HHS Public Access

Author manuscript

Alcohol. Author manuscript; available in PMC 2022 February 01.

Published in final edited form as:

Alcohol. 2022 February ; 98: 25–41. doi:10.1016/j.alcohol.2021.08.001.

Structural, Functional, and Behavioral Significance of Sex and Gonadal Hormones in the Basolateral Amygdala: A Review of Preclinical Literature

Michaela E. Price[1,2], **Brian A. McCool**[2]

[1]Neuroscience Graduate Training Program, Wake Forest School of Medicine, Winston-Salem, NC

[2]Dept. of Physiology & Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC

Abstract

The basolateral amygdala (BLA) is intimately involved in the development of neuropsychiatric disorders such as anxiety and alcohol use disorder (AUD). These disorders have clear sex biases with women more likely to develop an anxiety disorder and men more likely to develop AUD. Preclinical models have largely confirmed these sex-specific vulnerabilities and emphasize the effects of sex hormones on behaviors influenced by the BLA. This review will discuss sex differences in BLA-related behaviors and highlight potential mechanisms mediated by altered BLA structure and function, including the composition of GABAergic interneuron subpopulations, glutamatergic pyramidal neuron morphology, glutamate/GABA neurotransmission, and neuromodulators. Further, sex hormones differentially organize dimorphic circuits during sensitive developmental periods (organizational effects) and initiate more transient effects throughout adulthood (activational effects). Current literature indicates that estradiol and allopregnanolone, a neuroactive progestogen, generally reduce BLA-related behaviors through a variety of mechanisms including activation of estrogen receptors or facilitation of $GABA_A$-mediated inhibition, respectively. This enhanced GABAergic inhibition may protect BLA pyramidal neurons from the excitability associated with anxiety and alcohol withdrawal. Understanding sex differences and the effects of sex hormones on BLA structure and function may help explain sex-specific vulnerabilities in BLA-related behaviors and ultimately improve treatments for anxiety and AUD.

Keywords

sex differences; basolateral amygdala; anxiety; alcohol; estradiol; allopregnanolone

Introduction

Men and women are diagnosed with anxiety disorders and alcohol use disorders (AUD) at different rates. Women have a significantly higher risk for anxiety disorders than men, with a lifetime prevalence of 40.4% in adult women compared to 26.4% in adult men (Kessler et

Corresponding Author: Brian A. McCool, Ph.D., Professor, Dept. Physiology & Pharmacology, Wake Forest School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157, Ph: 336-716-8608, bmccool@wakehealth.edu.

al., 2005, 2012). The higher prevalence of anxiety disorders in women may be influenced by natural fluctuations in sex hormone levels over the course of the menstrual cycle. These fluctuations, as well as a dramatic reduction in hormone levels during postpartum periods, can exacerbate anxiety symptoms (van Veen et al., 2009). In comparison, men are nearly twice as likely to develop AUD according to the 2018 National Survey on Drug Use and Health (Substance Abuse and Mental Health Services Administration, 2019)(Substance Abuse and Mental Health Services Administration, 2019). Alcohol withdrawal symptoms, such as withdrawal-induced seizures, delirium tremens, and anxiety, are more common and often more severe in men (Deshmukh et al., 2003; Erol & Karpyak, 2015). Despite these outcomes, alcohol-dependent women escalate their alcohol consumption at a faster rate and are more at risk for alcohol-related diseases (Devaud et al., 2006; Erol & Karpyak, 2015; Giacometti & Barker, 2020). Importantly, exposure to traumatic events, with or without subsequent post-traumatic stress disorder, dramatically increases the risk for developing an AUD in women (Breslau et al., 1997, 2003). These data suggest that sex differences may underlie unique vulnerabilities to AUD-related neuropsychiatric disorders. Understanding the mechanisms for these sex-dependent vulnerabilities may uncover neurobiological mechanisms that therapeutics can exploit. This work will review preclinical literature to highlight potential anatomical and neurophysiological mechanisms for these sex differences.

