HHS Public Access

Author manuscript

Addict Neurosci. Author manuscript; available in PMC 2025 May 07.

Published in final edited form as:

Addict Neurosci. 2025 March ; 14: . doi:10.1016/j.addicn.2024.100194.

Central amygdala neuroimmune signaling in alcohol use disorder

Mariam Melkumyan[a], **Patrick A. Randall**[b,c,*], **Yuval Silberman**[a,**]

[a]Department of Neural and Behavioral Sciences, Penn State College of Medicine, United States

[b]Department of Anesthesiology, Penn State College of Medicine, United States

[c]Department of Pharmacology, Penn State College of Medicine, United States

Abstract

Alcohol Use Disorder (AUD) is a prevalent and debilitating condition characterized by an inability to control alcohol consumption despite adverse consequences. Current treatments for AUD, including FDA-approved medications such as naltrexone and acamprosate, have limited efficacy and compliance, underscoring the need for novel therapeutic approaches. The central amygdala (CeA) plays a crucial role in the development and maintenance of AUD, particularly aspects associated with stress and binge behaviors. Recent research indicates neuroimmune signaling in the CeA is emerging as a key factor in this process. Chronic alcohol consumption disrupts neuroimmune signaling, leading to altered cytokine expression and activation of glial cells, including astrocytes and microglia. These changes contribute to the dysregulation of neural circuits involved in reward and stress, perpetuating alcohol-seeking behavior and relapse. This review delves into how chronic alcohol exposure affects neuroimmune signaling in the CeA, contributing to the pathophysiology of AUD. By focusing on the impact of cytokine expression and glial cell activation, this review aims to elucidate the mechanisms by which neuroinflammation in the CeA influences alcohol-related behaviors. By providing a comprehensive overview of the current state of research, this review identifies potential therapeutic targets for AUD. Understanding the complex interplay between neuroimmune signaling and alcohol-related behaviors may pave the way for more effective treatments and improved outcomes for individuals struggling with AUD.

Keywords

Alcohol use disorder; Neuroinflammation; Central amygdala; Neuroimmune markers

*Corresponding author at: Department of Anesthesiology and Perioperative Medicine, Department of Pharmacology, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania 17033, United States. prandall@psu.edu (P.A. Randall).

**Corresponding author at: Department of Neural and Behavioral Sciences, College of Medicine, Pennsylvania State University, Hershey, Pennsylvania 17033, United States. yus72@psu.edu (Y. Silberman).

Introduction

Alcohol use disorder (AUD) is a highly prevalent medical condition characterized the inability to stop or control alcohol use, despite social, occupational, or health consequences. The risk of AUD is increased by various factors, including drinking at a young age, genetics and family history of alcohol problems, mental health conditions and trauma, and binge drinking or heavy alcohol use [1]. Currently, treatments for AUD include support groups, therapy, and medications. Three medications, naltrexone, acamprosate, and disulfiram, are approved by the Food and Drug Administration to help stop or control alcohol use. Naltrexone is an opioid antagonist, disulfiram is an acetaldehyde dehydrogenase inhibitor, while acamprosate acts on the $GABA_A$ and $GABA_B$ receptors and NMDA receptors, amongst other targets [2,3]. Of these medications, acamprosate and naltrexone seem to be more effective compared to disulfiram, which has major compliance issues due to the unpleasant symptoms when alcohol is ingested [2]. Both naltrexone and acamprosate are effective in patients who are not currently drinking alcohol [4]. Other therapeutics, including Nalmafene (an opioid receptor antagonist), Baclofen (a $GABA_B$ receptor agonist), Topimarate (an anticonvulsant medication), and others are emerging pharmacological approaches in the US and globally for treating AUD [5]. However, due to the various side effects and low efficacy/compliance of these medications, many studies have been searching for new therapeutics.

Alcohol differentially affects neurotransmission in various brain regions, such as the central amygdala (CeA), a finding that both allows for identification of novel therapeutics as well as challenges in determining which systems might be the most effective targets for clinical outcomes. Chronic heavy alcohol consumption can increase inflammatory activity in the body and the brain (for more details on the functioning of the neuroimmune system itself see [6–10]). A recent meta-analytical study explored the peripheral concentration of various cytokines in patients with AUD and found that, overall, cytokine concentrations increased in AUD patients, with the pro-inflammatory cytokines having the greatest effect size [11]. Interestingly, the concentration of peripheral cytokines vary, with the greatest cytokine concentrations being observed during active drinking and alcohol withdrawal [11]. There have only been two studies investigating the central cytokine concentrations through cerebrospinal fluid [12,13]. Both studies found elevations in inflammatory markers, including IL-6 [12] and MCP-1 [13]. In post-mortem samples of individuals with AUD, studies have found elevated levels of inflammatory markers, such as MCP1, NF-kB and HMGB1/Il-1b heterocomplexes [11,14]. These clinical findings validate the importance of inflammatory activity in alcohol use, not only peripherally, but also centrally. To further our understanding of the mechanisms of neuroinflammation in alcohol use, pre-clinical and foundational research are necessary.

This review will provide an overview of alcohol's effects on the neuroimmune system across the translational spectrum. We first discuss potential mechanisms by which alcohol may modulate neuroimmune signaling in multiple brain regions. We then focus on alcohol-neuroimmune interactions in the central amygdala (CeA), a brain system shown to be critical to stress, binge behaviors, and AUD development. We finally discuss current clinical literature on the use of neuroimmune modulators as potential treatments for AUD.

Understanding alcohol-neuroimmune interaction in relevant neurocircuits may uncover novel therapeutic approaches.

Neuroinflammation in AUD

Pre-clinical and foundational studies have extensively shown multiple roles of inflammation processes in AUD and AUD models. Chronic alcohol consumption has been shown to persistently increase neuroimmune gene expression [15]. A study examining the changes in gene expression in response to alcohol in the ventral tegmental area, a brain region critical for reward processing, found significant changes in several genes indicating an overall upregulation of pro-inflammatory response and neuronal damage after alcohol exposure [16]. Neuroimmune changes can also be found outside reward centers. For instance, chronic alcohol through alcohol drinking and chronic intermittent ethanol (CIE) vapor exposure, a model for inducing alcohol dependence, upregulates gene expression in the type 1 interferon and cytokine signaling pathways in the nucleus of the solitary tract [17], part of the dorsal vagal complex, a brainstem region regulating central and peripheral signaling interactions that has been critically understudied in the AUD field [18–21]. Additionally, studies have shown differential gene expression in inflammatory pathways in brain regions like the prefrontal cortex, including changes in *PDE4a* [22], interferon signaling, astrocyte-neuron communication, microglial homeostasis and immune surveillance [23]. Importantly, the method and access schedule of alcohol exposure may induce changes in distinct gene sets [24]. These and other studies exploring gene expression changes in response to alcohol have found alterations in genes associated with astrocytes and microglia as well [16,17,22–25], implicating the importance of these cells in alcohol responses. However, the broad nature of alcohol-induced neuroimmune changes creates difficulty in understanding which of these changes may make for the most efficacious treatment targets.

In addition to changes in gene expression, there are changes in the release of cytokines and chemokines in response to alcohol, and these changes vary based on the brain region and neuroimmune cell type studied. Alcohol's effects on microglial activity are complex. Microglia exist in a range of anti-inflammatory to pro-inflammatory states, and can release both pro- (e.g. TNFα, IL-1β, IL-6) and anti-inflammatory (e.g. IL-10) cytokines. Alcohol application to microglial cells in vitro increases microglia activity as examined by changes in morphology, phagocytic activity, and pro-inflammatory gene expression [26]. In alcohol-treated rats through the Majchrowicz AUD model, a model for inducing alcohol dependence, there is a reduction in the number of microglia in the hippocampus and an increase in the number of degenerating microglia [27]. The degenerating microglia are hypothesized to have impaired pro-inflammatory responses and reduced capacity to release anti-inflammatory factors which are vital for cellular repair [27,28]. It is possible that microglial activation and apoptosis are dependent on the length and severity of alcohol exposure. In adolescent mice, a single alcohol exposure led into increased microglia number and activation states [29]. However, after a 5-day alcohol exposure, there was an increase in the number of apoptotic microglia, while a 14-day alcohol exposure followed by a 21-day withdrawal reduced the number of microglia in the hippocampal dentate gyrus but showed the remaining microglia stayed in an activated state [29]. Together, these findings suggest that microglia may become pro-inflammatory after a short-term alcohol exposure [26,30,31], but with

chronic alcohol, the microglia may become apoptotic and decline in number [27,29]. The changes in microglia number may also be sex dependent, as a study exploring the effect of binge alcohol and exercise on microglia numbers in the hippocampus found that female mice have more microglia in the hippocampus compared to males after binge alcohol consumption [32].

Like microglia, astrocytes also produce and release cytokines, including, but not limited to IL-6, IL-8, IL-1β, and TNFα [33]. Alcohol causes increased glial fibrillary acidic protein (GFAP) synthesis, suggesting an increase in the number of pro-inflammatory astrocytes [34]. However, similar to microglia, alcohol can have a spectrum of effects on astrocytes, not only increasing the immunoreactivity of these cells, but also leading to a reduction in the number of astrocytes with long-term exposure [35]. The astrocyte density changes are dependent on the time, brain region, and method of alcohol administration [36]. Some studies show increased GFAP+ astrocyte density in the rat cerebral cortex following repeated gavage [34] and in a period of alcohol withdrawal [37], while others show a reduction in the number of GFAP+ astrocytes in the orbitofrontal cortex after 3 weeks of abstinence from operant alcohol self-administration [38]. Activating astrocytes in the prefrontal cortex (PFC) of alcohol-naïve mice via chemogenetic approaches led to an increase in alcohol consumption [39]. Additionally, reducing astrocyte calcium activity in the PFC resulted in reduced alcohol intake in alcohol-naïve mice [39]. Interestingly, chemogenetic PFC astrocyte activation did not alter alcohol intake in mice with established drinking behaviors [39].

Alcohol-induced cytokine expression varies by brain region

Some of the most common cytokines involved in AUD are TNFα, IL-1β, and IL-6 [11,27,40,41]. In pre-clinical models, chronic alcohol exposure has been shown to upregulate the expression of IL-1β and IL-6 in the serum and the hippocampus [42]. In the cerebellum of chronic alcohol-fed mice, the levels of TNFα and IL-1β were elevated, in a toll-like receptor 4 (TLR4) dependent mechanism [43]. Chronic vapor alcohol exposure also increases TNFα expression in the nucleus accumbens (NAc) and the expression of IL-6 in ventromedial prefrontal cortex (vmPFC) and the NAc in both males and females [44]. A single 14-hour vapor exposure led to an increase in TNFα expression not only in the NAc, but also the basolateral amygdala (BLA) and the ventral tegmental area (VTA) [44]. This single alcohol vapor exposure also led to an increase in IL-6 expression in the NAc and the VTA, but not in the vmPFC, and this upregulation was specific to males only [44]. Therefore, the effect of alcohol on cytokine expression throughout the brain may be dependent on sex, the timing of alcohol exposure, and the region of the brain.

