24 hours when neurons appear most plastic are critical for preventing negative affective behaviors from developing. It is the negative affective symptoms of depression and anxiety that often lead

abstinent individuals to resume alcohol seeking and relapse. If we can map when this peak efficacious window is in humans, we may be better able to better decide when to best administer either behavioral or pharmacotherapies to best aid the body in acclimating in the absence of alcohol. The ultimate goal would be to lessen the adverse toll that withdrawal takes on someone both physically and emotionally, maximizing an individual's chances of reaching and maintaining abstinence.

3.3 Therapeutic Options for AUD

In this final section I will summarize literature on treatments for AUD and voice my thoughts on best practices. As discussed above, my data in conjunction with data from Oliver Vranjkovic (Vranjkovic et al., 2018) showing that early intervention is key for mitigating harsh plasticity adaptations that result in negative affect/other negative withdrawal symptoms.

Various evidence-based behavioral therapies are effective in treating AUD. These therapies aim to help individuals change their drinking behaviors, develop coping skills, and address underlying psychological factors. Cognitive-Behavioral therapy helps individuals identify and modify unhealthy thoughts and behaviors related to alcohol use, develop coping strategies, and improve problem-solving skills. Motivational enhancement therapy aims to enhance an individual's motivation and commitment to change their drinking behaviors by exploring their values and goals, resolving ambivalence, and building self-efficacy. Contingency management uses positive reinforcement to encourage abstinence or reductions in alcohol use by providing tangible rewards for meeting treatment goals. 12-Step Facilitation involves participation in self-

help groups, such as Alcoholics Anonymous (AA), which provide social support and a structured recovery program.

3.3.1 Special consideration for underlying disorders

Several disorders can underlie or co-occur with addiction, contributing to its development and complexity. Common mental health disorders that may underlie AUD include depression, anxiety, post-traumatic stress disorder (PTDS), attention deficit hyperactivity disorder (ADHD) and others (Fergusson et al., 2009; Kathryn Mchugh & Weiss, 2019; Zhan et al., 2022).

Conditions such as major depression, generalized anxiety disorder, panic disorder, or social anxiety disorder can increase the risk of developing AUD as individuals may use substances to cope with or alleviate symptoms (Crum et al., 2013; Fergusson et al., 2009). PTSD is associated with an increased risk of AUD as individuals may misuse substances to self-medicate or numb emotional distress related to past traumatic experiences (Pietrzak et al., 2011). A review by Zhan and colleagues nicely outlines overlapping neurocircuitry including the norepinephrine system, HPA axis and reward processing regions effected in both disorders (Zhan et al., 2022). Finally, ADHD and bipolar disorder can contribute to an increased vulnerability to AUD due to impulsivity, risk-taking behavior, and difficulties with self-regulation (Grunze & Soyka, 2022; Luderer et al., 2021; Preuss et al., 2021).

It is important to recognize that the relationship between addiction and these underlying disorders is complex and can vary among individuals. Integrated treatment approaches that address both the addiction and underlying disorders simultaneously tend to

yield better outcomes for individuals with dual diagnoses. The harsh physical effects of withdrawal should make that a priority and then aggressive treatment of underlying disorders later to treat the root causes and prevent relapse.

3.3.2 Treatments aimed at targeting the salience network

Therapeutic treatments targeting the salience network aim to address dysregulation or dysfunction in this specific brain network. The salience network is involved in detecting and processing salient or important stimuli, as well as coordinating responses and attentional shifts. When the salience network is impaired, it can contribute to various psychiatric and neurological conditions (Schimmelpfennig et al., 2023)

Cognitive Behavioral Therapy (CBT) is a psychotherapeutic approach that focuses on identifying and modifying patterns of thinking and behavior. It can help individuals with salience network dysregulation by addressing maladaptive responses to salient stimuli. CBT techniques may involve cognitive restructuring, behavior activation, and emotion regulation strategies (Carson & McWhirter, 2022; Thoma et al., 2015). One strategy employed in CBT, mindfulness, is discussed in greater detail below.

3.3.2.1 Mindfulness and IC resting state connectivity: evidence for active coping

Mindfulness practices, such as mindfulness meditation, can help individuals regulate their attention and become more aware of their thoughts, emotions, and bodily sensations. Mindfulness-based interventions aim to enhance awareness of salient stimuli while fostering

non-reactivity and acceptance. Work by Kirk et al 2014 and Yu et al 2022 separately provide evidence that mindfulness can improve the regulation of the salience network.

In the first study, a subset of healthy individuals underwent mindfulness training (MT) where they were guided though breath monitoring, body scans, and attention to sounds, thoughts, feelings and bodily sensations. Another subset underwent active control training (ACT) where they were instructed on progressive muscle relaxation (Kirk et al., 2014). Each group practiced these techniques weekly for 8 weeks. Following the last session, everyone underwent a reward-based fMRI task. Interestingly, both groups showed similar engagement of the vmPFC, however only in the MT group do they see functional connectivity between this region and the IC (Kirk et al., 2014). The second publication investigates mindfulness using a neurofeedback task where participants cycled through "Focus-on-Breath" (mindfulness training), "Describe" (self-referential processing), and rest conditions (Yu et al., 2022). During these neurofeedback runs they found greater anterior IC activity and reduced posterior IC activity (Yu et al., 2022).

Unlike other more superficial regions, the IC may not be a good candidate for other commonly used therapies like transcranial magnetic stimulation. However, the ability of mindfulness to functionally change IC activity creates a new avenue in which to target this region. My work supports the validity of employing this strategy as good option for those seeking AUD treatment. Mindfulness training would be a great option to employ in outpatient clinics as the Kirk et al study demonstrated once weekly sessions for 8 weeks was sufficient to change functional connectivity (Kirk et al., 2014).

REFERENCES

Abdolahi, A., Williams, G. C., & van Wijngaarden, E. (2019). Implications of insular cortex laterality for treatment of nicotine addiction. *Drug and Alcohol Dependence*, *201*, 178–181. https://doi.org/10.1016/J.DRUGALCDEP.2019.04.017

Abrahao, K. P., Salinas, A. G., & Lovinger, D. M. (2017). Alcohol and the Brain: Neuronal Molecular Targets, Synapses, and Circuits. *Neuron*, *96*(6), 1223–1238. https://doi.org/10.1016/J.NEURON.2017.10.032

Airagnes, G., Ducoutumany, G., Laffy-Beaufils, B., Le Faou, A. L., & Limosin, F. (2019). Alcohol withdrawal syndrome management: Is there anything new? *La Revue de Medecine Interne*, *40*(6), 373–379. https://doi.org/10.1016/J.REVMED.2019.02.001

Alcohol Facts and Statistics | National Institute on Alcohol Abuse and Alcoholism (NIAAA). (n.d.). Retrieved June 30, 2023, from https://www.niaaa.nih.gov/alcohols-effects-health/alcohol-topics/alcohol-facts-and-statistics

Alheid, G. F. (2003). Extended amygdala and basal forebrain. *Annals of the New York Academy of Sciences*, *985*, 185–205. https://doi.org/10.1111/J.1749-6632.2003.TB07082.X

Allgaier, C. (2002). Ethanol sensitivity of NMDA receptors. *Neurochemistry International*, *41*(6), 377–382. https://doi.org/10.1016/S0197-0186(02)00046-3

Arakawa, H., Higuchi, Y., & Ozawa, A. (2023). Oxytocin neurons in the paraventricular nucleus of the hypothalamus circuit-dependently regulates social behavior, which malfunctions in BTBR mouse model of autism. *Research Square*. https://doi.org/10.21203/RS.3.RS-2621359/V1

Arinze, I., & Moorman, D. E. (2020). Selective impact of lateral orbitofrontal cortex inactivation on reinstatement of alcohol seeking in male Long-Evans rats. *Neuropharmacology*, *168*. https://doi.org/10.1016/J.NEUROPHARM.2020.108007

Arizzi-LaFrance, M. N., Correa, M., Aragon, C. M. G., & Salamone, J. D. (2006). Motor stimulant effects of ethanol injected into the substantia nigra pars reticulata: importance of catalase-mediated metabolism and the role of acetaldehyde. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *31*(5), 997–1008. https://doi.org/10.1038/SJ.NPP.1300849

Arnsten, A. F. T. (2009). Toward a new understanding of attention-deficit hyperactivity disorder pathophysiology: an important role for prefrontal cortex dysfunction. *CNS Drugs*, *23 Suppl 1*(SUPPL. 1), 33–41. https://doi.org/10.2165/00023210-200923000-00005

Avegno, E. M., Kasten, C. R., Snyder, W. B., Kelley, L. K., Lobell, T. D., Templeton, T. J., Constans, M., Wills, T. A., Middleton, J. W., & Gilpin, N. W. (2021). Alcohol dependence activates ventral tegmental area projections to central amygdala in male mice and rats. *Addiction Biology*, *26*(4). https://doi.org/10.1111/ADB.12990

Avery, S. N., Clauss, J. A., Winder, D. G., Woodward, N., Heckers, S., & Blackford, J. U. (2014). BNST neurocircuitry in humans. *NeuroImage*, *91*, 311–323. https://doi.org/10.1016/J.NEUROIMAGE.2014.01.017

Awasthi, S., Pan, H., LeDoux, J. E., Cloitre, M., Altemus, M., McEwen, B., Silbersweig, D., & Stern, E. (2020). The bed nucleus of the stria terminalis and functionally linked neurocircuitry modulate emotion processing and HPA axis dysfunction in posttraumatic stress disorder. *NeuroImage. Clinical*, *28*. https://doi.org/10.1016/J.NICL.2020.102442

Becker, H. C. (1999). Alcohol Withdrawal: Neuroadaptation and Sensitization. *CNS Spectrums*, *4*(1), 38-40,57-65. https://doi.org/10.1017/S1092852900011202

Becker, H. C. (2008). Alcohol Dependence, Withdrawal, and Relapse. *Alcohol Research & Health*, *31*(4), 348. https://doi.org/10.1016/B978-0-12-405941-2.00019-5

Bernhardt, B. C., & Singer, T. (2012). The neural basis of empathy. *Annual Review of Neuroscience*, *35*, 1–23. https://doi.org/10.1146/ANNUREV-NEURO-062111-150536

Bettinger, J. C., & Davies, A. G. (2014). The role of the BK channel in ethanol response behaviors: evidence from model organism and human studies. *Frontiers in Physiology*, *5*. https://doi.org/10.3389/FPHYS.2014.00346

Bian, X. L., Qin, C., Cai, C. Y., Zhou, Y., Tao, Y., Lin, Y. H., Wu, H. Y., Chang, L., Luo, C. X., & Zhu, D. Y. (2019). Anterior Cingulate Cortex to Ventral Hippocampus Circuit Mediates Contextual Fear Generalization. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(29), 5728–5739. https://doi.org/10.1523/JNEUROSCI.2739-18.2019

