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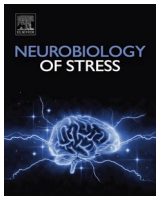

The role of brain serotonin signaling in excessive alcohol consumption and withdrawal: A call for more research in females

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ABSTRACT

Alcohol Use Disorder (AUD) is a leading cause of death and disability worldwide, but current treatments are insufficient in fully addressing the symptoms that often lead to relapses in alcohol consumption. The brain's serotonin system has been implicated in AUD for decades and is a major regulator of stress-related behaviors associated with increased alcohol consumption. This review will discuss the current literature on the association between neurobiological adaptations in serotonin systems and AUD in humans as well as the effectiveness of serotonin receptor manipulations on alcohol-related behaviors like consumption and withdrawal. We will further discuss how these findings in humans relate to findings in animal models, including a comparison of systemic pharmacological manipulations modulating alcohol consumption. We next provide a detailed overview of brain region-specific roles for serotonin and serotonin receptor signaling in alcohol-related behaviors in preclinical animal models, highlighting the complexity of forming a cohesive model of serotonin function in AUD and providing possible avenues for more effective therapeutic intervention. Throughout the review, we discuss what is known about sex differences in the sequelae of AUD and the role of serotonin in these sequelae. We stress a critical need for additional studies in women and female animals so that we may build a clearer path to elucidating sex-specific serotonergic mechanisms and develop better treatments.

1. Introduction

Alcohol Use Disorder (AUD) is a chronic, relapsing disorder characterized by the inability to control alcohol intake despite negative consequences of continued heavy use. Compared to all other licit and illicit drugs of abuse, alcohol is reported to cause the most harm to the self and others in terms of both mental and physical health (Nutt et al., 2010). These harms include disruption of social relationships, chronic health problems (cancer, stroke, liver disease), the development of mood disorders, loss of financial stability, accidental injury, and even death. While men have historically had higher rates of AUD than women, this gap is narrowing. From 2009 to 2019, rates of AUD rose 85% in women while rising only 35% in men (Grant et al., 2017). The onset of the COVID-19 pandemic in 2020 has accelerated increases in AUD diagnoses in women (Acuff et al., 2022; Kerr et al., 2022; Schmidt et al., 2021), highlighting an urgent need to identify the gender-specific psychosocial and neurobiological mechanisms driving AUD development and progression.

While the factors leading to this sharp increase in AUD in women are multitudinous, they include drinking to relieve negative affect or cope with symptoms of affective disorders, stress-induced drinking, and a short progression from initiation of binge drinking to alcohol dependence (referred to as "telescoping") (Agabio et al., 2016, 2017; Sharrett-Field et al., 2013). Women also show greater susceptibility to alcohol-related chronic health issues than men, including cardiovascular disease, stroke, anxiety, and major depression (Agabio et al., 2016). Finally, while AUD negatively affects sensorimotor arousal, aggressive behavior, social cognition, and mood in both men and women, men are generally more sensitive to the effects of alcohol on aggression and arousal and women are generally more sensitive to the effects of alcohol on social cognition and mood (Nolen-Hoeksema, 2004; Le Berre, 2019; Hoaken et al., 2000). These gender differences in the progression of AUD and the long-term effects of alcohol on behavior suggest that specific pharmacological and/or behavioral approaches for treating AUD in men and women may be necessary.

Serotonin (5-Hydroxytryptamine, 5HT) is a modulatory neurotransmitter whose actions in the CNS have been implicated in the pathophysiology of AUD for many decades. Despite this, the role of the

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brain's 5HT system in discrete alcohol-related neurobiological processes and behaviors remains incomplete, particularly in female subjects. This review will discuss what is known about the role of the brain's serotonin system in alcohol consumption and withdrawal, with a focus on preclinical studies in rodents indicating potential sex differences that may be relevant for future AUD treatment development in humans. We suggest that the influence of serotonergic manipulations on alcohol consumption may relate to their effects on motivation and negative affect, which contribute to heavy alcohol consumption and relapse behaviors. Critically, how serotonin modulates these behaviors appears to depend on the recruitment of discrete serotonin circuits mediating different aspects of alcohol abuse and AUD-related endophenotypes.

2. Alcohol abuse as a stress-related disorder

The progression from initial alcohol exposure to AUD is complex and involves neurobiological and behavioral processes related to reward, stress, and executive function. Early on in the development of alcohol abuse, the positive reinforcing properties of alcohol (euphoria, disinhibition, anxiolysis) are thought to drive escalated consumption via the brain's mesolimbic reward circuitry (Koob et al., 2016). This is often referred to as the "binge/intoxication" stage of alcohol addiction. However, following experiences of binge intoxication, individuals go through a process of withdrawal in which they experience negative emotional and physical symptoms that worsen with each repeated cycle. These worsening withdrawal symptoms are thought to arise from neuro-adaptations in brain stress systems, and eventually shift the primary driver of binge intoxication from positive to negative reinforcement; that is, from seeking reward to avoiding dysphoria (Koob et al., 2016). Indeed, repeated withdrawal from alcohol is associated with elevated signaling of stress-related molecules in the brain and body, including corticotropin releasing factor (CRF), dynorphin, and cortisol (Quadros et al., 2016; Karkhanis et al., 2020; Adinoff et al., 1998). Repeated cycles of intoxication and withdrawal further disrupt cognitive centers of the brain, which promotes future drinking through impaired decision-making and increased impulsivity. There is strong evidence that adverse childhood experiences greatly predispose individuals to AUD, with individuals experiencing four or more adverse childhood events 3-6x more likely to engage in heavy alcohol use, and 7x+ more likely to engage in problematic alcohol use (Hughes et al., 2017). Adverse childhood experiences also increase the risk of developing mood disorders, which frequently co-occur with AUD and can promote alcohol consumption through self-medication of mood symptoms (Bolton et al., 2009).

Sex differences in the relationship between stress and alcohol consumption are apparent throughout the literature, highlighting a further need for elucidating the underlying neurobiology in females. For example, adverse childhood experiences like emotional neglect, physical/sexual/emotional abuse, or witnessing domestic violence are more common in girls and escalate alcohol consumption more robustly in women than in men (Šulejová, 2022) (for extensive review of interactions between serotonin genes, AUD, and adverse experiences in humans, see (Enoch, 2012)). The serotonin system is intricately interconnected with brain stress systems impacted by stress and chronic alcohol consumption, poising it functionally and anatomically to mediate alcohol's effects on mood and emotion as well as the effects of stress on alcohol consumption. For example, serotonin neurons influence the activity of CRF neurons in the paraventricular nucleus of the hypothalamus, the primary cellular population by which the hypothalamic-pituitary-adrenal axis stress response is activated (Kawano et al., 1992). Furthermore, serotonin neurons densely express CRF receptors, which can modulate serotonin release in downstream regions when bound to CRF (Price et al., 2001). In addition, the hindbrain contains a small population of CRF-positive neurons that appear to co-localize with serotonin and project to the extended amygdala (Commons et al., 2003). Together, this evidence highlights a bidirectional and integrated relationship between serotonin and stress systems that could regulate physiology and behavior in the context of AUD. This relationship is especially relevant for women, as the co-expression of stress-related disorders and AUD is almost twice as high in women as it is in men (Brady et al., 1999).

3. Modeling alcohol consumption in rodents

While it is impossible to model every aspect of human AUD in animals, rodent models of various components of AUD have proven valuable in delineating the complex neurobiological mechanisms underlying AUD pathology. Using two-bottle choice drinking procedures, researchers can model acute and chronic voluntary alcohol consumption. In these procedures, rodents are given free or restricted access to both water and alcohol bottles in their home cages. Alcohol-related reward and aversion behaviors can be assessed using conditioned place preference/aversion (CPP/CPA) tests. In these tests, animals are trained to associate different contexts with doses of intraperitoneal alcohol injections and then tested (without alcohol) for contextual preference/aversion. Alcohol-related reward, motivation, and relapse can be modeled with operant alcohol self-administration procedures, in which animals are trained to associate an action (e.g. lever presses or nose pokes) with the outcome of receiving intravenous or oral alcohol rewards. Following a period of operant alcohol seeking and taking, experimenters can remove the alcohol from the apparatus to extinguish operant responding. Following extinction, researchers can promote reinstatement of operant alcohol responding with exposure to stress or alcohol itself as a model of relapse behavior. Finally, alcohol-related withdrawal and negative affect can be modeled with voluntary two-bottle choice drinking, alcohol vapor exposure, or forced alcohol injections followed by paradigms measuring depression-like behavior, anxiety-like behavior, pain, and social interaction.

A major discrepancy between human AUD and rodent models is that in humans males consume more alcohol than females, while in rodents females consume more alcohol than males. This appears to be the case for both operant alcohol self-administration as well as two-bottle choice consumption paradigms. However, stress-induced reinstatement of operant alcohol seeking is more robust in female rats compared to male rats (Bertholomey et al., 2016a, 2019), which is consistent with findings in humans suggesting that women are more likely to relapse on alcohol following stress than men. Interestingly, previous studies do not support a strong influence of estrous cycle on operant alcohol self-administration behavior in females, nor do they suggest that gonadal hormones can fully explain the sex differences in alcohol drinking behaviors in rodents (Bertholomey et al., 2019; Priddy et al., 2017; Roberts et al., 1998).

The idea that men tend to drink for sensation and reward seeking while women tend to drink to relieve negative emotions is recapitulated in data from current rodent models in some regards, but not in others. While male rodents do engage in greater risk-taking behaviors following alcohol consumption, female rodents show greater alcohol-related reward, reduced alcohol-related aversion, and greater acute alcohol-related anxiolysis (relief). For example, acutely intoxicated male rats will choose more large/uncertain rewards over small/certain rewards in a probabilistic learning task compared to females (Wallin-Miller et al., 2017). However, wild-type female rats readily display alcohol CPP while male rats do not (Torres et al., 2014). Estrogen may be important for increased alcohol reward in females, as ovariectomy blunts alcohol CPP and estrogen replacement enhances it (Hilderbrand et al., 2018). In addition, experimenter-administered alcohol results in more robust anxiolysis in the elevated plus maze in females compared to males (but this anxiolysis is short-lived and rebounds during withdrawal) (Tanchuck-Nipper et al., 2015). Female rodents also show increased resistance to quinine adulteration of alcohol and foot-shock punishment in operant alcohol self-administration paradigms (Sneddon et al., 2020, 2023), suggesting reduced alcohol-related aversion.

