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Sex differences in alcohol use: is it all about hormones?

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Abstract

Risky alcohol use and alcohol use disorders (AUD) are a rising problem in women, yet a major disparity in our understanding of what drives alcohol consumption in women remains. Historically biomedical research has focused on male subjects, however, recent increases in reporting of females, have highlighted major differences between the sexes. Here we review the current literature of the impact of gonadal steroid hormones (estrogens, androgens and progestins), neurosteroids and neurobiological factors on alcohol use in clinical and preclinical studies of both sexes. Further, we briefly discuss how fundamental sex differences in genetics, metabolism, neuroimmune and stress responses may influence sex differences in alcohol intake. Comparing the sexes could aid in the discovery of novel therapeutics to treat AUD, and implementation of current treatment options in women.

Keywords: Alcohol use disorder, sex, estrogen, progesterone, testosterone, neurosteroid

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38 **Introduction**

39 Alcohol use disorder (AUD) is one of the most prevalent and disabling disorders globally,
40 characterised by compulsive alcohol-seeking and consumption despite negative consequences (1).
41 An estimated 107 million people — 1.4% of the global population — currently suffer from AUD,
42 constituting ~5.3% of global deaths annually, with this percentage continuing to rise (2). Additionally,
43 there are notable differences between men and women in many facets of AUD. Historically, men have
44 had higher rates of alcohol abuse and AUD diagnosis, however in recent decades these rates have
45 risen significantly more in women, reducing this gender disparity (3). Indeed, between 2001 and 2012
46 alone, AUD increased in women by ~84%, compared to ~35% in males (3). Further, a meta-analysis
47 of >1.8 million people highlighted an increased prevalence of alcohol abuse and binge drinking
48 behaviours from 2000 to 2016 in women, but not in men (4). Consistently, there are also increased
49 rates of alcohol-related hospitalisations, injuries, and deaths in women compared to men (5). This
50 aligns with research suggesting that women are more susceptible to the negative health
51 consequences of alcohol including liver disease, breast cancer, cardiovascular disease, sleep
52 disruptions and mood disorders compared to men (6, 7).

53
54 Despite this, studies that incorporate sex as a factor remain limited. A recent review of
55 pharmacological treatment of AUD, highlights preliminary evidence to suggest that treatments
56 including naltrexone and varenicline are more effective in men, while baclofen may be more effective
57 in women (8). However, these studies are insufficient to provide conclusive evidence of potential sex-
58 differences in the efficacy of these pharmacotherapies. Another study examining the safety and
59 efficacy of naltrexone to treat AUD found that women experienced more adverse side-effects than
60 men, although sex-differences regarding its efficacy were inconclusive (9). Sex differences in
61 vulnerability to develop AUD, and treatment efficacy may be related to differences in underlying
62 biology. It should be noted that "sex" refers to a biological categorisation based on physiological and
63 anatomical characteristics. Sex is generally assigned male or female at birth according to sex
64 chromosome complement and genes that usually define gonad development, genitalia and relative
65 hormone concentrations. This differs from gender, which refers to a social categorisation based on a
66 complex interplay of psychosocial factors including self-perceptions, behaviours, attitudes, and
67 expectations held about humans based on their sex. Gender varies between cultures, exists on a
68 continuum, and can change. Here, we refer to "sex" differences across rodent and human studies,
69 noting the caveat that most studies in humans do not disentangle sex and gender.

70
71 Here we review evidence of how gonadal steroid hormones, neurosteroids and other factors may
72 contribute to sex differences in alcohol use and highlight how these findings may result in more
73 targeted treatment options for subpopulations of individuals with AUD.

74

75 **A spectrum of alcohol use**

It is important to highlight that alcohol use exists across a spectrum, from low risk drinking to AUD (10). The progression from low risk, or social/recreational alcohol use to AUD has been conceptualised in a three-stage addiction cycle framework (11). The first stage is the binge/intoxication phase, where alcohol intake elicits positive reinforcing effects that can lead to more frequent engagement of problematic alcohol use, such as binge drinking, which has been preclinically modelled through operant self-administration, conditioned place preference and binge drinking paradigms (12-14). The withdrawal/negative affect stage follows, where the cessation of alcohol use coincides with the experience of withdrawal symptoms, both physical and psychological, with the extended amygdala suggested to play a critical role during this phase (11, 15). This stage has been investigated preclinically using numerous models including, the alcohol deprivation effect, anxiety-like responses and conditioned place aversion (16, 17). Finally, the preoccupation/anticipation stage is where alcohol is sought again after a period of abstinence. This phase is commonly modelled preclinically through drug, cue and stress-induced reinstatement paradigms (14, 18).

The impact of gonadal steroid hormones in alcohol use

The interaction between naturally cycling gonadal hormones and alcohol use

The relationship between naturally circulating hormones and alcohol is not well-understood, with associations between specific cycle phases and alcohol use likely influenced by other factors (i.e. age, patterns/severity of alcohol use) (19-25). For example, studies report higher alcohol intake during the luteal phase (19, 20), while another found a reduced probability to binge drink during the late luteal phase compared to all other phases (25), and others have seen no difference across the cycle following a priming dose of alcohol (24). In preclinical models, estrous cycle phase does not consistently alter alcohol intake, nor aversion-resistant alcohol consumption (26-28), although one study did report increased intake during diestrous, when progesterone levels are low and estrogen levels are rising (29). However, these data are hard to interpret, likely due to the variability in fluctuating hormones within different phases of the cycle, and differences between menstrual cycle of species (Figure 1).

When studies have quantified hormone levels, either no effect, or a positive association between estradiol and alcohol use are observed in both sexes (summarised in Table 1) (20, 30-34). However, in women this may be dependent on age, with meta-analysis revealing a positive association between alcohol intake and estradiol in post-menopausal, but not pre-menopausal women (35). However, serum estradiol levels do not differ between alcohol-dependent and non-dependent women (34), however, postmenopausal women with AUD report higher levels of craving than premenopausal women with AUD (36). Conversely, low progesterone levels are generally associated with increased alcohol intake in women, while men with AUD have higher progesterone levels than men without AUD (summarised in Table 1) (35-38). Again, in women this may be influenced by menopause with progesterone concentration alone not associated with alcohol intake in pre-menopausal women (35).

114 Notably, menopause results in stable and greatly reduced levels of hormones, including serum levels
115 of progesterone (<0.5ng/mL) (39) and in postmenopausal women, lower baseline progesterone levels
116 correlated with increased cravings (36). Heightened levels of testosterone, are correlated with earlier
117 onset, increased risk and quantity of alcohol use, and alcohol dependence in men (30, 31, 38, 40,
118 41), with heightened testosterone levels correlating with alcohol use and alcohol dependence
119 compared to non-dependent controls (34, 42-45). This does not appear to be impacted by menopause
120 with alcohol intake positively associated with testosterone concentrations in both pre and
121 postmenopausal women (35). An important consideration is the relationship between progesterone
122 and estrogen levels, and how the balance of these hormones may interact. Higher
123 progesterone:estradiol ratio was associated with a reduced likelihood of binge drinking in women and
124 men, and of any alcohol use in men (25, 46). Interestingly, this ratio may influence the efficacy of
125 certain pharmacotherapies for problematic alcohol use, as the efficacy of naltrexone to improve
126 drinking outcomes in heavy-drinking women who smoke were enhanced in cycle phases with high
127 progesterone:estradiol ratios (47).

128

129 Overall, natural levels of gonadal hormones appear to influence, or be influenced by alcohol intake
130 (Table 1). However, further studies are needed to support the association of certain estrous cycle
131 phases, hormones, and hormonal ratios more robustly with alcohol use, as the current evidence is
132 inconsistent and subject to several limitations, including samples of narrow age ranges and
133 unstandardised cycle phase classifications (48, 49). Additionally, there are a lack of preclinical studies
134 investigating the association between specific natural hormone levels and alcohol use, which would
135 be of interest to assess whether clinical observations are also seen in rodents, particularly regarding
136 the influence of the progesterone:estradiol ratio.

137

138 ***Impact of manipulated gonadal hormone levels on alcohol consumption***

139 Examining the impact of manipulating natural hormonal levels on alcohol use could enhance our
140 understanding of the relationship between gonadal hormones and alcohol use. In postmenopausal
141 women undertaking hormone replacement therapy, there is a positive association between alcohol
142 consumption and estrone, estradiol and 2-hydroxyestrone estrogen metabolite (2-OHE)
143 concentrations (32), although oral intake of estradiol elicited no change on alcohol use in young
144 women (50). The human literature here is limited, however more extensive investigation has been
145 performed preclinically.

146

147 Most studies utilise gonadectomies to manipulate naturally circulating hormone levels preclinically,
148 then re-introduce a hormone of interest to examine how this may alter alcohol intake and related
149 behaviours (see Table 2 for summary of preclinical findings). This approach has been somewhat
150 consistent in showing a positive correlation between estrogen and alcohol intake across studies in
151 female rodents (27, 51-64); however, some studies report either an opposing or no effect (65-67). In

male rodents, estradiol administration increased ethanol intake in one study (68), but had a limited effect on ethanol preference (69) or reduced voluntary intake (70) in other studies. The central mechanisms through which estrogen may influence alcohol use have begun to be investigated. Knockdown of either estrogen receptor (ER) α or ER β in the ventral tegmental area (VTA) reduced binge-like alcohol intake in female, but not male, mice with a more pronounced effect after ER α knockdown (71). Additionally, female mice display heightened levels of chronic binge-like alcohol drinking compared to male mice (13, 62), associated with decreased expression of ER α mRNA in the dorsal raphe nucleus (DRN) in females and increased ER α and serotonin-related mRNA expression in males. Further, activation of either ER α or ER β expressing neurons in the DRN reduced binge-like alcohol drinking in female mice (62).

162

Administration of progesterone does not appear to influence alcohol intake in intact or gonadectomised rodents of either sex (64, 69, 72, 73) While chronic testosterone administration reduced alcohol intake in castrated male rats, but not stress and cue induced reinstatement (60, 74). Similarly, testosterone administration in male rats was also shown to reduce ethanol preference (80). Although, another study did report that the testosterone replacement in castrated male rats increased ethanol preference (69). Interestingly, testosterone administration in castrated male rats appears to modulate the effects of alcohol on μ -opioid binding kinetics (75). Further investigation into the effect that manipulating gonadal hormone levels may have on alcohol use is required, as it may assist in elucidating the functional role of these hormones, particularly in problematic alcohol use. However, most rodent studies manipulating normal hormone levels elevate hormones to supraphysiological levels, thus consideration must be taken in the interpretation and translational utility of this information.

174

175 **The impact of neurosteroids on alcohol use**

Another emerging avenue of investigation are neurosteroids, steroid hormones synthesised within the brain from either precursor circulating gonadal hormones or *de novo* synthesis from cholesterol (76-78). Neurosteroids are classified based on their structural characteristics: pregnanes, such as allopregnanolone and allotetrahydrodeoxycorticosterone (THDOC), derived from progesterone and deoxycorticosterone, respectively; androstanes, including androstanediol and etiocholanolone; or sulphated neurosteroids such as pregnenolone sulphate and dehydroepiandrosterone sulphate (DHEA-S) (see Figure 2). Sex differences in neurosteroid levels exist under basal physiological conditions, partly due to differences in the expression and activity of neurosteroidogenic machinery, including enzymes, transporters, and receptors within the brain (for reviews see (79, 80)). As observed in gonadectomised rodents, these differences are also influenced by gonadal hormones, as the nervous system adapts local neurosteroid levels to physiological changes in these hormones in a region-specific manner (81, 82). It is important to note that the identification of neurosteroid levels in the brain and nervous system does not provide direct information on the site of synthesis; both locally synthesised neurosteroids and neuroactive peripherally synthesised steroid hormones contribute to

190 neurosteroid levels within the brain. Consequently, the effects of locally synthesised neurosteroids
191 cannot be definitively isolated (79).

192

193 Neurosteroids exert chronic effects on the brain in part by modulating neurotransmitter-gated ion
194 channels and G-protein-coupled receptors, notably γ -aminobutyric acid (GABA)-A and N-methyl-D-
195 aspartate (NMDA) receptors (83, 84). GABA-A and NMDA receptor activity is potentiated or
196 attenuated depending on the neurosteroid binding site and chemical structure, respectively. For
197 instance, allopregnanolone, a progesterone derivative, acts as a potent positive allosteric modulator
198 of GABA-A receptors, promoting GABAergic signalling (85), while also attenuating the endocrine
199 response to stress, inhibiting the release of corticotrophin releasing factor (CRF), adrenocorticotrophic
200 hormone, and glucocorticoids *in vitro* and *in vivo* (86-88). The pathophysiology of AUD is complex,
201 involving factors such as the loss of GABAergic inhibition and dysregulation of the hypothalamic-
202 pituitary-adrenal (HPA) stress axis (for reviews see (89, 90)). Neurosteroids may offer a promising
203 therapeutic approach to restore homeostasis in these systems and are under exploration as novel
204 treatments for various neuropsychiatric disorders, including AUD.