Bianchi, P. C., Carneiro de Oliveira, P. E., Palombo, P., Leão, R. M., Cogo-Moreira, H., Planeta, C. da S., & Cruz, F. C. (2018). Functional inactivation of the orbitofrontal cortex disrupts context-induced reinstatement of alcohol seeking in rats. *Drug and Alcohol Dependence*, *186*, 102–112. https://doi.org/10.1016/J.DRUGALCDEP.2017.12.045

Bliss, T. V. P., Collingridge, G. L., Kaang, B. K., & Zhuo, M. (2016). Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nature Reviews. Neuroscience*, *17*(8), 485–496. https://doi.org/10.1038/NRN.2016.68

Bonelli, R. M., & Cummings, J. L. (2007). Frontal-subcortical circuitry and behavior. *Dialogues in Clinical Neuroscience*, *9*(2), 141. https://doi.org/10.31887/DCNS.2007.9.2/RBONELLI

Borsook, D., Edwards, R., Elman, I., Becerra, L., & Levine, J. (2013). Pain and analgesia: the value of salience circuits. *Progress in Neurobiology*, *104*, 93–105. https://doi.org/10.1016/J.PNEUROBIO.2013.02.003

Brandt, L., Liu, S., Heim, C., & Heinz, A. (2022). The effects of social isolation stress and discrimination on mental health. *Translational Psychiatry*, *12*(1). https://doi.org/10.1038/S41398-022-02178-4

Bremer, B., Wu, Q., Mora Álvarez, M. G., Hölzel, B. K., Wilhelm, M., Hell, E., Tavacioglu, E. E., Torske, A., & Koch, K. (2022). Mindfulness meditation increases default mode, salience, and central executive network connectivity. *Scientific Reports*, *12*(1). https://doi.org/10.1038/S41598-022-17325-6

Brengelmann, G. L., Johnson, J. M., Hermansen, L., & Rowell, L. B. (1977). Altered control of skin blood flow during exercise at high internal temperatures. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *43*(5), 790–794. https://doi.org/10.1152/JAPPL.1977.43.5.790

Brodie, M. S., Shefner, S. A., & Dunwiddie, T. V. (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Research*, *508*(1), 65–69. https://doi.org/10.1016/0006-8993(90)91118-Z

Bukiya, A. N., Kuntamallappanavar, G., Edwards, J., Singh, A. K., Shivakumar, B., & Dopico, A. M. (2014). An alcohol-sensing site in the calcium- and voltage-gated, large conductance potassium (BK) channel. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(25), 9313–9318. https://doi.org/10.1073/PNAS.1317363111/SUPPL_FILE/PNAS.201317363SI.PDF

Burnham, N. W., Chaimowitz, C. N., Vis, C. C., Segantine Dornellas, A. P., Navarro, M., & Thiele, T. E. (2021). Lateral hypothalamus-projecting noradrenergic locus coeruleus pathway modulates binge-like ethanol drinking in male and female TH-ires-cre mice. *Neuropharmacology*, *196*. https://doi.org/10.1016/J.NEUROPHARM.2021.108702

Cacioppo, J. T., Hawkley, L. C., Norman, G. J., & Berntson, G. G. (2011). Social isolation. *Annals of the New York Academy of Sciences*, *1231*(1), 17–22. https://doi.org/10.1111/J.1749-6632.2011.06028.X

Cain, D. P. (1997). LTP, NMDA, genes and learning. *Current Opinion in Neurobiology*, *7*(2), 235–242. https://doi.org/10.1016/S0959-4388(97)80012-8

Campagne, D. M. (2019). Stress and perceived social isolation (loneliness). *Archives of Gerontology and Geriatrics*, *82*, 192–199. https://doi.org/10.1016/J.ARCHGER.2019.02.007

Campbell, E. J., Flanagan, J. P. M., Walker, L. C., Hill, M. K. R. I., Marchant, N. J., & Lawrence, A. J. (2019). Anterior Insular Cortex is Critical for the Propensity to Relapse Following Punishment-Imposed Abstinence of Alcohol Seeking. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(6), 1077–1087. https://doi.org/10.1523/JNEUROSCI.1596-18.2018

Carson, A. J., & McWhirter, L. (2022). Cognitive Behavioral Therapy: Principles, Science, and Patient Selection in Neurology. *Seminars in Neurology*, *42*(2), 114–122. https://doi.org/10.1055/S-0042-1750851

Cassataro, D., Bergfeldt, D., Malekian, C., Van Snellenberg, J. X., Thanos, P. K., Fishell, G., & Sjulson, L. (2014). Reverse pharmacogenetic modulation of the nucleus accumbens reduces ethanol consumption in a limited access paradigm. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *39*(2), 283–290. https://doi.org/10.1038/NPP.2013.184

Centanni, S. W., Bedse, G., Patel, S., & Winder, D. G. (2019). Driving the Downward Spiral: Alcohol-Induced Dysregulation of Extended Amygdala Circuits and Negative Affect. *Alcoholism, Clinical and Experimental Research*, *43*(10), 2000–2013. https://doi.org/10.1111/ACER.14178

Centanni, S. W., Conley, S. Y., Luchsinger, J. R., Lantier, L., & Winder, D. G. (2022). The impact of intermittent exercise on mouse ethanol drinking and abstinence-associated affective behavior and physiology. *Alcoholism, Clinical and Experimental Research*, *46*(1), 114. https://doi.org/10.1111/ACER.14742

Centanni, S. W., Morris, B. D., Luchsinger, J. R., Bedse, G., Fetterly, T. L., Patel, S., & Winder, D. G. (2019). Endocannabinoid control of the insular-bed nucleus of the stria terminalis circuit regulates negative affective behavior associated with alcohol abstinence. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *44*(3), 526–537. https://doi.org/10.1038/S41386-018-0257-8

Chen, H., & Lasek, A. W. (2020). Perineuronal nets in the insula regulate aversion-resistant alcohol drinking. *Addiction Biology*, *25*(6). https://doi.org/10.1111/ADB.12821

Ch'ng, S., Fu, J., Brown, R. M., McDougall, S. J., & Lawrence, A. J. (2018). The intersection of stress and reward: BNST modulation of aversive and appetitive states. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *87*(Pt A), 108–125. https://doi.org/10.1016/J.PNPBP.2018.01.005

Chong, J. S. X., Ng, G. J. P., Lee, S. C., & Zhou, J. (2017). Salience network connectivity in the insula is associated with individual differences in interoceptive accuracy. *Brain Structure & Function*, *222*(4), 1635–1644. https://doi.org/10.1007/S00429-016-1297-7

Chung, T., & Clark, D. B. (2014). Insula white matter volume linked to binge drinking frequency through enhancement motives in treated adolescents. *Alcoholism, Clinical and Experimental Research*, *38*(7), 1932–1940. https://doi.org/10.1111/ACER.12461

Clauss, J. A., Avery, S. N., Benningfield, M. M., & Blackford, J. U. (2019). Social anxiety is associated with BNST response to unpredictability. *Depression and Anxiety*, *36*(8), 666. https://doi.org/10.1002/DA.22891

Cole, A. B., Montgomery, K., Bale, T. L., & Thompson, S. M. (2022). What the hippocampus tells the HPA axis: Hippocampal output attenuates acute stress responses via disynaptic inhibition of CRF+ PVN neurons. *Neurobiology of Stress*, *20*. https://doi.org/10.1016/J.YNSTR.2022.100473

Colonna, M., & Butovsky, O. (2017). Microglia Function in the Central Nervous System During Health and Neurodegeneration. *Annual Review of Immunology*, *35*, 441–468. https://doi.org/10.1146/ANNUREV-IMMUNOL-051116-052358

Conrad, K. L., Louderback, K. M., Gessner, C. P., & Winder, D. G. (2011). Stress-induced alterations in anxiety-like behavior and adaptations in plasticity in the bed nucleus of the stria terminalis. *Physiology & Behavior*, *104*(2), 248–256. https://doi.org/10.1016/J.PHYSBEH.2011.03.001

Conrad, K. L., & Winder, D. G. (2011). Altered anxiety-like behavior and long-term potentiation in the bed nucleus of the stria terminalis in adult mice exposed to chronic social isolation, unpredictable stress, and ethanol beginning in adolescence. *Alcohol (Fayetteville, N.Y.)*, *45*(6), 585–593. https://doi.org/10.1016/J.ALCOHOL.2010.11.002

Contestabile, A., Casarotto, G., Musardo, S., Espinosa, P., Maltese, F., Jiang, Y. hui, Bellone, C., & Glangetas, C. (2023). Shank3 deficits in the anteromedial bed nucleus of the stria terminalis trigger an anxiety phenotype in mice. *The European Journal of Neuroscience*. https://doi.org/10.1111/EJN.16043

Corbit, L. H., & Janak, P. H. (2016). Habitual alcohol seeking: Neural bases and possible relations to alcohol use disorders. *Alcoholism, Clinical and Experimental Research*, *40*(7), 1380. https://doi.org/10.1111/ACER.13094

Corbit, L. H., Nie, H., & Janak, P. H. (2012). Habitual alcohol seeking: time course and the contribution of subregions of the dorsal striatum. *Biological Psychiatry*, *72*(5), 389–395. https://doi.org/10.1016/J.BIOPSYCH.2012.02.024

Coria-Avila, G. A., Manzo, J., Garcia, L. I., Carrillo, P., Miquel, M., & Pfaus, J. G. (2014). Neurobiology of social attachments. *Neuroscience and Biobehavioral Reviews*, *43*, 173–182. https://doi.org/10.1016/J.NEUBIOREV.2014.04.004

Crabbe, J. C., Metten, P., Rhodes, J. S., Yu, C. H., Brown, L. L., Phillips, T. J., & Finn, D. A. (2009). A Line of Mice Selected for High Blood Ethanol Concentrations Shows Drinking in the Dark to Intoxication. *Biological Psychiatry*, *65*(8), 662. https://doi.org/10.1016/J.BIOPSYCH.2008.11.002

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews. Neuroscience*, *3*(8), 655–666. https://doi.org/10.1038/NRN894

Craig, A. D. (2009). How do you feel--now? The anterior insula and human awareness. *Nature Reviews. Neuroscience*, *10*(1), 59–70. https://doi.org/10.1038/NRN2555

Criswell, H. E., Simson, P. E., Knapp, D. J., Devaud, L. L., McCown, T. J., Duncan, G. E., Morrow, A. L., & Breese, G. R. (1995). Effect of zolpidem on gamma-aminobutyric acid (GABA)-induced inhibition predicts the interaction of ethanol with GABA on individual neurons in several rat brain regions. *The Journal of Pharmacology and Experimental Therapeutics*, *273*(1), 526–536. https://pubmed.ncbi.nlm.nih.gov/7714808/