Women are more susceptible to the effects of alcohol on mood, and

rodent studies generally reflect this sex difference (Bloch et al., 2022). However, stress or alcohol exposure promotes both alcohol consumption and anxiety-like behavior in rodents of both sexes, and this can be enhanced by repeated cycles of binge/withdrawal as well as repeated exposures to stress (Bloch et al., 2022; Szumlinski et al., 2019; Wille-Bille et al., 2017). For example, acute stress promotes anxiogenesis in the novelty suppressed feeding test in female mice with a history of adolescent alcohol, but not in those without (Kasten et al., 2020). In a recent paper, five days of restraint stress increased alcohol consumption in adolescent females, but not in adolescent males or adult males or females (Wille-Bille et al., 2017). In another study, exposure to stress increased alcohol consumption in adult mice of both sexes, but this effect was exacerbated in males with a history of alcohol drinking prior to stress exposure (Quadir et al., 2019). In a study investigating the effects of a variety of acute stressors on alcohol intake, female mice displayed greater increases in drinking following stress than males (Cozzoli et al., 2014). Often, stratification of individuals according to baseline alcohol consumption can reveal an effect of stress on drinking only in females exhibiting low baseline consumption (Finn et al., 2018), suggesting that prior negative results may have been due to ceiling effects. Relatedly, the specific tests that experimenters use to assess affective behaviors in rodents may influence whether males, females, or both sexes show a given behavioral response to alcohol exposure. This notion is supported by studies employing a wide variety of paradigms reporting that male and female rodents show only partially overlapping social/affective responses to alcohol exposure (Bloch et al., 2022; Flanigan et al., 2023a; Neira et al., 2022). For an in-depth review of sex differences in the relationships between alcohol and stress in human and animal models, see the following excellent review from Peltier and colleagues (Peltier et al., 2019).

Adverse childhood experiences are highly associated with AUD in humans, and efforts to develop animal models of this relationship have been largely successful. Both male and female rodents appear to increase their alcohol consumption following early life stress, but some studies suggest that their sensitivity may differ depending on the age at which stress is experienced and the type of stressor experienced. For example, in a recent study, early life adversity modeled by limited nest bedding during PND 2–9 enhanced the escalation of adult alcohol consumption following alcohol vapor exposure in male, but not female, mice (Okhuarobo et al., 2020). In addition, maternal separation stress from post-natal days (PNDs) 2–10 increases binge alcohol consumption and alcohol self-administration in adult male mice (Gondré et al., 2016). In contrast, a recent study found that both male and female rats that underwent maternal separation from PND2-21 displayed increased alcohol self-administration behavior in adulthood compared to unstressed rats (Bassey et al., 2023). Interestingly, chronic corticosterone exposure from PND30-50 increases cue-induced reinstatement of alcohol seeking on PND 60 to a greater degree in females than in males (Bertholomey et al., 2016b). More acute stressors, such as a single administration of 15 electric foot-shocks on PND 17, do not increase alcohol consumption during adulthood per se (Perry et al., 2023), but do reduce alcohol-related aversion in male and female mice (Radke et al., 2020). Thus, similar to humans, early life stressors in rodents may have additive effects on alcohol consumption later in life such that the more stressors an individual experiences the more likely they are to develop maladaptive alcohol consumption patterns. Importantly, these early life stress models also engender a variety of other maladaptive behaviors in adulthood related to mood, impulsivity, social interaction, and cognition, as well as disrupt the development and function of the brain's serotonin and stress hormone circuitry (Hodes et al., 2019; Forster et al., 2018). As a result, rodent models of early life stress are excellent for identifying the complex ways in which adverse childhood experiences can lead to AUD and should be employed more in future studies.

4. Functional and anatomical properties of serotonin circuits

5HT is synthesized primarily in neurons of the hindbrain median raphe nucleus (MRN) and dorsal raphe nucleus (DRN) (Deneris et al., 2018). 5HT neurons in the DRN and MRN project widely throughout the brain and are to some extent anatomically organized by efferent projection target and responses to behavioral stimuli. For example, DRN 5HT neurons projecting to cortical regions are localized to the ventral DRN, while DRN 5HT neurons projecting to subcortical regions are localized to the dorsal DRN (Ren et al., 2018). While cortically-projecting DRN 5HT neurons are activated by reward, suppressed by punishment, and promote active coping, subcortically-projecting DRN 5HT neurons are activated by both reward and punishment and promote passive coping [ibid]. Moreover, single neurons in the DRN can project to more than one downstream target to coordinate serotonin release in both regions simultaneously during behavior (Flanigan et al., 2023a).

The physiological effects of 5HT on downstream neural targets are dependent on a wide variety of 5HT receptors, of which there are four subtypes: GPCRs coupled to Gi/o (5HT1 and 5HT5), GPCRs coupled to Gq (5HT2), GPCRs coupled to Gs (5HT7, 5HT4, 5HT6), and ionotropic receptors that increase intracellular calcium and sodium (5HT3) (Raymond et al., 2001). However, depending on the cells and brain regions examined, there can be substantial variability in these canonical 5HT receptor/G-protein combinations. In addition, RNA-editing, alternative splicing, dimerization, subcellular localization, epigenetic regulation, and experience-dependent plasticity of 5HT receptors all add significantly to the complexity of delineating the biological and behavioral function of the serotonin system in alcohol-related behaviors. To date, a variety of 5HT receptors have been implicated in AUD in both clinical and pre-clinical studies. These receptors primarily include 5HT1a, 5HT1b, 5HT2c, 5HT2a, and 5HT3.

The 5HT1a receptor functions as a pre-synaptic auto-receptor on 5HT neurons in the DRN and MRN that negatively regulates their 5HT release (Polter et al., 2010; Andrade et al., 2015). On non-5HT neurons, the 5HT1a receptor is primarily located post-synaptically (Polter et al., 2010). Activation of 5HT1a receptors induces the opening of inwardly-rectifying potassium channels (GIRKs) to strongly hyperpolarize neuronal membranes and reduce neuronal firing (Araneda et al., 1991). In addition, 5HT1a receptors profoundly reduce calcium currents and evoked calcium influx (Bayliss et al., 1995). The 5HT1b receptor is a mostly pre-synaptic receptor that allows 5HT to negatively modulate synaptic transmission from surrounding neuronal inputs, although 5HT1b can function as an auto-receptor on 5HT neurons to regulate 5HT release as well (Sari, 2004). Like 5HT1a, 5HT1b activates GIRK channels to hyperpolarize neurons and reduce firing; however it also may also result in phosphorylation of ERK1/2 via interactions with beta-arrestins, which can increase intracellular calcium and firing activity (Berg et al., 2001). 5HT2a and 5HT2c are generally located post-synaptically in neurons and share many intracellular signaling mechanisms, but often exert opposing influences on neuronal circuits due to their disparate expression patterns. As mentioned above, 5HT2a/c predominantly couple to Gq proteins, which enact PLC and PKA-dependent signaling cascades to increase intracellular calcium, membrane potential, and neuronal firing (Millan et al., 2008). However, 5HT2a has been shown to couple to Gi in some brain regions, notably the cortex (Berg et al., 1998). 5HT2c is highly expressed in midbrain interneurons and thus dampens the activity of nearby dopamine neurons when activated (Giorgetti et al., 2004). On the other hand, 5HT2a is highly expressed on midbrain dopamine neurons themselves and thus enhances the activity of dopamine neurons when activated (Bubar et al., 2006). Notably, 5HT2c receptors are known to undergo RNA-editing, which results in over 30 different functional isoforms with different levels of constitutive activity and affinities for serotonin (Stamm et al., 2017). 5HT3 is unique in that it is the only ionotropic 5HT receptor. 5HT3 is a Cys-loop ion channel that is directly activated by both 5HT and alcohol to cause membrane

depolarization (Barnes et al., 2009). Although its subcellular localization patterns are not immediately clear (electrophysiological studies suggest both pre- and post-synaptic expression patterns), 5HT3 is involved in mediating the stimulatory dopamine response to rewards in the midbrain, where it is expressed at high levels (Campbell et al., 1996).

5. Sex differences in brain 5HT systems

5HT metabolism, reuptake, and receptor expression/function differ in some regards between males and females, which is critically relevant for understanding the sex-specific functions of this system in AUD. Briefly, serotonin is synthesized from the amino acid tryptophan by the enzyme tryptophan dehydrogenase (TPH) (Murphy et al., 1998). Once synthesized, serotonin is loaded into synaptic vesicles by the vesicular monoamine transporter 2 (VMAT2) for release (Murphy et al., 1998). Following release, serotonin is cleared from the synaptic cleft by the serotonin transporter (SERT) and broken down by the enzyme monoamine oxidase a (MAO-A) (Murphy et al., 1998). Studies in rodent models consistently report that brain serotonin is higher in females compared to males (Carlsson et al., 1988; Mitsushima et al., 2006); however, studies in humans report mixed findings (Nishizawa et al., 1997; Chugani et al., 1998). In both humans and animal models, however, females display lower SERT binding than males, which would increase the amount of synaptic serotonin available at any given time (Mitsushima et al., 2006; Mann et al., 2000; McEuen et al., 2009). In addition, female rodents display increased circulation of tryptophan and expression of TPH compared to male rodents, which also leads to increased biosynthesis of serotonin (Songtachalert et al., 2018). Female rodents are also reported to have less MAO-A than males, highlighting a third mechanism likely contributing to increased serotonin levels in female rodents.

While sex differences in the expression and function of serotonin receptors have not been widely explored, existing studies suggest that these differences are brain region-specific. For example, one study reported that expression of 5HT1a is higher in male rats in the hypothalamus and amygdala, but higher in female rats in the hippocampus (Zhang et al., 1999). Interestingly, this difference in expression does not coincide with a difference in 5HT1a receptor binding in any brain region. In humans, however, 5HT1a binding is higher in women across cortical and subcortical regions (Jovanovic et al., 2008). This sex difference in humans may be hormone-dependent, though, as studies that control for endogenous hormone fluctuations in men and women, particularly those related to estrogen, do not report differences in 5HT1a receptor expression or binding (Moses-Kolko et al., 2011). These estrogen fluctuations across the ovulation cycle and the lifespan in women could also contribute to the inconsistent findings regarding brain serotonin levels in men and women, especially given that estrogen can modulate many aspects of the serotonin system (see below). In mice, the 5HT1b receptor inhibits 5HT release to a greater extent in females than in males, suggesting that it serves as a more effective auto-receptor in females (Jones et al., 2005). This may have important consequences for behavior, as female 5HT1b knockout mice display a behavioral despair phenotype while male 5HT1b knockouts do not. However, if 5HT1b knockout is limited to 5HT1b auto-receptors, male mice display reduced anxiety-like behavior, illustrating the potentially opposing functions of 5HT1b receptors on serotonergic versus non-serotonergic neurons (Nautiyal et al., 2016). 5HT2a receptor binding is higher in the hippocampus of female rats (Zhang et al., 1999), but whether there are sex differences in 5HT2a expression in humans is unclear. One study reported that 5HT2a receptor binding does not differ in any brain region between men and women, even when controlling for hormonal fluctuations (Moses-Kolko et al., 2011). Another study in humans revealed higher 5HT2 receptor binding in the frontal and cingulate cortex of men compared to women (Biver et al., 1996). Using a more specific radio-ligand, a third group found higher 5HT2a binding in men in multiple brain regions, including the hippocampus, lateral orbitofrontal cortex, occipital cortex, and thalamus (Soloff et al., 2010). Unfortunately, sex differences in 5HT2c receptor expression and function have yet to be widely explored in human or animal models, and more work in this area is sorely needed. Interestingly, while there are not sex differences in expression of 5HT3 receptors, there are notable sex differences in 5HT3 function. For example, systemic administration of a 5HT3 agonist increases locomotion in female, but not male, mice (Brookshire et al., 2009). Similarly, antagonism of 5HT3 increases immobility in the forced swim test selectively in female rats (Bhatnagar et al., 2004). Whether these differences relate to disparate effects of 5HT3 drugs on dopaminergic signaling remains to be tested, but it is a plausible mechanism considering its dense expression on VTA dopamine neurons. As estradiol and progesterone act as functional antagonists of 5HT3 (Bethea et al., 2002), hormonal influences on the differential effects of 5HT3 drugs on behavior in males and females should also be explored. Together, these data suggest that nearly all 5HT receptor sub-types implicated in AUD display some level of sexual dimorphism in expression, function, or both.