A critical brain region for AUD development is the central amygdala (CeA). The CeA is not only integral to the emotional and motivational aspects of AUD, but also plays a pivotal role in binge behaviors and acts as an integrative hub for numerous alcohol related circuits [45]. The remainder of the review will focus specifically on the CeA, discussing the role of CeA in AUD development and CeA neuroimmune activity in alcohol behaviors and mechanisms of action.

The role of central amygdala mechanisms in alcohol behavior

To understand how the CeA neuroimmune activity is involved in alcohol related behaviors, it is important to first understand how alcohol affects CeA neurocircuit function and how these mechanisms lead to changes in alcohol behaviors.

CeA involvement in alcohol-related behaviors

Early studies implicating the CeA in alcohol consumption and alcohol-directed behaviors showed that $GABA_A$ receptor antagonism and opioid receptor antagonism in the CeA decrease alcohol self-administration [46–48]. Lesions of the CeA reduce alcohol intake, with no effect on overall fluid intake [49]. Intraperitoneal (i.p.) injections of alcohol lead to increased immediate early gene (IEG) activation, namely *c-fos* and *FosB*, in the CeA [50]. Further studies indicate potential sex difference in alcohol effects on IEGs, as males exposed to alcohol-containing diet had an increase in *FosB* activity, while females had an increase in *c-fos* activity in the CeA [51].

In addition to IEG activation, studies showed that neuronal ensembles in the CeA may be involved in alcohol seeking, especially in rats with a history of alcohol binge drinking [52,53]. Inactivation of CeA neuronal ensembles during alcohol abstinence from two-bottle choice and from chronic intermittent alcohol vapor exposure resulted in a reduction in alcohol drinking in both alcohol-dependent and non-dependent rats [53]. Further studies indicate meta-ensembles encoding alcohol and saccharin reward found that the CeA is one of the most important nodes of the alcohol network in rats that self-administered alcohol. Although the amygdala played a role in the saccharin network in rats, the CeA involvement in the alcohol network was ranked much higher than in the saccharin network [54]. This result suggests that the CeA is preferentially involved in the processing of alcohol-associated stimuli, as opposed to general reward-associated stimuli. Combined, these data suggest that alterations to CeA function is critical to alcohol intake in multiple models, and that these alterations likely occur via cellular and synaptic mechanisms. A deeper understanding of alcohol effects on CeA neurotransmission in cell-type dependent mechanisms may shed light on the mechanisms driving development of alcohol use disorder and uncover potential new treatment strategies.

CeA neurotransmission in alcohol-related behaviors and mechanisms

The CeA receives inputs from various cortical and subcortical regions and primarily contains GABAergic cells, with some glutamatergic cells [45,55], all of which have been shown to be modulated by alcohol exposure. To date, research on alcohol's effects on CeA neurotransmission has predominantly focused on GABAergic neurotransmission in the medial subdivision of the CeA. Studies in the medial subdivision of the CeA mostly show an increase in GABAergic transmission after acute bath application of alcohol to CeA-containing brain slices or following a history of alcohol consumption or alcohol dependence induction [56–59]. For instance, after chronic 14-day intragastric alcohol gavage to induce dependence phenotypes, alcohol withdrawal decreases GABAA receptor α1 subunit expression and increases δ subunit expression in the CeA in male rats [60]. This model of chronic alcohol exposure increases inhibitory transmission in males, which may

be specific to PKC δ and calbindin 2 cell populations [60]. In contrast, female rats were not shown to exhibit cell-type specific changes in inhibitory transmission after alcohol exposure but show increased excitability in somatostatin cells in the CeA [60]. The effect of GABAergic transmission in the lateral subdivision of the CeA on alcohol has yet to be determined.

Similar to GABAergic transmission, the effects of alcohol on glutamatergic transmission in the medial subdivision of the CeA may be dependent on sex, strain, type of electrophysiological recording, and history of alcohol exposure (for further review see [55]). Overall, alcohol exposure both acutely in a slice and in AUD models reduces glutamatergic signaling in the medial subdivision of the CeA [55]. The role of glutamatergic transmission in the lateral subdivision of the CeA is less explored. Studies from our lab show acute bath alcohol application increases presynaptic glutamatergic activity in the lateral subdivision of the CeA in alcohol naïve mice [61,62]. In addition to modulation by corticotropin releasing factor receptor (CRFR) antagonists [62], alcohol enhancement of glutamatergic transmission in the lateral CeA is mediated by astrocytes, but not microglia [61]. This result suggests that alcohol requires functional activation of astrocyte-specific signals to modulate CeA glutamatergic transmission, at least in alcohol-naïve mice. Combined, these findings suggest that the alcohol-induced changes in inhibitory transmission and neuronal excitability in the medial and lateral CeA are cell-type specific.

CeA cell-type specific changes in response to alcohol

The alcohol effect on the various CeA cell types and their effects on alcohol-directed behaviors has been widely explored. Notably, corticotropin-releasing factor (CRF)-containing neurons are one of the most well studied cell types in the CeA in alcohol-related research. CRF signaling is critical for regulation of alcohol effects on both GABAergic and glutamatergic neurotransmission in the CeA [45,59,63]. Studies found that repeated binge alcohol intake increased the excitability of CeA CRF neurons, particularly in a subset of CRF neurons that were activated pre-lick [64]. Rats in alcohol withdrawal had increased Fos+ and CRF neuron colocalization, suggesting that in alcohol withdrawal, CRF neurons are activated [65]. Additionally, optogenetic inactivation of CeA CRF neurons led to reductions in alcohol consumption in alcohol-dependent but not non-alcohol-dependent rats [65]. Chemogenetic inhibition of CRF neurons in the CeA reduces alcohol intake in a drinking-in-the-dark (DID) paradigm, a model of binge drinking and alcohol dependence [66]. However, chemogenetic activation or inhibition of CeA CRF neurons does not have an effect on escalation of alcohol intake in CIE-withdrawn mice [67], suggesting CeA CRF neuron activity may have distinct roles in various stages of the AUD cycle. However, CRF receptor 1 (CRFR1) inhibition did lead to reductions in alcohol intake this study [67]. Furthermore, it is known that alcohol acts on CeA excitatory and inhibitory neurotransmission. Studies from our lab have shown that in the lateral subdivision of the CeA, brain slice application of alcohol increases glutamatergic transmission [61,62]. CRFR antagonism reduces this increase in glutamatergic transmission, however, CRF neuron ablation in the CeA and in the whole brain does not alter this effect [62]. Together, these results suggest a potential dichotomy between CRF neurons and CRFRs in the effect of

alcohol in the CeA, potentially suggesting other CRF sources [68] or CRF analogues like urocortins [69] may be important regulators of CeA signaling in AUD.

Other CeA cell types/peptides involved in alcohol consumption include neuropeptide Y (NPY), protein kinase C (PKC), nociceptin/orphanin (NOP), neurotensin, dynorphin, and others. Briefly, infusion of NPY into the CeA significantly reduced alcohol intake in a two-bottle choice paradigm [70]. PKCe antagonist infusion into the CeA alone or in combination with metabotropic glutamate receptor antagonists reduces alcohol intake in the DID paradigm [71]. NOP antagonist microinjection into the CeA reduces voluntary alcohol drinking in a two-bottle choice paradigm [72]. Ablation of neurotensin neurons in the CeA significantly decreases alcohol intake, with no effect on sucrose, saccharin, or quinine intake, in a two-bottle choice paradigm [73]. The targeted chemogenetic silencing of dynorphin neurons and kappa opioid receptor antagonism in the CeA reduced stress-enhanced alcohol consumption in mice [74]. Overall, the manipulation of the various cell types in the CeA leads to changes in alcohol-motivated behaviors.

The question remains as to how these various cell-type and circuit specific mechanisms integrate into a unifying mechanism regulating alcohol intake. We posit that neuroimmune signaling may play a role in coordinating alcohol effects between CeA cell-types and circuits. The following section will focus on the specific roles of astrocytes, microglia, and neuroimmune signals in the effect of alcohol on CeA neurotransmission.

The role of CeA neuroinflammation on alcohol effects

Despite playing an important role in the brain's response to alcohol, there is limited research into how neuroimmune signaling in CeA impacts, or is impacted, by alcohol use. Recent studies have explored the role of neuroinflammation in the CeA in terms of alcohol-induced changes to neurotransmission, gene and protein expression, and alcohol-related behaviors. Overall, the results of these studies are highly dependent on strains of rodents used, subregions of the amygdala, model of alcohol exposure, and the heterogeneity of not only the neurons, but also the neuroimmune cells being studied. To parse out these differences, this section will go through findings on the roles of astrocytes, microglia, and cytokines on alcohol effects in the CeA.

Astrocytes

Studies from our lab indicate the critical importance of astrocytes in the effects of alcohol on glutamatergic transmission in the CeA, showing that both pharmacologic and chemogenetic inhibition of astrocytes attenuates bath alcohol-induced increase in glutamatergic transmission in the lateral subdivision of the CeA of naïve mice [61]. Expanding the scope of these findings, a recent study using the CIE vapor model found an overrepresentation of astrocyte-specific genes and overrepresentation of functional groups related to glial cell, endothelial cells and innate immune response regulation in rats exposed to CIE [75]. These findings suggest that after chronic alcohol, there is an overall upregulation of neuroimmune systems, particularly through astrocyte mediated processes [75]. In contrast, another study showed that 14 intake sessions via the every-other-day intermittent 2-bottle choice (a model for escalation of alcohol intake and alcohol preference)

or the DID models did not alter CeA GFAP+ cell numbers, an immunohistochemical marker of astrocytes [76], but did not assess functional relationships. Combined, we propose a mechanism of alcohol actions on the CeA, where acute alcohol exposure to the slice increases glutamatergic transmission in the lateral CeA through the involvement of astrocytes. These effects may be engaged in certain alcohol exposure models, and the mechanisms for this differentiation should be further examined. In the larger picture, lateral CeA neurons are known to provide GABAergic projections to the medial CeA. Therefore, increased excitatory glutamatergic transmission onto lateral CeA neurons may be directly related to the known effects of alcohol to increase GABAergic transmission in the medial subdivision [55]. Overall, further studies are needed to fully elucidate the role of CeA astrocytes in alcohol consumption and alcohol-induced effects in the CeA.