Crum, R. M., Mojtabai, R., Lazareck, S., Bolton, J., Robinson, J., Sareen, J., Green, K., Stuart, E. A., La Flair, L., Alvanzo, A. A. H., & Storr, C. L. (2013). A prospective assessment of reports of drinking to self-medicate mood symptoms with the incidence and persistence of alcohol dependence. *JAMA Psychiatry*, *70*(7), 718–726. https://doi.org/10.1001/JAMAPSYCHIATRY.2013.1098

Cushnie, A. K., Tang, W., & Heilbronner, S. R. (2023). Connecting Circuits with Networks in Addiction Neuroscience: A Salience Network Perspective. *International Journal of Molecular Sciences*, *24*(10). https://doi.org/10.3390/IJMS24109083

David, S., Jhelum, P., Ryan, F., Jeong, S. Y., & Kroner, A. (2022). Dysregulation of Iron Homeostasis in the Central Nervous System and the Role of Ferroptosis in Neurodegenerative Disorders. *Antioxidants & Redox Signaling*, *37*(1–3), 150–170. https://doi.org/10.1089/ARS.2021.0218

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *35*(1), 105–135. https://doi.org/10.1038/NPP.2009.109

De Guglielmo, G., Crawford, E., Kim, S., Vendruscolo, L. F., Hope, B. T., Brennan, M., Cole, M., Koob, G. F., & George, O. (2016). Recruitment of a Neuronal Ensemble in the Central Nucleus of the Amygdala Is Required for Alcohol Dependence. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *36*(36), 9446–9453. https://doi.org/10.1523/JNEUROSCI.1395-16.2016

de Guglielmo, G., Kallupi, M., Pomrenze, M. B., Crawford, E., Simpson, S., Schweitzer, P., Koob, G. F., Messing, R. O., & George, O. (2019). Inactivation of a CRF-dependent amygdalofugal pathway reverses addiction-like behaviors in alcohol-dependent rats. *Nature Communications*, *10*(1). https://doi.org/10.1038/S41467-019-09183-0

De Oliveira Sergio, T., Lei, K., Kwok, C., Ghotra, S., Wegner, S. A., Walsh, M., Waal, J., Darevsky, D., & Hopf, F. W. (2021). The role of anterior insula-brainstem projections and alpha-1 noradrenergic receptors for compulsion-like and alcohol-only drinking. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *46*(11), 1918–1926. https://doi.org/10.1038/S41386-021-01071-W

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5274–5278. https://doi.org/10.1073/PNAS.85.14.5274

Domi, E., Domi, A., Adermark, L., Heilig, M., & Augier, E. (2021). Neurobiology of alcohol seeking behavior. *Journal of Neurochemistry*, *157*(5), 1585–1614. https://doi.org/10.1111/JNC.15343

Dopico, A. M., Bukiya, A. N., Kuntamallappanavar, G., & Liu, J. (2016). Modulation of BK Channels by Ethanol. *International Review of Neurobiology*, *128*, 239–279. https://doi.org/10.1016/BS.IRN.2016.03.019

Dornellas, A. P. S., Burnham, N. W., Luhn, K. L., Petruzzi, M. V., Thiele, T. E., & Navarro, M. (2021). Activation of locus coeruleus to rostromedial tegmental nucleus (RMTg) noradrenergic pathway blunts binge-like ethanol drinking and induces aversive responses in mice. *Neuropharmacology*, *199*. https://doi.org/10.1016/J.NEUROPHARM.2021.108797

Droutman, V., Read, S. J., & Bechara, A. (2015). Revisiting the role of the insula in addiction. *Trends in Cognitive Sciences*, *19*(7), 414–420. https://doi.org/10.1016/J.TICS.2015.05.005

Du Preez, A., Onorato, D., Eiben, I., Musaelyan, K., Egeland, M., Zunszain, P. A., Fernandes, C., Thuret, S., & Pariante, C. M. (2021). Chronic stress followed by social isolation promotes depressive-like behaviour, alters microglial and astrocyte biology and reduces hippocampal neurogenesis in male mice. *Brain, Behavior, and Immunity*, *91*, 24–47. https://doi.org/10.1016/J.BBI.2020.07.015

Elder, J., Brieant, A., Lauharatanahirun, N., King-Casas, B., & Kim-Spoon, J. (2019). Insular Risk Processing Predicts Alcohol Use Via Externalizing Pathway in Male Adolescents. *Journal of Studies on Alcohol and Drugs*, *80*(6), 602–613. https://doi.org/10.15288/JSAD.2019.80.602

Elvig, S. K., McGinn, M. A., Smith, C., Arends, M. A., Koob, G. F., & Vendruscolo, L. F. (2021). Tolerance to alcohol: A critical yet understudied factor in alcohol addiction. *Pharmacology, Biochemistry, and Behavior*, *204*. https://doi.org/10.1016/J.PBB.2021.173155

Even a Little Alcohol Can Harm Your Health, Research Shows - The New York Times. (n.d.). Retrieved June 30, 2023, from https://www.nytimes.com/2023/01/13/well/mind/alcohol-health-effects.html

Evrard, H. C. (2019a). The Organization of the Primate Insular Cortex. *Frontiers in Neuroanatomy*, *13*. https://doi.org/10.3389/FNANA.2019.00043

Evrard, H. C. (2019b). The Organization of the Primate Insular Cortex. *Frontiers in Neuroanatomy*, *13*. https://doi.org/10.3389/FNANA.2019.00043

Feola, B., McHugo, M., Armstrong, K., Noall, M. P., Flook, E. A., Woodward, N. D., Heckers, S., & Blackford, J. U. (2021). BNST and amygdala connectivity are altered during threat anticipation in schizophrenia. *Behavioural Brain Research*, *412*. https://doi.org/10.1016/J.BBR.2021.113428

Fergusson, D. M., Boden, J. M., & Horwood, L. J. (2009). Tests of causal links between alcohol abuse or dependence and major depression. *Archives of General Psychiatry*, *66*(3), 260–266. https://doi.org/10.1001/ARCHGENPSYCHIATRY.2008.543

Fetterly, T. L., Basu, A., Nabit, B. P., Awad, E., Williford, K. M., Centanni, S. W., Matthews, R. T., Silberman, Y., & Winder, D. G. (2019). α2A-Adrenergic Receptor Activation Decreases Parabrachial Nucleus Excitatory Drive onto BNST CRF Neurons and Reduces Their Activity In Vivo. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(3), 472–484. https://doi.org/10.1523/JNEUROSCI.1035-18.2018

Flook, E. A., Feola, B., Avery, S. N., Winder, D. G., Woodward, N. D., Heckers, S., & Blackford, J. U. (2020). BNST-insula structural connectivity in humans. *NeuroImage*, *210*. https://doi.org/10.1016/J.NEUROIMAGE.2020.116555

Flook, E. A., Luchsinger, J. R., Silveri, M. M., Winder, D. G., Benningfield, M. M., & Blackford, J. U. (2021). Anxiety during abstinence from alcohol: A systematic review of rodent and human evidence for the anterior insula's role in the abstinence network. *Addiction Biology*, *26*(2). https://doi.org/10.1111/ADB.12861

Fox, H. C., Bergquist, K. L., Hong, K. I., & Sinha, R. (2007). Stress-induced and alcohol cue-induced craving in recently abstinent alcohol-dependent individuals. *Alcoholism, Clinical and Experimental Research*, *31*(3), 395–403. https://doi.org/10.1111/J.1530-0277.2006.00320.X

Francesconi, W., Berton, F., Olivera-Pasilio, V., & Dabrowska, J. (2021). Oxytocin excites BNST interneurons and inhibits BNST output neurons to the central amygdala. *Neuropharmacology*, *192*. https://doi.org/10.1016/J.NEUROPHARM.2021.108601

Gallay, D. S., Gallay, M. N., Jeanmonod, D., Rouiller, E. M., & Morel, A. (2012). The insula of Reil revisited: multiarchitectonic organization in macaque monkeys. *Cerebral Cortex (New York, N.Y. : 1991)*, *22*(1), 175–190. https://doi.org/10.1093/CERCOR/BHR104

Gardiner, C., Geldenhuys, G., & Gott, M. (2018). Interventions to reduce social isolation and loneliness among older people: an integrative review. *Health & Social Care in the Community*, *26*(2), 147–157. https://doi.org/10.1111/HSC.12367

Gardner, E. L. (2011). Addiction and brain reward and antireward pathways. *Advances in Psychosomatic Medicine*, *30*, 22–60. https://doi.org/10.1159/000324065

Gatto, G. J., McBride, W. J., Murphy, J. M., Lumeng, L., & Li, T. K. (1994). Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. *Alcohol (Fayetteville, N.Y.)*, *11*(6), 557–564. https://doi.org/10.1016/0741-8329(94)90083-3

Gehrlach, D. A., Dolensek, N., Klein, A. S., Roy Chowdhury, R., Matthys, A., Junghänel, M., Gaitanos, T. N., Podgornik, A., Black, T. D., Reddy Vaka, N., Conzelmann, K. K., & Gogolla, N. (2019). Aversive state processing in the posterior insular cortex. *Nature Neuroscience*, *22*(9), 1424–1437. https://doi.org/10.1038/S41593-019-0469-1

Gehrlach, D. A., Weiand, C., Gaitanos, T. N., Cho, E., Klein, A. S., Hennrich, A. A., Conzelmann, K. K., & Gogolla, N. (2020). A whole-brain connectivity map of mouse insular cortex. *ELife*, *9*, 1–78. https://doi.org/10.7554/ELIFE.55585

George, O., & Hope, B. T. (2017). Cortical and amygdalar neuronal ensembles in alcohol seeking, drinking and withdrawal. *Neuropharmacology*, *122*, 107–114. https://doi.org/10.1016/J.NEUROPHARM.2017.04.031

Gessa, G. L., Muntoni, F., Collu, M., Vargiu, L., & Mereu, G. (1985). Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Research*, *348*(1), 201–203. https://doi.org/10.1016/0006-8993(85)90381-6

Gogolla, N. (2017). The insular cortex. *Current Biology : CB*, *27*(12), R580–R586. https://doi.org/10.1016/J.CUB.2017.05.010

Gonzales, K. K., & Smith, Y. (2015). Striatal Cholinergic interneurons in the dorsal and ventral striatum: anatomical and functional considerations in normal and diseased conditions. *Annals of the New York Academy of Sciences*, *1349*(1), 1. https://doi.org/10.1111/NYAS.12762

Grant, J. A. (2014). Meditative analgesia: the current state of the field. *Annals of the New York Academy of Sciences*, *1307*(1), 55–63. https://doi.org/10.1111/NYAS.12282