Current evidence suggests that estrogen modulates serotonin synthesis and availability as well as serotonin receptor expression and function, providing some potential mechanisms for the documented sex differences in this system. For example, estrogen decreases SERT expression and binding (Mitsushima et al., 2006; Mann et al., 2000; McEuen et al., 2009), increases TPH expression (Sanchez et al., 2005), and decreases MAO-A expression and function (Holschneider et al., 1998). Accordingly, 5HT levels in the DRN are highest in the proestrous phase (when estrogen is highest) (Felton et al., 2005). However, this does not appear to result in an appreciable fluctuation of alcohol intake across the menstrual/estrous cycle in humans or rodents (Terner et al., 2006). Interestingly, glucocorticoids, which are higher in females due to suppression by testosterone in males (Viau et al., 1996), reduce TPH in mouse brain (Clark et al., 2005) but increase it in rat brain (Azmitia et al., 1969). In rats, estrogen treatment reduces 5HT1a expression and function in the cortex, DRN, and amygdala (D'Souza, 2004; Jackson et al., 1996; Mize et al., 2000; Osterlund et al., 1998; Raap et al., 2000). In non-human primates, estrogen reduces 5HT1a receptor expression and G-protein coupling in the DRN, but not the hypothalamus (Birzniece et al., 2001; Lu et al., 2002; Gundlah et al., 1999). Estrogen-mediated anxiolysis is mediated, at least in part, through 5HT1a, as 5HT1a antagonists block this behavioral effect of estrogen (Andrade et al., 2005; Estrada-Camarena et al., 2006). One study also reported that estrogen reduces 5HT1b mRNA expression in a sub-region of the DRN, and that this reduction was correlated with anxiety levels (Hiroi et al., 2009). Given that 5HT1a and 5HT1b function as auto-receptors in the DRN, this indicates that estrogen enhances the synaptic availability of serotonin through reduced 5HT1R-mediated pre-synaptic inhibition.

6. 5HT systems in humans with AUD: Histological, genetic, and pharmacological findings

Clinical research studies report that humans with AUD display broad alterations in 5HT-related markers. For example, AUD patients of both sexes display increased 5-HT and/or the 5HT metabolite 5HIAA in cerebrospinal fluid during intoxication, and this decreases to sub-baseline levels when individuals have been abstinent for three months (Borg et al., 1985; LeMarquand et al., 1994a). In addition, AUD is associated with elevated levels of TPH in the DRN in both sexes (Bonkale et al., 2006). SERT binding and expression is also reduced in men and women AUD patients, particularly those with Type 2 AUD (Mann et al., 2000; Storvik et al., 2007; Hammoumi et al., 1999; Hinckers et al., 2006; Laucht et al., 2009; Nilsson et al., 2005; Sander et al., 1997; Seneviratne et al., 2013). Notably, this subtype of AUD is far more common in men and is characterized by early onset alcohol problems, impulsivity, and interpersonal aggression in addition to high alcohol intake. On the other hand, Type 1 AUD is more common in women and is characterized by late onset alcohol problems, low novelty seeking behavior, and mood

dysregulation. Similarly, the S-allele of the 5HT transporter, which confers low transporter function, is associated with AUD and habitually violent behavior in men (Hallikainen et al., 1999). These studies highlight the behavioral and neurobiological overlaps between alcohol and aggression and suggest that increased 5HT signaling could possibly underlie the pathophysiology of both. However, medications that broadly alter brain 5HT signaling have shown mixed utility in treating humans with AUD and/or aggression. This may relate to the aforementioned sub-types of AUD, as selective serotonin reuptake inhibitors (SSRIs) have been shown to reduce alcohol consumption in late onset alcoholics (Type 1) but increase it in early onset alcoholics (Type 2) (Kranzler et al., 2011). Together, these data suggest that there is a "sweet spot" of serotonin function where either too much or too little can lead to AUD phenotypes. Future research that attempts to separate these AUD sub-groups may uncover important differences in the pathophysiology of each, which could lead to more tailored and effective treatments. The fact that Type 2 AUD patients are predominantly men may suggest that the biological differences observed between Type 1 and Type 2 AUD also reflect sex differences such that AUD in men is driven more by excess 5HT (Type 2) while AUD in women is driven by deficient 5HT (Type 1). This idea should be further explored in future research.

The morphology of the serotonin system is also substantially impacted by alcohol exposure, and this is true at various stages of brain development. Exposure to alcohol during gestation can predispose individuals to stress-related disorders and AUD later in life and is associated with impairments in the growth and maturation of the brain's serotonergic circuitry. For example, in-utero exposure to alcohol in female rats decreases the density of serotonin neurons as well as the levels of serotonin and TPH in the DRN and MRN (Sari et al., 2010). Prenatal exposure to stress induces a similar loss of 5HT neurons (St-Pierre et al., 2016), highlighting the shared actions of alcohol and stress on this system and the potential dangers of exposure to both during fetal development. Interestingly, studies in adults with AUD report both increased area of 5HT processes in the DRN (Underwood et al., 2007) and reduced 5HT cell number (Halliday et al., 1995), illustrating again that both high and low levels of 5HT may be related to AUD pathology. While this may depend on an individual's sex and/or AUD sub-type, this also likely depends on the sub-population of DRN neurons analyzed and their downstream projection targets, the latter of which are difficult to identify in humans.

Additional work suggests that alterations in 5HT1a, 5HT1b, 5HT2a, 5HT2c, and 5HT3 are associated with AUD in humans, and that pharmacological agents targeting some of these receptors individually or in combination could be viable clinical treatment options. For example, genetic variations in the 5HT1b receptor are associated with AUD in men with antisocial personality disorder (Lappalainen et al., 1998). Further, in both men and women with AUD, 5HT1b binding is increased in the ventral striatum compared to healthy controls (Hu et al., 2010). While alcohol-related aggression is reduced by zolmitriptan, a 5HT1b/1d agonist (Gowin et al., 2010), no studies to date have investigated the utility of selective 5HT1b agonists in reducing alcohol consumption in humans. Interestingly, 5HT1a binding in the cortex is reduced in men and women with AUD (Dillon et al., 1991), but agonists of 5HT1a do not reliably alter alcohol consumption in humans when considering the entire population of AUD patients (Malcolm et al., 1992; Bruno, 2010). However, 5HT1a agonists are effective in reducing anxiety, alcohol consumption, and alcohol craving in AUD patients with comorbid anxiety disorders (Malec et al., 1996). Interestingly, the comorbidity of these two disorders is substantially higher in women compared to men (Sonne et al., 2003), indicating 5HT1a agonists might have more therapeutic utility in women with AUD. Together, these data suggest that the viability of 5HT1a- and 5HT1b-targeted treatment approaches may hinge on clarifying the specific AUD-related endophenotypes that most effectively determine medication response.

Drugs targeting 5HT3 and 5HT2 receptors hold substantial promise for the treatment of AUD, but like 5HT1a- and 5HT1b-targeting drugs, our understanding of the factors that most effectively determine medication response is incomplete. Current evidence suggests that genetic factors and AUD-related phenotypes are important for effective responses to 5HT3 antagonists. In men, a gain-of-function allele of the 5HT3 receptor predicts alcohol dependence, suggesting that 5HT3 activation in humans promotes alcohol consumption (Enoch et al., 2011). Interestingly, the 5HT3 antagonist ondansetron appears to reduce alcohol consumption and alcohol-related problems in early onset (Type 2), but not late onset (Type 1), men with AUD (Kranzler et al., 2003; Johnson et al., 2000), indicating again that sub-types of AUD are highly relevant for treatment approaches. While some studies have suggested that 5HT transporter and 5HT3 genotypes can predict responses to ondansetron in AUD (Johnson et al., 2013; Kenna et al., 2009), some also suggest that ondansetron is not effective for treating AUD in humans regardless of genotype (Seneviratne et al., 2022; Sherwood Brown et al., 2021). Future studies are needed to further elucidate the relationship between 5HT3 genotype, AUD phenotype, and medication response. Agonists of the 5HT2a receptor, particularly classical psychedelic compounds like LSD and psilocybin, are reported to reduce alcohol consumption in men and women, especially when combined with psychological therapy (Bogenschutz et al., 2015, 2022; Krebs et al., 2012). However, the ethics and feasibility of this type of treatment strategy is of current debate, and appropriately regulating such a treatment strategy will be complex. Although the 5HT2c receptor agonist lorcaserin is effective in reducing binge alcohol consumption in men and women (Campbell et al., 2021), off-target effects of the drug that promote cancer have prevented its widespread use in humans. In addition, lorcaserin has agonist properties at 5HT2a and 5HT1a receptors, the former of which could pose risks for individuals with substance use disorders due to abuse potential (Serafine et al., 2015, 2016). The development of more safe and selective pharmacological compounds for targeting 5HT2c may offer a valuable path for treating AUD without posing the issues associated with lorcaserin. It must be mentioned, however, that while 5HT2c agonism does appear to reduce drinking in humans, it can also worsen excessive arousal symptoms in recently detoxified male patients and promote anxiety (Krystal et al., 1997). This may suggest different classes of 5HT2c receptor-based treatments may be necessary during specific stages of AUD progression and recovery.