Microglia

Similar to astrocytes, the role of microglia in alcohol-induced effects on CeA transmission is becoming increasingly studied. Microglia can exist in a spectrum of pro- and anti-inflammatory states which makes interpretation of various studies on alcohol effects on microglia often difficult to interpret. For instance, using flow cytometry, 4-day binge alcohol exposure increased markers of microglial activity in the hippocampus of adolescent male rats, however, the cell markers and the cytokine profile suggested that the activated microglia populations may have been in pro-inflammatory and anti-inflammatory states [77,78]. Using Iba-1 immunoreactivity to examine microglia morphology as an assessment of activation states, a study exploring the time course of microglial activation in response to CIE exposure and withdrawal found increased Iba-1 density in the amygdala immediately after the end of vapor, 1 day and 28 days into withdrawal compared to control rats [79]. Other types of alcohol exposures paradigms, such as 1–3 cycles of DID, may not produce significant effects on microglia function in the CeA, at least as measured by Iba-1 immunoreactivity [80], suggesting that microglia regulation of CeA function may be altered in various alcohol exposure models. In support of this hypothesis, a recent study showed that mice given CIE to mimic dependence phenotypes have a trending increase in Iba1-positive cells in the CeA [81]. The study also showed that microglia depletion through a special diet containing an inhibitor of colony-stimulating factor 1 receptor, a receptor important for microglia survival, reduced alcohol intake in alcohol dependent mice, with no effect on non-alcohol dependent mice [81]. In alcohol dependent mice, microglia depletion also reduced excitatory transmission in a presynaptic manner, with no differences in non-alcohol dependent mice compared to control diet [81]. Microglia depletion reduced inhibitory neurotransmission in both alcohol dependent and non-alcohol dependent mice in a presynaptic and a postsynaptic manner [81]. The dichotomy between the effect of microglial depletion on glutamatergic transmission in alcohol dependent and non-alcohol dependent mice aligns with previous findings from our lab showing that pharmacologic microglial inhibition does not significantly alter alcohol-induced increases in glutamatergic transmission in the CeA in naïve mice [61]. Taken together, these findings suggest that microglia may be more involved in the alcohol effect on CeA excitatory transmission after induction of alcohol dependence, but not in an acute or non-alcohol dependent state. Future studies will be needed to further explore this dichotomy of microglial involvement in alcohol consumption between alcohol dependence and non-alcohol dependence.

Regulators and mediators of neuroimmune signaling in AUD

Interleukins. Interleukins (ILs), one of the most prominent and widely expressed cytokines in the body, play an important role in nearly every aspect of immune signaling and can ultimately induce pro- and anti-inflammatory outcomes. Given the pro-inflammatory nature of chronic alcohol use, it is unsurprising that the ILs have been implicated in alcohol dependence, particularly in the CeA and can directly alter CeA neurotransmission without alcohol exposure as well as modulate the effects of alcohol on CeA neurotransmission. IL-1β has been widely studied in the CeA in alcohol dependence models. In alcohol exposed mice IL-1β levels were elevated in neurons and microglia compared to no alcohol exposure, and this effect was exacerbated in alcohol-dependent mice [82]. Conversely, long-term abstinence after chronic alcohol administration in rhesus macaques did not affect the levels of IL-1β or IL-1ra (interleukin-1 receptor 1 antagonist) in the CeA [83]. Electrophysiological recordings examining GABAergic activity in the CeA showed that macaques in alcohol abstinence are less sensitive to the dampening effect of IL-1β compared to controls [83]. IL-1β bath application on slices from naïve, non-alcohol dependent or from alcohol dependent mice led to dual effects, with some cells showing an increase in action-potential dependent inhibitory neurotransmission both pre- and post-synaptically, and others showing a decrease [82]. The same result was seen with IL-1ra. However, acute alcohol sensitivity to GABAergic synapses in the CeA does not seem to be modulated by IL-1β or IL-1ra in naïve, non-dependent or in dependent mice, since co-application of IL-1β and alcohol or IL-1ra and alcohol did not result in any significant changes or correlations in the neurons' response [82]. Conversely, an earlier study from the same group showed that alcohol's enhancement of action-potential independent inhibitory neurotransmission is lost in IL-1β responsive neurons [84]. However, the study did not find any effect of IL-1β on alcohol mediated increase in evoked inhibitory neurotransmission [84]. These effects appear to important across species, as studies in rhesus macaques also indicate significant alterations to IL-1β function following chronic alcohol exposure and abstinence [83]. These findings suggest an interplay between the IL-1 system and alcohol-induced facilitation of certain forms of inhibitory transmission in the CeA.

The pro-inflammatory cytokine IL-6 has also been implicated in alcohol effects in the CeA and may have interactions with IL-1 signaling discussed above. CeA inhibitory transmission is decreased in astrocytic IL-6 knockout mice compared to IL-6 homozygous mice [85]. These homozygous mice also have increased CNS excitability compared to knockout mice during alcohol withdrawal, as shown by an increased handling-induced convulsions score [85]. *IL-6* is also a significant differentially expressed gene in CIE vapor exposed mice [75]. These findings point to the significance of IL-6 in alcohol behaviors due to their effect on CeA neurotransmission and their involvement in alcohol dependence and withdrawal. A study investigating the impact of IL-6 and IL-1β on conditioned responses to alcohol (such as cues or odors) found that IL-6 levels increased while IL-1β levels decreased [86]. These changes in cytokine concentrations were observed only 3 h after the intubation of lemon-flavored alcohol, suggesting that the timing of intoxication may influence these effects [86]. It is worth noting that even though both IL-1β and IL-6 act as pro-inflammatory cytokines, IL-6 can also act in an anti-inflammatory manner [87]. Therefore, it is possible that when alcohol is first ingested, the neuroimmune system is

activated, and anti-inflammatory processes are triggered to battle the negative effects of alcohol on the neuroimmune system, but with more chronic alcohol consumption, the concentration of pro-inflammatory cytokines is increased.

Another pro-inflammatory cytokine, IL-18, has also been implicated in alcohol use and stress, although not many studies have been conducted in the CeA. Immediately after the last vapor exposure in CIE, rats had decreased serum IL-18 concentration [79]. However, this decrease was not evident 1 or 28 days into withdrawal [79]. A study explored the role of IL-18 on rats that received concurrent shock exposure in addition to 2-bottle choice in familiar and novel environments [88]. The study revealed that in male rats with access to alcohol but no stress, the expression of IL18r1 remained unchanged across different contexts [88]. However, exposure to stress in a familiar environment significantly reduced IL-18 binding protein expression and increased IL-18+ cells. This finding suggests that the role of IL-18 may be influenced by stress exposure and environmental context. Electrophysiological findings in the same study indicated that IL-18 decreases inhibitory neurotransmission in the alcohol-exposed, unstressed males, but not in the stressed males [88]. In contrast, females showed a reduction in inhibitory response in response to IL-18 across all conditions [88]. These findings suggest that the effect of IL-18 on neuroinflammatory responses to alcohol and stress is complex and sex dependent, warranting further investigation to understand the mechanisms and the potential therapeutic targets.

IL-10, an anti-inflammatory cytokine, presents mixed findings. Some studies show decreased levels of CeA IL-10 following chronic alcohol exposure and show that IL-10 exposure attenuates alcohol's effect on inhibitory neurotransmission [89]. Other studies show that 1 or 3 cycles of DID do not affect the IL-10 levels in the CeA, however, the levels of this cytokine are decreased in the basolateral amygdala [90]. When looking at protein expression through ELISA throughout the entire amygdala, the study found decreased IL-10 levels in mice that were exposed to alcohol through DID. Other work has found that prenatal alcohol exposure combined with early life adversity in rat models can lead to increased IL-10 levels in the amygdala at postnatal day 12 [91]. Therefore, the role of IL-10 may be dependent on the type of alcohol exposure and may be more evident in the basolateral amygdala instead of the CeA.

TNFa. TNFα, while widely studied in the CeA in terms of stress [92], has been less explored in the alcohol context compared to other cytokines such as interleukins. Though limited, there is some support for TNFα being involved in the effects of alcohol on CeA. Chronic alcohol exposure leads to increased CeA *TNFα* gene expression during withdrawal [93]. By contrast, acute ethanol intoxication leads to decreased *TNFα* in amygdala [94]. Microinjection of TNFα into CeA sensitized withdrawal-induced anxiety in rats compared to vehicle in a social interaction test [95]. Conversely, when paired with a familiar or novel stressor, alcohol led to a reduction in CeA *TNFα* levels in rats [86]. A gene expression study exploring the roles of various cytokines in response to intermittent alcohol exposure found no differences *TNFα* compared to the control group [96]. These contradictory findings point to the need to further study the role of CeA TNFα in alcohol use.

Toll-like receptors (TLRs). TLRs are a family of pathogen detecting receptors that modulate pro-inflammatory signaling cascades in both the body and the brain. Under healthy conditions, levels of these receptors are low in brain [97]. Alcohol has been shown to activate several TLR subtypes though the majority of its pro-inflammatory actions are linked to TLR4 [98,99]. In CeA electrophysiological recordings, a pretreatment with a TLR4 inhibitor had no effect on GABAergic neurotransmission on its own but blunted alcohol induced potentiation of GABAergic transmission [100]. Further work indicates that TLR4 inhibition leads to decreased alcohol intake with no effect on saccharin intake in both control and CIE mice [101]. Additionally, TLR4 deficiency is protective against alcohol-induced glial activation, induction of other inflammatory mediators, and eventual apoptosis [98]. TLR4 inhibition also leads to a reduced overall abundance of Iba-1 in the CeA and normalizes the number of microglia after CIE to a level similar to the control group [101]. The mechanism of TLR4 involvement in alcohol use may be downstream of the α2-subunit containing $GABA_A$ receptors (GABRA2), since the GABRA2 siRNA vector infusion in the CeA reduced alcohol binge drinking and significantly decreased the levels of GABRA2 and TLR4 in the CeA [102]. TLR4 siRNA in the CeA also reduced alcohol intake, further suggesting similarities, and potentially interplay, in the mechanisms of alcohol modulation of TLR4 and GABRA2 signaling [102]. It is important to note that TLR4 levels may also be dependent on patterns of alcohol intake, therefore the changes in TLR4 levels in response to alcohol may be due to various mechanisms [103]. For example, in naïve rats, bath alcohol exposure to the slice increases GABAergic activity in the CeA TLR4 KO rats. However, in CIE exposed rats, this effect is reversed, with TLR4 KO attenuating the increase in GABAergic transmission in response to bath alcohol exposure [104]. In alcohol preferring P rats TLR4 has been shown to colocalize with MCP-1 (Monocyte chemoattractant protein-1), a chemokine involved in recruitment and activation of microglia and playing an important role in the stimulation of inflammatory pathways in alcohol use [105]. MCP-1 siRNA vector infused into the CeA reduced binge alcohol consumption [106], similar to findings with TLR4 siRNA, suggesting that MCP-1 may be an important factor in alcohol effects on CeA function via TLR4 signaling. Interestingly, GABAA-TLR4-MCP1 interactions may be downstream of the critical CeA CRF system in alcohol intake and dependence models, as the CeA antagonism of CRFR1 has been shown to inhibit the expression of TLR4 in alcohol-drinking P rats [106].