205

206 Like with peripherally circulating hormones, the interaction between alcohol use and neurosteroids is
207 bidirectional and reciprocal (91). Alcohol can alter neurosteroidogenesis and neurosteroid levels, with
208 effects varying based on factors such as sex, severity, and duration of alcohol use (see (90, 92-94)).
209 In return, exogenous neurosteroids influence alcohol consumption and reinforcement in rodents.
210 Although, it should be noted that these neurosteroids are administered at supraphysiological doses,
211 which are not representative of regular physiological conditions. Allopregnanolone administration
212 selectively modulated ethanol intake in C57B/6J male mice, with low doses (3.2 mg/kg) increasing
213 consumption, while high doses (24 mg/kg) lead to a reduction in drinking (95-97). Similar dose specific
214 responses were observed with allopregnanolone (98) and its synthetic derivative, ganaxolone in male
215 rodents (99, 100). Conversely, allopregnanolone administration did not alter alcohol consumption in
216 female C57BL/6J mice (101). Preliminary results indicate that ganaxolone may decrease alcohol
217 consumption in female mice; however, higher dosages are required to achieve outcomes similar to
218 those observed in male mice (102). It is conceivable that exogenous neurosteroid administration may
219 have sex-dependent effects on alcohol consumption; however, many of the reports described here
220 lack studies involving both sexes.

221

222 In recent years, pregnane neurosteroids and their synthetic analogues have emerged as a promising
223 novel class of drugs for treating various neuropsychiatric disorders (103-105). An integrated analysis
224 of four randomized clinical trials on zuranolone, an orally available, synthetic form of
225 allopregnanolone, for post-partum depression, demonstrated significant and sustained treatment
226 benefits with minimal side effects, improving quality of life beyond the dosing window (106) and was
227 recently FDA approved. However, mixed outcomes were observed in phase 3 trials for major

depressive disorder, notably failure of 30 mg daily zuranolone to meet its primary endpoint and two patients experiencing serious adverse effects (107). To date, only one trial investigating the use of exogenous neurosteroids for treating AUD (NCT03872128) is completed with results, finding 300 mg, but not 500 mg, of pregnenolone significantly decreased the percent of heavy drinking days and number of drinks per day over 8 weeks. Analysis of a subset of these participants also revealed a reduction in alcohol craving and normalised HPA stress markers in response to stress and alcohol cues at both doses (108). This trial of 91 participants included both men (65.5%) and women (34.5%), however sex differences were not assessed. A clinical trial (NCT05781009) is currently recruiting for the treatment of AUD with pregnenolone, and published results are pending for the effects of allopregnanolone (NCT04015869) and pregnenolone (NCT02582905) on AUD. Future research is warranted to determine whether neurosteroid treatments will be more beneficial in targeted populations and their mechanism(s) of action.

240

241 **Neurobiological factors driving alcohol consumption**

There are basal sex differences in total brain volume (109, 110), developmental trajectories and variance in volumes of both specific, and clusters of, nuclei (111-117), and grey matter volumes (109, 118), highlight both structural and volumetric differences in humans. Conversely, there is no significant difference in total brain weight in mice (119), and minimal sex differences in volumes of discrete brain regions, excluding subregions of, and surrounding, the hypothalamus, that directly modulate sexual and reproductive behaviours (120-122). In humans, there are also notable basal sex differences in the excitability of cortical networks (123), and the organisation of intrinsic and functional connectivity in humans as shown by functional magnetic resonance imaging (fMRI) studies (124). Sex differences are apparent in the neurochemical composition of healthy individuals, with higher levels of dopamine, serotonin and GABA documented in women (125). Preclinically, sex differences in monoamine levels in the rat brain have been reported, although these findings are inconsistent, as within specific nuclei there are contrasting reports of sex differences in certain monoamine levels (for review see (126)). Notably, sex differences in brain structure, morphology and neurochemistry are not independent of gonadal hormones, rather there is extensive evidence suggesting that gonadal hormones have a significant role in the manifestation/presentation of these sex differences (127-130). Despite there still being a significant underrepresentation of female rodents and women in preclinical and clinical alcohol neuroimaging studies (131, 132), the limited evidence to date suggests alcohol has both shared and distinct effects on these factors between the sexes.

260

261 ***Sex differences in brain structure and volume resulting from alcohol use***

Problematic alcohol use has both sex-specific and independent effects on the brain (133, 134). MRI revealed that college-aged women who binge drink have increased, whilst men who binge drink have decreased, volumes in certain prefrontal and motor preparatory regions, and the left caudate, hippocampus and ventral striatum, compared to healthy same-sex controls (135). Similarly, another

study showed that adolescent women who engage in binge drinking have greater cortical thickness in four left frontal regions (frontal pole, pars orbitalis, medial orbital frontal and rostral anterior cingulate) compared to same-sex controls, whilst cortical thickness of these regions are reduced in adolescent men who binge drink (136). Although, as measurements were not taken prior to alcohol exposure in these studies, it is not known whether these sex differences are induced by alcohol itself or are a pre-existing factor that may contribute to the susceptibility to engage in risky alcohol drinking. In the context of AUD, women appear more susceptible to the harmful neurological effects of alcohol, with greater impact on grey and white matter volumes according to MRI and computed tomography studies (137-142). Interestingly, in a MRI study of 966 participants (643 with alcohol dependence and 323 controls), alcohol-dependent men displayed dose-dependently reduced total amygdala and basolateral amygdala volumes, an effect not seen in alcohol-dependent women (143). Recent preclinical MRI studies including both sexes have revealed that adolescent binge alcohol exposure reduced brain growth to a greater extent in female, compared to male mice (144). Whilst, operant alcohol self-administration during adolescence reduced myelinated fibre density in the medial prefrontal cortex (mPFC) of male, but not female, rats (145), and repeated cycles of chronic intermittent ethanol vapour exposure showed females to be more vulnerable to transient disruptions of white matter integrity compared to males (146). These studies are somewhat consistent with the clinical literature, positing that females are more susceptible to the detrimental effects of alcohol on brain structure, possibly due to greater initial blood alcohol levels. Thus, not only are there baseline sex differences in brain structure and volume, problematic alcohol use also appears to have both similar and disparate effects between sexes across species.

Sex differences in alcohol-induced neural activation

In addition to basal sex differences, numerous clinical studies have revealed sex differences in neural activation in response to alcohol and alcohol-associated cues. Interestingly, even prior to alcohol exposure, alcohol-associated cues increased blood-oxygen-level dependent (BOLD) signal in frontal brain regions of alcohol-naïve adolescent women compared to men, with this inverting following moderate to heavy drinking (147). Social drinking women also have greater alcohol cue-induced BOLD response in the superior and middle frontal gyrus compared to men (148), suggesting this level of alcohol use may not be sufficient to elicit the changes in patterns of BOLD signal seen in moderate to heavy drinkers. Furthermore, in social drinkers, acute alcohol administration decreased left anterior cingulate cortex (ACC) activity in women to a greater extent than men (149), although this was not replicated (150). Whole brain linear regression of error responses in a stop signal task correlated against Alcohol Use Disorder Identification Test (AUDIT) scores revealed decreased BOLD signal across several cortical and subcortical regions, including the thalamus and mPFC, were associated with problem drinking in women, but not men (151). In terms of problematic drinkers, men have greater activation of the striatum to visual alcohol cues in an event-related alcohol cue-reactivity fMRI paradigm (152) and the left amygdala to alcohol taste cues compared to women (153). Notably, no

sex differences in alcohol cue reactivity in the striatum, ACC, mPFC, insula, amygdala or hippocampus were reported in a sample of 166 treatment seeking in-patients with AUD (154). Interestingly however, reduced activation in several brain regions in response to emotionally salient stimuli in men with AUD have been reported, whilst increased activation in the superior frontal and supramarginal regions were seen in women with AUD (155). Thus, although the clinical literature assessing sex differences in alcohol-induced neural activation remains limited, the evidence to date suggests sex differences may be more prominent in social and problematic drinkers whilst response to alcohol-associated cues may converge across sexes.

312

Whilst few direct comparisons have been published, sex differences in neural activation have been reported following aversion-resistant alcohol intake, with increased activation of the VTA in female mice being associated with increased aversion-resistant alcohol intake compared to male mice (156). There are notable sex differences in rats in neural activation following acute withdrawal and prolonged abstinence, with increased activation in the PFC and nucleus accumbens (NAc) following acute withdrawal in females, whilst following prolonged abstinence, there were sex-specific reductions in neural activation in the PFC for females and lateral habenula (LHb) for males (157). Additionally, a positive correlation between neural activation in several brain regions (e.g. prelimbic cortex, NAc, PVT, LHb, ventral subiculum) with increased reinstatement of alcohol seeking following forced, compared to voluntary abstinence has been observed in male, but not female rats (158). Therefore, numerous sex differences exist in alcohol-induced neural activation, whether that be in response to alcohol itself or alcohol-associated cues, which appears to be heavily influenced by the alcohol-related behaviour being assessed and the degree of alcohol use. Given the fragmented nature of the literature, it is difficult to draw comparisons between the preclinical and clinical research, future studies facilitating this comparison would be beneficial for translational understanding.

328

Sex differences in neurochemistry and neurocircuitry

Alcohol use, particularly its misuse, is known to result in dysregulation of numerous neurochemical systems and neural circuits (159), with there being evidence of sex differences in these changes. Young social drinking men have heightened striatal dopamine release induced by oral alcohol intake compared to women, with the most significant dopamine release being in striatal regions (160). Interestingly, alcoholic women with and without liver cirrhosis have reduced GABA-A receptor $\alpha 5$ subunit expression in prefrontal and motor cortices, whilst this was only seen in alcoholic men with liver cirrhosis (161). Preclinical research has further revealed sex differences in several neurochemical systems (162-165), including dopamine (166, 167), serotonin (168), GABA (169-171), opioid (172, 173), glutamate (174, 175) and neuropeptides (13, 176). For example, there is a sex-specific increase in GABAergic input onto central amygdala (CeA) neurons in male, but not female, alcohol-dependent rats (171). Additionally, administration of exendin-4 (glucagon-like peptide-1 receptor agonist) suppressed reinstatement of alcohol seeking to extinction levels in male mice, whilst

having no effect in female mice (165). The neuropeptide cocaine- and amphetamine-regulated transcript appears to mediate binge drinking in a sexually dimorphic manner, which is not influenced by peripherally circulating gonadal hormones. In female, but not male mice, this involves modulation of bitter taste sensitivity, driven in part, by signalling in the CeA (13). Further, CeA neurons from female rats showed reduced sensitivity to the inhibitory effects of alcohol compared to males, dependent on phase of the estrous cycle (177). Notably, numerous studies have also found no sex differences in GABA (178), μ -opioid (173) and κ -opioid systems (179, 180) mediating alcohol consumption, and no significant difference in the effect of adolescent alcohol consumption on CRF peptide expression in the CeA (181), suggesting specific neurochemical systems may be differentially involved between the sexes in alcohol-related behaviours. Most of these studies have emerged since 2014 when the NIH enacted policies to ensure biological sex is considered as a variable (182). Indeed, these policies are having an effect, with over 50% of neuroscience studies published in 2017 including both sexes, however, only 15% compared between sexes (183), missing the opportunity to identify key factors that promote and prevent behaviour.

Other factors contributing to sex differences in alcohol consumption

While hormonal and neural factors may play important roles in driving alcohol consumption differently between sexes, other factors that may also influence this exist including underlying differences in genetics, metabolism, neuroimmune responses and stress as a motivator for drinking.

Sex differences in metabolism, neuroimmune and stress responses

The pharmacokinetics of alcohol in men and women differ considerably; when all variables are the same, women reach higher peak breath alcohol levels and these levels also decline more rapidly (7). Several factors may contribute to this, including the fact that women have a smaller volume distribution, less body water, and greater fat mass than men (7). Women also have lower activity of alcohol dehydrogenase (ADH), decreasing first pass metabolism (184), increased alcohol absorption and more rapid metabolism of alcohol compared to male counterparts (185). These factors are thought to contribute to the greater toxicity of alcohol in women, with women shown to develop alcoholic cirrhosis and alcoholic hepatitis at younger and after lower accumulated alcohol intake than men (186). Although whether some of these differences are attributable to gonadal hormone levels or changes to the menstrual cycle are less clear (7).