Together, these data and many others indicate that the serotonin system plays a complex role in human AUD that varies across brain region, gender, stage of disease progression, and AUD sub-type. Fortunately, pre-clinical studies allowing for the dissection of individual serotonergic circuits and receptor subtypes in discrete alcohol-related behaviors have enhanced our collective understanding of the causal mechanisms leading to excessive alcohol intake and associated emotional dysregulation. These studies have provided the vast majority of our current insights into the function of serotonin in AUD and are discussed below. Unfortunately, because very few of them have included female animals, conclusions regarding sex differences are difficult to formulate, but are made whenever possible. It is critical that future studies perform novel experiments in both sexes and repeat previous studies in females so that we may get a fuller picture of the serotonin-related mechanisms of AUD.

7. Effects of systemic pharmacological manipulations of 5HT receptors on alcohol-related behaviors in rodents

In a variety of rodent behavioral models, systemic manipulations of 5HT receptors have been shown to modulate alcohol consumption and withdrawal-related anxiety. These manipulations primarily involve intraperitoneal (i.p.) injections of brain-penetrant pharmacological compounds or whole-body receptor knockout approaches. While only moderately informative for determining mechanisms of serotonin's modulation of behavior, these studies are critical for subsequent

translational applications. For example, in many studies, agonism of 5HT1a reduces alcohol consumption in rodents of both sexes (Ko et al., 2023; Long et al., 1996; Wilde et al., 1994; Wilson et al., 1996). This is at odds with findings in humans, though, in which 5HT1a agonism does not reliably influence alcohol consumption unless individuals have a comorbid diagnosis of an anxiety disorder (see above). However, one study reports that agonism of 5HT1a increases alcohol self-administration at low doses but reduces it at high doses in male rats, which could be related to different sensitivities of auto-versus hetero--receptors to drug treatment (Schreiber et al., 1999). Yet this study also suggests that the observed effects of 5HT1a agonism on alcohol self-administration are non-specific, as 5HT1a agonism also reduced saccharin self-administration. Conversely, treatment with a 5HT1a antagonist, particularly when combined with an SSRI, has also been reported to reduce alcohol consumption (Zhou et al., 1998). Regardless of these mixed effects on alcohol consumption, agonism or partial agonism of 5HT1a does appear to prevent alcohol-induced social and anxiety-like symptoms in rodents of both sexes (Overstreet et al., 1997; Belmer et al., 2018a, 2018b). Together, these studies suggest that the role of rodent 5HT1a receptors in alcohol-induced mood dysfunction likely translates to humans, but that its role in alcohol consumption may only translate to anxious humans.

Although less studied, 5HT1b receptors have been implicated in alcohol consumption in rodents of both sexes and thus should be further explored in humans with AUD. For example, mice with genetic deletion of 5HT1b display increased aggression, impulsivity, and voluntary alcohol consumption (Brunner et al., 1997; Crabbe et al., 1996), and this is true for males and females. In addition, systemic agonism of 5HT1b reduces alcohol intake and self-administration in male rats (Maurel et al., 1999b; Tomkins et al., 2000). To date, no studies have investigated the effects of 5HT1b agonism on alcohol-related behaviors in females, so potential sex differences in these effects remained undetermined.

Like studies in humans, studies in rodents suggest the 5HT3 receptor influences alcohol consumption only under certain conditions or in certain individuals. Antagonism of 5HT3 reduces alcohol consumption and self-administration in males and females in some studies, but not others (Fadda et al., 1991; Hodge et al., 1993; McKinzie et al., 1998; Tomkins et al., 1995; Rodd-Henricks et al., 2000). This appears to be related to the specifics of the drinking conditions, as 5HT3 antagonists are largely effective in reducing alcohol consumption in long access, but not short access, models (McKinzie et al., 1998, 2000). 5HT3 antagonism also prevents stress-induced reinstatement of alcohol self-administration (Lê, 2006) and compulsive alcohol seeking (Barker et al., 2014), but does not affect alcohol conditioned taste aversion or conditioned place preference (Bienkowski et al., 1997). As compulsive alcohol seeking and relapse behaviors commonly require long-term consumption of alcohol prior to testing (while CPA and CPP do not), it is interesting to speculate that 5HT3-mediated increases in dopamine play a greater role in drinking when alcohol is chronic and predictably consumed than when it is novel and less predictably consumed. This notion stems from studies illustrating a stimulatory effect of 5HT3 activation on dopamine release from VTA neurons (Imperato et al., 1989). Though this could challenge the idea that dopamine signaling is more critically involved in the early stages of binge and intoxication than in the later stages of withdrawal and negative affect, it is also possible that 5HT3 receptors located outside of the mesolimbic reward pathway are mediating some of the effects of antagonism on alcohol-related behaviors. Together, these data indicate that 5HT3 antagonism in both humans and rodents is effective for reducing alcohol intake in both sexes of individuals that have had a prolonged history of alcohol abuse (humans: early-onset, rodents: long access models).

The 5HT2 receptors are attractive targets for future AUD-related medication development, as they have been shown to regulate alcohol consumption, reward, and withdrawal in rodent models. Agonism of 5HT2a reduces voluntary alcohol consumption, alcohol self-administration, and alcohol conditioned place preference in male rodents in the majority of studies (Maurel et al., 1999a; Berquist et al., 2021; Kimmey et al., 2022; Serra et al., 2022). Unfortunately, this manipulation has only been tested in female rodents in one study, but was effective in reducing alcohol consumption (Maurel et al., 1999a). Considering the attention that 5HT2a-targeting drugs have received recently for treating addiction, it is critical that future studies further explore this mode of treatment in female animals. While systemic agonism of 5HT2c reduces voluntary alcohol consumption in both sexes in most studies (Maurel et al., 1999a; Rezvani et al., 2014; Tabbara et al., 2021; Tomkins et al., 2002; Fu et al., 2020), 5HT2c antagonism also reduces voluntary alcohol consumption in males (Ko et al., 2023). 5HT2c antagonism also normalizes alcohol-induced social deficits in males (Marcinkiewcz et al., 2015; Overstreet et al., 2003), highlighting this receptor's importance in both alcohol consumption and the behavioral consequences of alcohol consumption. Clearly, further research on the role of the 5HT2c receptor in these behaviors is required to explain why both antagonists and agonists might be having similar effects, and whether there are sex differences in the effects of 5HT2c manipulations on withdrawal-related behaviors. For a visualization of 5HT receptor-targeting drugs that have been effective at reducing alcohol consumption in rodents and humans, please see Table 1. For a visualization of 5HT-related genetic, transcriptional, and protein changes in AUD and animal models of AUD, please see Table 2.

8. Effects of acute alcohol on 5HT dynamics in rodents

In rodents of both sexes, acute alcohol intoxication increases 5HT release in multiple brain sites along the mesocorticolimbic reward pathway and beyond. For example, systemic intraperitoneal injection of alcohol promotes 5HT release in the nucleus accumbens (NAc), prefrontal cortex (PFC), ventral tegmental area (VTA), caudate and putamen (CPu), suprichiasmatic nucleus (SCN), and central amygdala (CeA) (Jamal et al., 2016; Yan, 1999; Thielen et al., 2001, 2002; Yoshimoto et al., 1992a, 2000; Selim et al., 1996; Portas et al., 1994). 5HT also potentiates the acute effects of alcohol on neural activation of the NAc and ventral tegmental area (VTA) and potentiates alcohol-induced dopamine release in the NAc and anterior cingulate cortex (ACC) (Yoshimoto et al., 1992b; Blanchard et al., 1993). While 5HT levels are increased by acute alcohol in many brain regions, multiple studies report that acute alcohol decreases the firing rate of neurons in the DRN (Thielen et al., 2001; Pistis et al., 1997; Maguire et al., 2014). Thus, local presynaptic mechanisms in downstream regions may be involved in regulating acute alcohol-induced 5HT release. Rats that have been bred for high versus low alcohol drinking behaviors (Alcohol Preferring (P) vs. Alcohol Non-preferring (NP) rats, respectively) have also been valuable in studies examining 5HT dynamics during acute alcohol intoxication, as they can provide information on neurobiological mechanisms associated with high voluntary alcohol consumption. Male P rats, but not male NP rats, display increased 5HT release in the ventral hippocampus (vHipp) and the frontal cortex upon acute injection with alcohol (Thielen et al., 2002), suggesting that 5HT release in these regions could be involved in driving future high alcohol drinking behaviors. 5HT release upon alcohol intoxication in the vHipp of P rats likely requires MRN 5HT neurons, as this is the predominant 5HT input to this region (Ren et al., 2019). For longer periods of alcohol administration that may still be considered acute (1 week), alcohol increases 5HT levels in the lateral septum (LS) and lateral hypothalamus (LH), brain regions involved primarily in social behavior and homeostasis, respectively (Kelly, 1996a, 1996b). Interestingly, this effect was predominantly driven by females. One to seven weeks after exposure to alcohol, however, LS and LH 5HT levels were normalized in these animals. In the Bed Nucleus of the Stria Terminalis (BNST) and the lateral habenula (LHb), voluntary alcohol consumption in alcohol-naïve mice reduces 5HT release in both sexes (Flanigan et al., 2023a). These recordings were performed specifically in 5HT2c receptor-containing neurons, which are

Table 1
Effects of systemic pharmacological manipulations of serotonin receptors on alcohol consumption in humans and rodents.

Pharmacological Manipulation	Men with AUD	Women with AUD	Female Rodents	Male Rodents
5HT1a agonism	Reduces drinking in patients with comorbid anxiety disorder(s) (Malcolm et al., 1992; Bruno, 2010; Malec et al., 1996)	Reduces drinking in patients with comorbid anxiety disorder(s) (Malcolm et al., 1992; Bruno, 2010; Malec et al., 1996)	Reduces drinking (Ko et al., 2023; Zhou et al., 1998)	Reduces drinking (Ko et al., 2023; Long et al., 1996; Overstreet et al., 1997; Schreiber et al., 1999; Tomkins et al., 1994)
5HT1b agonism	Not tested	Not tested	Not tested	Reduces drinking (Maurel et al., 1999a, 1999b; Miczek et al., 2001; Tomkins et al., 2000)
5HT2c agonism	Reduces drinking and craving (Campbell et al., 2021; Krystal et al., 1997)	Reduces drinking and craving (Campbell et al., 2021; Krystal et al., 1997)	Reduces drinking (Maurel et al., 1999a; Rezvani et al., 2014)	Reduces drinking (Maurel et al., 1999a; Tabbara et al., 2021; Tomkins et al., 2002)
5HT3 antagonism	Reduces drinking in early-onset AUD (Kranzler et al., 2003, 2011; Johnson et al., 2000, 2013) Reduces drinking in patients drinking <10 drinks/day (Sellers et al., 1994)	Reduces drinking in early-onset AUD (Kranzler et al., 2003, 2011; Johnson et al., 2000, 2013)	Reduces drinking in long-access models (McKinzie et al., 2000)	Reduces drinking in long-access models (Barker et al., 2014; Bienkowski et al., 1997; Fadda et al., 1991; Hodge et al., 1993; McKinzie et al., 1998; Tomkins et al., 1995)
5HT2a agonism	Reduces drinking (Bogenschutz et al., 2015, 2022)	Reduces drinking (Bogenschutz et al., 2015, 2022)	Reduces drinking (Maurel et al., 1999a)	Reduces drinking (Maurel et al., 1999a; Berquist et al., 2021; Kimmey et al., 2022; Serra et al., 2022)
5HT2c antagonism	Not tested	Not tested	No effect (Ko et al., 2023)	Reduces drinking (Ko et al., 2023)

generally activated by 5HT. Notably, this study was the first to employ fluorescent sensors for in-vivo measurement of 5HT release during voluntary alcohol consumption. Future studies utilizing this technique will offer much greater temporal resolution of alcohol-induced 5HT dynamics than what was possible with older techniques like microdialysis. Together, these studies highlight the general stimulatory effects of acute alcohol exposure on 5HT release in both sexes, but future research in females will be necessary to confirm this for each brain region.