Downstream signaling factors in the TLR4 pathway may also be involved in alcohol effects in the CeA. Inhibitor of nuclear factor kappa-B kinase (IKKb) is crucial for the translocation of nuclear factor kappa B (NFκB) to the nucleus inducing pro-inflammatory cytokines and is directly modulated via TLR4 signaling. IKKb knockdown in the CeA decreased alcohol consumption and alcohol preference, with no changes in total fluid intake [107]. In an every-other-day 2-bottle choice paradigm, there was a decrease in IKKe and an increase in interferon regulatory factor 3 (IRF3) both of which are critical nodes in the TLR signaling pathway, 24 h after the removal of alcohol [108]. In addition to changes in IKKe and IRF3, there was a decrease in TLR4 transcript in the CeA 24 h after the removal of alcohol, with no changes immediately after the removal of alcohol. The increased IRF3 could have been a compensatory increase due to the decreased TLR signaling [108]. Together, these studies suggest CeA TLR signaling is a promising target for studying the effects of alcohol and

further studies are needed to understand the role of CeA TLRs in alcohol seeking/craving, consumption, and withdrawal.

CD11b and CD14. CD11b is a microglial activation marker and has been implicated in alcohol use, with binge alcohol intake increasing CD11b levels [77]. Even though CD11b has been widely studied for alcohol use, to our knowledge there has only been one study examining the effect of alcohol exposure on CeA CD11b levels. The study found that one time intragastric alcohol exposure in adult rats resulted in no changes in CD11b immunoreactivity compared to control [109]. However, in adolescent mice, through a chronic intragastric alcohol exposure on a 2-days on/2-days off schedule and following an acute stressor, there was a significant increase in CD11b immunoreactivity [109]. Further studies are needed to parse out these differences in CeA CD11b immunoreactivity between age and acute and chronic alcohol administration.

CD14 is a co-receptor for TLRs and is highly involved in immune regulation [110]. CD14 knockout (KO) mice had altered alcohol response in a dose-specific manner. Specifically, bath alcohol application (44 and 66 mM) to the CeA did not alter the inhibitory neurotransmission in the CeA in CD14 KO mice, but did increase inhibitory activity in WT mice [100]. However, the effect of alcohol was the same between CD14 KO and wildtype mice at the 100 mM concentration [100]. These results suggest that at physiologically relevant concentrations, alcohol's effect on GABAergic transmission is partially mediated by CD14, but that CD14 is not required for the effects of higher alcohol concentrations. Further studies are needed to understand the role of CD14 in alcohol use.

Cannabinoids

The endocannabinoid (eCB) system is highly involved in both GABAergic and glutamatergic transmission in the CeA. CB1 receptors are present on astrocytes in the CeA, and eCBs released from neurons in the medial subdivision of the CeA activate astrocytic CB1 receptors, leading to an increase in astrocytic calcium levels [111]. This rise in astrocytic calcium levels subsequently enhances inhibitory transmission in the CeA. This increase in inhibitory activity is abolished in the presence of a CB1 receptor antagonist or in mice lacking CB1 receptors in astrocytes [111]. Furthermore, the application of an adenosine A_{2A} receptor antagonist, a receptor important for astrocyte-neuron communication [112], also abolishes the enhancement of inhibitory activity [111]. Therefore, the activation of the CB1 receptors mediates the evoked increase in inhibitory currents and leads to the activation of adenosine A_{2A} receptors in astrocytes to potentiate the inhibitory synaptic transmission in the medial CeA subdivision [111]. Such results suggest CB signaling may mediate or modulate alcohol effects on CeA signaling via regulation of neuroimmune signaling.

CB1 receptor manipulations lead to divergent changes in inhibitory and excitatory transmission in the CeA. Activation of CB1 prevents alcohol-induced increase in GABAergic transmission, while antagonism of the CB1 receptor increases GABAergic transmission in both naïve and CIE rats [113,114]. In the medial subdivision of the CeA, CB1 agonist exacerbates the alcohol-induced decrease in evoked glutamatergic transmission

in a sex and strain specific manner [115]. In the lateral subdivision, CB1 suppresses the release of glutamate [116], although the interaction between CB1 and alcohol in the lateral CeA has not yet been extensively studied. To that end, our recent studies showed that cannabinoid treatment can reduce aspects of acute alcohol withdrawal induced anxiety-like behaviors in mice, which may be due to CB interactions with neuroimmune signaling in the lateral CeA [117]. Additional work will be needed to further test the mechanism(s) by which this occurs.

Further findings suggest that alcohol downregulates the CB1 function [114,115] in the CeA and that the CB1 receptor is an important target for the inhibition of alcohol-induced effects on CeA transmission. Considering the interplay between CB1 and alcohol to alter glutamatergic activity in the CeA, it is possible that astrocytic modulation of the alcohol effects on glutamatergic transmission in the CeA is in part due to CB1 receptor inhibition. Therefore, this interaction between the eCB system and astrocytes to mediate neurotransmission in the CeA needs to be further explored in the context of alcohol.

Clinical studies on neuroimmune modulators as treatment for AUD

Clinical studies have started to examine various neuroinflammatory system components as potential therapeutics for AUD. Here we provide an overview of recent clinical studies examining neuroimmune modulators as AUD treatments.

Phosphodiesterase inhibitors

Phosphodiesterases (PDEs) are enzymes that degrade second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [118]. These second messengers are involved in inflammatory activity, with low levels of cAMP increasing inflammation in the brain [118]. PDE4 inhibition, via the degradation of cyclic AMP, is correlated with decreased production of pro-inflammatory cytokines and an increase in anti-inflammatory cytokines [119]. PDEs are widely expressed in the brain, including in the amygdala [120,121]. Given positive results in preclinical studies [122], PDE4 inhibitors have been studied clinically for the treatment of AUD.

Ibudilast, a PDE inhibitor acting on PDE3, PDE4, PDE10, and PDE11, has been extensively studied in clinical trials in the context of AUD. In a clinical trial in 2017, after an infusion of alcohol, ibudilast did not affect subjective response to alcohol craving, stimulation, sedation, or mood [123]. In stress-induced alcohol craving, ibudilast promoted faster recovery to a positive mood compared to placebo, although the difference was not significant [123]. A follow up study in 2018 suggested that ibudilast modulates mood states, making happiness and depression a stronger predictor of alcohol craving compared to placebo [124]. In a further trial in 2021, ibudilast reduced the number of heavy drinking days compared to placebo and attenuated alcohol cue-elicited activation in the ventral striatum, although it did not affect mood, contrasting previous findings [125]. A secondary analysis of this clinical trial found that ibudilast reduced alcohol cue-induced functional connectivity between the ventral striatum and reward processing regions of the brain compared to control, potentially reducing alcohol reward processing [126]. A separate analysis of this study found no effect of ibudilast on levels of stimulation and sedation during alcohol drinking [127]. However,

participants on ibudilast reported consuming more alcohol when feeling more stimulated or sedated. Related studies showed participants with ibudilast had significantly lower levels of both peripheral and central neuroimmune markers compared to placebo [128]. Overall, ibudilast modulates the relationship between mood and alcohol craving and consumption, suggesting neuroimmune signaling may be an important target for treatment development but increased selectivity is needed.

Apremilast, a PDE4 specific inhibitor, has been tested in a phase IIa double-blind clinical trial for reduced alcohol intake in AUD patients. The study found that apremilast was mostly well-tolerated and significantly reduced the number of drinks per day compared to placebo during an 11-day period [129]. Participants who were given apremilast had a greater decline in drinking, with individuals with higher baseline craving scores reducing their drinking at a faster rate [129]. Further studies, with larger study population are needed to explore the efficacy of apremilast in alcohol seeking and drinking long-term. Overall, these studies suggest that targeting pathways to mitigate inflammation may show promise in reducing alcohol intake but that further studies are needed to better understand the mechanisms by which this occurs to increase target specificity.

Minocycline

Minocycline is an FDA approved tetracycline antibiotic. Minocycline is also commonly used to inhibit microglial activation in preclinical studies [130]. There have been a limited number of clinical trials exploring the efficacy of minocycline for alcohol use. In a double-blind, placebo-controlled trial, even though minocycline administration did not result in many side effects, the participants did not feel any change in subjective response to alcohol or alcohol-induced craving after minocycline treatment at two different doses for 10 days [131]. At the time of this review, a robust phase 1 clinical trial to explore the efficacy and mechanisms of action of minocycline for AUD was recently completed but the findings have not yet been published (ClinicalTrials.gov ID: NCT04210713).

These clinical studies indicate the therapeutic potential of targeting neuroimmune signaling in AUD. To improve on these medications, it is imperative to understand how the neuroimmune system interacts with critical neurocircuits in the development of AUD. We posit such studies, particularly in brain regions heavily involved in alcohol use such as the CeA, will help determine novel neuroimmune-related treatment targets.

Conclusions

Alcohol and inflammation go hand in hand. This review discussed the numerous clinical and preclinical studies implicating the neuroimmune system in alcohol use and alcohol-related behaviors, with an emphasis on the understudied role of CeA neuroimmune signaling in alcohol use. Significant gaps remain in our understanding of the mechanisms underlying interactions between CeA neuroimmune signaling, CeA neurotransmission, and alcohol-related behaviors. As such, it is imperative to further explore the various neuroimmune factors, including glial cells, cytokines, and inflammatory pathway signaling factors, and the interaction of these neuroimmune factors with neurotransmission in the CeA in healthy and in alcohol-exposed brain. The mixed findings on alcohol's effect on neuroimmune

activity suggest a diversity in neuroimmune response, similar to the diversity in CeA neurotransmission across different cell types and subregions. It is possible that microglia and astrocytes heterogeneously respond to the actions of alcohol based on subtypes and/or activation states, and this gap in knowledge should be directly examined in future studies. Differentiating immune cell subtypes by their cytokine profiles and involvement in synaptic transmission may help uncover novel mechanisms of alcohol in the CeA. Increased examination of pharmacologic manipulations on CeA neuroimmune function and impacts on CeA neuronal subtypes is also critical. The understanding of the mechanism behind alcohol use in terms of neuroimmune functions should also extend beyond the CeA, exploring other cortical and subcortical regions in the brain implicated in alcohol use. Advancing research on these neuroimmune factors in alcohol use could provide new insights for effective AUD treatments.

Acknowledgements

Funding by PA Options for Wellness Academic Clinical Research Center (ACRC) Student Award and NIH grant AA026865.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

YS has minimal ownership of FLORA, a Vermont adult-use cannabis retailer. FLORA was not involved in any portion of any study, their funding, or the decision to submit the article for publication.