The role of neuroimmune signalling following chronic alcohol use has received significant attention recently and several studies have highlighted genetic, epigenetic, cytokine and glial differences in which females appear more reactive to neuroinflammatory changes after chronic alcohol exposure (for reviews see (187, 188)). For example, binge drinking alters hormone signalling and neuroimmune pathways specifically in the NAc of female mice (189) and using a model of lipopolysaccharide-induced neuroinflammation, both sexes showed increased alcohol consumption, however female

380 mice showed greater cytokine upregulation, compared to males (190). Targeting the neuroimmune
381 system may be a promising avenue for the treatment of AUD, however, whether this is more beneficial
382 for women is unknown, with studies using minocycline (tetracycline antibiotic) reducing alcohol
383 consumption in both sexes (191).

384

385 Another prominent feature of alcohol use in women is the telescoping effect; where the transition to
386 AUD, onset and progression of health-related outcomes, and admission into treatment occur faster in
387 women compared to men (192). This is consistent in preclinical literature where female rodents
388 develop addictive-like behaviours more rapidly than males, including compulsive-like consumption,
389 as well as heightened craving, motivation, and vulnerability to relapse (193). Stress plays an important
390 role in the initiation, development and maintenance of AUD in both sexes (194), the role is especially
391 critical in women (195). Indeed, women with AUD report higher levels of co-occurring psychiatric
392 conditions than men with AUD. Large scale studies, including the Epidemiological Catchment Area
393 study showed over 13% of women met 6-month criteria for a DSM-III anxiety disorder, compared to
394 6% of men (196). However, the underlying drivers of such behaviour are not well defined (see (197,
395 198) for review). Importantly, stress and anxiety are major precipitants of alcohol use, and relapse to
396 alcohol use in humans and preclinical models (15, 199, 200) and sex differences in stress responses
397 are observed across the lifespan (for reviews see (197, 201)). Importantly sex differences in stress
398 responses have been linked to sex chromosome complement, as well as organisational and
399 activational effects of gonadal hormones in a bidirectional manner. Indeed, gonadal hormones
400 influence the stress response, through the HPA axis, and in turn stress hormones can alter the
401 hypothalamic-pituitary-gonadal (HPG) axis (202). These specific pathways are yet to be linked to
402 AUD.

403

404 ***The impact of sex chromosome complement and organisational effects of gonadal hormones***

405 How sex differences in alcohol-related behaviours are shaped through environment and sociocultural
406 factors, as well as experience, practice, and neural plasticity cannot be ruled out and likely interact
407 with hormones during development (203). In humans, a critical period for brain organisation is thought
408 to be between week 8 and 24 of gestation (204). Males have a sex specific peak in testosterone levels
409 between week 12 and 18, highlighting the likelihood that the brain undergoes a differential
410 developmental trajectory prenatally (205). A second surge of hormones occurs during puberty,
411 typically, beginning at age 10 in girls and age 12 in boys, puberty results in substantial increases in
412 the respective sex steroid hormones estrogen and testosterone (206). This period is critical for brain
413 development and organisation and coincides with the maturation of cognitive processes and
414 behaviour, and the development of anxiety disorders (207, 208). A recent study has examined the
415 impact of neonatal gonadal hormone exposure on subsequent ethanol intake (63). Neonatal exposure
416 to a single supraphysiological dose of estradiol valerate or testosterone propionate increased ethanol
417 intake in both male and female rats in adulthood. Of note, neonatal estradiol valerate showed a greater

effect on alcohol intake than testosterone propionate, however differences between sexes were not directly compared, and baseline alcohol consumption was low in control rats (63).

While the developmental effects of hormones appear important for alcohol use, others argue this is predetermined with genetics, and cerebral sexual dimorphism precedes gonadal hormone exposure (209). Development of gonadal sex (ovaries vs. testes) is typically determined chromosomal sex (XX vs. XY) driven by the presence or absence of the Y chromosome linked *Sry* gene making it hard to detangle the contributions of gonadal sex and sex chromosome complement to behaviour. In this regard, the four core genotypes model generates XX and XY gonadal males, and XX and XY gonadal females allowing investigation onto the influence of gonadal hormones and sex chromosome complement (210). Indeed, this model has shown sex chromosome complement contribute to differences in alcohol consumption. Mice with XY complement show greater habitual responding for ethanol than XX mice, regardless of gonadal phenotype (211). While mice with ovaries (*Sry*-) showed greater alcohol consumption in an operant procedure compared to mice with testes, irrespective of sex chromosome complement (211). Further, XY mice with testes (*Sry*+) showed greater alcohol consumption in a limited access procedure compared to XX mice with testes (*Sry*+), while preference was increased in XY males irrespective of gonads (212). In the same study authors showed XY chromosomes promoted quinine-resistant drinking in mice with ovaries (*Sry*-) (212). Using continuous home cage two-bottle choice procedures, another study showed heightened alcohol consumption in mice with ovaries (*Sry*-) compared to those with testes (*Sry*+); and in XX genotype compared to XY (213). Together these studies suggest a role for X and Y chromosome linked genes and highlight a complex relationship between chromosomal complement and gonadal hormones in alcohol related behaviours that requires greater examination. Further, given epigenetic factors serve as an interface of environmental influence over the genome, play a role in sexual differentiation of the brain (214) and AUD is linked to sex-specific DNA methylation in monoamine oxidase (215) and prodynorphin genes (216), epigenetic regulation of sex differences underpinning AUD should be further explored.

Conclusions

The complex interplay of genetic, epigenetic, environmental and hormonal interactions likely each contribute to underlying sexual dimorphisms in neural structure and function, which then in turn may predispose the sexes differently to future hormonal sensitivity and subsequent behaviour, including alcohol use – however this interplay is not well understood. Further, while recent advances have been made to ensure both sexes are accounted for in research, researchers should be implored to explore sex differences in biological, societal and environmental impacts on alcohol use, and how they can be harnessed for treatment. Future studies that not only include sex as a biological variable, but also probe the underpinnings of these difference are of utmost importance. Ultimately understanding these differences may lead to better treatment options for both sexes.

456

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463

464 **Author contributions**

465 All authors contributed to the conceptualisation, original drafting and editing of the manuscript.

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467 **Data availability**

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470 **References:**

- 471 1. Luscher C, Robbins TW, Everitt BJ. The transition to compulsion in addiction. *Nat Rev*
472 *Neurosci.* 2020;21(5):247-63.
- 473 2. Manthey J, Shield KD, Rylett M, Hasan OSM, Probst C, Rehm J. Global alcohol exposure
474 between 1990 and 2017 and forecasts until 2030: a modelling study. *Lancet.* 2019;393(10190):2493-
475 502.
- 476 3. Grant BF, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, et al. Prevalence of 12-
477 Month Alcohol Use, High-Risk Drinking, and DSM-IV Alcohol Use Disorder in the United States, 2001-
478 2002 to 2012-2013: Results From the National Epidemiologic Survey on Alcohol and Related
479 Conditions. *JAMA Psychiatry.* 2017;74(9):911-23.
- 480 4. Grucza RA, Sher KJ, Kerr WC, Krauss MJ, Lui CK, McDowell YE, et al. Trends in Adult Alcohol
481 Use and Binge Drinking in the Early 21st-Century United States: A Meta-Analysis of 6 National Survey
482 Series. *Alcohol Clin Exp Res.* 2018;42(10):1939-50.
- 483 5. White AM. Gender Differences in the Epidemiology of Alcohol Use and Related Harms in the
484 United States. *Alcohol Res.* 2020;40(2):01.
- 485 6. Arnedt JT, Rohsenow DJ, Almeida AB, Hunt SK, Gokhale M, Gottlieb DJ, Howland J. Sleep
486 following alcohol intoxication in healthy, young adults: effects of sex and family history of alcoholism.
487 *Alcohol Clin Exp Res.* 2011;35(5):870-8.
- 488 7. Agabio R, Pisanu C, Gessa GL, Franconi F. Sex Differences in Alcohol Use Disorder. *Curr*
489 *Med Chem.* 2017;24(24):2661-70.
- 490 8. Kirsch DE, Belnap MA, Burnette EM, Grodin EN, Ray LA. Pharmacological Treatments for
491 Alcohol Use Disorder: Considering the Role of Sex and Gender. *Curr Addict Rep.* 2024;11(1):81-93.
- 492 9. Agabio R, Pani PP, Preti A, Gessa GL, Franconi F. Efficacy of Medications Approved for the
493 Treatment of Alcohol Dependence and Alcohol Withdrawal Syndrome in Female Patients: A
494 Descriptive Review. *Eur Addict Res.* 2016;22(1):1-16.
- 495 10. MacKillop J, Agabio R, Feldstein Ewing SW, Heilig M, Kelly JF, Leggio L, et al. Hazardous
496 drinking and alcohol use disorders. *Nat Rev Dis Primers.* 2022;8(1):80.
- 497 11. Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology.*
498 2010;35(1):217-38.
- 499 12. Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC. Evaluation of a simple model of ethanol
500 drinking to intoxication in C57BL/6J mice. *Physiol Behav.* 2005;84(1):53-63.
- 501 13. Maddern XJ, Letherby B, Ch'ng SS, Pearl A, Gogos A, Lawrence AJ, Walker LC. Cocaine and
502 amphetamine regulated transcript (CART) mediates sex differences in binge drinking through central
503 taste circuits. *Neuropsychopharmacology.* 2024;49(3):541-50.

504 14. Walker LC, Berizzi AE, Chen NA, Rueda P, Perreau VM, Huckstep K, et al. Acetylcholine
505 Muscarinic M(4) Receptors as a Therapeutic Target for Alcohol Use Disorder: Converging Evidence
506 From Humans and Rodents. *Biol Psychiatry*. 2020;88(12):898-909.

507 15. Walker LC. A balancing act: the role of pro- and anti-stress peptides within the central
508 amygdala in anxiety and alcohol use disorders. *J Neurochem*. 2021;157(5):1615-43.

509 16. Becker HC, Lopez MF. Animal Models of Excessive Alcohol Consumption in Rodents. *Curr*
510 *Top Behav Neurosci*. 2024.

511 17. Walker LC, Kastman HE, Krstew EV, Gundlach AL, Lawrence AJ. Central amygdala relaxin-
512 3/relaxin family peptide receptor 3 signalling modulates alcohol seeking in rats. *Br J Pharmacol*.
513 2017;174(19):3359-69.

514 18. Walker LC, Kastman HE, Koeleman JA, Smith CM, Perry CJ, Krstew EV, et al. Nucleus
515 incertus corticotrophin-releasing factor 1 receptor signalling regulates alcohol seeking in rats. *Addict*
516 *Biol*. 2017;22(6):1641-54.

517 19. Harvey SM, Beckman LJ. Cyclic fluctuation in alcohol consumption among female social
518 drinkers. *Alcohol Clin Exp Res*. 1985;9(5):465-71.

519 20. Muti P, Trevisan M, Micheli A, Krogh V, Bolelli G, Sciajno R, et al. Alcohol consumption and
520 total estradiol in premenopausal women. *Cancer Epidemiol Biomarkers Prev*. 1998;7(3):189-93.

521 21. Sutker PB, Libet JM, Allain AN, Randall CL. Alcohol use, negative mood states, and menstrual
522 cycle phases. *Alcohol Clin Exp Res*. 1983;7(3):327-31.

523 22. Pastor AD, Evans SM. Alcohol outcome expectancies and risk for alcohol use problems in
524 women with and without a family history of alcoholism. *Drug Alcohol Depend*. 2003;70(2):201-14.

525 23. Mello NK, Mendelson JH, Lex BW. Alcohol use and premenstrual symptoms in social drinkers.
526 *Psychopharmacology*. 1990;101:448-55.

527 24. Holdstock L, de Wit H. Effects of ethanol at four phases of the menstrual cycle.
528 *Psychopharmacology*. 2000;150:374-82.

529 25. Hoffmann S, Gerhardt S, Mühle C, Reinhard I, Reichert D, Bach P, et al. Associations of
530 Menstrual Cycle and Progesterone-to-Estradiol Ratio With Alcohol Consumption in Alcohol Use
531 Disorder: A Sex-Separated Multicenter Longitudinal Study. *Am J Psychiatry*. 2024.