The amygdala is one of several brain regions that has been implicated in a variety of neuropsychiatric disorders, including anxiety and AUD (Christian et al., 2012, 2013; Diaz et al., 2011a, 2011b; McGinnis et al., 2020a, 2020b; Morales et al., 2018). The amygdala is comprised of 13 subnuclei including the prominent subdivisions lateral amygdala (LA), basolateral amygdala (BA), central amygdala (CeA), and medial amygdala (Sah et al., 2003). The LA and BA (together the BLA), in particular, have unique positions within aversion- and reward-related circuitry and together form the primary input nucleus of the amygdala, receiving sensory, executive, and memory-related information (Figure 1). Glutamatergic inputs arriving through the external capsule (‘lateral’ inputs) provide executive and processed sensory information from more lateralized cortical sources such as the temporal, insular, and lateral prefrontal cortices (Leichnetz & Astruc, 1977; McDonald, 1998; McDonald et al., 1996; Sah et al., 2003). The *stria terminalis*, a white matter tract located just medial to the BLA, provides glutamatergic inputs arriving from more midline brain structures including the medial prefrontal cortex (mPFC) and polymodal sensory thalamus. The BLA processes and consolidates information from these distinct inputs and relays it to downstream regions. For example, BLA neurons projecting to reward-related regions like the nucleus accumbens (NAC) are preferentially responsive to reward-predictive cues, suggesting that they encode positive valence (Beyeler et al., 2016). The BLA-NAC projection promotes self-stimulation (Britt et al., 2012; Namburi et al., 2015; Stuber et al., 2011) as well as reward-seeking (Stuber et al., 2011) and is strengthened by reward conditioning (Namburi et al., 2015). BLA neurons also project to fear and anxiety-related regions like the CeA and bed nucleus of the stria terminalis (BNST). These neurons are preferentially responsive to aversive cues (Beyeler et al., 2016) and the BLA-CeA projection is strengthened by fear conditioning (Namburi et al., 2015). BLA neurons also send reciprocal projections back to medial and lateral frontal cortical areas to influence executive processes in the context of emotionally relevant stimuli.

This review discusses potential structural and functional mechanisms underlying sex differences in anxiety and AUD. We will begin by describing the organizational and activational effects of sex steroids, as well as how sex hormones are synthesized. Then we will cover baseline sex differences and the effects of sex hormones on behaviors that the BLA influences, including anxiety, fear conditioning and stress interactions, and alcohol consumption/withdrawal. Finally, we will detail sex differences in BLA structure and function, as well as the effects of sex hormones, stress, fear conditioning, and alcohol exposure. The sections on BLA structure and function are as follows: cellular composition; cellular morphology; glutamate, GABA, and excitability; dopamine system; and serotonin system.

Organizational and Activational Effects of Sex Steroids

As will be detailed throughout this review, structural and functional differences in the BLA may underlie sexually divergent behaviors, particularly differential responding to stress and anxiety interactions with alcohol. Sex differences generally arise from genes encoded within the sex chromosomes (genotypically XX or XY within individual animals). These genes can directly influence developmental processes (organizational effects) as well as transiently influence neuronal activity via circulating sex hormones across the life span (activational effects). For example, the testes-determining gene (Sry) and related genes drive the development of gonads and ultimately the production of gonadal hormones during early development (Puralewski et al., 2016). Exposure to these hormones during sensitive periods of embryonic and postnatal development organizes sexually dimorphic neural circuits. These organizational effects are considered relatively permanent and were first highlighted in the 1959 study by Phoenix, Goy, Gerall, and Young (Phoenix et al., 1959) which related how sex hormones can organize tissues mediating mating behavior. In adults, the activational effects of circulating sex hormones, particularly during the menstrual/estrous cycle, modulate these dimorphic neural circuits to initiate transient sex-specific neural and ultimately behavioral responses (see Arnold, 2009; Schulz & Sisk, 2016; Wallen, 2009 for review on organizational and activational effects of sex hormones).

Sex hormones represent distinct families of cellular modulators, including progestogens, androgens, and estrogens. These are produced in varying quantities in both males and females. The neuroactive progestogen allopregnanolone (also referred to as 3α,5α-tetrahydroprogesterone or 3α-hydroxy-5α-pregnan-20-one) is synthesized from progesterone by isozymes of the enzyme 5alpha-reductase (5α-reductase) and by the enzyme 3alpha-hydroxysteroid dehydrogenase (3α-HSD). Importantly, 5α-reductase type I and 3α-HSD are expressed in the BLA suggesting that allopregnanolone is locally synthesized (Agís-Balboa et al., 2006). In the LA nucleus of the BLA, allopregnanolone immunoreactivity is localized near both vesiclular glutamate and GABA transporter immunoreactivity suggesting it could influence both synapses (Maldonado-Devincci et al., 2014a). These studies were conducted in male mice (Agís-Balboa et al., 2006; Maldonado-Devincci et al., 2014a), but females are expected to show similar expression and colocalization patterns. Progestogens also serve as substrates for androgen biosynthesis, including testosterone and dihydrotestosterone, that bind to androgen receptors (AR). The enzyme cytochrome P450 aromatase (AROM) can then synthesize estrogens from

androgens. Estradiol is the primary estrogen expressed in females, although other estrogens like estrone and estriol are also present. BLA neurons in both sexes express AROM, AR, the classic nuclear estrogen receptors alpha (ERα) and beta (ERβ), and the transmembrane G protein-coupled estrogen receptor (GPR30) (Bender et al., 2017; Blurton-Jones & Tuszynski, 2002; Osterlund et al., 1998; Simerly et al., 1990). Notably, ERα is the predominant estrogen receptor in the BLA whereas ERβ is predominant in the CeA and medial amygdala of female rats (Osterlund et al., 1998). Thus, sexually dimorphic, BLA-dependent behaviors can be influenced differential steroid receptor activation within BLA neurons.