9. Effects of chronic alcohol on 5HT dynamics in rodents

Chronic alcohol consumption is also associated with alterations in 5HT dynamics in multiple brain regions. The majority of studies investigating the effects of chronic alcohol on 5HT take measurements during a withdrawal period whereby animals have been exposed to alcohol chronically but are currently abstinent. This withdrawal period can vary from acute (0–72 h) to protracted (72 h-beyond). Studies investigating the effects of chronic alcohol exposure and withdrawal on serotonin neuron physiology have yielded inconsistent results. In one study, male mice chronically exposed to alcohol vapor displayed increased excitability of DRN neurons 24 h and 7 days after exposure (Lowery-Gionta et al., 2015). On the other hand, a separate study reported that 12 h into withdrawal from chronic alcohol, DRN neurons showed reduced firing rates in male mice (Pistis et al., 1997). The reasons for these discrepancies may relate to the alcohol exposure paradigms used, the withdrawal time points chosen, the sub-region of the DRN recorded from, and the DRN cell types recorded from. Clearly, additional work is required to tease apart the effects of chronic alcohol on serotonin neuron physiology, and these future studies should be sure to include females. It may be that chronic alcohol exposure can either decrease or increase the activity of DRN serotonin neurons depending on the sub-type of AUD, the projection target(s) of the 5HT neurons, and the time from last alcohol exposure. Indeed, SSRIs increase alcohol consumption in Type 2 AUD patients but decrease it in Type 1 AUD patients, and chronic alcohol exposure increases or decreases 5HT signaling in rodents in some brain regions depending on the withdrawal time-point chosen and the length of the history of alcohol consumption. For example, male and female rats in acute withdrawal display reduced 5HT in the NAc that is restored following alcohol consumption (Weiss et al., 1996; Thielen et al., 2004; Esteban et al., 2002; Luessen et al., 2019), but male mice in protracted withdrawal show increased 5HT release in the NAc during social interactions with novel conspecifics (Wang et al., 2023). Similarly, in the BNST, protracted withdrawal in females is associated with increased serotonin release upon social interaction with a novel conspecific (Flanigan et al., 2023a). Therefore, future studies should consider recording from populations of serotonin neurons defined by projection target at both acute and protracted withdrawal time-points, as this will help disentangle the current data on the effects of chronic alcohol and subsequent abstinence on serotonin dynamics and behavior.

10. Effects of chronic alcohol on 5HT circuits in rodents

As mentioned above, much of the complexity of the serotonin system arises from diverse expression of 5HT receptor sub-types at different sites throughout the brain. These receptors can be co-expressed in the same cells, but can have completely different signal transduction pathways that are activated by serotonin. On the other hand, brain regions can also express the same receptors in different cell types that release opposing fast neurotransmitters (GABA or Glutamate). This likely means that the effects of 5HT on a given cell type or brain region depends on the coordinated actions of multiple receptors and cell types at once. To ascertain the contributions of each of these receptors and cell types to alcohol-related behaviors, researchers have utilized a combination of site-specific behavioral pharmacology and site- and/or cell-specific genetic manipulations. Both approaches are valuable for our understanding of serotonin in AUD, as behavioral pharmacology provides better temporal resolution while genetic manipulations provide better spatial resolution. However, recent advances in techniques for manipulating neuronal circuits have allowed researchers to gain unprecedented insights into the functional role of serotonin signaling in AUD. These advances primarily include opto- and chemo-genetics, which allow for both spatially and temporally refined control of cell-type specific neural activity during behavior. A combination of the few recent studies using these modern techniques and the numerous historical studies using behavioral pharmacology approaches are discussed below.

Table 2
Genetic, transcriptional, and protein changes related to 5HT in humans with AUD and in rodent models of chronic alcohol intake.

Serotonin pathway component	Men with AUD	Women with AUD	Female Rodents	Male Rodents
5-HT (or metabolites)	Reduced 5HIAA in CSF (Borg et al., 1985; LeMarquand et al., 1994b)	Reduced 5HIAA in CSF (LeMarquand et al., 1994b)	Higher 5HT in Hyp in adults exposed to adolescent alcohol (Kelly, 1996a) Lower in NAc during acute withdrawal (Weiss et al., 1996)	Lower 5HT content in cortex, striatum, NAc, septum of High Alcohol Drinking (HAD) compared to Low Alcohol Drinking (LAD) rat lines (Gongwer et al., 1989; Devoto et al., 1998) Higher 5HT in Hyp in adults exposed to adolescent alcohol (Kelly, 1996a) Lower 5HT in NAc during acute withdrawal (Weiss et al., 1996) Higher 5HT in NAc during protracted withdrawal (Wang et al., 2023) Fewer 5HT neurons in P-rats (Zhou et al., 1994) Higher 5HT and 5HIAA in P-rats (BANO et al., 1998)
5-HT Transporter	Reduced binding and surface expression in DRN (Andreas Heinz et al., 1998) S-allele increased in AUD (Hammoumi et al., 1999; Hinckers et al., 2006; Sander et al., 1997; Hallikainen et al., 1999; McHugh et al., 2010) S-allele unrelated to AUD and comorbid depression (Gorwood et al., 2000) Interaction between AUD and 5HTT genotype on 5HTT availability (Heinz et al., 2000)	Reduced binding and surface expression in DRN (Andreas Heinz et al., 1998) S-allele increased in AUD (Hammoumi et al., 1999; Hinckers et al., 2006; Sander et al., 1997; McHugh et al., 2010)		
Tryptophan Hydroxylase	Increased protein in dorsal DRN (Bonkale et al., 2006) Mutation associated with AUD (Nielsen et al., 1998a, 1998b) Increased density in caudal DRN with comorbid Depression (Bach-Mizrachi et al., 2008)	Increased protein in dorsal DRN (Bonkale et al., 2006) Increased density in caudal DRN with comorbid Depression (Bach-Mizrachi et al., 2008)		TPH2 mRNA correlates positively with voluntary alcohol intake; voluntary alcohol intake does not change TPH mRNA expression (Zaniewska et al., 2022)
Monoamine oxidase	Increased binding in PFC (Matthews et al., 2014)	Increased binding in PFC (Matthews et al., 2014) Polymorphism associated with AUD (Guindalini et al., 2005)		No difference in MAO activity (Sherif et al., 1993) Reduced expression in striatum (Bendre et al., 2015)
5HT1a	Reduced binding in cortex (Dillon et al., 1991; Storvik, 2008) Reduced binding in cortex with comorbid Depression/Anxiety (Storvik, 2008) Increased mRNA in cortex (Thompson et al., 2012)	Reduced binding in cortex (Dillon et al., 1991; Storvik, 2008) Reduced binding in cortex with comorbid Depression/Anxiety (Storvik, 2008)		Increased binding and function in DRN (Kela et al., 2008) Increased binding in cortex and Hipp (Wong et al., 1990) No change in receptor expression in BLA (Patkar et al., 2019a)
5HT1b	Increased binding in NAc in NAc (Hu et al., 2010) Mutation associated with antisocial AUD (Lappalainen et al., 1998)	Increased binding in NAc (Hu et al., 2010)		Reduced mRNA expression in BLA (McBride et al., 1997)
5HT2a	Binding reduced in cortex (Underwood et al., 2008) Polymorphism associated with AUD (Nakamura et al., 1999)	Binding reduced in cortex (Underwood et al., 2008) Polymorphism associated with AUD (Nakamura et al., 1999)		Decreased protein in cortex (Popova et al., 2020)
5HT2c	No association between AUD and promoter variants (Mottagui-Tabar, 2004) No association between AUD and polymorphism (Samochowiec et al., 1999)	No association between AUD and promoter variants (Mottagui-Tabar, 2004)	No change in mRNA in LHb (Flanigan et al., 2023a)	Increased mRNA and protein in LHb (Flanigan et al., 2023a; Fu et al., 2020) Increased mRNA in NAc, ACC, BLA, DRN, CPU, LH, Hipp (Yoshimoto et al., 2012) Increased protein in NAc (Yoshimoto et al., 2012) Increased protein in Hipp and Amyg of P-rats (Pandey et al., 1996) Increased RNA editing in DRN and NAc (Watanabe et al., 2014)
5HT3	Mutation increased in AUD and predicts antagonist response (Seneviratne et al., 2013; Enoch et al., 2011)	Mutation increased in AUD and predicts antagonist response (Seneviratne et al., 2013)		Reduced density in P rats (Ciccocioppo et al., 1998) Compulsive alcohol consumption associated with epigenetic adaptations in 5HT3 in Amygdala (Barker et al., 2014)

Dorsal and Median Raphe Nuclei (DRN/MRN): Exposure to alcohol modulates serotonin receptor expression and function in the DRN and MRN. Furthermore, functional manipulations of 5HT receptors and 5HT neurons in the DRN and MRN have revealed important roles for 5HT signaling in alcohol-related behaviors. For example, chronic alcohol consumption increases 5HT1a availability in the DRN of male monkeys (Hillmer et al., 2014) and increases DRN 5HT1a sensitivity in male mice (Kela et al., 2008). Consistent with these findings, injection of a 5HT1a agonist into the DRN or MRN increases voluntary alcohol consumption and induces reinstatement of operant alcohol seeking in male rats

(Tomkins et al., 1994; Lê, 2002a). As 5HT1a is an inhibitory auto-receptor, this manipulation would reduce 5HT release from DRN and MRN neurons, suggesting that dampened 5HT tone can drive alcohol consumption. However, there is also evidence that activation of DRN and MRN 5HT neurons leads to enhanced alcohol consumption. Interestingly, DRN and MRN 5HT neurons seem to differ with regard to their capacity to promote alcohol consumption at various stages of exposure. In male mice that have been consuming alcohol for 6 weeks, chemogenetic inhibition of the DRN, but not the MRN, reduced voluntary alcohol consumption (Belmer et al., 2022). However, in mice that have been drinking for 12 weeks, chemogenetic inhibition of the MRN, but not the DRN, reduced voluntary alcohol consumption. These effects are recapitulated with 5HT1a auto-receptor agonism, where 5HT1a agonism in the DRN reduced drinking at 6 weeks (but not 12), and 5HT1a auto-receptor agonism in the MRN reduced drinking at 12 weeks (but not 6). To get an idea of when and how this switch to DRN versus MRN-based control over alcohol consumption might occur, future studies should employ modern cell-type specific in-vivo recording techniques like fiber photometry or one-photon *in-vivo* calcium imaging to longitudinally track the activity of DRN and MRN 5HT neurons across the development of alcohol dependence and withdrawal in males **and** females. It will be important for these studies to consider the potential heterogeneity of 5HT neurons with regards to location within the DRN/MRN, genetic markers, and projection targets.