532 26. Ford MM, Eldridge J, Samson HH. Microanalysis of ethanol self-administration: estrous cycle
533 phase-related changes in consumption patterns. *Alcohol Clin Exp Res*. 2002;26(5):635-43.

534 27. Satta R, Hilderbrand ER, Lasek AW. Ovarian hormones contribute to high levels of binge-like
535 drinking by female mice. *Alcohol Clin Exp Res*. 2018;42(2):286-94.

536 28. Fulenwider HD, Nennig SE, Price ME, Hafeez H, Schank JR. Sex differences in aversion-
537 resistant ethanol intake in mice. *Alcohol Alcohol*. 2019;54(4):345-52.

538 29. Roberts AJ, Smith A, Weiss F, Rivier C, Koob G. Estrous cycle effects on operant responding
539 for ethanol in female rats. *Alcohol Clin Exp Res*. 1998;22(7):1564-9.

540 30. de Water E, Braams BR, Crone EA, Peper JS. Pubertal maturation and sex steroids are
541 related to alcohol use in adolescents. *Horm Behav*. 2013;63(2):392-7.

542 31. Costello EJ, Sung M, Worthman C, Angold A. Pubertal maturation and the development of
543 alcohol use and abuse. *Drug Alcohol Depend*. 2007;88:S50-S9.

544 32. Playdon MC, Coburn SB, Moore SC, Brinton LA, Wentzensen N, Anderson G, et al. Alcohol
545 and oestrogen metabolites in postmenopausal women in the Women's Health Initiative Observational
546 Study. *Br J Cancer*. 2018;118(3):448-57.

547 33. Heinz A, Rommelspacher H, Gräf K-J, Kürten I, Otto M, Baumgartner A. Hypothalamic-
548 pituitary-gonadal axis, prolactin, and cortisol in alcoholics during withdrawal and after three weeks of
549 abstinence: comparison with healthy control subjects. *Psychiatry Res*. 1995;56(1):81-95.

550 34. Ho AM-C, Pozsonyiova S, Waller TC, Song Y, Geske JR, Karpyak VM, Winham SJ. Associations of sex-related steroid hormones and proteins with alcohol dependence: A United
551 Kingdom Biobank study. *Drug Alcohol Depend*. 2023;244:109781.

552 35. Tin ST, Smith-Byrne K, Ferrari P, Rinaldi S, McCullough ML, Teras LR, et al. Alcohol intake
553 and endogenous sex hormones in women: meta-analysis of cohort studies and Mendelian
554 randomization. *Res Sq*. 2023.

555 36. Weinland C, Mühle C, Kornhuber J, Lenz B. Progesterone serum levels correlate negatively
556 with craving in female postmenopausal in-patients with alcohol use disorder: A sex-and menopausal
557 status-separated study. *Prog Neuropsychopharmacol Biol Psychiatry*. 2021;110:110278.

37. Holzhauser CG, Wemm SE, Wulfert E, Cao ZT. Fluctuations in progesterone moderate the relationship between daily mood and alcohol use in young adult women. *Addict Behav.* 2020;101:106146.

38. Ho AM-C, Geske JR, Bakalkin G, Winham SJ, Karpayak VM. Correlations between sex-related hormones, alcohol dependence and alcohol craving. *Drug Alcohol Depend.* 2019;197:183-90.

39. Henderson V. Progesterone and human cognition. *Climacteric.* 2018;21(4):333-40.

40. Eriksson CP, Kaprio J, Pulkkinen L, Rose RJ. Testosterone and alcohol use among adolescent male twins: testing between-family associations in within-family comparisons. *Behav Genet.* 2005;35:359-68.

41. Stålenheim EG, Eriksson E, von Knorring L, Wide L. Testosterone as a biological marker in psychopathy and alcoholism. *Psychiatry Res.* 1998;77(2):79-88.

42. Braams BR, Peper JS, Van Der Heide D, Peters S, Crone EA. Nucleus accumbens response to rewards and testosterone levels are related to alcohol use in adolescents and young adults. *Dev Cogn Neurosci.* 2016;17:83-93.

43. La Grange L, Jones TD, Erb L, Reyes E. Alcohol consumption: biochemical and personality correlates in a college student population. *Addict Behav.* 1995;20(1):93-103.

44. Tin Tin S, Key TJ, Reeves GK. Alcohol intake and endogenous hormones in pre-and postmenopausal women: findings from the UK Biobank. *Cancer Epidemiol Biomarkers Prev.* 2021;30(12):2294-301.

45. Lenz B, Mühle C, Braun B, Weinland C, Bouna-Pyrrou P, Behrens J, et al. Prenatal and adult androgen activities in alcohol dependence. *Acta Psychiatrica Scandinavica.* 2017;136(1):96-107.

46. Martel MM, Eisenlohr-Moul T, Roberts B. Interactive effects of ovarian steroid hormones on alcohol use and binge drinking across the menstrual cycle. *J Abnorm Psychol.* 2017;126(8):1104.

47. Green R, Roche DJ, Ray LA. The effects of menstrual cycle hormones on responses to varenicline and naltrexone among female heavy drinking smokers. *Alcohol Alcohol.* 2022;57(5):609-14.

48. Warren JG, Fallon VM, Goodwin L, Gage SH, Rose AK. Menstrual cycle phase, hormonal contraception, and alcohol consumption in premenopausal females: a systematic review. *Front Glob Womens Health.* 2021;2:745263.

49. Joyce KM, Good KP, Tibbo P, Brown J, Stewart SH. Addictive behaviors across the menstrual cycle: a systematic review. *Arch Womens Ment Health.* 2021:1-14.

50. Little R, Moore D, Guzinski G, Perez A. Absence of effect of exogenous estradiol on alcohol consumption in women. *Subst Alcohol Actions Misuse.* 1980;1(5-6):551-6.

51. Forger NG, Morin L. Reproductive state modulates ethanol intake in rats: effects of ovariectomy, ethanol concentration, estrous cycle and pregnancy. *Pharmacol Biochem Behav.* 1982;17(2):323-31.

52. Reid LD, Marinelli PW, Bennett SM, Fiscale LT, Narciso SP, Oparowski CJ, et al. One injection of estradiol valerate induces dramatic changes in rats' intake of alcoholic beverages. *Pharmacol Biochem Behav.* 2002;72(3):601-16.

53. Ford MM, Eldridge JC, Samson HH. Ethanol consumption in the female Long-Evans rat: a modulatory role of estradiol. *Alcohol.* 2002;26(2):103-13.

54. Ford MM, Eldridge J, Samson HH. Determination of an estradiol dose-response relationship in the modulation of ethanol intake. *Alcohol Clin Exp Res.* 2004;28(1):20-8.

55. Marinelli PW, Quirion R, Gianoulakis C. Estradiol valerate and alcohol intake: a comparison between Wistar and Lewis rats and the putative role of endorphins. *Behav Brain Res.* 2003;139(1-2):59-67.

56. Reid ML, Hubbell CL, Reid LD. A pharmacological dose of estradiol can enhance appetites for alcoholic beverages. *Pharmacol Biochem Behav.* 2003;74(2):381-8.

57. Quirarte GL, Reid LD, de la Teja ISL, Reid ML, Sánchez MA, Díaz-Trujillo A, et al. Estradiol valerate and alcohol intake: dose-response assessments. *BMC Pharmacol.* 2007;7:1-14.

58. Sherrill LK, Koss WA, Foreman ES, Gulley JM. The effects of pre-pubertal gonadectomy and binge-like ethanol exposure during adolescence on ethanol drinking in adult male and female rats. *Behav Brain Res.* 2011;216(2):569-75.

59. Torres OV, Walker EM, Beas BS, O'Dell LE. Female rats display enhanced rewarding effects of ethanol that are hormone dependent. *Alcohol Clin Exp Res.* 2014;38(1):108-15.

60. Bertholomey ML, Torregrossa MM. Gonadal hormones affect alcohol drinking, but not cue+ yohimbine-induced alcohol seeking, in male and female rats. *Physiol Behav.* 2019;203:70-80.

61. Hilderbrand ER, Lasek AW. Estradiol enhances ethanol reward in female mice through activation of ER α and ER β . *Horm Behav.* 2018;98:159-64.

62. Torres Irizarry VC, Feng B, Yang X, Patel N, Schaul S, Ibrahimi L, et al. Estrogen signaling in the dorsal raphe regulates binge-like drinking in mice. *Transl Psychiatry.* 2024;14(1):122.

63. Venegas FC, Rosas D, Delgado N, Estay-Olmos C, Iturriaga-Vasquez P, Rivera-Meza M, et al. Early-life exposure to sex hormones promotes voluntary ethanol intake in adulthood. A vulnerability factor to drug addiction. *Prog Neuropsychopharmacol Biol Psychiatry.* 2024;133:111001.

64. Arnold ME, Decker Ramirez EB, Beugelsdyk LA, Siano Kuzolitz MV, Jiang Q, Schank JR. Estradiol mediates sex differences in aversion-resistant alcohol intake. *Front Neurosci.* 2023;17:1282230.

65. Sandberg D, Stewart J. Effects of estradiol benzoate and MER-25 on ethanol consumption in the ovariectomized rat. *J Comp Physiol Psychol.* 1982;96(4):635.

66. Cailhol S, Mormède P. Sex and strain differences in ethanol drinking: effects of gonadectomy. *Alcohol Clin Exp Res.* 2001;25(4):594-9.

67. Vetter-O'Hagen CS, Spear LP. The Effects of Gonadectomy on Age- and Sex-Typical Patterns of Ethanol Consumption in Sprague-Dawley Rats. *Alcohol Clin Exp Res.* 2011;35(11):2039-49.

68. Hilakivi-Clarke L. Role of estradiol in alcohol intake and alcohol-related behaviors. *J Stud Alcohol.* 1996;57(2):162-70.

69. Lakoza G, Barkov N. The role of testosterone in the development of experimental alcoholism. *Bull Narc.* 1980;32(2):41-8.

70. Juárez J, De Tomasi EB, Virgen M. Effects of estradiol treatment on voluntary and forced alcohol consumption in male rats. *Pharmacol Biochem Behav.* 2002;71(1-2):259-68.

71. Vandegrift BJ, Hilderbrand ER, Satta R, Tai R, He D, You C, et al. Estrogen receptor α regulates ethanol excitation of ventral tegmental area neurons and binge drinking in female mice. *J Neurosci.* 2020;40(27):5196-207.

72. Almeida OF, Shoaib M, Deicke J, Fischer D, Darwish MH, Patchev VK. Gender differences in ethanol preference and ingestion in rats. The role of the gonadal steroid environment. *J Clin Invest.* 1998;101(12):2677-85.

73. Wolfe JR, Means LW, McMillen BA. Effects of pregnancy and progesterone on the consumption of ethanol by the high ethanol preferring (HEP) rat. *Alcohol Alcohol.* 2000;35(4):344-50.

74. Vetter-O'Hagen CS, Sanders KW, Spear LP. Evidence for suppressant effects of testosterone on sex-typical ethanol intake in male Sprague-Dawley rats. *Behav Brain Res.* 2011;224(2):403-7.

75. Khalil R, Humann J. Testosterone modulation of ethanol effects on the μ -opioid receptor kinetics in castrated rats. *Biomed Rep.* 2019;11(3):103-9.

76. Paul SM, Purdy RH. Neuroactive steroids. *FASEB J.* 1992;6(6):2311-22.

77. Agís-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *P Natl Acad Sci USA.* 2006;103(39):14602-7.

78. Reddy DS. Neurosteroids: Endogenous role in the human brain and therapeutic potentials. *Sex Differences in the Human Brain, Their Underpinnings and Implications.* 1862010. p. 113-37.

79. Giatti S, Diviccaro S, Serafini MM, Caruso D, Garcia-Segura LM, Viviani B, Melcangi RC. Sex differences in steroid levels and steroidogenesis in the nervous system: Physiopathological role. *Front Neuroendocrin.* 2020;56.

80. Panzica G, Melcangi RC. Structural and molecular brain sexual differences: A tool to understand sex differences in health and disease. *Neurosci Biobehav Rev.* 2016;67:2-8.

81. Caruso D, Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Melcangi RC. Effect of short- and long-term gonadectomy on neuroactive steroid levels in the central and peripheral nervous system of male and female rats. *J Neuroendocrinol.* 2010;22(11):1137-47.

82. Giatti S, Diviccaro S, Garcia-Segura LM, Melcangi RC. Sex differences in the brain expression of steroidogenic molecules under basal conditions and after gonadectomy. *J Neuroendocrinol.* 2019;31(6):e12736.