Estrogen and progesterone levels fluctuate naturally during the primate menstrual cycle and the rodent estrous cycle. The primate menstrual and rodent estrous cycles are closely analogous despite the fact that female rodents do not have a functional corpus luteum and therefore do not have a phase analogous to the primate luteal phase (Finn, 2020). The rodent estrous cycle lasts 4–5 days and consists of four phases: proestrus, estrus, metestrus (diestrus I), and diestrus (II). Estradiol and progesterone levels peak during proestrus and then plummet to their lowest levels during estrus (Becker et al., 2005; Blume et al., 2017; Butcher et al., 1974; Vetter-O'Hagen & Spear, 2012). Progesterone levels have a small, secondary peak midway through diestrus I and II while estrogen levels rise later to peak as the rodents reenter proestrus. The phase of the estrous cycle can be experimentally determined by measuring serum estradiol and progesterone levels or by evaluating changes in vaginal cytology (Becker et al., 2005). Hormonal fluctuations during the estrous cycle have the same pattern in younger female rodents beginning puberty as they do in older females, but the overall increase in sex hormones during adolescence and early adulthood allows for more pronounced changes in adults (Vetter-O'Hagen & Spear, 2012). In male rats, serum testosterone levels also fluctuate over a 4-day cycle and peak every 8–12 hours in a 24-hour period (Diatroptov, 2011; Diatroptov et al., 2017; Waite et al., 2009). The activational effects of sex hormones, driven by natural hormone fluctuations, are often examined experimentally by performing a gonadectomy (referred to as an ovariectomy in females, orchiectomy/castration in males) and supplying exogenous circulating sex hormones or vehicle.

Sex Differences in BLA-Related Behaviors

Sex Differences in Anxiety

Baseline Sex Differences—Women are more likely to develop anxiety disorders than men (Kessler et al., 1994; Seedat et al., 2009), and dramatic changes in sex hormone levels influence the severity of anxiety symptoms (Maeng & Milad, 2015; van Veen et al., 2009). Preclinical models of anxiety were developed and validated decades ago including the elevated plus maze (EPM), light-dark box, open field test (OFT), social interaction test, and Vogel conflict test. Since then, studies examining how sex and sex hormones influence anxiety-like behavior have yielded inconsistent results. These studies are summarized in Table 1. In the EPM, studies have reported that female rodents exhibit less anxiety-like behavior than males (Domonkos et al., 2017; Frye et al., 2000; Knight et al., 2021; Scholl et al., 2019; Xiang et al., 2011) or no significant sex differences (Marcondes et al., 2001). Similarly, in the OFT, female rodents show less anxiety-like behavior than males

(Domonkos et al., 2017; Knight et al., 2021) or there are no sex differences (Scholl et al., 2019). In contrast, female rodents exhibit more anxiety-like behavior than males in the Vogel conflict test (De Jesus-Burgos et al., 2016) and social interaction test (Carrier & Kabbaj, 2012; Johnston & File, 1991; Stack et al., 2010). Given that these models were validated at a time when it was common to only use male rodents, sex differences observed in these models may also reflect differences in coping strategies. For instance, locomotor activity appears to impact the activity levels of female rodents exploring the EPM more so than anxiety (Fernandes et al., 1999).