The remaining authors declare that the manuscript was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

[1]. Understanding Alcohol Use Disorder | National Institute on Alcohol Abuse and Alcoholism (NIAAA), (n.d.). https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/understanding-alcohol-use-disorder (accessed May 4, 2021).

[2]. Winslow BT, Onysko M, Hebert M, Medications for alcohol use disorder, Am. Fam. Physician 93 (2016) 457–465. [PubMed: 26977830]

[3]. Stokes M, Patel P, Abdijadid S, Disulfiram, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2024. http://www.ncbi.nlm.nih.gov/books/NBK459340/(accessed October 29, 2024).

[4]. Maisel NC, Blodgett JC, Wilbourne PL, Humphreys K, Finney JW, Meta-analysis of naltrexone and acamprosate for treating alcohol use disorders: when are these medications most helpful? Addict. Abingdon Engl 108 (2013) 275–293, 10.1111/j.1360-0443.2012.04054.x.

[5]. Morley KC, Perry CJ, Watt J, Hurzeler T, Leggio L, Lawrence AJ, Haber P, New approved and emerging pharmacological approaches to alcohol use disorder: a review of clinical studies, Expert Opin. Pharmacother 22 (2021) 1291–1303, 10.1080/14656566.2021.1892641. [PubMed: 33615945]

[6]. DiSabato D, Quan N, Godbout JP, Neuroinflammation: the devil is in the details, J. Neurochem 139 (2016) 136, 10.1111/jnc.13607. [PubMed: 26990767]

[7]. Woodburn SC, Bollinger JL, Wohleb ES, The semantics of microglia activation: neuroinflammation, homeostasis, and stress, J. Neuroinflammation 18 (2021) 258, 10.1186/s12974-021-02309-6. [PubMed: 34742308]

[8]. Nimmerjahn A, Kirchhoff F, Helmchen F, Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo, Science 308 (2005) 1314–1318, 10.1126/science.1110647. [PubMed: 15831717]

[9]. Lawrence JM, Schardien K, Wigdahl B, Nonnemacher MR, Roles of neuropathology-associated reactive astrocytes: a systematic review, Acta Neuropathol. Commun 11 (2023) 42, 10.1186/s40478-023-01526-9. [PubMed: 36915214]

[10]. Savtchouk I, Volterra A, Gliotransmission: beyond black-and-white, J. Neurosci 38 (2018) 14–25, 10.1523/JNEUROSCI.0017-17.2017. [PubMed: 29298905]

[11]. Adams C, Perry N, Conigrave J, Hurzeler T, Stevens J, Yacou Dunbar KP, Sweeney A, Lee K, Sutherland G, Haber P, Morley KC, Central markers of neuroinflammation in alcohol use disorder: a meta-analysis of neuroimaging, cerebral spinal fluid, and postmortem studies, Alcohol Clin. Exp. Res 47 (2023) 197–208, 10.1111/acer.14992.

[12]. Guest J, Grant R, Mori TA, Croft KD, Changes in oxidative damage, inflammation and [NAD(H)] with age in cerebrospinal fluid, PLoS One 9 (2014) e85335, 10.1371/journal.pone.0085335. [PubMed: 24454842]

[13]. Umhau JC, Schwandt M, Solomon MG, Yuan P, Nugent A, Zarate CA, Drevets WC, Hall SD, George DT, Heilig M, Cerebrospinal fluid monocyte chemoattractant protein-1 in alcoholics: support for a neuroinflammatory model of chronic alcoholism, Alcohol. Clin. Exp. Res 38 (2014) 1301–1306, 10.1111/acer.12367. [PubMed: 24689518]

[14]. He J, Crews FT, Increased MCP-1 and microglia in various regions of the human alcoholic brain, Exp. Neurol 210 (2008) 349–358, 10.1016/j.expneurol.2007.11.017. [PubMed: 18190912]

[15]. Crews FT, Sarkar DK, Qin L, Zou J, Boyadjieva N, Vetreno RP, Neuroimmune function and the consequences of alcohol exposure, Alcohol Res. Curr. Rev 37 (2015) 331–351.

[16]. McBride WJ, Kimpel MW, McClintick JN, Ding Z-M, Hauser SR, Edenberg HJ, Bell RL, Rodd ZA, Changes in gene expression within the ventral tegmental area following repeated excessive binge-like alcohol drinking by alcohol-preferring (P) rats, Alcohol 47 (2013) 367–380, 10.1016/j.alcohol.2013.04.002. [PubMed: 23714385]

[17]. Grantham EK, Tiwari GR, Ponomereva O, Harris RA, Lopez MF, Becker HC, Mayfield RD, Transcriptome changes in the nucleus of the solitary tract induced by repeated stress, alcohol dependence, or stress-induced drinking in dependent mice, Neuropharmacology (2023) 109768, 10.1016/j.neuropharm.2023.109768. [PubMed: 37865137]

[18]. Keller BN, Hajnal A, Browning KN, Arnold AC, Silberman Y, Involvement of the dorsal vagal complex in alcohol-related behaviors, Front. Behav. Neurosci 16 (2022) 801825, 10.3389/fnbeh.2022.801825. [PubMed: 35330845]

[19]. Keller BN, Snyder AE, Coker CR, Aguilar EA, O'Brien MK, Lookfong NA, Bingaman SS, Arnold AC, Hajnal A, Silberman Y, Vagus nerve damage increases alcohol intake and preference in a nonpreferring rat line: relationship to vagal regulation of the hypothalamic-pituitary-adrenal axis, Alcohol Clin. Exp. Res 48 (2024) 488–498, 10.1111/acer.15264.

[20]. Aguilar LA, Coker CR, McCullers Z, Evans A, Showemimo O, Melkumyan M, Keller BN, Snyder AE, Bingaman SS, Randall PA, Hajnal A, Browning KN, Arnold AC, Silberman Y, Adolescent alcohol disrupts development of noradrenergic neurons in the nucleus of the tractus solitarius and enhances stress behaviors in adulthood in mice in a sex specific manner, Addict. Neurosci 9 (2023) 100132, 10.1016/j.addicn.2023.100132. [PubMed: 38162404]

[21]. Keller BN, Randall PA, Arnold AC, Browning KN, Silberman Y, Ethanol inhibits pancreatic projecting neurons in the dorsal motor nucleus of the vagus, Brain Res. Bull 189 (2022) 121–129, 10.1016/j.brainresbull.2022.08.020. [PubMed: 35998791]

[22]. Farris SP, Tiwari GR, Ponomareva O, Lopez MF, Mayfield RD, Becker HC, Transcriptome analysis of alcohol drinking in non-dependent and dependent mice following repeated cycles of forced swim stress exposure, Brain Sci. 10 (2020) 275, 10.3390/brainsci10050275. [PubMed: 32370184]

[23]. Erickson EK, Blednov YA, Harris RA, Mayfield RD, Glial gene networks associated with alcohol dependence, Sci. Rep 9 (2019) 10949, 10.1038/s41598-019-47454-4. [PubMed: 31358844]

[24]. Osterndorff-Kahanek E, Ponomarev I, Blednov YA, Harris RA, Gene expression in brain and liver produced by three different regimens of alcohol consumption in mice: comparison with immune activation, PLoS One 8 (2013) e59870, 10.1371/journal.pone.0059870. [PubMed: 23555817]

[25]. Holloway KN, Pinson MR, Douglas JC, Rafferty TM, Kane CJM, Miranda RC, Drew PD, Cerebellar transcriptomic analysis in a chronic plus binge mouse model of alcohol use disorder demonstrates ethanol-induced neuroinflammation and altered glial gene expression, Cells 12 (2023) 745, 10.3390/cells12050745. [PubMed: 36899881]

[26]. Fernandez-Lizarbe S, Pascual M, Guerri C, Critical role of TLR4 response in the activation of microglia induced by Ethanol1, J. Immunol 183 (2009) 4733–4744, 10.4049/jimmunol.0803590. [PubMed: 19752239]

[27]. Marshall SA, McClain JA, Wooden JI, Nixon K, Microglia dystrophy following binge-like alcohol exposure in adolescent and adult male rats, Front. Neuroanat 14 (2020). https://www.frontiersin.org/articles/10.3389/fnana.2020.00052 (accessed September 7, 2023).

[28]. Streit WJ, Xue Q-S, Life and death of microglia, J. Neuroimmune Pharmacol 4 (2009) 371–379, 10.1007/s11481-009-9163-5. [PubMed: 19680817]

[29]. Hu P, Wang D, Zhang Y, Cai Z, Ye T, Tong L, Xu X, Lu J, Liu F, Lu X, Huang C, Apoptosis-triggered decline in hippocampal microglia mediates adolescent intermittent alcohol exposure-induced depression-like behaviors in mice, Neuropharmacology 170 (2020) 108054, 10.1016/j.neuropharm.2020.108054. [PubMed: 32217088]

[30]. Cruz C, Meireles M, Silva SM, Chronic ethanol intake induces partial microglial activation that is not reversed by long-term ethanol withdrawal in the rat hippocampal formation, Neurotoxicology 60 (2017) 107–115, 10.1016/j.neuro.2017.04.005. [PubMed: 28408342]

[31]. Nixon K, Kim DH, Potts EN, He J, Crews FT, Distinct cell proliferation events during abstinence after alcohol dependence: microglia proliferation precedes neurogenesis, Neurobiol. Dis 31 (2008) 218–229, 10.1016/j.nbd.2008.04.009. [PubMed: 18585922]

[32]. Karoly HC, Mueller RL, Andrade CC, Hutchison KE, Investigating relationships between alcohol and cannabis use in an online survey of cannabis users: a focus on cannabinoid content and cannabis for medical purposes, Front. Psychiatry 11 (2020) 613243, 10.3389/fpsyt.2020.613243. [PubMed: 33408657]

[33]. Choi SS, Lee HJ, Lim I, Satoh J, Kim SU, Human astrocytes: secretome profiles of cytokines and chemokines, PLoS One 9 (2014) e92325, 10.1371/journal.pone.0092325. [PubMed: 24691121]

[34]. Dalçik H, Yardimoglu M, Fi˙liż S, Gonca S, Dalçik C, Erden BF, Chronic Ethanol-Induced Glial Fibrillary Acidic Protein (GFAP) immunoreactivity: an immunocytochemical observation in various regions of adult rat brain, Int. J. Neurosci 119 (2009) 1303–1318, 10.1080/00207450802333672. [PubMed: 19922358]

[35]. Carlson ER, Guerin SP, Nixon K, Fonken LK, The neuroimmune system – where aging and excess alcohol intersect, Alcohol 107 (2023) 153–167, 10.1016/j.alcohol.2022.08.009. [PubMed: 36150610]

[36]. Adermark L, Bowers MS, Disentangling the role of astrocytes in alcohol use disorder, Alcohol. Clin. Exp. Res 40 (2016) 1802–1816, 10.1111/acer.13168. [PubMed: 27476876]

[37]. Miguel-Hidalgo JJ, Withdrawal from free-choice ethanol consumption results in increased packing density of glutamine synthetase-immunoreactive astrocytes in the prelimbic cortex of alcohol-preferring rats, Alcohol Alcohol. Oxf. Oxfs 41 (2006) 379–385, 10.1093/alcalc/agl006.