Graybiel, A. M., & Grafton, S. T. (2015). The Striatum: Where Skills and Habits Meet. *Cold Spring Harbor Perspectives in Biology*, *7*(8), a021691. https://doi.org/10.1101/CSHPERSPECT.A021691

Gremel, C. M., & Cunningham, C. L. (2008). Roles of the nucleus accumbens and amygdala in the acquisition and expression of ethanol-conditioned behavior in mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(5), 1076–1084. https://doi.org/10.1523/JNEUROSCI.4520-07.2008

Grinband, J., Hirsch, J., & Ferrera, V. P. (2006). A neural representation of categorization uncertainty in the human brain. *Neuron*, *49*(5), 757–763. https://doi.org/10.1016/J.NEURON.2006.01.032

Grodin, E. N., & Momenan, R. (2017). Decreased subcortical volumes in alcohol dependent individuals: effect of polysubstance use disorder. *Addiction Biology*, *22*(5), 1426–1437. https://doi.org/10.1111/ADB.12421

Grunze, H., & Soyka, M. (2022). The pharmacotherapeutic management of comorbid bipolar disorder and alcohol use disorder. *Expert Opinion on Pharmacotherapy*, *23*(10), 1181–1193. https://doi.org/10.1080/14656566.2022.2083500

Gruß, M., Henrich, M., König, P., Hempelmann, G., Vogel, W., & Scholz, A. (2001). Ethanol reduces excitability in a subgroup of primary sensory neurons by activation of BK(Ca) channels. *The European Journal of Neuroscience*, *14*(8), 1246–1256. https://doi.org/10.1046/J.0953-816X.2001.01754.X

Gu, X., Hof, P. R., Friston, K. J., & Fan, J. (2013). Anterior insular cortex and emotional awareness. *The Journal of Comparative Neurology*, *521*(15), 3371–3388. https://doi.org/10.1002/CNE.23368

Gupta, A., Khan, H., Kaur, A., & Singh, T. G. (2021). Novel Targets Explored in the Treatment of Alcohol Withdrawal Syndrome. *CNS & Neurological Disorders Drug Targets*, *20*(2), 158–173. https://doi.org/10.2174/1871527319999201118155721

Haber, P. S., Riordan, B. C., Winter, D. T., Barrett, L., Saunders, J., Hides, L., Gullo, M., Manning, V., Day, C. A., Bonomo, Y., Burns, L., Assan, R., Curry, K., Mooney-Somers, J., Demirkol, A., Monds, L., McDonough, M., Baillie, A. J., Clark, P., … Morley, K. C. (2021). New Australian guidelines for the treatment of alcohol problems: an overview of recommendations. *Medical Journal of Australia*, *215*(S7), S3–S32. https://doi.org/10.5694/MJA2.51254

Haggerty, D. L., Munoz, B., Pennington, T., Di Prisco, G. V., Grecco, G. G., & Atwood, B. K. (2022a). The role of anterior insular cortex inputs to dorsolateral striatum in binge alcohol drinking. *ELife*, *11*. https://doi.org/10.7554/ELIFE.77411

Haggerty, D. L., Munoz, B., Pennington, T., Di Prisco, G. V., Grecco, G. G., & Atwood, B. K. (2022b). The role of anterior insular cortex inputs to dorsolateral striatum in binge alcohol drinking. *ELife*, *11*. https://doi.org/10.7554/ELIFE.77411

Hajek, A., & König, H. H. (2022). Climate Anxiety, Loneliness and Perceived Social Isolation. *International Journal of Environmental Research and Public Health*, *19*(22). https://doi.org/10.3390/IJERPH192214991

Handforth, A., Kosoyan, H. P., Kadam, P. A., & Singh, R. P. (2023). Alcohol and Ganaxolone Suppress Tremor via Extra-Synaptic GABAA Receptors in the Harmaline Model of Essential

Tremor. *Tremor and Other Hyperkinetic Movements (New York, N.Y.)*, *13*, 18. https://doi.org/10.5334/TOHM.760

Harris, J. L. (2011). Cortical–Subcortical Loop. *Encyclopedia of Clinical Neuropsychology*, 723–723. https://doi.org/10.1007/978-0-387-79948-3_310

Hernandez, J. S., Binette, A. N., Rahman, T., Tarantino, J. D., & Moorman, D. E. (2020). Chemogenetic Inactivation of Orbitofrontal Cortex Decreases Cue-induced Reinstatement of Ethanol and Sucrose Seeking in Male and Female Wistar Rats. *Alcoholism, Clinical and Experimental Research*, *44*(9), 1769–1782. https://doi.org/10.1111/ACER.14407

Herring, B. E., Mayfield, R. D., Camp, M. C., & Alcantara, A. A. (2004). Ethanol-induced Fos immunoreactivity in the extended amygdala and hypothalamus of the rat brain: focus on cholinergic interneurons of the nucleus accumbens. *Alcoholism, Clinical and Experimental Research*, *28*(4), 588–597. https://doi.org/10.1097/01.ALC.0000122765.58324.6D

Hillmer, A. T., Mason, G. F., Fucito, L. M., O'Malley, S. S., & Cosgrove, K. P. (2015). How Imaging Glutamate, γ-Aminobutyric Acid, and Dopamine Can Inform the Clinical Treatment of Alcohol Dependence and Withdrawal. *Alcoholism, Clinical and Experimental Research*, *39*(12), 2268–2282. https://doi.org/10.1111/ACER.12893

Hiser, J., Schneider, B., & Koenigs, M. (2021). Uncertainty Potentiates Neural and Cardiac Responses to Visual Stimuli in Anxiety Disorders. *Biological Psychiatry. Cognitive Neuroscience and Neuroimaging*, *6*(7), 725–734. https://doi.org/10.1016/J.BPSC.2021.02.003

Hitzemann, B., & Hitzemann, R. (1997). Genetics ethanol and the Fos response: a comparison of the C57BL/6J and DBA/2J inbred mouse strains. *Alcoholism, Clinical and Experimental Research*, *21*(8), 1497–1507. https://doi.org/10.1111/j.1530-0277.1997.tb04482.x

Hodge, C. W., Haraguchi, M., Erickson, H., & Samson, H. H. (1993). Ventral tegmental microinjections of quinpirole decrease ethanol and sucrose-reinforced responding. *Alcoholism, Clinical and Experimental Research*, *17*(2), 370–375. https://doi.org/10.1111/J.1530-0277.1993.TB00778.X

Hodge, C. W., Samson, H. H., & Chappelle, A. M. (1997). Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. *Alcoholism, Clinical and Experimental Research*, *21*(6), 1083–1091. https://doi.org/10.1111/J.1530-0277.1997.TB04257.X

Hoffman, P. L., Rabe, C. S., Moses, F., & Tabakoff, B. (1989). N-methyl-D-aspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production. *Journal of Neurochemistry*, *52*(6), 1937–1940. https://doi.org/10.1111/J.1471-4159.1989.TB07280.X

Holleran, K. M., Wilson, H. H., Fetterly, T. L., Bluett, R. J., Centanni, S. W., Gilfarb, R. A., Rocco, L. E. R., Patel, S., & Winder, D. G. (2016). Ketamine and MAG Lipase Inhibitor-Dependent Reversal of Evolving Depressive-Like Behavior During Forced Abstinence From Alcohol Drinking. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *41*(8), 2062–2071. https://doi.org/10.1038/NPP.2016.3

Holleran, K. M., & Winder, D. G. (2017). Preclinical voluntary drinking models for alcohol abstinence-induced affective disturbances in mice. *Genes, Brain, and Behavior*, *16*(1), 8–14. https://doi.org/10.1111/GBB.12338

Holt-Lunstad, J., Smith, T. B., Baker, M., Harris, T., & Stephenson, D. (2015). Loneliness and social isolation as risk factors for mortality: a meta-analytic review. *Perspectives on*

Psychological Science : A Journal of the Association for Psychological Science, *10*(2), 227–237. https://doi.org/10.1177/1745691614568352

Holzhauer, C. G., Epstein, E. E., Cohn, A. M., McCrady, B. S., Graff, F. S., & Cook, S. (2017). Heterogeneity in pathways to abstinence among women in treatment for alcohol use disorder. *Journal of Substance Abuse Treatment*, *75*, 1–9. https://doi.org/10.1016/J.JSAT.2017.01.002

Hong, S. I., Kang, S., Chen, J. F., & Choi, D. S. (2019). Indirect Medium Spiny Neurons in the Dorsomedial Striatum Regulate Ethanol-Containing Conditioned Reward Seeking. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(36), 7206–7217. https://doi.org/10.1523/JNEUROSCI.0876-19.2019

Huang, H., Chen, C., Rong, B., Wan, Q., Chen, J., Liu, Z., Zhou, Y., Wang, G., & Wang, H. (2022). Resting-state functional connectivity of salience network in schizophrenia and depression. *Scientific Reports*, *12*(1). https://doi.org/10.1038/S41598-022-15489-9

Hulsman, A. M., Terburg, D., Roelofs, K., & Klumpers, F. (2021). Roles of the bed nucleus of the stria terminalis and amygdala in fear reactions. *Handbook of Clinical Neurology*, *179*, 419–432. https://doi.org/10.1016/B978-0-12-819975-6.00027-3

Jang, J. H., Kim, J. H., Yun, J. Y., Choi, S. H., An, S. C., & Kang, D. H. (2018). Differences in Functional Connectivity of the Insula Between Brain Wave Vibration in Meditators and Non-meditators. *Mindfulness*, *9*(6), 1857. https://doi.org/10.1007/S12671-018-0928-X

Jaramillo, A. A., Agan, V. E., Makhijani, V. H., Pedroza, S., McElligott, Z. A., & Besheer, J. (2018). Functional role for suppression of the insular-striatal circuit in modulating interoceptive effects of alcohol. *Addiction Biology*, *23*(5), 1020–1031. https://doi.org/10.1111/ADB.12551

Jaramillo, A. A., Randall, P. A., Frisbee, S., & Besheer, J. (2016). Modulation of sensitivity to alcohol by cortical and thalamic brain regions. *The European Journal of Neuroscience*, *44*(8), 2569–2580. https://doi.org/10.1111/EJN.13374

Jaramillo, A. A., Van Voorhies, K., Randall, P. A., & Besheer, J. (2018). Silencing the insular-striatal circuit decreases alcohol self-administration and increases sensitivity to alcohol. *Behavioural Brain Research*, *348*, 74–81. https://doi.org/10.1016/J.BBR.2018.04.007

Jasinska, A. J., Stein, E. A., Kaiser, J., Naumer, M. J., & Yalachkov, Y. (2014). Factors modulating neural reactivity to drug cues in addiction: a survey of human neuroimaging studies. *Neuroscience and Biobehavioral Reviews*, *38*(1), 1–16. https://doi.org/10.1016/J.NEUBIOREV.2013.10.013