83. Reddy DS. Is there a physiological role for the neurosteroid THDOC in stress-sensitive conditions? *Trends Pharmacol Sci.* 2003;24(3):103-6.

84. Lester RAJ, Jahr CE. Nmda Channel Behavior Depends on Agonist Affinity. *J Neurosci.* 1992;12(2):635-43.

85. Purdy RH, Morrow AL, Blinn JR, Paul SM. Synthesis, Metabolism, and Pharmacological Activity of 3-Alpha-Hydroxy Steroids Which Potentiate Gaba-Receptor-Mediated Chloride-Ion Uptake in Rat Cerebral Cortical Synaptoneurosomes. *J Med Chem.* 1990;33(6):1572-81.

86. Owens MJ, Ritchie JC, Nemeroff CB. 5-Alpha-Pregnane-3-Alpha,21-Diol-20-One (Thdoc) Attenuates Mild Stress-Induced Increases in Plasma-Corticosterone Via a Nonglucocorticoid Mechanism - Comparison with Alprazolam. *Brain Res.* 1992;573(2):353-5.

87. Patchev VK, Hassan AHS, Holsboer F, Almeida OFX. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. *Neuropsychopharmacology.* 1996;15(6):533-40.

88. Patchev V, Shoaib M, Holsboer F, Almeida O. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience.* 1994;62(1):265-71.

89. Morrow AL, Boero G, Balan I. Emerging evidence for endogenous neurosteroid modulation of pro-inflammatory and anti-inflammatory pathways that impact neuropsychiatric disease. *Neurosci Biobehav Rev.* 2024;158:105558.

90. Morrow AL, Boero G, Porcu P. A Rationale for Allopregnanolone Treatment of Alcohol Use Disorders: Basic and Clinical Studies. *Alcohol Clin Exp Res.* 2020;44(2):320-39.

91. Finn DA, Jimenez VA. Dynamic Adaptation in Neurosteroid Networks in Response to Alcohol. *Handb Exp Pharmacol.* 2018;248:55-78.

92. Gatta E, Camussi D, Auta J, Guidotti A, Pandey SC. Neurosteroids (allopregnanolone) and alcohol use disorder: From mechanisms to potential pharmacotherapy. *Pharmacol Ther.* 2022;240:108299.

93. Peltier MR, Verplaetse TL, Altemus M, Zakiniaieiz Y, Ralevski EA, Mineur YS, et al. The role of neurosteroids in posttraumatic stress disorder and alcohol use disorder: A review of 10 years of clinical literature and treatment implications. *Front Neuroendocrinol.* 2024;101119.

94. Peltier MR, Verplaetse TL, Mineur YS, Gueorguieva R, Petrakis I, Cosgrove KP, et al. Sex differences in progestogen- and androgen-derived neurosteroids in vulnerability to alcohol and stress-related disorders. *Neuropharmacology.* 2021;187:108499.

95. Ford MM, Mark GP, Nickel JD, Phillips TJ, Finn DA. Allopregnanolone influences the consummatory processes that govern ethanol drinking in C57BL/6J mice. *Behav Brain Res.* 2007;179(2):265-72.

96. Ford MM, Nickel JD, Phillips TJ, Finn DA. Neurosteroid modulators of GABA(A) receptors differentially modulate Ethanol intake patterns in male C57BL/6J mice. *Alcohol Clin Exp Res.* 2005;29(9):1630-40.

97. Ford MM, Nickel JD, Finn DA. Treatment with and withdrawal from finasteride alter ethanol intake patterns in male C57BL/6J mice: potential role of endogenous neurosteroids? *Alcohol.* 2005;37(1):23-33.

98. Janak PH, Redfern JE, Samson HH. The reinforcing effects of ethanol are altered by the endogenous neurosteroid, allopregnanolone. *Alcohol Clin Exp Res.* 1998;22(5):1106-12.

99. Ramaker MJ, Ford MM, Fretwell AM, Finn DA. Alteration of Ethanol Drinking in Mice via Modulation of the GABAA Receptor with Ganaxolone, Finasteride, and Gaboxadol. *Alcohol Clin Exp Res.* 2011;35(11):1994-2007.

100. Besheer J, Lindsay TG, O'Buckley TK, Hodge CW, Morrow AL. Pregnenolone and ganaxolone reduce operant ethanol self-administration in alcohol-preferring p rats. *Alcohol Clin Exp Res.* 2010;34(12):2044-52.

101. Sinnott RS, Phillips TJ, Finn DA. Alteration of voluntary ethanol and saccharin consumption by the neurosteroid allopregnanolone in mice. *Psychopharmacology (Berl).* 2002;162(4):438-47.

102. Finn DA. The Endocrine System and Alcohol Drinking in Females. *Alcohol Res.* 2020;40(2):02.

103. Marecki R, Kaluska J, Kolanek A, Hakalo D, Waszkiewicz N. Zuranolone - synthetic neurosteroid in treatment of mental disorders: narrative review. *Front Psychiatry.* 2023;14:1298359.

104. Patterson R, Balan I, Morrow AL, Meltzer-Brody S. Novel neurosteroid therapeutics for post-partum depression: perspectives on clinical trials, program development, active research, and future directions. *Neuropsychopharmacology.* 2024;49(1):67-72.

105. Wang M, Hu S, Fu X, Zhou H, Yang S, Yang C. Neurosteroids: A potential target for neuropsychiatric disorders. *J Steroid Biochem Mol Biol.* 2024;239:106485.

106. Clayton AH, Suthoff E, Jain R, Kosinski M, Fridman M, Deligiannidis KM, et al. The magnitude and sustainability of treatment benefit of zuranolone on function and well-being as assessed by the SF-36 in adult patients with MDD and PPD: An integrated analysis of 4 randomized clinical trials. *J Affect Disord.* 2024;351:904-14.

107. Clayton AH, Lasser R, Nandy I, Sankoh AJ, Jonas J, Kanesh SJ. Zuranolone in Major Depressive Disorder: Results From MOUNTAIN-A Phase 3, Multicenter, Double-Blind, Randomized, Placebo-Controlled Trial. *J Clin Psychiatry.* 2023;84(2).

108. Milivojevic V, Sullivan L, Tiber J, Fogelman N, Simpson C, Hermes G, Sinha R. Pregnenolone effects on provoked alcohol craving, anxiety, HPA axis, and autonomic arousal in individuals with alcohol use disorder. *Psychopharmacology (Berl).* 2023;240(1):101-14.

109. Ruigrok AN, Salimi-Khorshidi G, Lai M-C, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J. A meta-analysis of sex differences in human brain structure. *Neurosci Biobehav Rev.* 2014;39:34-50.

110. Ritchie SJ, Cox SR, Shen X, Lombardo MV, Reus LM, Alloza C, et al. Sex differences in the adult human brain: evidence from 5216 UK biobank participants. *Cereb Cortex.* 2018;28(8):2959-75.

111. Group BDC. Total and regional brain volumes in a population-based normative sample from 4 to 18 years: the NIH MRI Study of Normal Brain Development. *Cereb Cortex.* 2012;22(1):1-12.

112. Good CD, Johnsrude I, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. *Neuroimage.* 2001;14(3):685-700.

113. Koolschijn PCM, Crone EA. Sex differences and structural brain maturation from childhood to early adulthood. *Dev Cogn Neurosci.* 2013;5:106-18.

114. Lenroot RK, Gogtay N, Greenstein DK, Wells EM, Wallace GL, Clasen LS, et al. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage.* 2007;36(4):1065-73.

115. Li W, van Tol MJ, Li M, Miao W, Jiao Y, Heinze HJ, et al. Regional specificity of sex effects on subcortical volumes across the lifespan in healthy aging. *Hum Brain Mapp.* 2014;35(1):238-47.

116. Pfefferbaum A, Rohlfing T, Rosenbloom MJ, Chu W, Colrain IM, Sullivan EV. Variation in longitudinal trajectories of regional brain volumes of healthy men and women (ages 10 to 85 years) measured with atlas-based parcellation of MRI. *Neuroimage.* 2013;65:176-93.

117. Wierenga LM, Sexton JA, Laake P, Giedd JN, Tamnes CK, Pediatric Imaging N, Study G. A key characteristic of sex differences in the developing brain: greater variability in brain structure of boys than girls. *Cereb Cortex.* 2018;28(8):2741-51.

118. Lotze M, Domin M, Gerlach FH, Gaser C, Lueders E, Schmidt CO, Neumann N. Novel findings from 2,838 adult brains on sex differences in gray matter brain volume. *Sci Rep.* 2019;9(1):1671.

119. Hager R, Lu L, Rosen GD, Williams RW. Genetic architecture supports mosaic brain evolution and independent brain-body size regulation. *Nat Commun.* 2012;3(1):1079.

120. McCarthy M, De Vries G, Forger N. Sexual Differentiation of the Brain: A Fresh Look at Mode, Mechanisms, and Meaning. 2017.

121. McCarthy MM. A new view of sexual differentiation of mammalian brain. *J Comp Physiol Psychol.* 2020;206(3):369-78.

122. Gorski RA, Gordon JH, Shryne JE, Southam AM. Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res.* 1978;148(2):333-46.

123. Jaušovec N, Jaušovec K. Resting brain activity: differences between genders. *Neuropsychologia.* 2010;48(13):3918-25.

124. Filippi M, Valsasina P, Misci P, Falini A, Comi G, Rocca MA. The organization of intrinsic brain activity differs between genders: A resting-state fMRI study in a large cohort of young healthy subjects. *Hum Brain Mapp.* 2013;34(6):1330-43.

125. Cosgrove KP, Mazure CM, Staley JK. Evolving knowledge of sex differences in brain structure, function, and chemistry. *Biol Psychiatry.* 2007;62(8):847-55.

126. Simpson J, Kelly JP. An investigation of whether there are sex differences in certain behavioural and neurochemical parameters in the rat. *Behav Brain Res.* 2012;229(1):289-300.

127. Witte AV, Savli M, Holik A, Kasper S, Lanzenberger R. Regional sex differences in grey matter volume are associated with sex hormones in the young adult human brain. *Neuroimage.* 2010;49(2):1205-12.

128. Pletzer B, Kronbichler M, Aichhorn M, Bergmann J, Ladurner G, Kerschbaum HH. Menstrual cycle and hormonal contraceptive use modulate human brain structure. *Brain Res.* 2010;1348:55-62.

784 129. Marrocco J, McEwen BS. Sex in the brain: hormones and sex differences. *Dialogues Clin*
785 *Neurosci.* 2016;18(4):373-83.

786 130. Barth C, Villringer A, Sacher J. Sex hormones affect neurotransmitters and shape the adult
787 female brain during hormonal transition periods. *Front Neurosci.* 2015;9:37.

788 131. Verplaetse TL, Cosgrove KP, Tanabe J, McKee SA. Sex/gender differences in brain function
789 and structure in alcohol use: A narrative review of neuroimaging findings over the last 10 years. *J*
790 *Neurosci Res.* 2021;99(1):309-23.

791 132. Fritz M, Klawonn AM, Zahr NM. Neuroimaging in alcohol use disorder: From mouse to man. *J*
792 *Neurosci Res.* 2022;100(5):1140-58.

793 133. Maggioni E, Rossetti MG, Allen NB, Batalla A, Bellani M, Chye Y, et al. Brain volumes in
794 alcohol use disorder: Do females and males differ? A whole-brain magnetic resonance imaging mega-
795 analysis. *Hum Brain Mapp.* 2023;44(13):4652-66.

796 134. Pfefferbaum A, Rohlfing T, Pohl KM, Lane B, Chu W, Kwon D, et al. Adolescent development
797 of cortical and white matter structure in the NCANDA sample: role of sex, ethnicity, puberty, and
798 alcohol drinking. *Cereb Cortex.* 2016;26(10):4101-21.

799 135. Kvamme TL, Schmidt C, Strelchuk D, Chang-Webb YC, Baek K, Voon V. Sexually dimorphic
800 brain volume interaction in college-aged binge drinkers. *Neuroimage Clin.* 2016;10:310-7.

801 136. Squeglia LM, Sorg SF, Schweinsburg AD, Wetherill RR, Pulido C, Tapert SF. Binge drinking
802 differentially affects adolescent male and female brain morphometry. *Psychopharmacology.*
803 2012;220:529-39.

804 137. Agartz I, Shoaf S, Rawlings RR, Momenan R, Hommer DW. CSF monoamine metabolites and
805 MRI brain volumes in alcohol dependence. *Psychiatry Res Neuroimaging.* 2003;122(1):21-35.