[38]. Bull C, Syed WA, Minter SC, Bowers MS, Differential response of GFAP-positive astrocytes in the rat prefrontal cortex following ethanol self-administration, Alcohol. Clin. Exp. Res 39 (2015) 650–658, 10.1111/acer.12683. [PubMed: 25833026]

[39]. Erickson EK, DaCosta AJ, Mason SC, Blednov YA, Mayfield RD, Harris RA, Cortical astrocytes regulate ethanol consumption and intoxication in mice, Neuropsychopharmacology 46 (2021) 500–508, 10.1038/s41386-020-0721-0. [PubMed: 32464636]

[40]. Mednova IA, Levchuk LA, Boiko AS, Roschina OV, Simutkin GG, Bokhan NA, Loonen AJM, Ivanova SA, Cytokine level in patients with mood disorder, alcohol use disorder and their comorbidity, World J. Biol. Psychiatry 24 (2023) 243–253, 10.1080/15622975.2022.2095439. [PubMed: 35818961]

[41]. Moura HF, Hansen F, Galland F, Silvelo D, Rebelatto FP, Ornell F, Massuda R, Scherer JN, Schuch F, Kessler FH, von Diemen L, Inflammatory cytokines and alcohol use disorder: systematic review and meta-analysis, Rev. Bras. Psiquiatr. Sao Paulo Braz 44 (2022) 548–556, 10.47626/1516-4446-2021-1893, 1999.

[42]. Jiang C, Zhang Y, Tang X, Jing C, Qiu S, Li B, Li Y, IL-6 and IL-1β upregulation and tau protein phosphorylation in response to chronic alcohol exposure in the mouse hippocampus, NeuroReport 32 (2021) 851, 10.1097/WNR.0000000000001661. [PubMed: 34029285]

[43]. Lippai D, Bala S, Csak T, Kurt-Jones EA, Szabo G, Chronic alcohol-induced microRNA-155 contributes to neuroinflammation in a TLR4-dependent manner in mice, PLoS One 8 (2013) e70945, 10.1371/journal.pone.0070945. [PubMed: 23951048]

[44]. Baxter-Potter LN, Henricks AM, Berger AL, Bieniasz KV, Lugo JM, McLaughlin RJ, Alcohol vapor exposure differentially impacts mesocorticolimbic cytokine expression in a sex-, region-, and duration-specific manner, Neuroscience 346 (2017) 238–246, 10.1016/j.neuroscience.2017.01.015. [PubMed: 28131626]

[45]. Gilpin NW, Herman MA, Roberto M, The central amygdala as an integrative hub for anxiety and alcohol use disorders, Biol. Psychiatry 77 (2015) 859–869, 10.1016/j.biopsych.2014.09.008. [PubMed: 25433901]

[46]. Hyytiä P, Koob GF, GABAA receptor antagonism in the extended amygdala decreases ethanol self-administration in rats, Eur. J. Pharmacol 283 (1995) 151–159, 10.1016/0014-2999(95)00314-B. [PubMed: 7498304]

[47]. Roberts AJ, Cole M, Koob GF, Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats, Alcohol. Clin. Exp. Res 20 (1996) 1289–1298, 10.1111/j.1530-0277.1996.tb01125.x. [PubMed: 8904984]

[48]. Heyser CJ, Roberts AJ, Schulteis G, Koob GF, Central administration of an opiate antagonist decreases oral ethanol self-administration in rats, Alcohol. Clin. Exp. Res 23 (1999) 1468–1476, 10.1111/j.1530-0277.1999.tb04669.x. [PubMed: 10512312]

[49]. Möller C, Wiklund L, Sommer W, Thorsell A, Heilig M, Decreased experimental anxiety and voluntary ethanol consumption in rats following central but not basolateral amygdala lesions, Brain Res. 760 (1997) 94–101, 10.1016/S0006-8993(97)00308-9. [PubMed: 9237523]

[50]. Ryabinin AE, Wang Y-M, Repeated alcohol administration differentially affects c-Fos and FosB protein immunoreactivity in DBA/2J mice, Alcohol. Clin. Exp. Res 22 (1998) 1646–1654, 10.1111/j.1530-0277.1998.tb03962.x. [PubMed: 9835277]

[51]. Retson TA, Hoek JB, Sterling RC, Van Bockstaele EJ, Amygdalar neuronal plasticity and the interactions of alcohol, sex, and stress, Brain Struct. Funct 220 (2015) 3211–3232, 10.1007/s00429-014-0851-4. [PubMed: 25081549]

[52]. George O, Hope BT, Cortical and amygdalar neuronal ensembles in alcohol seeking, drinking and withdrawal, Neuropharmacology 122 (2017) 107–114, 10.1016/j.neuropharm.2017.04.031. [PubMed: 28435008]

[53]. de Guglielmo G, Crawford E, Kim S, Vendruscolo LF, Hope BT, Brennan M, Cole M, Koob GF, George O, Recruitment of a neuronal ensemble in the central nucleus of the amygdala is required for alcohol dependence, J. Neurosci. Off. J. Soc. Neurosci 36 (2016) 9446–9453, 10.1523/JNEUROSCI.1395-16.2016.

[54]. Wandres M, Pfarr S, Molnár B, Schöllkopf U, Ercsey-Ravasz M, Sommer WH, Körber C, Alcohol and sweet reward are encoded by distinct meta-ensembles, Neuropharmacology 195 (2021) 108496, 10.1016/j.neuropharm.2021.108496. [PubMed: 33582149]

[55]. Melkumyan M, Silberman Y, Subregional differences in alcohol modulation of central amygdala neurocircuitry, Front. Mol. Neurosci 15 (2022) 1–23.

[56]. Roberto M, Gilpin NW, Siggins GR, The central amygdala and alcohol: role of γ-aminobutyric acid, glutamate, and neuropeptides, Cold Spring Harb. Perspect. Med 2 (2012), 10.1101/cshperspect.a012195.

[57]. Roberto M, Madamba SG, Stouffer DG, Parsons LH, Siggins GR, Increased GABA release in the central amygdala of ethanol-dependent rats, J. Neurosci 24 (2004) 10159–10166, 10.1523/JNEUROSCI.3004-04.2004. [PubMed: 15537886]

[58]. Herman MA, Kallupi M, Luu G, Oleata CS, Heilig M, Koob GF, Ciccocioppo R, Roberto M, Enhanced GABAergic transmission in the central nucleus of the amygdala of genetically selected Marchigian Sardinian rats: alcohol and CRF effects, Neuropharmacology 67 (2013) 337–348, 10.1016/j.neuropharm.2012.11.026. [PubMed: 23220399]

[59]. Roberto M, Kirson D, Khom S, The role of the central amygdala in alcohol dependence, Cold Spring Harb. Perspect. Med (2020) a039339, 10.1101/cshperspect.a039339.

[60]. Crofton EJ, O'Buckley TK, Bohnsack JP, Morrow AL, Herman MA, Divergent population-specific effects of chronic ethanol exposure on excitability and inhibitory transmission in male and female rat central amygdala, J. Neurosci (2023), 10.1523/JNEUROSCI.0717-23.2023. JN-RM-0717–23.

[61]. Melkumyan M, Snyder AE, Bingaman SS, Arnold AC, Silberman Y, Astrocytes play a critical role in mediating the effect of acute ethanol on central amygdala glutamatergic transmission, Neuropharmacology 205 (2022) 108918, 10.1016/j.neuropharm.2021.108918. [PubMed: 34896402]

[62]. Silberman Y, Fetterly TL, Awad EK, Milano EJ, Usdin TB, Winder DG, Ethanol produces corticotropin releasing factor receptor-dependent enhancement of spontaneous glutamatergic transmission in the mouse central amygdala, Alcohol. Clin. Exp. Res 39 (2015) 2154–2162, 10.1111/acer.12881. [PubMed: 26503065]

[63]. Agoglia AE, Herman MA, The center of the emotional universe: alcohol, stress, and CRF1 amygdala circuitry, Alcohol 72 (2018) 61–73, 10.1016/j.alcohol.2018.03.009. [PubMed: 30220589]

[64]. Aroni S, Marino RAM, Girven KS, Irving JM, Cheer JF, Sparta DR, Repeated binge ethanol drinking enhances electrical activity of central amygdala corticotropin releasing factor neurons in vivo, Neuropharmacology 189 (2021) 108527, 10.1016/j.neuropharm.2021.108527. [PubMed: 33741403]

[65]. de Guglielmo G, Kallupi M, Pomrenze MB, Crawford E, Simpson S, Schweitzer P, Koob GF, Messing RO, George O, Inactivation of a CRF-dependent amygdalofugal pathway reverses addiction-like behaviors in alcohol-dependent rats, Nat. Commun 10 (2019) 1238, 10.1038/s41467-019-09183-0. [PubMed: 30886240]

[66]. Marshall SA, Robinson SL, Ebert SE, Companion MA, Thiele TE, Chemogenetic inhibition of corticotropin-releasing factor neurons in the central amygdala alters binge-like ethanol consumption in male mice, Behav. Neurosci 136 (2022) 541–550, 10.1037/bne0000522. [PubMed: 35771510]

[67]. Kreifeldt M, Herman MA, Sidhu H, Okhuarobo A, Macedo GC, Shahryari R, Gandhi PJ, Roberto M, Contet C, Central amygdala corticotropin-releasing factor neurons promote hyponeophagia but do not control alcohol drinking in mice, Mol. Psychiatry 27 (2022) 2502–2513, 10.1038/s41380-022-01496-9. [PubMed: 35264727]

[68]. Simpson S, Shankar K, Kimbrough A, George O, Role of corticotropin-releasing factor in alcohol and nicotine addiction, Brain Res. 1740 (2020) 146850, 10.1016/j.brainres.2020.146850. [PubMed: 32330519]

[69]. Zuniga A, Ryabinin AE, Involvement of centrally projecting edinger-westphal nucleus neuropeptides in actions of addictive drugs, Brain Sci. 10 (2020) 67, 10.3390/brainsci10020067. [PubMed: 31991932]

[70]. Zhang H, Sakharkar AJ, Shi G, Ugale R, Prakash A, Pandey SC, Neuropeptide Y signaling in the central nucleus of amygdala regulates alcohol-drinking and anxiety-like behaviors of alcohol-preferring rats, Alcohol. Clin. Exp. Res 34 (2010) 451–461, 10.1111/j.1530-0277.2009.01109.x. [PubMed: 20028368]