The Effects of the Estrous Cycle and Sex Hormones—Preclinical studies utilizing the EPM have found that anxiety-like behavior decreases during proestrus compared to diestrus, suggesting that estradiol or progesterone may diminish anxiety-like behavior in female rats relative to that measured in males (Bitran & Dowd, 1996; Brunton & Russell, 2010; Frye et al., 2000; Marcondes et al., 2001). Indeed, estradiol is anxiolytic in female rodents (Koss et al., 2004; Marcondes et al., 2001; Tian et al., 2013; Walf & Frye, 2005a; Wang et al., 2019) and estrogen withdrawal, typical of the postpartum period, increases anxiety-like behavior in the EPM (Yang et al., 2017), consistent with epidemiological reports of increased symptom severity during the postpartum period in humans. Although, estradiol is generally anxiolytic in the EPM, some studies have failed to find an effect of estradiol on anxiety-like behavior in female rodents (Anchan et al., 2014; Renczés et al., 2020). Similarly, in the OFT, estradiol decreases (Renczés et al., 2020; Walf & Frye, 2005a) or has no effect (Anchan et al., 2014) on anxiety-like behavior in female rodents. Thus, estradiol may explain how female rodents are typically less anxious in the EPM and OFT than their male counterparts (Domonkos et al., 2017; Frye et al., 2000; Knight et al., 2021; Scholl et al., 2019; Xiang et al., 2011). In the social interaction test, where females rodents typically have higher anxiety-like behavior than males, estradiol appears to increase anxiety-like behavior (Koss et al., 2004) although that is not always the case (Stack et al., 2010). Estradiol's impact on anxiety-like behavior could be mediated through the classical estrogen receptors ERα and ERβ, or GPR30. The anxiolytic effects of estradiol are dependent on ERβ, not ERα, activation in the OFT, EPM, light-dark box, and vogel conflict test in ovariectomized rats (Lund et al., 2005; Walf & Frye, 2005b). Moreover, female ERβ knockout mice have more anxiety-like behavior compared to their wildtype counterparts (Imwalle et al., 2005). GPR30 activation is also reported to be anxiolytic in female mice exploring the EPM and OFT (Anchan et al., 2014; Tian et al., 2013).

Progesterone and allopregnanolone levels peak during proestrus as well, coinciding with a decrease in anxiety-like behavior in female rats (Frye et al., 2000). This suggests that progestogens are anxiolytic in female rodents, and indeed they are in the burying behavior task and EPM (Bitran et al., 1995; Bitran & Dowd, 1996; Picazo & Fernández-Guasti, 1995). Conversely, progestogen withdrawal increases anxiety-like behavior in the EPM (Smith et al., 1998). Progesterone is converted to neuroactive progestogens like allopregnanolone which act as positive allosteric modulators of $GABA_A$ receptors (Belelli & Lambert, 2005; Nuss, 2015). The potentiation of $GABA_A$ receptors produces the anxiolytic effects of neuroactive progestogens (Nuss, 2015). Altogether, estradiol and progestogens

generally reduce anxiety-like behaviors through the activation of ERβ and GPR30 for estradiol and the potentiation of $GABA_A$ receptors for progestogens.

Few studies have investigated how androgens alter anxiety-like behavior. Testosterone treatment typically decreases anxiety-like behavior in the EPM, OFT, and burying behavior test through AR activation and through its aromatase-derived metabolites like estradiol (Bitran et al., 1993; Carrier et al., 2015; Fernández-Guasti & Martínez-Mota, 2005). Conversely, androgen-insensitive male mice have higher anxiety levels than wildtype controls in the EPM (Hamson et al., 2014). These data would suggest that testosterone is anxiolytic; however, prenatal exposure to testosterone in female rats increases anxiety-like behavior in the EPM (Rankov Petrovic et al., 2019). Altogether, testosterone appears to be anxiolytic in male rodents, but prenatal exposure to testosterone in female rodents engenders a male-phenotype and is anxiogenic in the EPM.

Sex Differences in Fear Conditioning and Stress-Enhanced Fear Conditioning

Baseline Sex Differences—Sex differences in fear conditioning and extinction, as well as stress-mediated changes to fear learning, depend on the type of conditioned stimulus used to establish the fear-memory (Table 1). During fear conditioning, animals are presented with a neutral stimulus paired with an aversive stimulus like footshock. After repeatedly pairing, animals ‘learn’ that the originally neutral stimulus now predicts the aversive stimulus (unconditioned stimulus or US). At this point, the neutral stimulus has become a conditioned stimulus (CS) and will elicit a fear response. In cued fear conditioning, the CS is typically a simple sensory cue, most commonly a distinct auditory stimulus. In contextual fear conditioning, the CS is represented by a complex environment composed of novel tactile and visual stimuli. Fear conditioning paradigms have traditionally measured freezing to assess fear behaviors, but rodents can also express fear through escape-like darting behavior (Gruene et al., 2015; Ribeiro et al., 2010) or ultrasonic vocalizations (Kosten et al., 2006). Female rodents generally exhibit more darting behavior and less ultrasonic vocalizations during fear conditioning compared to males (Gruene et al., 2015; Kosten et al., 2006; Ribeiro et al., 2010). During extinction trials, the CS is repeatedly presented without the US. Once animals ‘learn’ that the neutral stimulus no longer predicts the aversive stimulus, the expression of conditioned responses like freezing and darting decrease.