806 138. Hommer DW, Momenan R, Kaiser E, Rawlings RR. Evidence for a gender-related effect of
807 alcoholism on brain volumes. *Am J Psychiatry.* 2001;158(2):198-204.

808 139. Mann K, Ackermann K, Croissant B, Mundle G, Nakovics H, Diehl A. Neuroimaging of gender
809 differences in alcohol dependence: are women more vulnerable? *Alcohol Clin Exp Res.*
810 2005;29(5):896-901.

811 140. Momenan R, Steckler LE, Saad ZS, van Rafelghem S, Kerich MJ, Hommer DW. Effects of
812 alcohol dependence on cortical thickness as determined by magnetic resonance imaging. *Psychiatry*
813 *Res.* 2012;204(2-3):101-11.

814 141. Monnig MA, Tonigan JS, Yeo RA, Thoma RJ, McCrady BS. White matter volume in alcohol
815 use disorders: a meta-analysis. *Addict Biol.* 2013;18(3):581-92.

816 142. Thayer RE, Hagerty SL, Sabbineni A, Claus ED, Hutchison KE, Weiland BJ. Negative and
817 interactive effects of sex, aging, and alcohol abuse on gray matter morphometry. *Hum Brain Mapp.*
818 2016;37(6):2276-92.

819 143. Grace S, Rossetti MG, Allen N, Batalla A, Bellani M, Brambilla P, et al. Sex differences in the
820 neuroanatomy of alcohol dependence: hippocampus and amygdala subregions in a sample of 966
821 people from the ENIGMA Addiction Working Group. *Transl Psychiatry.* 2021;11(1):156.

822 144. Piekarski DJ, Zahr NM, Zhao Q, Sullivan EV, Pfefferbaum A. Alcohol's effects on the mouse
823 brain are modulated by age and sex. *Addict Biol.* 2022;27(5).

824 145. Tavares ER, Silva-Gotay A, Riad WV, Bengston L, Richardson HN. Sex Differences in the
825 Effect of Alcohol Drinking on Myelinated Axons in the Anterior Cingulate Cortex of Adolescent Rats.
826 *Brain Sci.* 2019;9(7).

827 146. Zahr NM, Lenart AM, Karpf JA, Casey KM, Pohl KM, Sullivan EV, Pfefferbaum A. Multi-modal
828 imaging reveals differential brain volumetric, biochemical, and white matter fiber responsivity to
829 repeated intermittent ethanol vapor exposure in male and female rats. *Neuropharmacology.*
830 2020;170:108066.

831 147. Nguyen-Louie TT, Courtney KE, Squeglia LM, Bagot K, Eberson S, Migliorini R, et al.
832 Prospective changes in neural alcohol cue reactivity in at-risk adolescents. *Brain Imaging Behav.*
833 2018;12:931-41.

834 148. Seo D, Jia Z, Lacadie CM, Tsou KA, Bergquist K, Sinha R. Sex differences in neural responses
835 to stress and alcohol context cues. *Hum Brain Mapp.* 2011;32(11):1998-2013.

836 149. Marinkovic K, Rickenbacher E, Azma S, Artsy E. Acute alcohol intoxication impairs top-down
837 regulation of Stroop incongruity as revealed by blood oxygen level-dependent functional magnetic
838 resonance imaging. *Hum Brain Mapp.* 2012;33(2):319-33.

839 150. Marinkovic K, Rickenbacher E, Azma S, Artsy E, Lee AK. Effects of acute alcohol intoxication
840 on saccadic conflict and error processing. *Psychopharmacology (Berl).* 2013;230(3):487-97.

151. Ide JS, Zhornitsky S, Chao HH, Zhang S, Hu S, Wang W, et al. Thalamic Cortical Error-Related Responses in Adult Social Drinkers: Sex Differences and Problem Alcohol Use. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2018;3(10):868-77.

152. Kaag AM, Wiers RW, de Vries TJ, Pattij T, Goudriaan AE. Striatal alcohol cue-reactivity is stronger in male than female problem drinkers. *Eur J Neurosci*. 2019;50(3):2264-73.

153. Claus ED, Ewing SW, Filbey FM, Sabbineni A, Hutchison KE. Identifying neurobiological phenotypes associated with alcohol use disorder severity. *Neuropsychopharmacology*. 2011;36(10):2086-96.

154. Gerhardt S, Hoffmann S, Tan H, Gerchen MF, Kirsch P, Vollstadt-Klein S, et al. Neural cue reactivity is not stronger in male than in female patients with alcohol use disorder. *Front Behav Neurosci*. 2022;16:1039917.

155. Sawyer KS, Maleki N, Urban T, Marinkovic K, Karson S, Ruiz SM, et al. Alcoholism gender differences in brain responsivity to emotional stimuli. *Elife*. 2019;8:e41723.

156. Arnold ME, Butts AN, Erlenbach TR, Amico KN, Schank JR. Sex differences in neuronal activation during aversion-resistant alcohol consumption. *Alcohol Clin Exp Res*. 2023;47(2):240-50.

157. Li J, Chen P, Han X, Zuo W, Mei Q, Bian EY, et al. Differences between male and female rats in alcohol drinking, negative affects and neuronal activity after acute and prolonged abstinence. *Int J Physiol Pathophysiol Pharmacol*. 2019;11(4):163.

158. Maddern XJ, Lawrence AJ, Campbell EJ. Electric barrier-induced voluntary abstinence reduces alcohol seeking in male, but not female, iP rats. *Behav Neurosci*. 2023.

159. Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology*. 2010;35(1):217-38.

160. Urban NB, Kegeles LS, Slifstein M, Xu X, Martinez D, Sakr E, et al. Sex differences in striatal dopamine release in young adults after oral alcohol challenge: a positron emission tomography imaging study with [11C] raclopride. *Biol Psychiatry*. 2010;68(8):689-96.

161. Janeczek P, Colson N, Dodd PR, Lewohl JM. Sex Differences in the Expression of the $\alpha 5$ Subunit of the GABAA Receptor in Alcoholics with and without Cirrhosis of the Liver. *Alcohol Clin Exp Res*. 2020;44(2):423-34.

162. Henricks AM, Berger AL, Lugo JM, Baxter-Potter LN, Bieniasz KV, Craft RM, McLaughlin RJ. Sex differences in alcohol consumption and alterations in nucleus accumbens endocannabinoid mRNA in alcohol-dependent rats. *Neuroscience*. 2016;335:195-206.

163. Lovelock DF, Randall PA, Van Voorhies K, Vetreno RP, Crews FT, Besheer J. Increased alcohol self-administration following repeated Toll-like receptor 3 agonist treatment in male and female rats. *Pharmacol Biochem Behav*. 2022;216:173379.

164. Blednov YA, Black M, Benavidez JM, Stamatakis EE, Harris RA. PPAR agonists: II. Fenofibrate and tesaglitazar alter behaviors related to voluntary alcohol consumption. *Alcohol Clin Exp Res*. 2016;40(3):563-71.

165. Díaz-Megido C, Thomsen M. Sex-dependent divergence in the effects of GLP-1 agonist exendin-4 on alcohol reinforcement and reinstatement in C57BL/6J mice. *Psychopharmacology*. 2023;240(6):1287-98.

166. Blanchard B, Steindorf S, Wang S, Glick S. Sex differences in ethanol-induced dopamine release in nucleus accumbens and in ethanol consumption in rats. *Alcohol Clin Exp Res*. 1993;17(5):968-73.

167. Sauton P, Jeanblanc J, Benzerouk F, Gierski F, Naassila M. Sex-specific decision-making impairments and striatal dopaminergic changes after binge drinking history in rats. *Front Pharmacol*. 2023;14:1076465.

168. Flanigan M, Hon O, D'Ambrosio S, Boyt K, Hassanein L, Castle M, et al. Subcortical serotonin 5HT2c receptor-containing neurons sex-specifically regulate binge-like alcohol consumption, social, and arousal behaviors in mice. *Nat Commun*. 2023;14(1):1800.

169. Devaud LL, Fritschy J-M, Morrow AL. Influence of gender on chronic ethanol-induced alterations in GABAA receptors in rats. *Brain Res*. 1998;796(1-2):222-30.

170. Devaud LL, Matthews DB, Morrow AL. Gender impacts behavioral and neurochemical adaptations in ethanol-dependent rats. *Pharmacol Biochem Behav*. 1999;64(4):841-9.

171. Kirson D, Khom S, Rodriguez L, Wolfe SA, Varodayan FP, Gandhi PJ, et al. Sex differences in acute alcohol sensitivity of naïve and alcohol dependent central amygdala GABA synapses. *Alcohol Alcohol*. 2021;56(5):581-8.

172. Moriya Y, Kasahara Y, Hall FS, Sakakibara Y, Uhl GR, Tomita H, Sora I. Sex differences in the effects of adolescent social deprivation on alcohol consumption in μ -opioid receptor knockout mice. *Psychopharmacology*. 2015;232:1471-82.

173. Matzeu A, Terenius L, Martin-Fardon R. Exploring sex differences in the attenuation of ethanol drinking by naltrexone in dependent rats during early and protracted abstinence. *Alcohol Clin Exp Res*. 2018;42(12):2466-78.

174. Devaud LL, Morrow AL. Gender-selective effects of ethanol dependence on NMDA receptor subunit expression in cerebral cortex, hippocampus and hypothalamus. *Eur J Pharmacol*. 1999;369(3):331-4.

175. Devaud L, Alele P, editors. Alterations in protein levels involved in GABA and glutamate neurotransmission during ethanol withdrawal in male and female rats. *ALCOHOLISM-CLINICAL AND EXPERIMENTAL RESEARCH*; 2004: LIPPINCOTT WILLIAMS & WILKINS 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA.

176. Nentwig TB, Wilson DE, Rhinehart EM, Grisel JE. Sex differences in binge-like EtOH drinking, corticotropin-releasing hormone and corticosterone: effects of β -endorphin. *Addict Biol*. 2019;24(3):447-57.

177. Logrip ML, Oleata C, Roberto M. Sex differences in responses of the basolateral-central amygdala circuit to alcohol, corticosterone and their interaction. *Neuropharmacology*. 2017;114:123-34.

178. Lorrain I, Contini A, Gessa GL, Mugnaini C, Corelli F, Colombo G, Maccioni P. Operant, oral alcohol self-administration: Sex differences in Sardinian alcohol-preferring rats. *Alcohol*. 2019;79:147-62.

179. Haun HL, Griffin WC, Lopez MF, Becker HC. Kappa opioid receptors in the bed nucleus of the stria terminalis regulate binge-like alcohol consumption in male and female mice. *Neuropharmacology*. 2020;167:107984.

180. Zhou Y, Kreek MJ. Kappa opioid receptors and mu opioid receptors as combined targets for medication development for alcoholism. *Biol Psychiatry*. 2019;86(11):809-10.

181. Karanikas CA, Lu YL, Richardson HN. Adolescent drinking targets corticotropin-releasing factor peptide-labeled cells in the central amygdala of male and female rats. *Neuroscience*. 2013;249:98-105.

182. Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. *Nature*. 2014;509(7500):282-3.

183. Mamlouk GM, Dorris DM, Barrett LR, Meitzen J. Sex bias and omission in neuroscience research is influenced by research model and journal, but not reported NIH funding. *Front Neuroendocrinol*. 2020;57:100835.

184. Frezza M, di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS. High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. *N Engl J Med*. 1990;322(2):95-9.

185. Pizon AF, Becker CE, Bikin D. The clinical significance of variations in ethanol toxicokinetics. *J Med Toxicol*. 2007;3(2):63-72.

186. Gavalier JS. Sex-related differences in ethanol-induced liver disease: artifactual or real? *Alcohol Clin Exp Res*. 1982;6(2):186-96.

187. Cruz B, Borgonetti V, Bajo M, Roberto M. Sex-dependent factors of alcohol and neuroimmune mechanisms. *Neurobiol Stress*. 2023;26:100562.

188. Hitzemann R, Bergeson SE, Berman AE, Bubier JA, Chesler EJ, Finn DA, et al. Sex Differences in the Brain Transcriptome Related to Alcohol Effects and Alcohol Use Disorder. *Biol Psychiatry*. 2022;91(1):43-52.

189. Finn DA, Hashimoto JG, Cozzoli DK, Helms ML, Nipper MA, Kaufman MN, et al. Binge Ethanol Drinking Produces Sexually Divergent and Distinct Changes in Nucleus Accumbens Signaling Cascades and Pathways in Adult C57BL/6J Mice. *Front Genet*. 2018;9:325.