Ventral Tegmental Area (VTA): The ventral tegmental area (VTA) is the hub of the mesolimbic dopamine pathway, a neural substrate of reward processing in the brain that is important for the reinforcing properties of alcohol. The VTA receives serotonin primarily from the DRN, which acts on both dopaminergic and non-dopaminergic neurons there (Gervais et al., 2000). Serotonin actions in the VTA are classically thought to inhibit dopamine cell firing by stimulating 5HT2c receptors expressed on local GABAergic interneurons (Di Giovanni et al., 2008), but recent evidence calls this idea into question. Indeed, some evidence suggests that 5HT in the VTA actually activates a greater proportion of dopamine neurons than it inhibits (Gervais et al., 2000). VTA dopamine neurons express 5HT3 as well as 5HT2a, both of which result in neural activation when bound to serotonin. In addition, optogenetic stimulation of DRN 5HT terminals in the VTA appears to promote reward-seeking behavior, not reduce it (Liu et al., 2014). Further study revealed that many of the DRN serotonin neurons projecting to the VTA also release glutamate and promote reward-seeking behavior when activated, an effect driven via both glutamate receptors and 5HT3 receptors expressed on dopamine neurons (Wang et al., 2019). In female P rats, local infusion of a 5HT3 antagonist into the VTA prevents the acquisition of operant alcohol self-administration but increases responding during the maintenance phase (Rodd et al., 2010). Moreover, 5HT3 antagonism in the posterior VTA reduces operantly self-administered intra-VTA alcohol infusions in female P rats (Rodd et al., 2005). Notably, antagonism of VTA 5HT3 receptors is associated with reduced dopamine efflux in the male nucleus accumbens (NAc), the primary downstream target of VTA dopamine neurons (Dremencov et al., 2006). Consequently, this may suggest that 5HT3-induced stimulation of VTA dopamine release drives alcohol seeking in early stages of exposure but suppresses it at later stages. Unfortunately, formulating conclusions on potential sex differences in these effects is difficult due to a lack of operant studies performing intra-VTA 5HT3 manipulations in males.

There is also evidence that VTA 5HT2a receptors are involved in alcohol-related neuroplasticity and behaviors. In female Wistar rats, intra-VTA infusion of a 5HT2a, but not 5HT1b, antagonist reduced operantly self-administered alcohol infusions into the VTA (Ding et al., 2009). As 5HT2a receptors are mainly expressed on dopamine neurons in the VTA, this suggests that serotonin release onto these neurons promotes alcohol seeking and dopamine release via activation of 5HT2a. However, chronic alcohol consumption also impairs chloride homeostasis in VTA GABA neurons, and this is reversed with a systemic and/or ex-vivo 5HT2a agonist in male mice (Kimmey et al., 2022). Together, these data suggests that 5HT2a signaling in multiple VTA cell types may be involved in the effects of alcohol on physiology and behavior, and potential sex differences in these effects should be explored.

Nucleus Accumbens (NAc): The NAc is the primary target of VTA dopamine neurons and has been identified as an important regulator of alcohol intake and alcohol-related behavioral dysregulation. It is primarily comprised of GABAergic medium spiny neurons, which are separated into dopamine receptor 1 (DR1) expressing and dopamine receptor 2 (DR2) expressing sub-types. NAc neurons express a variety of 5HT receptors, including 5HT6 (Helboe et al., 2015), 5HT2a (Mijnster et al., 1997), 5HT2c (Eberle-Wang et al., 1997), 5HT4 (Jean et al., 2007), 5HT7 (Clissold et al., 2013), and 5HT1b (Muramatsu et al., 1998). Furthermore, pre-synaptic 5HT1b receptors on glutamatergic inputs to the NAc mediate serotonin-induced inhibition of excitatory transmission from the thalamus, amygdala, and vHipp (Dö et al., 2013; Christoffel et al., 2021).

Current evidence implicating NAc serotonin signaling in AUD relates primarily to 5HT2c receptors. Male mice chronically exposed to alcohol vapor display increased basal expression of 5HT2c in the NAc, and intra-NAc infusion of a 5HT2c antagonist blocks escalations in voluntary alcohol intake resulting from vapor exposure (Yoshimoto et al., 2012). Also in male mice, chronic alcohol exposure induces a shift in the balance of 5HT2c isoforms, a process driven by RNA-editing (Watanabe et al., 2014). Over-expression of the unedited isoform of 5HT2c, which displays reduced constitutive activity, in these mice also prevents escalations in alcohol intake resulting from vapor exposure. In a more recent study, Wang et al. report that chronic alcohol vapor exposure in male mice upregulates 5HT2c, but not 5HT2a or 5HT1b, expression in dynorphin-expressing (another marker for DR1) NAc neurons (Wang et al., 2023). Social behavior deficits induced by this chronic alcohol exposure were prevented by both chemogenetic inhibition of DRN-NAc serotonin neurons and genetic knockdown of 5HT2c in dynorphin neurons, indicating that this projection and these receptors are functionally important for driving the effects of alcohol on social behavior. Taking it a step further, the authors finally showed that chemogenetic inhibition of NAc dynorphin neurons prevented social deficits induced by activation of DRN-NAc serotonin neurons, which was associated with increased NAc dopamine efflux during social interaction. Whether these same mechanisms are at play in females remains untested.

There is also some indication that NAc 5HT1b receptors are involved in alcohol-related behaviors. Site-directed infusion of a 5HT1b agonist into the NAc core reduces alcohol-seeking behavior in male rats (Czachowski, 2005), which is thought to occur via presynaptic inhibition of glutamatergic NAc inputs from the thalamus, hippocampus, and/or amygdala. On the other hand, viral over-expression of 5HT1b on projection neurons of the NAc shell increases alcohol-seeking behavior in male rats by altering the micro-structure of drinking bouts at different phases of alcohol exposure (Furay et al., 2011). Specifically, during the initiation of drinking, 5HT1b over-expression increased alcohol consumption by increasing the number of drinking bouts per day and the frequency of licking during bouts. During drinking maintenance, 5HT1b over-expression did not change the amount of total alcohol consumed, but promoted longer drinking bouts. Because 5HT1b receptors are primarily expressed pre-synaptically and NAc projection neurons target the VTA, this could suggest that serotonin-mediated activation of 5HT1b on NAc neurons in the VTA promotes alcohol seeking by inhibiting GABA release onto dopamine neurons. This idea is supported by studies reporting that antagonism of 5HT1b in the VTA increases extracellular dopamine concentrations stimulated by alcohol in both the VTA and NAc (Yan et al., 2005). Together, the studies discussed in this section highlight critical interactions between the serotonin, glutamate, opioid, and dopamine systems in the NAc that may be involved in the pathophysiology of AUD.

Amygdala and Extended Amygdala: The amygdala and the extended amygdala have been strongly implicated in AUD, particularly in

behaviors related to withdrawal, negative affect, and stress-induced relapse. Despite this, very little research has investigated the role of serotonin signaling in these regions in the context of alcohol.

Although the CeA expresses a wide variety of serotonin receptors and plays a pivotal role in AUD, only one study has investigated CeA serotonin systems in alcohol-related behaviors. This study suggests that alterations in 5HT2c signaling in the CeA are critical for inhibitory plasticity induced by alcohol dependence. In naïve male rats, bath application of serotonin in the CeA increased the frequency of inhibitory post-synaptic currents without altering amplitude, suggesting increased GABA release (Khom et al., 2020). However, alcohol dependence induced by chronic alcohol vapor attenuates this effect of serotonin on GABA release during acute withdrawal, and this is partially recovered after two weeks of protracted withdrawal. The attenuated effect of serotonin on CeA inhibitory plasticity depended on the desensitization of pre-synaptic 5HT2c receptors, as a 5HT2c receptor agonist stimulated CeA GABA release in naïve, but not dependent, animals, while 5HT2c expression remained unchanged. Notably, 5HT1a function and expression were also unchanged in dependent animals in this study. Given that alcohol exposure increases the editing of 5HT2c mRNA to reduce G-protein coupling with intracellular signaling cascades, it is possible that this process is occurring in the CeA, and this should be investigated directly. Whether this loss of 5HT2c-mediated loss of inhibition is functionally required for alcohol-related behavioral dysregulation or alcohol consumption should be tested as well.

In the basolateral amygdala (BLA), 5HT3, 5HT2c, 5HT1a, and 5HT1b have been investigated in the context of AUD. However, much work remains to be done to delineate the functional role of these receptors in discrete alcohol-related phenotypes. For example, one study found that male P rats display increased binding density of 5HT2c in the BLA compared to NP rats, suggesting that this receptor's actions in the BLA may contribute to increased voluntary alcohol consumption (Pandey et al., 1996). In another study, two different 5HT3 receptor antagonists infused into the BLA of male rats were each effective in reducing voluntary binge alcohol intake in male rats (Dyr et al., 1995). In a third study, intra-BLA injection of a 5HT1a/5HT1b partial agonist reduced alcohol consumption in male mice that had been drinking for 12 weeks, but not in male mice that had been drinking for 4 weeks (Patkar et al., 2019b). In line with this, male P rats display reduced expression of 5HT1b in the basolateral amygdala (McBride et al., 2004). Together, these data demonstrate that signaling via multiple serotonin receptors in the BLA play functional roles in alcohol-related behaviors in males. Whether these functional roles extend to females should be tested in future studies.