Jeanblanc, J., Rolland, B., Gierski, F., Martinetti, M. P., & Naassila, M. (2019). Animal models of binge drinking, current challenges to improve face validity. *Neuroscience and Biobehavioral Reviews*, *106*, 112–121. https://doi.org/10.1016/J.NEUBIOREV.2018.05.002

Joutsa, J., Moussawi, K., Siddiqi, S. H., Abdolahi, A., Drew, W., Cohen, A. L., Ross, T. J., Deshpande, H. U., Wang, H. Z., Bruss, J., Stein, E. A., Volkow, N. D., Grafman, J. H., van Wijngaarden, E., Boes, A. D., & Fox, M. D. (2022). Brain lesions disrupting addiction map to a common human brain circuit. *Nature Medicine*, *28*(6), 1249–1255. https://doi.org/10.1038/S41591-022-01834-Y

June, H. L., Foster, K. L., McKay, P. F., Seyoum, R., Woods, J. E., Harvey, S. C., Eiler, W. J. A., Grey, C., Carroll, M. R., McCane, S., Jones, C. M., Yin, W., Mason, D., Cummings, R., Garcia, M., Ma, C., Sarma, P. V. V. S., Cook, J. M., & Skolnick, P. (2003). The reinforcing properties of

alcohol are mediated by GABA(A1) receptors in the ventral pallidum. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *28*(12), 2124–2137. https://doi.org/10.1038/SJ.NPP.1300239

Jupp, B., Krivdic, B., Krstew, E., & Lawrence, A. J. (2011). The orexin$_1$ receptor antagonist SB-334867 dissociates the motivational properties of alcohol and sucrose in rats. *Brain Research*, *1391*, 54–59. https://doi.org/10.1016/J.BRAINRES.2011.03.045

Kathryn Mchugh, R., & Weiss, R. D. (2019). Alcohol Use Disorder and Depressive Disorders. *Alcohol Research : Current Reviews*, *40*(1), e1–e8. https://doi.org/10.35946/ARCR.V40.1.01

Kavaliers, M., Ossenkopp, K. P., Tyson, C. D., Bishnoi, I. R., & Choleris, E. (2022). Social factors and the neurobiology of pathogen avoidance. *Biology Letters*, *18*(2). https://doi.org/10.1098/RSBL.2021.0371

Kemppainen, H., Raivio, N., Suo-Yrjo, V., & Kiianmaa, K. (2012). Opioidergic modulation of ethanol self-administration in the ventral pallidum. *Alcoholism, Clinical and Experimental Research*, *36*(2), 286–293. https://doi.org/10.1111/J.1530-0277.2011.01611.X

Kirk, U., Gu, X., Harvey, A. H., Fonagy, P., & Montague, P. R. (2014). Mindfulness training modulates value signals in ventromedial prefrontal cortex through input from insular cortex. *NeuroImage*, *100*, 254–262. https://doi.org/10.1016/J.NEUROIMAGE.2014.06.035

Klein, A. S., Dolensek, N., Weiand, C., & Gogolla, N. (2021). Fear balance is maintained by bodily feedback to the insular cortex in mice. *Science (New York, N.Y.)*, *374*(6570), 1010–1015. https://doi.org/10.1126/SCIENCE.ABJ8817

Koob, G. F. (2009). Brain stress systems in the amygdala and addiction. *Brain Research*, *1293*, 61–75. https://doi.org/10.1016/J.BRAINRES.2009.03.038

Koob, G. F., & Schulkin, J. (2019). Addiction and stress: An allostatic view. *Neuroscience and Biobehavioral Reviews*, *106*, 245–262. https://doi.org/10.1016/J.NEUBIOREV.2018.09.008

Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: a neurocircuitry analysis. *The Lancet. Psychiatry*, *3*(8), 760–773. https://doi.org/10.1016/S2215-0366(16)00104-8

Kreifeldt, M., Herman, M. A., Sidhu, H., Okhuarobo, A., Macedo, G. C., Shahryari, R., Gandhi, P. J., Roberto, M., & Contet, C. (2022). Central amygdala corticotropin-releasing factor neurons promote hyponeophagia but do not control alcohol drinking in mice. *Molecular Psychiatry*, *27*(5), 2502–2513. https://doi.org/10.1038/S41380-022-01496-9

Kulijewicz-Nawrot, M., Syková, E., Chvátal, A., Verkhratsky, A., & Rodríguez, J. J. (2013). Astrocytes and glutamate homoeostasis in Alzheimer's disease: a decrease in glutamine synthetase, but not in glutamate transporter-1, in the prefrontal cortex. *ASN NEURO*, *5*(4), 273–282. https://doi.org/10.1042/AN20130017

Kupchik, Y. M., Brown, R. M., Heinsbroek, J. A., Lobo, M. K., Schwartz, D. J., & Kalivas, P. W. (2015). Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nature Neuroscience*, *18*(9), 1230–1232. https://doi.org/10.1038/NN.4068

Labrakakis, C. (2023). The Role of the Insular Cortex in Pain. *International Journal of Molecular Sciences*, *24*(6), 5736. https://doi.org/10.3390/IJMS24065736

Lamm, C., & Singer, T. (2010). The role of anterior insular cortex in social emotions. *Brain Structure & Function*, *214*(5–6), 579–591. https://doi.org/10.1007/S00429-010-0251-3

Lanz, T. A., Reinhart, V., Sheehan, M. J., Rizzo, S. J. S., Bove, S. E., James, L. C., Volfson, D., Lewis, D. A., & Kleiman, R. J. (2019). Postmortem transcriptional profiling reveals widespread

increase in inflammation in schizophrenia: a comparison of prefrontal cortex, striatum, and hippocampus among matched tetrads of controls with subjects diagnosed with schizophrenia, bipolar or major depressive disorder. *Translational Psychiatry*, *9*(1), 1234567890. https://doi.org/10.1038/S41398-019-0492-8

Le Berre, A. P. (2019). Emotional processing and social cognition in alcohol use disorder. *Neuropsychology*, *33*(6), 808–821. https://doi.org/10.1037/NEU0000572

Le, T. M., Malone, T., & Li, C. S. R. (2022). Positive alcohol expectancy and resting-state functional connectivity of the insula in problem drinking. *Drug and Alcohol Dependence*, *231*. https://doi.org/10.1016/J.DRUGALCDEP.2021.109248

Ledda, R., Battagliese, G., Attilia, F., Rotondo, C., Pisciotta, F., Gencarelli, S., Greco, A., Fiore, M., Ceccanti, M., & Attilia, M. L. (2019). Drop-out, relapse and abstinence in a cohort of alcoholic people under detoxification. *Physiology & Behavior*, *198*, 67–75. https://doi.org/10.1016/J.PHYSBEH.2018.10.009

Leigh-Hunt, N., Bagguley, D., Bash, K., Turner, V., Turnbull, S., Valtorta, N., & Caan, W. (2017). An overview of systematic reviews on the public health consequences of social isolation and loneliness. *Public Health*, *152*, 157–171. https://doi.org/10.1016/J.PUHE.2017.07.035

Leriche, M., Méndez, M., Zimmer, L., & Bérod, A. (2008). Acute ethanol induces Fos in GABAergic and non-GABAergic forebrain neurons: a double-labeling study in the medial prefrontal cortex and extended amygdala. *Neuroscience*, *153*(1), 259–267. https://doi.org/10.1016/J.NEUROSCIENCE.2008.01.069

Levine, O. B., Skelly, M. J., Miller, J. D., Rivera-Irizarry, J. K., Rowson, S. A., DiBerto, J. F., Rinker, J. A., Thiele, T. E., Kash, T. L., & Pleil, K. E. (2021). The paraventricular thalamus provides a polysynaptic brake on limbic CRF neurons to sex-dependently blunt binge alcohol drinking and avoidance behavior in mice. *Nature Communications*, *12*(1). https://doi.org/10.1038/S41467-021-25368-Y

Leyrer-Jackson, J. M., Hood, L. E., & Olive, M. F. (2021). Alcohol consumption preferentially activates a subset of pro-opiomelanocortin (POMC) producing neurons targeting the amygdala. *Neuropharmacology*, *195*. https://doi.org/10.1016/J.NEUROPHARM.2021.108674

Liang, J., & Olsen, R. W. (2014). Alcohol use disorders and current pharmacological therapies: the role of GABA(A) receptors. *Acta Pharmacologica Sinica*, *35*(8), 981–993. https://doi.org/10.1038/APS.2014.50

Liston, C., McEwen, B. S., & Casey, B. J. (2009). Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(3), 912–917. https://doi.org/10.1073/PNAS.0807041106

Liu, S., Wang, N., Long, Y., Wu, Z., & Zhou, S. (2023). Zinc Homeostasis: An Emerging Therapeutic Target for Neuroinflammation Related Diseases. *Biomolecules*, *13*(3), 416. https://doi.org/10.3390/BIOM13030416

Lovinger, D. M., & Alvarez, V. A. (2017). Alcohol and basal ganglia circuitry: Animal models. *Neuropharmacology*, *122*, 46–55. https://doi.org/10.1016/J.NEUROPHARM.2017.03.023

Lovinger, D. M., & Roberto, M. (2013). Synaptic effects induced by alcohol. *Current Topics in Behavioral Neurosciences*, *13*, 31–86. https://doi.org/10.1007/7854_2011_143

Lovinger, D. M., White, G., & Weight, F. F. (1989). Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science (New York, N.Y.)*, *243*(4899), 1721–1724. https://doi.org/10.1126/SCIENCE.2467382

Luchsinger, J. R., Fetterly, T. L., Williford, K. M., Salimando, G. J., Doyle, M. A., Maldonado, J., Simerly, R. B., Winder, D. G., & Centanni, S. W. (2021). Delineation of an insula-BNST circuit engaged by struggling behavior that regulates avoidance in mice. *Nature Communications*, *12*(1). https://doi.org/10.1038/S41467-021-23674-Z

Luderer, M., Ramos Quiroga, J. A., Faraone, S. V., Zhang James, Y., & Reif, A. (2021). Alcohol use disorders and ADHD. *Neuroscience and Biobehavioral Reviews*, *128*, 648–660. https://doi.org/10.1016/J.NEUBIOREV.2021.07.010

Macey, P. M., Wu, P., Kumar, R., Ogren, J. A., Richardson, H. L., Woo, M. A., & Harper, R. M. (2012). Differential Responses of the Insular Cortex Gyri to Autonomic Challenges. *Autonomic Neuroscience : Basic & Clinical*, *168*(0), 72. https://doi.org/10.1016/J.AUTNEU.2012.01.009

Maldonado, J. R., Sher, Y., Ashouri, J. F., Hills-Evans, K., Swendsen, H., Lolak, S., & Miller, A. C. (2014). The "Prediction of Alcohol Withdrawal Severity Scale" (PAWSS): systematic literature review and pilot study of a new scale for the prediction of complicated alcohol withdrawal syndrome. *Alcohol (Fayetteville, N.Y.)*, *48*(4), 375–390. https://doi.org/10.1016/J.ALCOHOL.2014.01.004