At baseline, male and female rodents differ in their fear conditioning response and extinction depending on the CS. In cued fear conditioning paradigms, male and female rats freeze similarly during conditioning, but males extinguish freezing behavior more quickly than females during repeated CS presentations (Baran et al., 2009). In contrast, female rodents freeze less and extinguish more quickly than males in contextual fear conditioning paradigms (Daviu et al., 2014; Gupta et al., 2001; Maren et al., 1994; Ribeiro et al., 2010). In both paradigms, female rats engage in far more escape-like darting compared to males (Gruene et al., 2015; Ribeiro et al., 2010). In fact, female rats are four times more likely to exhibit escape-like darting behaviors during cued fear conditioning compared to males with approximately 40% of females are classified as “darters” compared to only 10% of males (Gruene et al., 2015). This suggests that females may favor the escape-like darting coping strategy as opposed to freezing.

Stress models including chronic variable stress, restraint stress, maternal separation, and social isolation can also alter fear conditioning and extinction. In chronic variable stress models, animals are exposed to multiple stressors including forced swim, vibration, restraint, cold temperature, ultrasound, crowding, and isolation stress. The animals are exposed to two stressors per day for seven days with each stressor being experienced twice over the 7-day treatment. In cued fear conditioning paradigms, chronic variable stress enhances freezing behavior in female mice but has no effect in males (Sanders et al., 2010). Ovariectomized females also express stress-enhanced freezing, suggesting this sex-dependent response reflects organizational differences in fear circuitry established during development (Sanders et al., 2010). During contextual fear conditioning, chronic variable stress increases freezing exclusively in males (McGuire et al., 2010; Sanders et al., 2010), and impairs fear extinction in males (McGuire et al., 2010). These findings illustrate that the effects of chronic variable stress rely heavily on the CS.

Chronic restraint stress lasting at least 7 days has mixed effects on fear conditioning in both sexes. In male rodents, restraint stress increases freezing behavior during cued fear conditioning in some studies (Blume et al., 2019; Zhang & Rosenkranz, 2013), but not others (Baran et al., 2009; Negrón-Oyarzo et al., 2014; Sanders et al., 2010). Likewise, studies have shown that restraint stress impairs (Zhang & Rosenkranz, 2013) or has no effect on (Baran et al., 2009; Blume et al., 2019; Negrón-Oyarzo et al., 2014) cued fear extinction, and may impair cued fear extinction recall in males (Baran et al., 2009; Negrón-Oyarzo et al., 2014). Restraint stress does not appear to affect freezing responses in male mice conditioned to context (Sanders et al., 2010). With similarly mixed results, chronic restraint stress has no effect on freezing during cued fear conditioning in intact female rodents (Blume et al., 2019; Sanders et al., 2010; Takuma et al., 2012), and either increases (Hoffman et al., 2010) or decreases (Takuma et al., 2012) freezing in ovariectomized females. Furthermore, studies have found that restraint stress either impairs (Blume et al., 2019; Hoffman et al., 2010) or facilitates (Baran et al., 2009) cued fear extinction, and facilitates cued fear extinction recall (Baran et al., 2009) in female rodents. In contextual fear conditioning paradigms, restraint stress does not affect freezing in intact females, but may actually reduce freezing in ovariectomized females (Sanders et al., 2010; Takuma et al., 2012). The source of the inconsistent outcomes related to chronic restraint stress are not known but may involve procedural differences like the duration of restraint, species/strain contributions, or the rodents' age. More experiments are necessary to fully elucidate how restraint stress alters fear conditioning.

Social stress can also impact cued and contextual fear conditioning. Although maternal separation has no effect on freezing behaviors, it reduces ultrasonic vocalizations in both sexes during cued and contextual fear conditioning (Kosten et al., 2006). In contrast, social isolation significantly increases contextual freezing in male mice (Pibiri et al., 2008) and decreases freezing (Egashira et al., 2016; Pereda-Pérez et al., 2013) or has no effect (Martin & Brown, 2010) in females. Social isolation has no effect on cued fear conditioning for either sex (Martin & Brown, 2010; Pereda-Pérez et al., 2013; Pibiri et al., 2008; Skelly et al., 2015), but may impair cued fear extinction in male rats (Skelly et al., 2015). Thus, it appears that maternal separation alters fear conditioning independent of sex and CS, whereas

social isolation enhances fear conditioning specifically in male rodents during contextual fear conditioning.