The Bed Nucleus of the Stria Terminalis (BNST) has been highly implicated in alcohol consumption and withdrawal-related negative affect and expresses a wide variety of 5HT receptors, including 5HT1a, 5HT1b, 5HT2c, and 5HT2a, 5HT3, and 5HT7 (Hazra et al., 2012). The BNST receives its 5HT input from the DRN (Phelix et al., 1992), and activation of 5HT inputs to the BNST promotes fear and anxiety-related behaviors in male mice (Marcinkiewcz et al., 2016). Activation of 5HT2c receptors in the BNST depolarizes neurons and promotes anxiety-like behavior, while activation of 5HT1a in the BNST does the opposite (Kash, 2012; Levita et al., 2004). As would be expected, patterns of 5HT receptor expression differ among BNST cell types, with some being inhibited by 5HT, some being excited by 5HT, and some showing no responses to 5HT (Guo et al., 2009). Studies investigating the role of the BNST 5HT2c receptor in alcohol consumption and withdrawal-related behaviors demonstrate a complex role of this receptor in AUD-related pathology and marked sex differences in its modulation of alcohol consumption. Unfortunately, other BNST 5HT receptors have not been explored in alcohol-related behaviors. Chronic intermittent alcohol vapor exposure in male mice enhances BNST excitability in a 5HT2c receptor-dependent fashion and increases the sensitivity of BNST 5HT2c receptors to agonists (Marcinkiewcz et al., 2015). Furthermore, these physiological adaptations in the BNST are accompanied by social deficits, which can be reversed by systemic treatment with a 5HT2c receptor antagonist [ibid]. Interestingly, the activity of neurons expressing this receptor is reduced in both sexes during acute alcohol consumption, and serotonin release is increased onto these neurons in females with a history of binge alcohol consumption (Flanigan et al., 2023a). Genetic deletion of 5HT2c in the BNST partially normalizes female-specific alcohol-induced social recognition deficits, indicating that this receptor is also important for the behavioral effects of alcohol during abstinence in females (Flanigan et al., 2023a). Altogether, these studies suggest the 5HT2c receptor promotes negative social states associated with alcohol withdrawal and abstinence in both sexes, but whether these social states are induced in males and females may depend on the level of alcohol exposure. The influence of the BNST 5HT2c receptor on alcohol consumption depends largely on the alcohol consumption paradigm used. For example, chemogenetic activation of 5HT2c receptor-containing neurons in the BNST reduces home cage binge alcohol consumption in females, but not males (Flanigan et al., 2023a). Chemogenetic inhibition of these neurons has no effect on home cage alcohol consumption in either sex (Flanigan et al., 2023a), but increases alcohol self-administration and consumption early in training in both sexes (Flanigan et al., 2023b). Concerning the receptor itself, genetic deletion of 5HT2c in the BNST increases home cage alcohol consumption and lever pressing for quinine-adulterated alcohol in females but not males (Flanigan et al., 2023a, 2023b). Together, these data indicate the 5HT2c receptor in the BNST modulates alcohol consumption-related behaviors more strongly in females than in males, and this effect may involve an influence on alcohol-related aversion.

Lateral Habenula: Both functionally and anatomically, the LHb is well poised to regulate alcohol-related behaviors, particularly those related to negative emotional states. It is reciprocally connected with both the VTA and the DRN and strongly regulates the activity of both serotonergic and dopaminergic neurons. While the LHb indirectly inhibits VTA dopamine neurons via its excitation of the GABAergic rostromedial tegmental nucleus (RMTg) (Christoph et al., 1986), LHb neurons projecting to the DRN have been shown to activate GABAergic, glutamatergic, and serotonergic neurons there (Pollak Dorocic et al., 2014; Takahashi et al., 2022; Zhou et al., 2017). In addition to this complex influence over serotonergic neurotransmission, the LHb densely expresses a variety of 5HT receptors, including 5HT1a, 5HT1b, 5HT2c, 5HT3, 5HT5, and 5HT7 (Wallace et al., 2020; Tchenio et al., 2016). While 5HT2c is primarily post-synaptically expressed in LHb neurons, 5HT1b is primarily localized to pre-synaptic inputs to the LHb from upstream regions like the entopeduncular nucleus (EPN) (Hwang et al., 2014; Xie et al., 2016; Zuo et al., 2016). Thus, serotonin release in the LHb simultaneously inhibits spontaneous glutamatergic transmission from incoming glutamatergic synapses via 5HT1b but also directly excites LHb neurons via 5HT2c. Unfortunately, other 5HT sub-types expressed in LHb have not been well characterized.

Though the LHb has not been widely studied in the context of AUD, emerging evidence suggests that 5HT2c signaling in the LHb may be important for both alcohol consumption and withdrawal-associated negative affective behaviors. In male mice, three weeks of binge alcohol drinking followed by one week of abstinence, which is associated with increased anxiety-like behavior, increases the expression of 5HT2c in the LHb (Flanigan et al., 2023a). This increase in 5HT2c expression was not observed in females, but there was a trend for increased LHb 5HT release in binge drinking females during social interactions, suggesting potentially different effects of alcohol on LHb serotonin circuitry in males and females. Increases in LHb 5HT2c expression and 5HT metabolites are also observed in male rats following eight weeks of intermittent alcohol consumption (Fu et al., 2020). Further, antagonism of LHb 5HT2c receptors reduces alcohol self-administration, withdrawal-related anxiety, and withdrawal-related pain by reducing the excitability of LHb neurons in male rats through a combination of downstream effects on CAMKII and K + -sensitive M-channels (Fu et al., 2020; Zuo et al., 2019). Interestingly, genetic

deletion of LHb 5HT2c in male mice only modestly influences binge alcohol consumption and withdrawal-related anxiety (Flanigan et al., 2023a), highlighting a potentially important species difference in the role of LHb 5HT2c in behavior. It is also possible that acute (pharmacological) versus chronic (genetic) manipulation of 5HT2c induces different molecular and physiological effects in LHb neurons, which could explain the disparate findings of the mouse and rat studies. In female mice, the effect of LHb 5HT2c deletion on binge alcohol consumption and alcohol-induced social dysfunction is also relatively modest (Flanigan et al., 2023a). However, chemogenetic inhibition of LHb neurons containing the 5HT2c receptor in this same study normalized alcohol-induced social dysfunction and anxiety-like behavior in mice both sexes. Interestingly, this manipulation also increased voluntary alcohol consumption selectively in females. This sex difference is consistent with studies in rats reporting that chemogenetic inhibition of LHb neurons (non-selectively) does not affect alcohol consumption in males (Nentwig et al., 2022). Taken together, these findings suggest that a history of alcohol consumption alters 5HT2c-related expression and signaling in the LHb to promote sex-specific negative emotional states during abstinence. Critically, increased activation of LHb neurons containing 5HT2c is also critical for these effects. Future studies should investigate how a history of alcohol consumption alters the responses of these neurons to 5HT and whether adaptations in other serotonin receptor sub-types, many of which are co-expressed with 5HT2c, are involved.

Cortex: The frontal cortex is important for mediating the deleterious effects of alcohol on cognitive and social behavior, including those related to increased aggression and impulsivity. Translationally, these phenotypes are most commonly observed in Type 2 AUD patients. High alcohol consumption is associated with reduced serotonin release in cortical regions, which may be important for the display of alcohol-heightened aggression. For example, male mice displaying alcohol-heightened aggression show reduced serotonin release in the PFC during attacks (van Erp et al., 2000). This alcohol-heightened aggression is reduced by intra-DRN infusion of a CRF receptor 1 antagonist, which increases serotonin release in the PFC (Quadros et al., 2014). Simultaneous delivery of a 5HT1a agonist into the DRN blocks the effects of DRN CRF receptor 1 antagonism on aggression, suggesting that reduced serotonin output to the PFC as a consequence of alcohol exposure promotes escalated aggressive behavior. This data is supported by another study, which found that male P rats display higher densities of 5HT1a receptors in the cortex compared to NP rats. This indicates that reduced cortical serotonin release in the cortex is associated with increased alcohol drinking behaviors (Wong et al., 1990). In addition, high alcohol drinking rats display reduced serotonin levels in the frontal cortex compared to low alcohol drinking rats (Gongwer et al., 1989). On the other hand, humans with comorbid major depression and AUD display reduced postmortem expression and/or in-vivo binding of 5HT1a, 5HT1b, and 5HT2a in the cortex, particularly the prefrontal cortex (Storvik et al., 2007, Storvik, 2008; Thompson et al., 2012; Underwood et al., 2004, 2008). Depending on whether the 5HT1a and 5HT1b receptors implicated are auto- or hetero-receptors, this could suggest that AUD and comorbid depression are associated with increased or reduced 5HT function in the cortex, respectively. Together, these findings could suggest that there are disparate adaptations in in 5HT1a and 5HT1b auto-receptors versus hetero-receptors in the cortex as a consequence of alcohol, which function to synergistically modulate 5HT-mediated cortical inhibition to dysregulate behavior.

11. Linking stress and alcohol consumption: CRF and 5HT interactions

As mentioned in previous sections, there is a bidirectional relationship between stress and alcohol consumption such that stress can promote excessive alcohol intake and excessive alcohol intake can promote enhanced stress reactivity and negative affect. Current evidence suggests that this bidirectional relationship may be mediated, at least in part, by interactions between serotonin and CRF-related circuits. The DRN is a major site of CRF and serotonin interactions, with CRF neurons originating in the paraventricular hypothalamus, the CeA, and the BNST and synapsing onto both GABAergic and serotonergic neurons in the DRN (Fox et al., 2013). Both CRF1 and CRF2 receptors are expressed on serotonergic and non-serotonergic DRN neurons, leading to a complex interplay of activity dynamics between cell types based on receptor expression and local connectivity. Generally, CRF1 receptor activation in the DRN decreases, while CRF2 receptor activation in the DRN increases, 5HT neuron activity and 5HT release in downstream regions. For example, CRF2 receptor activation in the DRN promotes 5HT release in various amygdala sub-regions, the prefrontal cortex, and the NAc (Kirby et al., 2000; Forster et al., 2008; Lukkes et al., 2008). CRF1 receptor activation in the DRN, on the other hand, reduces 5HT release in the NAc (Forster et al., 2008). Furthermore, CRF1 and CRF2 receptors in the DRN mount differential responses to stress, with CRF1 receptors being internalized in response to stress and CRF2 receptors increasing their surface expression in response to stress (Waselus et al., 2009).