Mantsch, J. R., Baker, D. A., Funk, D., Lê, A. D., & Shaham, Y. (2016). Stress-Induced Reinstatement of Drug Seeking: 20 Years of Progress. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *41*(1), 335–356. https://doi.org/10.1038/NPP.2015.142

Marino, R. A. M., Girven, K. S., Figueiredo, A., Navarrete, J., Doty, C., & Sparta, D. R. (2021). Binge ethanol drinking associated with sex-dependent plasticity of neurons in the insula that project to the bed nucleus of the stria terminalis. *Neuropharmacology*, *196*. https://doi.org/10.1016/J.NEUROPHARM.2021.108695

Martin, G. E., Hendrickson, L. M., Penta, K. L., Friesen, R. M., Pietrzykowski, A. Z., Tapper, A. R., & Treistman, S. N. (2008). Identification of a BK channel auxiliary protein controlling molecular and behavioral tolerance to alcohol. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(45), 17543. https://doi.org/10.1073/PNAS.0801068105

McBride, W. J., & Li, T. K. (1998). Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Critical Reviews in Neurobiology*, *12*(4), 339–369. https://doi.org/10.1615/CRITREVNEUROBIOL.V12.I4.40

McGinnis, M. M., Parrish, B. C., Chappell, A. M., Alexander, N. J., & McCool, B. A. (2020). Chronic Ethanol Differentially Modulates Glutamate Release from Dorsal and Ventral Prefrontal Cortical Inputs onto Rat Basolateral Amygdala Principal Neurons. *ENeuro*, *7*(2). https://doi.org/10.1523/ENEURO.0132-19.2019

Meier, M. L., Brügger, M., Ettlin, D. A., Luechinger, R., Barlow, A., Jäncke, L., & Lutz, K. (2012). Brain activation induced by dentine hypersensitivity pain--an fMRI study. *Journal of Clinical Periodontology*, *39*(5), 441–447. https://doi.org/10.1111/J.1600-051X.2012.01863.X

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Structure & Function*, *214*(5–6), 655–667. https://doi.org/10.1007/S00429-010-0262-0

Mereu, G., Fadda, F., & Gian Luigi Gessa. (1984). Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. *Brain Research*, *292*(1), 63–69. https://doi.org/10.1016/0006-8993(84)90890-4

Michel, M. (2017). A role for the anterior insular cortex in the global neuronal workspace model of consciousness. *Consciousness and Cognition*, *49*, 333–346. https://doi.org/10.1016/J.CONCOG.2017.02.004

Miura, I., Sato, M., Overton, E. T. N., Kunori, N., Nakai, J., Kawamata, T., Nakai, N., & Takumi, T. (2020). Encoding of social exploration by neural ensembles in the insular cortex. *PLoS Biology*, *18*(9). https://doi.org/10.1371/JOURNAL.PBIO.3000584

Moorman, D. E. (2018). The role of the orbitofrontal cortex in alcohol use, abuse, and dependence. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *87*(Pt A), 85–107. https://doi.org/10.1016/J.PNPBP.2018.01.010

Morton, G. J., Cummings, D. E., Baskin, D. G., Barsh, G. S., & Schwartz, M. W. (2006). Central nervous system control of food intake and body weight. *Nature*, *443*(7109), 289–295. https://doi.org/10.1038/NATURE05026

Muñoz, B., Fritz, B. M., Yin, F., & Atwood, B. K. (2018). Alcohol exposure disrupts mu opioid receptor-mediated long-term depression at insular cortex inputs to dorsolateral striatum. *Nature Communications*, *9*(1). https://doi.org/10.1038/S41467-018-03683-1

Murphy, J. M., Stewart, R. B., Bell, R. L., Badia-Elder, N. E., Carr, L. G., McBride, W. J., Lumeng, L., & Li, T. K. (2002). Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behavior Genetics*, *32*(5), 363–388. https://doi.org/10.1023/A:1020266306135

Mutschler, I., Ball, T., Wankerl, J., & Strigo, I. A. (2012). Pain and emotion in the insular cortex: evidence for functional reorganization in major depression. *Neuroscience Letters*, *520*(2), 204–209. https://doi.org/10.1016/J.NEULET.2012.03.095

Nagai, M., Scheper, V., Lenarz, T., & Förster, C. Y. (2021). The insular cortex as a vestibular area in relation to autonomic function. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society*, *31*(2), 179–185. https://doi.org/10.1007/S10286-020-00744-8

Nall, R. W., Heinsbroek, J. A., Nentwig, T. B., Kalivas, P. W., & Bobadilla, A. C. (2021). Circuit selectivity in drug versus natural reward seeking behaviors. *Journal of Neurochemistry*, *157*(5), 1450–1472. https://doi.org/10.1111/JNC.15297

Naqvi, N. H., & Bechara, A. (2010). The insula and drug addiction: an interoceptive view of pleasure, urges, and decision-making. *Brain Structure & Function*, *214*(5–6), 435–450. https://doi.org/10.1007/S00429-010-0268-7

Naqvi, N. H., Rudrauf, D., Damasio, H., & Bechara, A. (2007). Damage to the Insula Disrupts Addiction to Cigarette Smoking. *Science (New York, N.Y.)*, *315*(5811), 531. https://doi.org/10.1126/SCIENCE.1135926

Nestoros, J. N. (1980). Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. *Science (New York, N.Y.)*, *209*(4457), 708–710. https://doi.org/10.1126/SCIENCE.7394531

Nieuwenhuys, R. (2012). The insular cortex: a review. *Progress in Brain Research*, *195*, 123–163. https://doi.org/10.1016/B978-0-444-53860-4.00007-6

Nordman, J. C., Ma, X., Gu, Q., Potegal, M., Li, H., Kravitz, A. V., & Li, Z. (2020). Potentiation of Divergent Medial Amygdala Pathways Drives Experience-Dependent Aggression Escalation. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *40*(25), 4858–4880. https://doi.org/10.1523/JNEUROSCI.0370-20.2020

Nose, H., Kamijo, Y. ichiro, & Masuki, S. (2018). Interactions between body fluid homeostasis and thermoregulation in humans. *Handbook of Clinical Neurology*, *156*, 417–429. https://doi.org/10.1016/B978-0-444-63912-7.00025-4

Novembre, G., Zanon, M., & Silani, G. (2015). Empathy for social exclusion involves the sensory-discriminative component of pain: a within-subject fMRI study. *Social Cognitive and Affective Neuroscience*, *10*(2), 153–164. https://doi.org/10.1093/SCAN/NSU038

Nowak, K. L., McBride, W. J., Lumeng, L., Li, T. K., & Murphy, J. M. (2000). Involvement of dopamine D2 autoreceptors in the ventral tegmental area on alcohol and saccharin intake of the alcohol-preferring P rat. *Alcoholism, Clinical and Experimental Research*, *24*(4), 476–483. https://doi.org/10.1111/j.1530-0277.2000.tb02014.x

Nudelman, J. L., & Waltz, J. A. (2022). Acute and Lifetime Stress and Psychotic Illness: The Roles of Reward and Salience Networks. *Journal of Psychiatry and Brain Science*, *7*(6). https://doi.org/10.20900/JPBS.20220012

O'Tousa, D., & Grahame, N. (2014). Habit formation: implications for alcoholism research. *Alcohol (Fayetteville, N.Y.)*, *48*(4), 327–335. https://doi.org/10.1016/J.ALCOHOL.2014.02.004

Packard, A. E. B., Egan, A. E., & Ulrich-Lai, Y. M. (2016). HPA axis- Interaction with Behavioral Systems. *Comprehensive Physiology*, *6*(4), 1897. https://doi.org/10.1002/CPHY.C150042

Palacio, S., Velázquez-Marrero, C., Marrero, H. G., Seale, G. E., Yudowski, G. A., & Treistman, S. N. (2015). Time-Dependent Effects of Ethanol on BK Channel Expression and Trafficking in Hippocampal Neurons. *Alcoholism, Clinical and Experimental Research*, *39*(9), 1619–1631. https://doi.org/10.1111/ACER.12808

Palaniyappan, L., White, T. P., & Liddle, P. F. (2012). The concept of salience network dysfunction in schizophrenia: from neuroimaging observations to therapeutic opportunities. *Current Topics in Medicinal Chemistry*, *12*(21), 2324–2338. https://doi.org/10.2174/156802612805289881

Palmer, B. F., & Clegg, D. J. (2016). Physiology and pathophysiology of potassium homeostasis. *Advances in Physiology Education*, *40*(4), 480–490. https://doi.org/10.1152/ADVAN.00121.2016

Pang, T. Y., Du, X., Catchlove, W. A., Renoir, T., Lawrence, A. J., & Hannan, A. J. (2013). Positive environmental modification of depressive phenotype and abnormal hypothalamic-pituitary-adrenal axis activity in female C57BL/6J mice during abstinence from chronic ethanol consumption. *Frontiers in Pharmacology*, *4*. https://doi.org/10.3389/FPHAR.2013.00093

Paulus, M. P. (2007). Neural basis of reward and craving--a homeostatic point of view. *Dialogues in Clinical Neuroscience*, *9*(4), 379–387. https://doi.org/10.31887/DCNS.2007.9.4/MPAULUS

Paulus, M. P., & Khalsa, S. S. (2021). When You Don't Feel Right Inside: Homeostatic Dysregulation and the Mid-Insular Cortex in Psychiatric Disorders. *The American Journal of Psychiatry*, *178*(8), 683–685. https://doi.org/10.1176/APPI.AJP.2021.21060622

[PDF] Diagnostic And Statistical Manual Of Mental Disorders DSM-IV American Psychiatric Association-pdf download free book CLICK HERE FOR DOWNLOAD. (n.d.).