The Effects of Sex Hormones and the Estrous Cycle—Males may be more susceptible to stess-enhanced freezing during contextual fear conditioning compared to females because some stressors dysregulate sex hormones exclusively in males. Indeed, in socially-isolated male mice, there is a 50% decrease in 5α-reductase type I mRNA expression and a 75% decrease in allopregnanolone levels in corticolimbic regions like the amygdala that coincides with enhanced contextual fear responses (Pibiri et al., 2008). Systemic inhibition of 5α-reductase type I in unstressed animals mimics both the stress-induced increase in freezing and the reduction in amygdala allopregnanolone levels. Conversely, systemic allopregnanolone reverses stress-induced freezing (Pibiri et al., 2008). In females, social isolation stress does not impact allopregnanolone in cortical regions unless they were exposed to chronic testosterone treatment (Pinna et al., 2005); and social isolation does not enhance freezing behavior in females (Egashira et al., 2016; Martin & Brown, 2010; Pereda-Pérez et al., 2013). These data suggest that social isolation causes sex-specific reductions in allopregnanolone synthesis that may control enhanced contextual fear conditioning in male rodents.

Estrogen and progestogens modulate fear conditioning/extinction across the estrous cycle and appear to be ‘protective’ in both cued and contextual conditioning paradigms. During proestrus, there is a transient reduction in freezing behavior and an enhancement of fear extinction that mirror rising estrogen and progesterone levels (Blume et al., 2019; Milad et al., 2009). Moreover, female rats that were exposed to the initial extinction trials during proestrus exhibited enhanced recall of extinction memories 24 hours later (Milad et al., 2009). Given that fear learning dysregulates cortical-BLA circuits (Arruda-Carvalho & Clem, 2014; Clem & Huganir, 2010; Skelly et al., 2017; Tsvetkov et al., 2002), estrogen and progesterone may be ‘protective’ during fear learning by altering synaptic plasticity in cortical-BLA circuits. Unlike freezing responses, the rat estrous cycle does not impact female-specific darting behaviors (Gruene et al., 2015). Importantly, stressors like chronic restraint can alter estrous cycle modulation of fear conditioning and extinction. For example, chronic restraint both increases freezing behavior and reduces fear extinction during proestrus when reduced freezing/enhanced extinction are more typical (Blume et al., 2019). The normally protective effects of proestrus likely rely on circulating estrogens and progestogens. Estradiol decreases freezing during contextual fear conditioning (Gupta et al., 2001; Hoffman et al., 2010) and, in some cases, enhances extinction learning in cued paradigms, possibly through through ERβ and NMDA receptor activation (Graham & Scott, 2018; Zeidan et al., 2011). Furthermore, increasing allopregnanolone levels in the BLA is known to reduce cued and contextual fear conditioning in male rats (Acca et al., 2017), suggesting that progestogens may have similar ‘protective’ effects in females and that these effects are mediated by the BLA.

Sex Differences in Alcohol-Related Behaviors

Baseline Sex Differences and the Effects of Sex Hormones on Alcohol Intake—The majority of studies have shown that non-dependent female rodents consume more

ethanol than non-dependent males using continuous-access two-bottle choice (Almeida et al., 1998; Lorrai et al., 2019; Priddy et al., 2017), intermittent-access two-bottle choice (Amodeo et al., 2018; Morales et al., 2015; Priddy et al., 2017; Scott et al., 2020; Vetter-O'Hagen et al., 2009; Vetter-O'Hagen & Spear, 2011), and operant self-administration paradigms (Logrip & Gainey, 2020). There are some showing that male rodents have higher alcohol intake compared to females (Fernandes et al., 2020; Vetter-O'Hagen et al., 2009) or there were no significant sex differences in alcohol intake (Albrechet-Souza et al., 2020; Fulenwider et al., 2019; Lorrai et al., 2019; Priddy et al., 2017; Randall et al., 2017; Tavares et al., 2019). The source of these inconsistences is not clear.

By using the four core genotype (FCG) mouse model, it is possible to uncouple the effects of sex chromosomes and developmental gonadal hormones (Finn, 2020; Puralewski et al., 2016) and their influence over ethanol drinking. In FCG mice, the testes-determining gene is excised from the Y chromosome and reincorporated into the genome as an autosomal transgene. The Y sex chromosome is thus decoupled from the development of gonads and production of gonadal hormones. Using the FCG model, gonadal females consume more alcohol than gonadal males in an operant self-administration paradigm, independent of the sex chromosome complement (Barker et al., 2010; Finn, 2020). This suggests that the higher alcohol consumption in females can be attributed to the organizational effects of developmental gonadal hormones on neural circuits. Moreover, neonatal exposure to testosterone facilitates male-like differentiation through its organizational effects. In female rodents, neonatal testosterone is quickly aromatized to estrogen, and this exposure to testosterone-derived estrogen reduces alcohol intake to mimic the lower alcohol consumption in intact males (Almeida et al., 1998; Finn, 2020). These studies suggest that the organizational effects of neonatal testosterone is critical for reducing alcohol intake in non-dependent males.