190. Ramirez EBD, Arnold ME, McConnell KT, Solomon MG, Amico KN, Schank JR. The effects of lipopolysaccharide exposure on social interaction, cytokine expression, and alcohol consumption in male and female mice. *Physiol Behav*. 2023;265.

191. Agrawal RG, Hewetson A, George CM, Syapin PJ, Bergeson SE. Minocycline reduces ethanol drinking. *Brain Behav Immun*. 2011;25 Suppl 1(Suppl 1):S165-9.

192. Fama R, Le Berre AP, Sullivan EV. Alcohol's Unique Effects on Cognition in Women: A 2020 (Re)view to Envision Future Research and Treatment. *Alcohol Res*. 2020;40(2):03.

954 193. Towers EB, Williams IL, Qillawala EI, Rissman EF, Lynch WJ. Sex/Gender Differences in the
955 Time-Course for the Development of Substance Use Disorder: A Focus on the Telescoping Effect.
956 *Pharmacol Rev.* 2023;75(2):217-49.

957 194. Blaine SK, Sinha R. Alcohol, stress, and glucocorticoids: From risk to dependence and relapse
958 in alcohol use disorders. *Neuropharmacology.* 2017;122:136-47.

959 195. Guinle MIB, Sinha R. The Role of Stress, Trauma, and Negative Affect in Alcohol Misuse and
960 Alcohol Use Disorder in Women. *Alcohol Res.* 2020;40(2):05.

961 196. Pigott TA. Anxiety disorders in women. *Psychiatr Clin North Am.* 2003;26(3):621-72, vi-vii.

962 197. Bangasser DA, Cuarenta A. Sex differences in anxiety and depression: circuits and
963 mechanisms. *Nat Rev Neurosci.* 2021;22(11):674-84.

964 198. Jalnapurkar I, Allen, M., & Pigott, T. Sex differences in anxiety disorders: A review. *J*
965 *Psychiatry Depress Anxiety.* 2018;4(12):3-16.

966 199. Sinha R. How does stress lead to risk of alcohol relapse? *Alcohol Res.* 2012;34(4):432-40.

967 200. Walker LC, Hand LJ, Letherby B, Huckstep KL, Campbell EJ, Lawrence AJ. Cocaine and
968 amphetamine regulated transcript (CART) signalling in the central nucleus of the amygdala modulates
969 stress-induced alcohol seeking. *Neuropsychopharmacology.* 2021;46(2):325-33.

970 201. Bale TL, Epperson CN. Sex differences and stress across the lifespan. *Nat Neurosci.*
971 2015;18(10):1413-20.

972 202. Oyola MG, Handa RJ. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal
973 axes: sex differences in regulation of stress responsivity. *Stress.* 2017;20(5):476-94.

974 203. Becker JB, McClellan M, Reed BG. Sociocultural context for sex differences in addiction.
975 *Addict Biol.* 2016;21(5):1052-9.

976 204. Collaer ML, Hines M. Human behavioral sex differences: a role for gonadal hormones during
977 early development? *Psychol Bull.* 1995;118(1):55-107.

978 205. Wallen K. Hormonal influences on sexually differentiated behavior in nonhuman primates.
979 *Front Neuroendocrinol.* 2005;26(1):7-26.

980 206. Butler GE, Walker RF, Walker RV, Teague P, Riadfahmy D, Ratcliffe SG. Salivary
981 Testosterone Levels and the Progress of Puberty in the Normal Boy. *Clin Endocrinol.* 1989;30(5):587-
982 96.

983 207. Herting MM, Maxwell EC, Irvine C, Nagel BJ. The impact of sex, puberty, and hormones on
984 white matter microstructure in adolescents. *Cereb Cortex.* 2012;22(9):1979-92.

985 208. Powers A, Casey BJ. The Adolescent Brain and the Emergence and Peak of
986 Psychopathology. *J Infant Child Adolesc Psychother.* 2015;14(1):3-15.

987 209. Arnold AP, Xu J, Grisham W, Chen X, Kim YH, Itoh Y. Minireview: Sex chromosomes and
988 brain sexual differentiation. *Endocrinology.* 2004;145(3):1057-62.

989 210. De Vries GJ, Rissman EF, Simerly RB, Yang LY, Scordalakes EM, Auger CJ, et al. A model
990 system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. *J*
991 *Neurosci.* 2002;22(20):9005-14.

992 211. Barker JM, Torregrossa MM, Arnold AP, Taylor JR. Dissociation of genetic and hormonal
993 influences on sex differences in alcoholism-related behaviors. *J Neurosci.* 2010;30(27):9140-4.

994 212. Sneddon EA, Masters BM, Ream KD, Fennell KA, DeMedio JN, Cash MM, et al. Sex
995 chromosome and gonadal hormone contributions to binge-like and aversion-resistant ethanol drinking
996 behaviors in Four Core Genotypes mice. *Front Psychiatry.* 2023;14:1098387.

997 213. Sneddon EA, Rasizer LN, Cavalco NG, Jaymes AH, Ostlie NJ, Minshall BL, et al. Gonadal
998 hormones and sex chromosome complement differentially contribute to ethanol intake, preference,
999 and relapse-like behaviour in four core genotypes mice. *Addict Biol.* 2022;27(5):e13222.

1000 214. Piferrer F. Epigenetics of sex determination and gonadogenesis. *Dev Dyn.* 2013;242(4):360-
1001 70.

1002 215. Philibert RA, Gunter TD, Beach SR, Brody GH, Madan A. MAOA methylation is associated
1003 with nicotine and alcohol dependence in women. *Am J Med Genet B Neuropsychiatr Genet.*
1004 2008;147B(5):565-70.

1005 216. D'Addario C, Shchetynsky K, Pucci M, Cifani C, Gunnar A, Vukojevic V, et al. Genetic variation
1006 and epigenetic modification of the prodynorphin gene in peripheral blood cells in alcoholism. *Prog*
1007 *Neuropsychopharmacol Biol Psychiatry.* 2017;76:195-203.

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Figure Legends:

Figure 1. The (A) human menstrual and (B) rodent estrous cycles with illustration of estrogen and progesterone fluctuations and approximate concentrations. Image made with Biorender.com

Figure 2. Neurosteroids are synthesised from precursor circulating gonadal hormones or *de novo* synthesis from cholesterol within the brain by steroidogenic enzymes (dark grey, italicised). Neurosteroids are classified based on their structural characteristics: pregnanes (red), androstanes (blue), or sulphated (pink). DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; P450scc, cholesterol side-chain cleavage enzyme; THDOC, tetrahydrodeoxycorticosterone; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 3 α -HSOR, 3 α -hydroxysteroid oxoreductase; 3 α -HSD, 3 α -hydroxysteroid dehydrogenase; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; STS, Sulphotransferase; 5 α -R, 5 α -reductase.

Table 1. Summary of human studies examining the relationship between hormones and alcohol use.*Abbreviations: MHT, menopausal hormone therapy; W, women; M, men; n.d, no difference*

Hormone	Study	Sample	Measure	Result/association	High hormone level effect on alcohol intake or craving
Estrogen	(31)	Adolescent men/women	Alcohol intake	No association between alcohol use and blood estradiol levels in women.	W (n.d)
	(30)	Adolescent men/women	Onset and amount of alcohol use	Increased salivary estradiol associated with onset and amount of alcohol use in men only.	M ^ W (n.d)
	(20)	Premenopausal women	Alcohol intake	Positive association between alcohol intake and blood estradiol levels.	W ^
	(32)	Postmenopausal menopausal hormone therapy (MHT) users, or non-users.	Alcohol intake	Positive association between alcohol intake and oestrone, estradiol and 2-2-hydroxyestrone estrogen metabolite concentrations in postmenopausal women on menopausal hormone therapy (MHT). Positive association between alcohol intake and serum estrogen in postmenopausal women not using menopausal hormone therapy (non-MHT) that consume more alcohol than menopausal therapy users.	MHT W ^ non-MHT W ^
	(33)	Adult men with or without AUD	Association with AUD	Men with AUD had elevated blood estradiol levels compared to control subjects, which declined following 21 days of abstinence.	M ^
	(34)	Adult men and women, with or without alcohol dependence	Association with alcohol dependence	Alcohol-dependent men had elevated serum estradiol levels compared to control men, whilst no difference between alcohol-dependent and non-dependent women.	M ^ W (n.d)
	(35)	Pre and postmenopausal women	Alcohol intake	Positive association between alcohol intake and estradiol specifically in post-menopausal women. Positive association between oestrone concentrations and alcohol in both pre- and post-menopausal women.	postmenopausal W ^ pre and postmenopausal W ^
	(44)	Pre- and post-menopausal women	Habitual alcohol intake	No association between habitual alcohol intake and estradiol.	pre and postmenopausal W (n.d)
	(50)	Young adult women	Alcohol intake	No difference in alcohol intake following oral intake of estradiol (4mg/kg).	W (n.d)
Progesterone	(37)	Young adult women	Likelihood to consume alcohol	Likelihood to consume alcohol increased on days of low progesterone levels and negative mood.	W v
	(35)	Pre-menopausal women	Alcohol intake	No association between progesterone concentration and alcohol intake.	postmenopausal W (n.d)
	(36)	Men and women with or without AUD	Craving and AUD	Lower baseline progesterone levels correlated with increased cravings in postmenopausal women. Men with AUD had elevated progesterone levels, which declined during withdrawal, compared to men without AUD.	postmenopausal W v M ^
	(38)	Men with or without alcohol dependence	Craving	Positive correlation between progesterone levels and cravings.	M ^
Progesterone:estrogen ratio	(46)	Young adult women	Likelihood of drinking and binge drinking	Correlation between a reduced progesterone:estradiol and a higher risk of drinking and binge drinking.	W v

	(25)	Men and women with AUD	Alcohol use and binge drinking	Higher progesterone:estradiol associated with reduced likelihood to binge drinking in women. Higher progesterone:estradiol ratio associated with reduced likelihood of any alcohol use, or to binge drink, in men.	W ∇ M ∇
	(47)	Premenopausal women	Percentage of days abstinent	Higher progesterone:estradiol levels enhanced efficacy of naltrexone to improve drinking outcomes, increasing percent of days abstinent.	premenopausal W ∇
Testosterone	(30)	Adolescent men/women	Onset of alcohol use	Higher testosterone levels correlated with earlier onset of alcohol use in men.	M $\wedge$ W (n.d)
	(31)	Adolescent men/women	Alcohol use	Higher testosterone levels associated with increased risk of alcohol use in men.	M $\wedge$ W (n.d)
	(40)	Adolescent twin brothers	Alcohol drinking patterns	Positive association between testosterone levels and alcohol dependence diagnosis.	M $\wedge$
	(42)	Adolescent and young adult men and women	Alcohol use	Higher testosterone levels predicted increased alcohol use two years later in both sexes.	M $\wedge$ F $\wedge$
	(43)	Young adult men and women	Alcohol use	Positive correlation between blood testosterone levels and alcohol use in both sexes, with a stronger correlation in men	M $\wedge$ F $\wedge$
	(35)	Pre and postmenopausal women	Alcohol intake	Positive association between testosterone and alcohol intake in both pre and postmenopausal women.	pre and postmenopausal W $\wedge$
	(41)	Men previously detoxified and undergoing forensic examination	AUD	Higher levels of testosterone positively associated with early onset AUD, but not pure alcohol dependence.	M $\wedge$
	(38)	Men with or without alcohol dependence	Craving	Positive correlations between testosterone levels and alcohol cravings in alcohol-dependent men.	M $\wedge$
	(34)	Men and women with or without alcohol dependence	Alcohol dependence	Alcohol-dependent men and women have elevated total testosterone levels compared to same-sex controls.	M $\wedge$ F $\wedge$
	(44)	Pre and postmenopausal women	Habitual alcohol intake	Positive association between total testosterone and habitual alcohol intake in pre and postmenopausal women.	pre and postmenopause W $\wedge$
	(45)	Men with or without alcohol dependence	Alcohol dependence and withdrawal	Alcohol-dependent men have elevated levels of blood dihydrotestosterone compared to controls, with these levels reducing after 12-months of abstinence.	M $\wedge$ abstinent M ∇