Peng, B., Yang, Q., Joshi, R. B., Liu, Y., Akbar, M., Song, B. J., Zhou, S., & Wang, X. (2020). Role of Alcohol Drinking in Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis. *International Journal of Molecular Sciences*, *21*(7). https://doi.org/10.3390/IJMS21072316

Perez, S., Cui, Y., Vignoud, G., Perrin, E., Mendes, A., Zheng, Z., Touboul, J., & Venance, L. (2022). Striatum expresses region-specific plasticity consistent with distinct memory abilities. *Cell Reports*, *38*(11). https://doi.org/10.1016/J.CELREP.2022.110521

Perini, I., Kämpe, R., Arlestig, T., Karlsson, H., Löfberg, A., Pietrzak, M., Zangen, A., & Heilig, M. (2020). Repetitive transcranial magnetic stimulation targeting the insular cortex for reduction of heavy drinking in treatment-seeking alcohol-dependent subjects: a randomized controlled trial. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *45*(5), 842–850. https://doi.org/10.1038/S41386-019-0565-7

Peyron, R., & Fauchon, C. (2019). The posterior insular-opercular cortex: An access to the brain networks of thermosensory and nociceptive processes? *Neuroscience Letters*, *702*, 34–39. https://doi.org/10.1016/J.NEULET.2018.11.042

Peyron, R., Laurent, B., & García-Larrea, L. (2000). Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiologie Clinique = Clinical Neurophysiology*, *30*(5), 263–288. https://doi.org/10.1016/S0987-7053(00)00227-6

Phillips, K. B., & Sarter, M. (2020). Addiction vulnerability and the processing of significant cues: Sign-, but not goal-, tracker perceptual sensitivity relies on cue salience. *Behavioral Neuroscience*, *134*(2), 133–143. https://doi.org/10.1037/BNE0000353

Pietrzak, R. H., Goldstein, R. B., Southwick, S. M., & Grant, B. F. (2011). Prevalence and Axis I comorbidity of full and partial posttraumatic stress disorder in the United States: results from Wave 2 of the National Epidemiologic Survey on Alcohol and Related Conditions. *Journal of Anxiety Disorders*, *25*(3), 456–465. https://doi.org/10.1016/J.JANXDIS.2010.11.010

Pina, M. M., & Cunningham, C. L. (2017). Ethanol-seeking behavior is expressed directly through an extended amygdala to midbrain neural circuit. *Neurobiology of Learning and Memory*, *137*, 83–91. https://doi.org/10.1016/J.NLM.2016.11.013

Pina, M. M., Pati, D., Hwa, L. S., Wu, S. Y., Mahoney, A. A., Omenyi, C. G., Navarro, M., & Kash, T. L. (2020). The kappa opioid receptor modulates GABA neuron excitability and synaptic transmission in midbrainprojections from the insular cortex. *Neuropharmacology*, *165*. https://doi.org/10.1016/J.NEUROPHARM.2019.107831

Preuss, U. W., Schaefer, M., Born, C., & Grunze, H. (2021). Bipolar Disorder and Comorbid Use of Illicit Substances. *Medicina (Kaunas, Lithuania)*, *57*(11). https://doi.org/10.3390/MEDICINA57111256

Pushparaj, A., Kim, A. S., Musiol, M., Zangen, A., Daskalakis, Z. J., Zack, M., Winstanley, C. A., & Le Foll, B. (2015). Differential Involvement of the Agranular vs Granular Insular Cortex in

the Acquisition and Performance of Choice Behavior in a Rodent Gambling Task. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *40*(12), 2832–2842. https://doi.org/10.1038/NPP.2015.133

RASSNICK, S., D'AMICO, E., RILEY, E., PULVIRENTI, L., ZIEGLGÄNSBERGER, W., & KOOB, G. F. (1992). GABA and nucleus accumbens glutamate neurotransmission modulate ethanol self-administration in rats. *Annals of the New York Academy of Sciences*, *654*(1), 502–505. https://doi.org/10.1111/J.1749-6632.1992.TB26013.X

Renteria, R., Baltz, E. T., & Gremel, C. M. (2018). Chronic alcohol exposure disrupts top-down control over basal ganglia action selection to produce habits. *Nature Communications*, *9*(1). https://doi.org/10.1038/S41467-017-02615-9

Renteria, R., Cazares, C., Baltz, E. T., Schreiner, D. C., Yalcinbas, E. A., Steinkellner, T., Hnasko, T. S., & Gremel, C. M. (2021). Mechanism for differential recruitment of orbitostriatal transmission during actions and outcomes following chronic alcohol exposure. *ELife*, *10*. https://doi.org/10.7554/ELIFE.67065

Rinker, J. A., Marshall, S. A., Mazzone, C. M., Lowery-Gionta, E. G., Gulati, V., Pleil, K. E., Kash, T. L., Navarro, M., & Thiele, T. E. (2017). Extended Amygdala to Ventral Tegmental Area Corticotropin-Releasing Factor Circuit Controls Binge Ethanol Intake. *Biological Psychiatry*, *81*(11), 930–940. https://doi.org/10.1016/J.BIOPSYCH.2016.02.029

Roberto, M., Kirson, D., & Khom, S. (2021). The Role of the Central Amygdala in Alcohol Dependence. *Cold Spring Harbor Perspectives in Medicine*, *11*(2), 1–23. https://doi.org/10.1101/CSHPERSPECT.A039339

Robins, M. T., Chiang, T., Mores, K. L., Alongkronrusmee, D., & van Rijn, R. M. (2018). Critical Role for Gi/o-Protein Activity in the Dorsal Striatum in the Reduction of Voluntary Alcohol Intake in C57Bl/6 Mice. *Frontiers in Psychiatry*, *9*(APR). https://doi.org/10.3389/FPSYT.2018.00112

Robinson, D. L., Howard, E. C., McConnell, S., Gonzales, R. A., & Wightman, R. M. (2009). Disparity between tonic and phasic ethanol-induced dopamine increases in the nucleus accumbens of rats. *Alcoholism, Clinical and Experimental Research*, *33*(7), 1187–1196. https://doi.org/10.1111/J.1530-0277.2009.00942.X

Rodd, Z. A., Melendez, R. I., Bell, R. L., Kuc, K. A., Zhang, Y., Murphy, J. M., & McBride, W. J. (2004). Intracranial self-administration of ethanol within the ventral tegmental area of male Wistar rats: evidence for involvement of dopamine neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(5), 1050–1057. https://doi.org/10.1523/JNEUROSCI.1319-03.2004

Rodríguez-Sierra, O. E., Goswami, S., Turesson, H. K., & Pare, D. (2016). Altered responsiveness of BNST and amygdala neurons in trauma-induced anxiety. *Translational Psychiatry*, *6*(7). https://doi.org/10.1038/TP.2016.128

Rolls, E. T. (2000). The orbitofrontal cortex and reward. *Cerebral Cortex (New York, N.Y. : 1991)*, *10*(3), 284–294. https://doi.org/10.1093/CERCOR/10.3.284

Rolls, E. T. (2019). The cingulate cortex and limbic systems for emotion, action, and memory. *Brain Structure & Function*, *224*(9), 3001–3018. https://doi.org/10.1007/S00429-019-01945-2

Rolls, E. T., Cheng, W., Gong, W., Qiu, J., Zhou, C., Zhang, J., Lv, W., Ruan, H., Wei, D., Cheng, K., Meng, J., Xie, P., & Feng, J. (2019). Functional Connectivity of the Anterior Cingulate Cortex

in Depression and in Health. *Cerebral Cortex (New York, N.Y. : 1991)*, *29*(8), 3617–3630. https://doi.org/10.1093/CERCOR/BHY236

Ryabinin, A. E., Criado, J. R., Henriksen, S. J., Bloom, F. E., & Wilson, M. C. (1997). Differential sensitivity of c-Fos expression in hippocampus and other brain regions to moderate and low doses of alcohol. *Molecular Psychiatry*, *2*(1), 32–43. https://doi.org/10.1038/SJ.MP.4000206

Samson, H. H., Hodge, C. W., Tolliver, G. A., & Haraguchi, M. (1993). Effect of dopamine agonists and antagonists on ethanol-reinforced behavior: the involvement of the nucleus accumbens. *Brain Research Bulletin*, *30*(1–2), 133–141. https://doi.org/10.1016/0361-9230(93)90049-H

Schimmelpfennig, J., Topczewski, J., Zajkowski, W., & Jankowiak-Siuda, K. (2023). The role of the salience network in cognitive and affective deficits. *Frontiers in Human Neuroscience*, *17*. https://doi.org/10.3389/FNHUM.2023.1133367

Seeley, W. W. (2019). The Salience Network: A Neural System for Perceiving and Responding to Homeostatic Demands. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(50), 9878–9882. https://doi.org/10.1523/JNEUROSCI.1138-17.2019

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., Reiss, A. L., & Greicius, M. D. (2007). Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive Control. *The Journal of Neuroscience*, *27*(9), 2349. https://doi.org/10.1523/JNEUROSCI.5587-06.2007

Segovia, K. N., Vontell, R., López-Cruz, L., Salamone, J. D., & Correa, M. (2013a). c-Fos immunoreactivity in prefrontal, basal ganglia and limbic areas of the rat brain after central and peripheral administration of ethanol and its metabolite acetaldehyde. *Frontiers in Behavioral Neuroscience*, *7*(MAY). https://doi.org/10.3389/FNBEH.2013.00048

Segovia, K. N., Vontell, R., López-Cruz, L., Salamone, J. D., & Correa, M. (2013b). c-Fos immunoreactivity in prefrontal, basal ganglia and limbic areas of the rat brain after central and peripheral administration of ethanol and its metabolite acetaldehyde. *Frontiers in Behavioral Neuroscience*, *7*(MAY). https://doi.org/10.3389/FNBEH.2013.00048

Seif, T., Chang, S. J., Simms, J. A., Gibb, S. L., Dadgar, J., Chen, B. T., Harvey, B. K., Ron, D., Messing, R. O., Bonci, A., & Hopf, F. W. (2013). Cortical activation of accumbens hyperpolarization-active NMDARs mediates aversion-resistant alcohol intake. *Nature Neuroscience*, *16*(8), 1094–1100. https://doi.org/10.1038/NN.3445

Seiler, J. L., Cosme, C. V., Sherathiya, V. N., Schaid, M. D., Bianco, J. M., Bridgemohan, A. S., & Lerner, T. N. (2022). Dopamine signaling in the dorsomedial striatum promotes compulsive behavior. *Current Biology : CB*, *32*(5), 1175-1188.e5. https://doi.org/10.1016/J.CUB.2022.01.055

Sharp, P. B., Sutton, B. P., Paul, E. J., Sherepa, N., Hillman, C. H., Cohen, N. J., Kramer, A. F., Prakash, R. S., Heller, W., Telzer, E. H., & Barbey, A. K. (2018). Mindfulness training induces structural connectome changes in insula networks. *Scientific Reports 2018 8:1*, *8*(1), 1–10. https://doi.org/10.1038/s41598-018-26268-w

Shin, L. M., & Liberzon, I. (2010a). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*, *35*(1), 169–191. https://doi.org/10.1038/npp.2009.83

Shin, L. M., & Liberzon, I. (2010b). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *35*(1), 169–191. https://doi.org/10.1038/NPP.2009.83

Siciliano, C. A., Noamany, H., Chang, C. J., Brown, A. R., Chen, X., Leible, D., Lee, J. J., Wang, J., Vernon, A. N., Vander Weele, C. M., Kimchi, E. Y., Heiman, M., & Tye, K. M. (2019). A cortical-brainstem circuit predicts and governs compulsive alcohol drinking. *Science (New York, N.Y.)*, *366*(6468), 1008–1012. https://doi.org/10.1126/SCIENCE.AAY1186