The activational effects of sex homones on ethanol drinking are also evident (Table 1). In gonadectomized adult male rodents, dihydrotestosterone reduces alcohol intake in two-bottle choice paradigms whereas estradiol increases alcohol intake (Almeida et al., 1998; Hilakivi-Clarke, 1996). Studies investigating how the estrous cycle affects alcohol intake, as well as the activational effects of estradiol and progesterone in females, have yielded mixed findings. Generally, alcohol intake does not fluctuate over the estrous cycle in two-bottle choice and operant self-administration paradigms in rodents (Ford et al., 2002; Fulenwider et al., 2019; Lorrai et al., 2019; Priddy et al., 2017; Scott et al., 2020). In non-human primates however, alcohol self-administration is significantly higher during the luteal phase of the menstrual cycle compared to the follicular phase (Dozier et al., 2019). The peak alcohol intake follows the progesterone peak during the luteal phase when progesterone levels are rapidly decreasing, suggesting that progesterone may impact alcohol intake in female monkeys (Dozier et al., 2019). In contrast, progesterone treatment does not affect alcohol self-administration in ovariectomized female rats (Almeida et al., 1998). Similarly, serum estradiol levels do not correlate with ethanol intake during self-administration in female monkeys (Dozier et al., 2019); but estradiol reduces two-bottle choice alcohol intake in female rodents (Almeida et al., 1998; Hilakivi-Clarke, 1996). This is unlikely to be related to the rewarding properties of ethanol since estradiol facilitates ethanol-conditioned place preference (Almeida et al., 1998; Finn, 2020; Hilderbrand & Lasek, 2018). Notably, while

ethanol intake doesn't change during the rat estrous cycle, bout frequency increases and bout size decreases during proestrus in self-administration paradigms (Ford et al., 2002). Thus, the activational effects of sex hormones can modulate ethanol-related behaviors as well.

Baseline Sex Differences and Sex Hormones During Alcohol Withdrawal— Perhaps more intriguing are the consistent and profound sex differences observed during alcohol withdrawal, most notably seizure susceptibility and anxiety. Withdrawal symptoms are more common and more severe in alcohol-dependent men than women, including an increased risk for withdrawal-induced seizures and delirium tremens (Deshmukh et al., 2003; Erol & Karpyak, 2015; Finn, 2020). Preclinical models demonstrate that female rats require longer alcohol exposures to increase seizure susceptibility compared to males (Devaud & Chadda, 2001), and that seizure susceptibility during withdrawal declines more quickly in female rats (Alele & Devaud, 2007; Devaud & Chadda, 2001). Exogenous delivery of neuroactive progestogens, such as allopregnanolone (Bitran et al., 1995; Devaud et al., 1995, 1996), pregnanolone (Alele & Devaud, 2007), and the synthetic neuroactive steroid and $GABA_A$ modulator alphaxalone (Cagetti et al., 2004), decrease seizure susceptibility and severity in both male and female rodents, although females are more sensitive to their anticonvulsant effects (Devaud et al., 1995). These findings suggest that females are both more resilient to withdrawal symptoms compared to males and more sensitive to the protective effects of neuroactive progestogens.

Although a single ethanol injection does not impact allopregnanolone immunoreactivity in the BLA of male rats (Cook et al., 2014), chronic ethanol reduces allopregnanolone immunoreactivity in the LA nucleus, but not BA nucleus, of adult male mice (Maldonado-Devincci et al., 2014b). Chronic ethanol self-administration also reduces allopregnanolone immunoreactivity in the LA, particularly in male monkeys characterized as heavy drinkers, and the BA of both heavy and non-heavy drinkers (Beattie et al., 2017). These reductions in allopregnanolone immunoreactivity in the amygdala mimic the dramatic decrease in the plasma allopregnanolone levels of male monkeys (Beattie et al., 2017). Conversely, chronic ethanol self-administration does not affect serum allopregnanolone levels in female monkeys (Dozier et al., 2019), suggesting that females may also be resilient to the reduction in allopregnanolone immunoreactivity. In support of this, social isolation reduces corticolimbic allopregnanolone levels in male but not female mice (Pibiri et al., 2008; Pinna et al., 2005). If females can maintain normal allopregnanolone levels during chronic ethanol as well, sex-specific facilitation of GABAergic function by allopregnanolone could explain why females experience less severe withdrawal symptoms.