[71]. Cozzoli DK, Courson J, Rostock C, Campbell RR, Wroten MG, McGregor H, Caruana AL, Miller BW, Hu J-H, Wu Zhang P, Xiao B, Worley PF, Crabbe JC, Finn DA, Szumlinski KK, Protein kinase C Epsilon activity in the nucleus accumbens and central nucleus of the amygdala mediates binge alcohol consumption, Biol. Psychiatry 79 (2016) 443–451, 10.1016/j.biopsych.2015.01.019. [PubMed: 25861702]

[72]. Borruto AM, Fotio Y, Stopponi S, Brunori G, Petrella M, Caputi FF, Romualdi P, Candeletti S, Narendran R, Rorick-Kehn LM, Ubaldi M, Weiss F, Ciccocioppo R, NOP receptor antagonism reduces alcohol drinking in male and female rats through mechanisms involving the central amygdala and ventral tegmental area, Br. J. Pharmacol 177 (2020) 1525–1537, 10.1111/bph.14915. [PubMed: 31713848]

[73]. Torruella-Suárez ML, Vandenberg JR, Cogan ES, Tipton GJ, Teklezghi A, Dange K, Patel GK, McHenry JA, Hardaway JA, Kantak PA, Crowley NA, DiBerto JF, Faccidomo SP, Hodge

CW, Stuber GD, McElligott ZA, Manipulations of central amygdala neurotensin neurons alter the consumption of ethanol and sweet fluids in mice, J. Neurosci 40 (2020) 632–647, 10.1523/JNEUROSCI.1466-19.2019. [PubMed: 31744862]

[74]. Haun HL, Lebonville CL, Solomon MG, Griffin WC, Lopez MF, Becker HC, Dynorphin/Kappa opioid receptor activity within the extended amygdala contributes to stress-enhanced alcohol drinking in mice, Biol. Psychiatry 91 (2022) 1019–1028, 10.1016/j.biopsych.2022.01.002. [PubMed: 35190188]

[75]. Kisby BR, Farris SP, McManus MM, Varodayan FP, Roberto M, Harris RA, Ponomarev I, Alcohol dependence in rats is associated with global changes in gene expression in the central amygdala, Brain Sci. 11 (2021) 1149, 10.3390/brainsci11091149. [PubMed: 34573170]

[76]. Brewton HW, Robinson SL, Thiele TE, Astrocyte expression in the extended amygdala of C57BL/6J mice is sex-dependently affected by chronic intermittent and binge-like ethanol exposure, Alcohol 108 (2023) 55–64, 10.1016/j.alcohol.2022.12.001. [PubMed: 36539069]

[77]. Peng H, Nixon K, Microglia phenotypes following the induction of alcohol dependence in adolescent rats, Alcohol. Clin. Exp. Res 45 (2021) 105–116, 10.1111/acer.14504. [PubMed: 33164228]

[78]. Peng H, Nickell CRG, Chen KY, McClain JA, Nixon K, Increased expression of M1 and M2 phenotypic markers in isolated microglia after four-day binge alcohol exposure in male rats, Alcohol Fayettev. N 62 (2017) 29–40, 10.1016/j.alcohol.2017.02.175.

[79]. Sanchez-Alavez M, Nguyen W, Mori S, Wills DN, Otero D, Ehlers CL, Conti B, Time course of microglia activation and brain and blood cytokine/chemokine levels following chronic ethanol exposure and protracted withdrawal in rats, Alcohol Fayettev. N 76 (2019) 37–45, 10.1016/j.alcohol.2018.07.005.

[80]. Nelson JC, Greengrove E, Nwachukwu KN, Grifasi IR, Marshall SA, Repetitive binge-like consumption based on the drinking-in-the-dark model alters the microglial population in the mouse hippocampus, J. Integr. Neurosci 20 (2021) 933–943, 10.31083/j.jin2004094. [PubMed: 34997716]

[81]. Warden AS, Wolfe SA, Khom S, Varodayan FP, Patel RR, Steinman MQ, Bajo M, Montgomery SE, Vlkolinsky R, Nadav T, Polis I, Roberts AJ, Mayfield RD, Harris RA, Roberto M, Microglia control escalation of drinking in alcohol-dependent mice: genomic and synaptic drivers, Biol. Psychiatry 88 (2020) 910–921, 10.1016/j.biopsych.2020.05.011. [PubMed: 32680583]

[82]. Patel RR, Khom S, Steinman MQ, Varodayan FP, Kiosses WB, Hedges DM, Vlkolinsky R, Nadav T, Polis I, Bajo M, Roberts AJ, Roberto M, IL-1β expression is increased and regulates GABA transmission following chronic ethanol in mouse central amygdala, Brain. Behav. Immun 75 (2019) 208–219, 10.1016/j.bbi.2018.10.009. [PubMed: 30791967]

[83]. Patel RR, Varodayan FP, Herman MA, Jimenez V, Agnore R, Gao L, Bajo M, Cuzon Carlson VC, Walter NA, Fei SS, Grant KA, Roberto M, Synaptic effects of IL-1β and CRF in the central amygdala after protracted alcohol abstinence in male rhesus macaques, Neuropsychopharmacology 47 (2022) 847–856, 10.1038/s41386-021-01231-y. [PubMed: 34837077]

[84]. Bajo M, Varodayan FP, Madamba SG, Robert AJ, Casal LM, Oleata CS, Siggins GR, Roberto M, IL-1 interacts with ethanol effects on GABAergic transmission in the mouse central amygdala, Front. Pharmacol 6 (2015) 49, 10.3389/fphar.2015.00049. [PubMed: 25852553]

[85]. Roberts AJ, Khom S, Bajo M, Vlkolinsky R, Polis I, Cates-Gatto C, Roberto M, Gruol DL, Increased IL-6 expression in astrocytes is associated with emotionality, alterations in central amygdala GABAergic transmission, and excitability during alcohol withdrawal, Brain. Behav. Immun 82 (2019) 188–202, 10.1016/j.bbi.2019.08.185. [PubMed: 31437534]

[86]. Gano A, Pautassi RM, Doremus-Fitzwater TL, Deak T, Conditioned effects of ethanol on the immune system, Exp. Biol. Med 242 (2017) 718–730, 10.1177/1535370217694097.

[87]. Luo Y, Zheng SG, Hall of fame among pro-inflammatory cytokines: interleukin-6 gene and its transcriptional regulation mechanisms, Front. Immunol 7 (2016). https://www.frontiersin.org/articles/10.3389/fimmu.2016.00604 (accessed September 30, 2023).

[88]. Borgonetti V, Cruz B, Vozella V, Khom S, Steinman MQ, Bullard R, D'Ambrosio S, Oleata CS, Vlkolinsky R, Bajo M, Zorrilla EP, Kirson D, Roberto M, IL-18 signaling in the rat central

amygdala is disrupted in a comorbid model of post-traumatic stress and alcohol use disorder, Cells 12 (2023) 1943, 10.3390/cells12151943. [PubMed: 37566022]

[89]. Patel RR, Wolfe SA, Bajo M, Abeynaike S, Pahng A, Borgonetti V, D'Ambrosio S, Nikzad R, Edwards S, Paust S, Roberts AJ, Roberto M, IL-10 normalizes aberrant amygdala GABA transmission and reverses anxiety-like behavior and dependence-induced escalation of alcohol intake, Prog. Neurobiol 199 (2021) 101952, 10.1016/j.pneurobio.2020.101952. [PubMed: 33197496]

[90]. Marshall SA, McKnight KH, Blose AK, Lysle DT, Thiele TE, Modulation of binge-like ethanol consumption by IL-10 signaling in the basolateral amygdala, J. Neuroimmune Pharmacol. Off. J. Soc. NeuroImmune Pharmacol 12 (2017) 249–259, 10.1007/s11481-016-9709-2.

[91]. Raineki C, Bodnar TS, Holman PJ, Baglot SL, Lan N, Weinberg J, Effects of early-life adversity on immune function are mediated by prenatal environment: role of prenatal alcohol exposure, Brain. Behav. Immun 66 (2017) 210–220, 10.1016/j.bbi.2017.07.001. [PubMed: 28698116]

[92]. Ming Z, Criswell HE, Breese GR, Evidence for TNFα action on excitatory and inhibitory neurotransmission in the central amygdala: a brain site influenced by stress, Brain. Behav. Immun 33 (2013) 102, 10.1016/j.bbi.2013.06.001. [PubMed: 23770090]

[93]. Freeman K, Brureau A, Vadigepalli R, Staehle MM, Brureau MM, Gonye GE, Hoek JB, Hooper DC, Schwaber JS, Temporal changes in innate immune signals in a rat model of alcohol withdrawal in emotional and cardiorespiratory homeostatic nuclei, J. Neuroinflamm 9 (2012) 97, 10.1186/1742-2094-9-97.

[94]. Gano A, Doremus-Fitzwater TL, Deak T, Sustained alterations in neuroimmune gene expression after daily, but not intermittent, alcohol exposure, Brain Res. 1646 (2016) 62–72, 10.1016/j.brainres.2016.05.027. [PubMed: 27208497]

[95]. Knapp DJ, Whitman BA, Wills TA, Angel RA, Overstreet DH, Criswell HE, Ming Z, Breese GR, Cytokine involvement in stress may depend on corticotrophin releasing factor to sensitize ethanol withdrawal anxiety, Brain. Behav. Immun 25 (2011) S146–S154, 10.1016/j.bbi.2011.02.018. [PubMed: 21377524]

[96]. Sanchez-Marin L, Pavon FJ, Decara J, Suarez J, Gavito A, Castilla-Ortega E, Rodriguez de Fonseca F, Serrano A, Effects of intermittent alcohol exposure on emotion and cognition: a potential role for the endogenous cannabinoid system and neuroinflammation, Front. Behav. Neurosci 11 (2017) 15, 10.3389/fnbeh.2017.00015. [PubMed: 28223925]

[97]. Crews FT, Vetreno RP, Mechanisms of neuroimmune gene induction in alcoholism, Psychopharmacology 233 (2016) 1543–1557, 10.1007/s00213-015-3906-1. [PubMed: 25787746]

[98]. Alfonso-Loeches S, Pascual-Lucas M, Blanco AM, Sanchez-Vera I, Guerri C, Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage, J. Neurosci. Off. J. Soc. Neurosci 30 (2010) 8285–8295, 10.1523/JNEUROSCI.0976-10.2010.