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1036 **Table 2.** Summary of rodent studies assessing the relationship between hormones and alcohol use.
1037 Abbreviations: OVX, ovariectomy; GDX, gonadectomy; F, female; M, male; E2, estradiol, P4, progesterone, T, testosterone; n.d, no difference
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Hormone	Study	Sample	Surgical intervention	Hormonal manipulation	Measure	Result/association	Effect on ethanol measure
Estrogen	(51)	Female Sprague-Dawley rats	OVX or SHAM surgery prior to ethanol exposure	N/A	Ethanol intake and preference	Ovariectomy reduced ethanol intake and preference, associated with a reduction in endogenous estrogen.	F OVX v
	(52)	Male and female Sprague-Dawley rats	N/A	Single i.m. injection of 1mg or 2mg of estradiol valerate 1 or 2 months prior to, or during, ethanol exposure.	Ethanol intake	A single injection of estradiol valerate was associated with persistent increased intake of alcohol in females, and a decrease in males.	M E2 v F E2 ^
	(53)	Female Long-Evans rats	OVX or SHAM surgery after 2-weeks continuous-access to ethanol prior to hormonal manipulation.	Implantation of 17 β -estradiol replacement pellet (0.25mg; 21-day release), or placebo s.c.	Ethanol intake	Ovariectomised rats had a persistent reduction in ethanol intake compared to control mice, whilst estradiol replacement restored ethanol intake to baseline levels.	OVX v OVX+E2 (n.d)
	(54)	Female Long-Evans rats	OVX or SHAM surgery after 2-weeks continuous access to ethanol prior to hormonal manipulation.	Daily s.c. injections of E ₂ (0.05 μ g/kg, 0.15 μ g/kg, 0.5 μ g/kg, 1.5 μ g/kg, or 5 μ g/kg), or vehicle, for 25 consecutive days with continuous ethanol access.	Ethanol intake	Positive dose-response association between estradiol and ethanol intake in ovariectomised rats limited to estradiol administration within physiological ranges.	OVX+E2^
	(55)	Female Wistar and Lewis rats	N/A	Single 2mg i.m. injection of β -estradiol 17-valerate, first ethanol presentation 9-weeks post-injection.	Ethanol intake	Administration of estradiol valerate increased ethanol intake in both strains.	F E2 ^
	(56)	Female Sprague-Dawley rats	N/A	Single 2mg i.m. injection of estradiol valerate administered 15-days prior to first ethanol exposure.	Ethanol intake	Administration of estradiol valerate increased ethanol intake in all conditions assessed.	F E2 ^
	(57)	Female Wistar rats	OVX or no surgery prior to ethanol exposure.	Single i.m. injection of estradiol valerate (0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1 or 2mg) administered 6-days prior to first ethanol exposure.	Ethanol intake	Administration of estradiol valerate (2mg) increased ethanol intake, with ovariectomised mice displaying similar effects to gonadally-intact rats.	E2 ^ OVX+E2 ^
	(58)	Male and female Long-Evans rats	GDX or SHAM surgery 2-weeks or 80-days prior to first ethanol exposure.	N/A	Ethanol intake	Gonadally-intact females consumed more alcohol than males, although ovariectomy reversed this Whilst gonadectomised males consumed more alcohol than gonadally-intact males.	M GDX ^ F OVX v
	(59)	Male and female Wistar rats	OVX or SHAM/no surgery ~3-weeks prior to first ethanol exposure.	N/A	Aversive and rewarding effects of ethanol	An intermediate dose of ethanol produced rewarding effects in female, but not male or ovariectomised females.	F ^ F OVX (n.d)
	(27)	Male and female mice	OVX or no surgery prior to ethanol exposure.	Daily s.c. administration of 17 β -Estradiol-3-benzoate (~10 μ g/kg) 2-weeks prior to and during ethanol exposure.	Ethanol intake	Gonadally-intact female consumed more ethanol than male and ovariectomised female mice, with estradiol benzoate increasing ethanol intake in ovariectomised females.	F OVX v F OVX+E2 (n.d)

(60)	Male and female Sprague-Dawley rats	GDX or SHAM surgery prior to ethanol exposure.	Females administered s.c. with 17- β estradiol (50 μ g/kg) ~7 days prior to, and daily during, ethanol self-administration post-session, or 30 minutes prior to cue+stress-induced reinstatement.	Ethanol intake and reinstatement of ethanol seeking	Chronic estradiol replacement in ovariectomised females increased ethanol intake. Acute and chronic estradiol administration had no effect on cue+stress-induced reinstatement, however estrogen receptor antagonism tended to reduce reinstatement in gonadally-intact females.	F OVX+E2 [^] F OVX+E2 (<i>n.d</i>)
(65)	Female Wistar rats	OVX prior to ethanol exposure.	Single or daily s.c. injections of estradiol benzoate (5 μ g)	Ethanol intake	Administration of estradiol benzoate in ovariectomised rats led to transient reductions in ethanol intake, which returned to control animal levels despite continued hormone administration.	Initial OVX+E2 ^v OVX+E2 (<i>n.d</i>)
(66)	Male and female Wistar-Kyoto rats	GDX or SHAM surgery 12-16 days prior to ethanol self-administration.	N/A	Ethanol intake	No difference in the maintenance of ethanol self-administration behaviour between gonadectomised and control rats.	M GDX (<i>n.d</i>) F OVX (<i>n.d</i>)
(67)	Male and female Sprague-Dawley rats	GDX, SHAM or no surgery either pre-pubertally or post-pubertally prior to ethanol exposure.	N/A	Ethanol intake	Ovariectomy had no effect on ethanol intake in females, whilst GDX increased intake in males.	M GDX [^] F OVX (<i>n.d</i>)
(61)	Female mice	OVX surgery prior to ethanol surgery	Daily s.c. administration of 17 β -Estradiol-3-benzoate (~10 μ g/kg) beginning four days post-OVX surgery and delivered 4 hours prior to each conditioning session.	Ethanol conditioned place preference	Administration of 17 β -estradiol, or coactivation of ER α and ER β , enhanced ethanol conditioned place preference in ovariectomised mice.	OVX+E2 [^]
(69)	Albino male rats	Castration one month prior to ethanol exposure.	Weekly s.c. injections of estradiol benzoate, 1 μ g/kg for three and a half weeks, then 3 μ g/kg for next three and a half weeks.	Ethanol preference	There was no effect of estradiol benzoate administration of ethanol preference.	GDX +E2 (<i>n.d</i>)
(68)	Male and female mice	GDX or SHAM surgery prior to ethanol exposure.	Implantation of pellets releasing 0.1 or 0.25mg 17 β -estradiol, or 1.5 or 5mg anti-estrogen tamoxifen over a 60-day period.	Ethanol intake	Male mice treated with 17 β -estradiol increased voluntary ethanol intake compared to tamoxifen-treated males. Tamoxifen-treated OVX females increased drinking compared to 17 β -estradiol OVX females.	M E2 [^] F TAM+OVX [^]
(70)	Male Wistar rats	GDX or SHAM surgery following ethanol exposure.	Daily s.c. administration of estradiol benzoate (5 μ g) ~2 weeks post-surgery, for 8-days during ethanol exposure period.	Ethanol intake and pattern of drinking	Male rats administered with estradiol benzoate reduced ethanol intake, and the number and size of drinking bouts.	M E2 ^v
(71)	Male and female mice	Lentivirus-expressing short hairpin RNAs injected into the VTA targeting either the ER α or ER β , mice were tested for binge ethanol drinking 3-weeks post-surgery.	N/A	Binge drinking	Knockdown of ER α and ER β in the VTA reduced binge-like ethanol intake in female, but not male, mice.	M (<i>n.d</i>) F ^v
(62)	Esr1-Cre Male and female mice	Injected hM3Dq designer receptor exclusive activated by designer drug into dorsal raphe nucleus, and used clozapine N-oxide as the inert ligand.	N/A	Binge drinking	Elevated binge-like ethanol intake in female mice associated with decreased ER α mRNA expression in the DRN, whilst in male mice, there was increased ER α and 5HT-related genes in DRN. Chemogenetic activation of DRN neurons expressing either ER α or ER β reduced binge-like alcohol intake in female mice.	M ER α mRNA [^] F ER α mRNA ^v F ^v

	(63)	Male and female Sprague-Dawley rats	N/A	Single dose s.c. of 0.1mg estradiol valerate on postnatal day 1, with ethanol exposure beginning ~31 days post-administration.	Ethanol intake	Neonatal exposure to a single supraphysiological dose of estradiol valerate increased ethanol intake from adolescence to early adulthood.	M E2 [^] F E2 [^]
	(64)	Male and female mice	OVX or SHAM surgery ~10-14 days prior to ethanol exposure	No implantation or implantation of a subcutaneous 90-day slow releasing 0. 32mg 17 β -estradiol pellet or placebo.	Ethanol intake and aversion-resistant ethanol intake	Ovariectomy reduced ethanol intake and aversion-resistant ethanol intake in female mice compared to SHAM surgery. 17 β -estradiol supplementation returned aversion-resistant ethanol intake to SHAM+placebo levels.	F OVX v F OVX+E2 [^] v. OVX
Progesterone	(69)	Albino male rats	Castration one month prior to ethanol exposure.	Weekly s.c. injections of progesterone, 1mg/kg for three and a half weeks, then 3mg/kg for next three and a half weeks.	Ethanol preference	There was no effect of progesterone administration on ethanol preference.	M P4 (<i>n.d</i>)
	(72)	Male and female Wistar rats	GDX or SHAM surgery 7-10 days prior to ethanol exposure.	Daily s.c. administration of progesterone (1mg), delivered one day prior and during ethanol exposure paradigm.	Ethanol intake	Progesterone administration in ovariectomised female rats had no effect on ethanol drinking patterns when compared to gonadally intact female rats.	F OVX+P4 (<i>n.d</i>) M GDX + P4 (<i>n.d</i>)
	(73)	Female Meyers Higher Ethanol Preferring rats	N/A.	Daily s.c. administration of increasing doses of progesterone (1mg/kg days 1-2; 2mg/kg days 3-4; 3mg/kg days 5-20), during continuous 24hr ethanol access.	Ethanol intake	Progesterone administration had no effect on ethanol intake.	F P4 (<i>n.d</i>)
	(64)	Male and female mice	OVX or SHAM surgery ~10-14 days prior to ethanol exposure	No implantation or implantation of a subcutaneous 90-day slow releasing 5mg progesterone pellet or placebo.	Ethanol intake and aversion-resistant ethanol intake	Ovariectomy reduced ethanol intake and aversion-resistant ethanol intake in female mice compared to SHAM surgery counterparts. Whilst, progesterone replacement in ovariectomised mice had no effect on ethanol intake or aversion-resistant ethanol intake.	F OVX v F OVX+P4 v. OVX (<i>n.d</i>)
Testosterone	(60)	Male and female Sprague-Dawley rats	GDX or SHAM surgery prior to ethanol exposure.	Males administered s.c. with testosterone propionate (2mg/kg) ~7 days prior to, and daily during, ethanol self-administration post-session, or 30 minutes prior to cue+stress-induced reinstatement.	Ethanol intake and reinstatement of ethanol seeking	Chronic testosterone administration in castrated male rats reduced ethanol intake. Chronic testosterone administration had no effect on cue+stress-induced reinstatement.	M GDX [^] M GDX (<i>n.d</i>)
	(69)	Albino male rats	Castration one month prior to ethanol exposure.	Weekly s.c. injections of testosterone proportionate, 1mg/kg for three and a half weeks, then 3mg/kg for next three and a half weeks.	Ethanol preference	Testosterone administration in castrated male rats increased ethanol preference.	M GDX [^]
	(75)	Male Sprague-Dawley rats	Castrated two weeks prior to ethanol exposure	Daily injections of ethanol (3g/kg, i.p.), testosterone (2.5mg, s.c.), or both for 7-days.	Ethanol μ -opioid binding kinetics	Administration of testosterone was shown to have a regulatory role on ethanol-induced changes on μ -opioid binding kinetics.	M GDX [^]
	(72)	Male and female Wistar rats	GDX or SHAM surgery 7-10 days prior to ethanol exposure.	Daily s.c. administration of dihydrotestosterone (100 μ g), delivered one day prior to, and during, ethanol exposure paradigm.	Ethanol intake	Administration of dihydrotestosterone in castrated male rats reduced ethanol intake.	M GDX v
	(74)	Male Sprague-Dawley rats	GDX or SHAM surgery prior to ethanol exposure.	Implantation of pellet releasing 5mg testosterone propionate over a 21-	Ethanol preference	Testosterone replacement in castrated male rats reduced ethanol preference.	M GDX+T v

			day period, five days prior to ethanol exposure.			
(63)	Male and female Sprague-Dawley rats	N/A	Single dose s.c. of 1mg testosterone propionate on postnatal day 1, with ethanol exposure beginning ~31 days post-administration.	Ethanol intake	Neonatal exposure to a single supraphysiological dose of testosterone propionate increased ethanol intake from adolescence to early adulthood.	M ^ F ^

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