Although no studies have directly linked CRF2 receptor signaling in 5HT neurons to the effects of stress on alcohol consumption, converging lines of evidence suggest that stress-induced activation of CRF2 receptors expressed on 5HT neurons and consequent alterations in downstream 5HT release could be an important mechanism promoting excessive alcohol intake. Similarly, serving as a stressor itself, alcohol consumption could alter serotonergic neurotransmission in target brain regions via alcohol-induced activation of CRF2 receptors. Consistent with this, CRF infusion into the MRN promotes reinstatement of operant alcohol seeking in male rats, while dual antagonism of CRF1 and CRF2 receptors blocks stress-induced reinstatement of operant alcohol seeking (Lê, 2002b). Furthermore, CRF2 receptor activation in the DRN promotes 5HT release in the NAc, and this effect is enhanced by prior exposure to stress in early life (Lukkes et al., 2008, 2009). As mentioned above, chronic alcohol consumption also enhances 5HT transmission in the NAc during withdrawal to disrupt social preference in male mice (Wang et al., 2023). Together, these findings highlight the possibility that, like stress, alcohol could increase 5HT release in the NAc to disrupt behavior as a result of CRF2 receptor activation in the DRN. In addition, these results also raise the likely possibility that exposure to stress and alcohol together may additively contribute to enhanced 5HT transmission in the NAc to promote the development of AUD via activation of CRF2 receptors in the DRN. Interestingly, CRF2 receptor expression in DRN sub-regions projecting to the NAc is higher in females compared to males (Lukkes et al., 2016); however, whether this results in differences in stress- or alcohol-related 5HT transmission in downstream target regions is not currently known. Future studies should investigate these mechanisms in females to determine whether sex differences in CRF/5HT interactions confer differential susceptibility to the effects of drugs or stress on behavior across development. These studies should also investigate how CRF/5HT interactions may differ depending on the downstream projection target of the 5HT neuronal population.

12. The role of 5HT in other addictive disorders

It is critical to note that many, but not all, of the serotonergic adaptations that occur as a result of alcohol exposure also occur as a result of exposure to other drugs of abuse. For example, like alcohol, acute intoxication with cocaine or opioid drugs stimulates 5HT release in many sites throughout the brain, particularly in subcortical structures like the striatum and amygdala (Müller and Homberg, 2015, Müller, 2020). Interestingly, this is contrary to what is observed with acute cannabis administration, in which 5HT levels are reduced throughout the brain (Egashira et al., 2002; Sano et al., 2008). On the other hand, withdrawal from chronic cocaine, or opioids induces a reduction in basal 5HT that is restored by drug intake (Parsons et al., 1995; Tao et al., 1998). This hypo-serotonergic state, particularly in the NAc, is

associated with social deficits that accompany opioid withdrawal, and restoration of NAc 5HT release can be achieved with blockade of kappa opioid receptors or suppression of dynorphin release from DRN 5HT neurons (Wang et al., 2023; Pomrenze et al., 2022). Thus, alcohol and opioids may divergently regulate NAc 5HT release during withdrawal to promote similar behavioral dysregulations, but similarly stimulate 5HT release during acute intoxication.

Alcohol abuse also shares some common serotonin receptor-mediated mechanisms with other types of drug abuse, particularly cocaine and opioid abuse. For example, cocaine and opioid exposure sensitize 5HT1a receptors to promote drug-induced hyper-locomotion (Perret et al., 1998; Cunningham et al., 1992; Sastre-Coll et al., 2002). This sensitization also occurs with alcohol exposure and is thought to reduce serotonin release from raphe neurons during abstinence. The 5HT3 receptor is involved in the rewarding and stimulatory properties of cocaine, opioids, and alcohol, but this role is highly specific to experimental conditions and the substance history of the individual. Notably, delta-9-tetracannabidiol (THC) acts as an antagonist at 5HT3 whereas some opioids like morphine act as agonists at 5HT3 (Müller and Homberg, 2015). Treatment with the 5HT3 antagonist odansetron reduces CPP for cocaine, opioids, and alcohol, but its effects on self-administration and reinstatement are mixed (Müller and Homberg, 2015, Müller, 2020; Hui et al., 1993). While these findings suggest that 5HT3 receptors are involved in mediating the rewarding properties of some drugs and alcohol, clinical studies have failed to support the use of 5HT3 antagonists like odansetron in treating drug addiction in humans (Engleman et al., 2008). Unlike the 5HT3 receptor, the 5HT2c receptor has emerged as a shared mechanism for suppressing drug and alcohol consumption in rodents and humans. Stimulation of the 5HT2c receptor reduces a wide variety of addiction-like behaviors in rodent models of cocaine, alcohol, nicotine, cannabis, and opioid abuse (Müller and Homberg, 2015, Müller, 2020; Neelakantan et al., 2017). In humans, agonism of the 5HT2c receptor with the drug lorcaserin was found ineffective in treating Cocaine Use Disorder but effective in treating Cannabis, Methamphetamine, Alcohol, and Nicotine Use Disorders (Campbell et al., 2021; Negus et al., 2020; Shanahan et al., 2017; Johns et al., 2021). Thus, the 5HT2c receptor remains an incredibly promising target for the treatment of many addictive disorders, and more safe and selective 5HT2c-targeting compounds should be developed for future testing.

Finally, there are also some notable ways in which the serotonergic mechanisms mediating alcohol abuse are distinct from those mediating abuse of other drugs. Unlike alcohol consumption, which is generally reduced by 5HT2a agonism, cocaine consumption is reduced by 5HT2a antagonism (Nic Dhonnchadha et al., 2009a). Cocaine cue-induced reinstatement and cue-reactivity are also reduced by 5HT2a antagonism (Sholler et al., 2019; Nic Dhonnchadha et al., 2009b). Opioid self-administration, on the other hand, is similar to alcohol in that 5HT2a agonists reduce opioid motivation and consumption (Martin et al., 2021). This suggests that the use of psychedelic drugs targeting 5HT2a receptors to treat addiction, as has been recently proposed, may require substance-specific considerations.

13. Conclusions

The brain's serotonin system modulates the activity of a variety of brain circuits that are impacted by alcohol exposure, including those related to reward, stress, and mood. The effects of serotonin signaling on alcohol drinking behaviors are dependent on a multitude of factors at genetic, molecular, anatomical, temporal, and behavioral levels. For example, serotonin's influence over alcohol drinking behavior may change over the course of the exposure and addiction process, and this can result from alcohol-induced changes of specific receptors in specific brain regions. Broadly, we propose that AUD can arise from both excessive serotonin and deficient serotonin, and evidence suggests that the classification of human AUD patients as Type 1 or Type 2 is relevant for this distinction. While excessive serotonin has been associated with Type 2 AUD, deficient serotonin has been associated with Type 1 AUD. Type 2 AUD patients are predominantly men, which indicates that sex is also important for distinguishing the overall function of serotonin in AUD pathophysiology. Considering the sexual dimorphism of the serotonin system, it is critical that future studies further elucidate how known sex differences in serotonin metabolism, receptor expression, receptor function, and neuronal circuit architecture contribute to the effects of serotonin signaling on alcohol-related behaviors in males and females. This is especially needed in light of the paucity of clinical and preclinical studies on serotonin and alcohol that include females or analyze sex differences in outcomes.

Preclinical research studies, primarily in rodents, are critical for delineating the roles of individual 5HT receptor sub-types on specific aspects of AUD pathology because they permit refined temporal and spatial manipulation of 5HT receptor biology. Unfortunately, a major weakness of the current literature on 5HT receptors and AUD-related behaviors is that much of it fails to incorporate the modern neuroscientific tools that facilitate this more granular assessment of serotonin-related pathophysiology in AUD. Moving forward, it will be critical that these modern neuroscientific tools are employed in cell-type, brain-region, and projection-specific ways to enhance our understanding of serotonin's complex role in this disease. While current research findings do not support a strongly sex-specific role for discrete serotonergic mechanisms in AUD, future studies performing these more granular assessments of 5HT systems will likely uncover important sex differences that can be harnessed to develop more effective treatments.

In terms of what current studies (mostly in males) have yielded, 5HT1a and 5HT1b auto-receptors localized to pre-synaptic DRN and MRN 5HT terminals appear to be important for regulating 5HT efflux to downstream regions in early and late stages of alcohol drinking, respectively. Whether these receptors upregulate or downregulate their expression/function to influence serotonin release following alcohol exposure is likely dependent on the brain region targeted by a particular population of serotonin neurons, which tend to segregate into cortically and subcortically projecting groups. It is possible that disparate responses of cortically and subcortically projecting serotonin neurons underlie the failure of 5HT1a-related compounds to reliably modulate alcohol consumption in humans; however, this could also be related to selective effectiveness of these compounds in AUD patients with comorbid anxiety disorders or whether the drug/dosage used primarily auto- or hetero-receptors. While there is substantial evidence that decreased 5HT1a-related signaling promotes alcohol-induced anxiety, the specific cell types and circuits involved have not been widely explored. The majority of data on the 5HT2c receptor stresses its importance in modulating both alcohol consumption and related negative affect via its actions in subcortical brain regions, including the VTA, BNST, CeA, LHb, and NAc. Repeated cycles of heavy alcohol intoxication and withdrawal upregulate the expression of 5HT2c in many of these regions to promote subsequent social and arousal-related behavioral disturbances. Whether compounds targeting the 5HT2c receptor will prove valuable in treating AUD in humans largely depends on the development of more safe and selective molecules for human use. The majority of brain-region specific work on the 5HT3 receptor has focused on its role in mediating alcohol's rewarding properties via its actions in the VTA. Although antagonists of this receptor are effective in some individuals with AUD, these compounds have failed to prove effective enough in the broader population of AUD patients to warrant widespread use. However, personalized medical approaches that take patient genotype into account may prove valuable in identifying a clear subpopulation that will respond to 5HT3-related drugs. Finally, while the 5HT2a receptor is an attractive target for AUD treatment given its role in mediating the effects of psychedelics, we know very little about the specific brain regions and cell types that are important for mediating some of the effects of 5HT2a-targeting drugs on alcohol consumption and related behaviors. Further investigation using newly generated tools

will be necessary to determine whether 5HT2a, like 5HT2c, exerts the majority of its effects on drinking via signaling in subcortical brain regions implicated in reward and negative affect.

In conclusion, the role of brain serotonin signaling in AUD pathophysiology and behavioral dysfunction depends on an intricate interplay between a wide variety of serotonin receptors in multiple brain regions. Adding further to this intricacy, many cell types co-express multiple 5HT receptors that drive opposing changes in physiology and behavior. Thus, it would behoove researchers to begin to investigate the coordinated actions of multiple 5HT receptors in AUD and how they may work together to produce aspects of the AUD disease state by acting in multiple brain regions. Simultaneous treatment with different compounds targeting individual serotonin receptor sub-types could potentially provide a path forward for more precise modulation of affect and behavior in AUD that could be personalized to the individual depending on considerations like sex, age, genotype, AUD symptoms, and co-morbid mental health conditions.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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