[99]. Fernandez-Lizarbe S, Montesinos J, Guerri C, Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells, J. Neurochem 126 (2013) 261–273, 10.1111/jnc.12276. [PubMed: 23600947]

[100]. Bajo M, Madamba SG, Roberto M, Blednov YA, Sagi VN, Roberts E, Rice KC, Harris RA, Siggins GR, Innate immune factors modulate ethanol interaction with GABAergic transmission in mouse central amygdala, Brain. Behav. Immun 40 (2014) 191–202, 10.1016/j.bbi.2014.03.007. [PubMed: 24675033]

[101]. Bajo M, Montgomery SE, Cates LN, Nadav T, Delucchi AM, Cheng K, Yin H, Crawford EF, Roberts AJ, Roberto M, Evaluation of TLR4 inhibitor, T5342126, in modulation of ethanol-drinking behavior in alcohol-dependent mice, Alcohol Alcohol. Oxf. Oxfs 51 (2016) 541–548, 10.1093/alcalc/agw026.

[102]. Liu J, Yang AR, Kelly T, Puche A, Esoga C, June HL, Elnabawi A, Merchenthaler I, Sieghart W, June HL, Aurelian L, Binge alcohol drinking is associated with GABAA 2-regulated Toll-like receptor 4 (TLR4) expression in the central amygdala, Proc. Natl. Acad. Sci 108 (2011) 4465–4470, 10.1073/pnas.1019020108. [PubMed: 21368176]

[103]. Karoly HC, Ellingson JM, Hutchison KE, Interactions between TLR4 methylation and alcohol consumption on subjective responses to an alcohol infusion, Alcohol Alcohol. 53 (2018) 650–658, 10.1093/alcalc/agy046. [PubMed: 29982285]

[104]. Harris RA, Bajo M, Bell RL, Blednov YA, Varodayan FP, Truitt JM, de Guglielmo G, Lasek AW, Logrip ML, Vendruscolo LF, Roberts AJ, Roberts E, George O, Mayfield J, Billiar TR, Hackam DJ, Mayfield RD, Koob GF, Roberto M, Homanics GE, Genetic and pharmacologic manipulation of TLR4 has minimal impact on ethanol consumption in rodents, J. Neurosci 37 (2017) 1139, 10.1523/JNEUROSCI.2002-16.2016. [PubMed: 27986929]

[105]. Zhang K, Luo J, Role of MCP-1 and CCR2 in alcohol neurotoxicity, Pharmacol. Res 139 (2019) 360–366, 10.1016/j.phrs.2018.11.030. [PubMed: 30472461]

[106]. June HL, Liu J, Warnock KT, Bell KA, Balan I, Bollino D, Puche A, Aurelian L, CRF-amplified neuronal TLR4/MCP-1 signaling regulates alcohol self-administration, Neuropsychopharmacology 40 (2015) 1549–1559, 10.1038/npp.2015.4. [PubMed: 25567426]

[107]. Truitt JM, Blednov YA, Benavidez JM, Black M, Ponomareva O, Law J, Merriman M, Horani S, Jameson K, Lasek AW, Harris RA, Mayfield RD, Inhibition of IKKβ reduces ethanol consumption in C57BL/6J mice, eNeuro 3 (2016), 10.1523/ENEURO.0256-16.2016.

[108]. McCarthy GM, Warden AS, Bridges CR, Blednov YA, Harris RA, Chronic ethanol consumption: role of TLR3/TRIF-dependent signaling, Addict. Biol 23 (2018) 889–903, 10.1111/adb.12539. [PubMed: 28840972]

[109]. Walter TJ, Vetreno RP, Crews FT, Alcohol and stress activation of microglia and neurons: brain regional effects, Alcohol. Clin. Exp. Res 41 (2017) 2066–2081, 10.1111/acer.13511. [PubMed: 28941277]

[110]. Zanoni I, Granucci F, Role of CD14 in host protection against infections and in metabolism regulation, Front. Cell. Infect. Microbiol 3 (2013) 32, 10.3389/fcimb.2013.00032. [PubMed: 23898465]

[111]. Martin-Fernandez M, Jamison S, Robin LM, Zhao Z, Martin ED, Aguilar J, Benneyworth MA, Marsicano G, Araque A, Synapse-specific astrocyte gating of amygdala-related behavior, Nat. Neurosci 20 (2017) 1540–1548, 10.1038/nn.4649. [PubMed: 28945222]

[112]. Costenla AR, Diógenes MJ, Canas PM, Rodrigues RJ, Nogueira C, Maroco J, Agostinho PM, Ribeiro JA, Cunha RA, de Mendonça A, Enhanced role of adenosine A2A receptors in the modulation of LTP in the rat hippocampus upon ageing, Eur. J. Neurosci 34 (2011) 12–21, 10.1111/j.1460-9568.2011.07719.x. [PubMed: 21615561]

[113]. Varodayan FP, Bajo M, Soni N, Luu G, Madamba SG, Schweitzer P, Roberto M, Chronic alcohol exposure disrupts CB1 regulation of GABAergic transmission in the rat basolateral amygdala, Addict. Biol 22 (2017) 766–778, 10.1111/adb.12369. [PubMed: 26786379]

[114]. Varodayan FP, Soni N, Bajo M, Luu G, Madamba SG, Schweitzer P, Parsons LH, Roberto M, Chronic ethanol exposure decreases CB1 receptor function at GABAergic synapses in the rat central amygdala, Addict. Biol 21 (2016) 788–801, 10.1111/adb.12256. [PubMed: 25940135]

[115]. Kirson D, Oleata CS, Parsons LH, Ciccocioppo R, Roberto M, CB1 and ethanol effects on glutamatergic transmission in the central amygdala of male and female msP and Wistar rats, Addict. Biol 23 (2018) 676–688, 10.1111/adb.12525. [PubMed: 28656627]

[116]. Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, Mackie K, Watanabe M, Katona I, Patel S, Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses, Neuron 81 (2014) 1111–1125, 10.1016/j.neuron.2014.01.012. [PubMed: 24607231]

[117]. Melkumyan M, Annaswamy VM, Evans AM, Showemimo OF, McCullers ZE, Sun D, Murphy TE, Vrana KE, Arnold AC, Raup-Konsavage WM, Silberman Y, Effects of cannabidiol, with and without Δ9-tetrahydrocannabinol, on anxiety-like behavior following alcohol withdrawal in mice, Front. Neurosci 18 (2024), 10.3389/fnins.2024.1375440.

[118]. Delhaye S, Bardoni B, Role of phosphodiesterases in the pathophysiology of neurodevelopmental disorders, Mol. Psychiatry 26 (2021) 4570–4582, 10.1038/s41380-020-00997-9. [PubMed: 33414502]

[119]. Li H, Zuo J, Tang W, Phosphodiesterase-4 inhibitors for the treatment of inflammatory diseases, Front. Pharmacol (2018) 9. https://www.frontiersin.org/articles/10.3389/fphar.2018.01048 (accessed October 16, 2023). [PubMed: 29422861]

[120]. Tibbo AJ, Baillie GS, Phosphodiesterase 4B: master Regulator of Brain Signaling, Cells 9 (2020) 1254, 10.3390/cells9051254. [PubMed: 32438615]

[121]. Duman RS, Nestler EJ, Cyclic Nucleotide Phosphodiesterases, in: Basic Neurochem. Mol. Cell. Med. Asp 6th Ed., Lippincott-Raven, 1999. https://www.ncbi.nlm.nih.gov/books/NBK27996/ (accessed November 7, 2023).

[122]. Blednov YA, Benavidez JM, Black M, Harris RA, Inhibition of phosphodiesterase 4 reduces ethanol intake and preference in C57BL/6J mice, Front. Neurosci 8 (2014) 129, 10.3389/fnins.2014.00129. [PubMed: 24904269]

[123]. Ray LA, Bujarski S, Shoptaw S, Roche DJ, Heinzerling K, Miotto K, Development of the neuroimmune modulator ibudilast for the treatment of alcoholism: a randomized, placebo-controlled, human laboratory trial, Neuropsychopharmacology 42 (2017) 1776–1788, 10.1038/npp.2017.10. [PubMed: 28091532]

[124]. Meredith LR, Green R, Grodin EN, Chorpita M, Miotto K, Ray LA, Ibudilast moderates the effect of mood on alcohol craving during stress exposure, Exp. Clin. Psychopharmacol 30 (2022) 620–631, 10.1037/pha0000458. [PubMed: 36102596]

[125]. Grodin EN, Bujarski S, Towns B, Burnette E, Nieto S, Lim A, Lin J, Miotto K, Gillis A, Irwin MR, Evans C, Ray LA, Ibudilast, a neuroimmune modulator, reduces heavy drinking and alcohol cue-elicited neural activation: a randomized trial, Transl. Psychiatry 11 (2021) 355, 10.1038/s41398-021-01478-5. [PubMed: 34120149]

[126]. Burnette EM, Ray LA, Irwin MR, Grodin EN, Ibudilast attenuates alcohol cue–elicited frontostriatal functional connectivity in alcohol use disorder, Alcohol. Clin. Exp. Res 45 (2021) 2017–2028, 10.1111/acer.14696. [PubMed: 34585396]

[127]. Meredith LR, Grodin EN, Montoya AK, Miranda R, Squeglia LM, Towns B, Evans C, Ray LA, The effect of neuroimmune modulation on subjective response to alcohol in the natural environment, Alcohol. Clin. Exp. Res 46 (2022) 876–890, 10.1111/acer.14821. [PubMed: 35362101]

[128]. Grodin EN, Nieto SJ, Meredith LR, Burnette E, O'Neill J, Alger J, London ED, Miotto K, Evans CJ, Irwin MR, Ray LA, Effects of ibudilast on central and peripheral markers of inflammation in alcohol use disorder: a randomized clinical trial, Addict. Biol 27 (2022) e13182, 10.1111/adb.13182. [PubMed: 35754106]

[129]. Grigsby KB, Mangieri RA, Roberts AJ, Lopez MF, Firsick EJ, Townsley KG, Beneze A, Bess J, Eisenstein TK, Meissler JJ, Light JM, Miller J, Quello S, Shadan F, Skinner M, Aziz HC, Metten P, Morrisett RA, Crabbe JC, Roberto M, Becker HC, Mason BJ, Ozburn AR, Preclinical and clinical evidence for suppression of alcohol intake by apremilast, J. Clin. Invest 133 (2023) e159103, 10.1172/JCI159103. [PubMed: 36656645]

[130]. Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, Hirakawa A, Takeuchi H, Suzumura A, Ishiguro N, Kadomatsu K, Minocycline selectively inhibits M1 polarization of microglia, Cell Death Dis. 4 (2013) e525, 10.1038/cddis.2013.54. [PubMed: 23470532]

[131]. Petrakis IL, Ralevski E, Gueorguieva R, Sloan ME, Devine L, Yoon G, Arias AJ, Sofuoglu M, Targeting neuroinflammation with minocycline in heavy drinkers, Psychopharmacology 236 (2019) 3013–3021, 10.1007/s00213-019-05205-3. [PubMed: 30919006]