Silberman, Y., Matthews, R. T., & Winder, D. G. (2013). A corticotropin releasing factor pathway for ethanol regulation of the ventral tegmental area in the bed nucleus of the stria terminalis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(3), 950–960. https://doi.org/10.1523/JNEUROSCI.2949-12.2013

Sinha, R., Fox, H. C., Hong, K. A., Bergquist, K., Bhagwagar, Z., & Siedlarz, K. M. (2009). Enhanced Negative Emotion and Alcohol Craving, and Altered Physiological Responses Following Stress and Cue Exposure in Alcohol Dependent Individuals. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *34*(5), 1198. https://doi.org/10.1038/NPP.2008.78

Smith, R. J., Anderson, R. I., Haun, H. L., Mulholland, P. J., Griffin, W. C., Lopez, M. F., & Becker, H. C. (2020). Dynamic c-Fos changes in mouse brain during acute and protracted withdrawal from chronic intermittent ethanol exposure and relapse drinking. *Addiction Biology*, *25*(6). https://doi.org/10.1111/ADB.12804

Snelleman, M., Schoenmakers, T. M., & van de Mheen, D. (2014). The relationship between perceived stress and cue sensitivity for alcohol. *Addictive Behaviors*, *39*(12), 1884–1889. https://doi.org/10.1016/J.ADDBEH.2014.07.024

Sridharan, D., Levitin, D. J., & Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(34), 12569–12574. https://doi.org/10.1073/PNAS.0800005105

Szmitko, P. E., & Verma, S. (2005). Cardiology patient pages. Red wine and your heart. *Circulation*, *111*(2). https://doi.org/10.1161/01.CIR.0000151608.29217.62

Tang, Y. Y., Hölzel, B. K., & Posner, M. I. (2015). The neuroscience of mindfulness meditation. *Nature Reviews. Neuroscience*, *16*(4), 213–225. https://doi.org/10.1038/NRN3916

Teng, C., Liu, T., Zhang, N., Zhong, Y., & Wang, C. (2022). Cognitive behavioral therapy may rehabilitate abnormally functional communication pattern among the triple-network in major depressive disorder: A follow-up study. *Journal of Affective Disorders*, *304*, 28–39. https://doi.org/10.1016/J.JAD.2022.02.050

Thakkar, M. M., Sharma, R., & Sahota, P. (2015). Alcohol disrupts sleep homeostasis. *Alcohol (Fayetteville, N.Y.)*, *49*(4), 299–310. https://doi.org/10.1016/J.ALCOHOL.2014.07.019

Thiele, T. E., & Navarro, M. (2014). "Drinking in the dark" (DID) procedures: a model of binge-like ethanol drinking in non-dependent mice. *Alcohol (Fayetteville, N.Y.)*, *48*(3), 235–241. https://doi.org/10.1016/J.ALCOHOL.2013.08.005

Thoma, N., Pilecki, B., & McKay, D. (2015). Contemporary Cognitive Behavior Therapy: A Review of Theory, History, and Evidence. *Psychodynamic Psychiatry*, *43*(3), 423–462. https://doi.org/10.1521/PDPS.2015.43.3.423

Thomason, M. E., Hamilton, J. P., & Gotlib, I. H. (2011). Stress-induced activation of the HPA axis predicts connectivity between subgenual cingulate and salience network during rest in adolescents. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *52*(10), 1026. https://doi.org/10.1111/J.1469-7610.2011.02422.X

Tsurugizawa, T., Uematsu, A., Uneyama, H., & Torii, K. (2010). The role of the GABAergic and dopaminergic systems in the brain response to an intragastric load of alcohol in conscious rats. *Neuroscience*, *171*(2), 451–460. https://doi.org/10.1016/J.NEUROSCIENCE.2010.09.016

Tuerke, K. J., Limebeer, C. L., Fletcher, P. J., & Parker, L. A. (2012). Double dissociation between regulation of conditioned disgust and taste avoidance by serotonin availability at the 5-HT(3) receptor in the posterior and anterior insular cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(40), 13709–13717. https://doi.org/10.1523/JNEUROSCI.2042-12.2012

Vena, A. A., Zandy, S. L., Cofresí, R. U., & Gonzales, R. A. (2020). Behavioral, Neurobiological, and Neurochemical Mechanisms of Ethanol Self-Administration: A Translational Review. *Pharmacology & Therapeutics*, *212*, 107573. https://doi.org/10.1016/J.PHARMTHERA.2020.107573

Vogt, B. A. (2016). Midcingulate cortex: Structure, connections, homologies, functions and diseases. *Journal of Chemical Neuroanatomy*, *74*, 28–46. https://doi.org/10.1016/J.JCHEMNEU.2016.01.010

Vogt, B. A., Berger, G. R., & Derbyshire, S. W. G. (2003). Structural and functional dichotomy of human midcingulate cortex. *The European Journal of Neuroscience*, *18*(11), 3134–3144. https://doi.org/10.1111/J.1460-9568.2003.03034.X

Volkow, N. D., Michaelides, M., & Baler, R. (2019). The Neuroscience of Drug Reward and Addiction. *Physiological Reviews*, *99*(4), 2115. https://doi.org/10.1152/PHYSREV.00014.2018

Vranjkovic, O., Winkler, G., & Winder, D. G. (2018). Ketamine administration during a critical period after forced ethanol abstinence inhibits the development of time-dependent affective disturbances. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *43*(9), 1915–1923. https://doi.org/10.1038/S41386-018-0102-0

Walker, D. L., Toufexis, D. J., & Davis, M. (2003). Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *European Journal of Pharmacology*, *463*(1–3), 199–216. https://doi.org/10.1016/S0014-2999(03)01282-2

Wallner, M., & Olsen, R. W. (2008). Physiology and pharmacology of alcohol: the imidazobenzodiazepine alcohol antagonist site on subtypes of GABAA receptors as an opportunity for drug development? *British Journal of Pharmacology*, *154*(2), 288. https://doi.org/10.1038/BJP.2008.32

Wang, N., Zhang, Y. H., Wang, J. Y., & Luo, F. (2021). Current Understanding of the Involvement of the Insular Cortex in Neuropathic Pain: A Narrative Review. *International Journal of Molecular Sciences*, *22*(5), 1–17. https://doi.org/10.3390/IJMS22052648

Weight, F. F., Aguayo, L. G., White, G., Lovinger, D. M., & Peoples, R. W. (1992). GABA- and glutamate-gated ion channels as molecular sites of alcohol and anesthetic action.

Advances in Biochemical Psychopharmacology, *47*, 335–347. https://pubmed.ncbi.nlm.nih.gov/1354918/

Whylings, J., Rigney, N., Peters, N. V., de Vries, G. J., & Petrulis, A. (2020). Sexually dimorphic role of BNST vasopressin cells in sickness and social behavior in male and female mice. *Brain, Behavior, and Immunity*, *83*, 68–77. https://doi.org/10.1016/J.BBI.2019.09.015

Widmer, H., Lemos, J. R., & Treistman, S. N. (1998). Ethanol reduces the duration of single evoked spikes by a selective inhibition of voltage-gated calcium currents in acutely dissociated supraoptic neurons of the rat. *Journal of Neuroendocrinology*, *10*(6), 399–406. https://doi.org/10.1046/J.1365-2826.1998.00221.X

Wirkner, K., Poelchen, W., Köles, L., Mühlberg, K., Scheibler, P., Allgaier, C., & Illes, P. (1999). Ethanol-induced inhibition of NMDA receptor channels. *Neurochemistry International*, *35*(2), 153–162. https://doi.org/10.1016/S0197-0186(99)00057-1

Wright, H., Li, X., Fallon, N. B., Crookall, R., Giesbrecht, T., Thomas, A., Halford, J. C. G., Harrold, J., & Stancak, A. (2016). Differential effects of hunger and satiety on insular cortex and hypothalamic functional connectivity. *The European Journal of Neuroscience*, *43*(9), 1181–1189. https://doi.org/10.1111/EJN.13182

Yang, Q., Luo, C., Zhang, X., Liu, Y., Wang, Z., Cacciamani, P., Shi, J., Cui, Y., Wang, C., Sinha, B., Peng, B., Tong, G., Das, G., Shah, E., Gao, Y., Li, W., Tu, Y., Qian, D., Shah, K., … Wang, X. (2020). Tartary buckwheat extract alleviates alcohol-induced acute and chronic liver injuries through the inhibition of oxidative stress and mitochondrial cell death pathway. *American Journal of Translational Research*, *12*(1), 70. /pmc/articles/PMC7013218/

Yu, X., Cohen, Z. P., Tsuchiyagaito, A., Cochran, G., Aupperle, R. L., Stewart, J. L., Singh, M. K., Misaki, M., Bodurka, J., Paulus, M. P., & Kirlic, N. (2022). Neurofeedback-Augmented Mindfulness Training Elicits Distinct Responses in the Subregions of the Insular Cortex in Healthy Adolescents. *Brain Sciences*, *12*(3). https://doi.org/10.3390/BRAINSCI12030363

Zakhari, S., & Gordis, E. (1999). Moderate drinking and cardiovascular health. *Proceedings of the Association of American Physicians*, *111*(2), 148–158. https://doi.org/10.1046/J.1525-1381.1999.09250.X

Zhan, B., Zhu, Y., Xia, J., Li, W., Tang, Y., Beesetty, A., Ye, J. H., & Fu, R. (2022). Comorbidity of Post-Traumatic Stress Disorder and Alcohol Use Disorder: Animal Models and Associated Neurocircuitry. *International Journal of Molecular Sciences*, *24*(1). https://doi.org/10.3390/IJMS24010388

Zhang, R., & Volkow, N. D. (2019). Brain default-mode network dysfunction in addiction. *NeuroImage*, *200*, 313–331. https://doi.org/10.1016/J.NEUROIMAGE.2019.06.036

Zhao, D., Wang, D., Wang, W., Dai, J., Cui, M., Wu, M., Liu, C., Liu, J., Meng, F., Wang, K., Hu, F., Liu, D., Qiu, C., Li, W., & Li, C. (2022). The altered sensitivity of acute stress induced anxiety-related behaviors by modulating insular cortex-paraventricular thalamus-bed nucleus of the stria terminalis neural circuit. *Neurobiology of Disease*, *174*, 105890. https://doi.org/10.1016/J.NBD.2022.105890

Zhou, X., Li, A., Mi, X., Li, Y., Ding, Z., An, M., Chen, Y., Li, W., Tao, X., Chen, X., & Li, Y. (2023). Hyperexcited limbic neurons represent sexual satiety and reduce mating motivation. *Science (New York, N.Y.)*, *379*(6634), 820–825. https://doi.org/10.1126/SCIENCE.ABL4038