Men are also more likely than women to report anxiety during alcohol withdrawal (Deshmukh et al., 2003). Although withdrawal-induced anxiety-like behavior has been demonstrated in male and female rats using the EPM and social interaction test (Morales et al., 2015, 2018; Overstreet et al., 2004), females may require longer or more intense ethanol exposures to produce anxiogenisis during withdrawal (Overstreet et al., 2004). In the novelty-suppressed feeding task, withdrawal-induced anxiety-like behavior is observed exclusively in male mice (Jury et al., 2017). Withdrawal-induced anxiety is also associated with neurobiological shifts in the balance between excitatory and inhibitory neurotransmission. Chronic ethanol and withdrawal reduces GABAergic transmission onto

BLA neurons in male rats (Diaz et al., 2011b) and elevates glutamatergic transmission in rats of both sexes (Christian et al., 2012, 2013; McGinnis et al., 2020a, 2020b; Morales et al., 2018; Sizer et al., 2021). Similar to seizure susceptibility, female rats require longer alcohol exposures to induce these neurophysiological changes (Morales et al., 2018); and, females may recover more quickly compared to males (*unpublished observations* by M Price). Given that ethanol dependence disrupts menstrual/estrous cycles (Finn, 2020; Morales et al., 2018), sex hormones may be initially 'protective' during chronic ethanol exposure in females. While there are numerous reports demonstrating the anxiolytic properties of estradiol and neuroactive progestogens in ethanol naïve rats (Bitran et al., 1995; Bitran & Dowd, 1996; Marcondes et al., 2001; Picazo & Fernández-Guasti, 1995), estradiol is not an effective anxiolytic in the EPM after chronic alcohol exposure (Henricks et al., 2017). Importantly in male rats, alphaxalone remains an effective anxiolytic after chronic alcohol, but it is unclear if it would remain anxiolytic in females (Cagetti et al., 2004).

Sex Differences in BLA Structure

Cellular Composition

The BLA contains glutamatergic pyramidal cells and a variety of GABAergic interneuron subpopulations. Glutamatergic pyramidal cells account for approximately 80% of BLA neurons and are the primary drivers of BLA signaling to downstream brain regions (Sah et al., 2003). At least two anatomically distinct GABAergic subpopulations regulate pyramidal cell activity: GABAergic lateral paracapsular cells (LPCs) and 'local' interneurons. GABAergic LPCs are clustered near the external capsule along the lateral boundary of the BLA and provide feedforward inhibition to glutamatergic pyramidal cells (Marowsky et al., 2005). GABAergic 'local' interneurons are dispersed throughout the BLA and supply feedback inhibition to the pyramidal cells (Spampanato et al., 2011). These 'local' GABAergic interneurons are a heterogeneous population that differ with respect to the expression of calcium-binding proteins, neuropeptides, and synaptic targets (McDonald & Mascagni, 2001; McDonald & Pearson, 1989; Prager et al., 2016). The calcium-binding proteins parvalbumin (PV) and calbindin (CB) are co-expressed in 40–60% of BLA GABAergic interneurons (Mascagni et al., 2009; McDonald & Betette, 2001; McDonald & Mascagni, 2001). PV+ interneurons receive excitatory input from and are the main source of perisomatic feedback inhibition to BLA pyramidal cells (McDonald et al., 2005; Muller et al., 2006; Smith et al., 2000). In contrast, the calcium-binding protein calretinin (CR) has almost no colocalization with PV or CB in the BLA (McDonald & Mascagni, 2001). Projections from CR+ interneurons target other interneurons, including CB+ interneurons, and make up 20–30% of GABAergic interneurons in the BLA (Mascagni et al., 2009; McDonald & Mascagni, 2001; Sorvari et al., 1998). A minority of GABAergic interneurons within the BLA also express one or more neuropeptides including somatostatin, neuropeptide Y, vasointestinal polypeptide, and cholecystokinin (McDonald & Pearson, 1989).

Sex and sex hormones differentially affect subpopulations of GABAergic interneurons expressing calcium-binding proteins (summarized Table 2). Female guinea pigs have a higher density of CB+ interneurons (Równiak et al., 2015), suggesting BLA principal

neurons in females may be more influenced by feedback inhibition relative to males. In addition, the vast majority of interneurons expressing ERβ also coexpress PV in the LA, and the number of PV+ interneurons increases during diestrus in female rats (Blume et al., 2017; Blurton-Jones & Tuszynski, 2002). PV+ interneurons play a pivotal role in regulating BLA-dependent behaviors like fear conditioning. In male mice, PV+ interneuron activity is suppressed during the delivery of the footshock, and exogenous activation of these cells during a footshock directly inhibits pyramidal neurons and impairs fear learning (Wolff et al., 2014). Thus, fluctuations in sex hormone levels can potentially regulate ERβ-expressing PV+ interneurons and therefore alter the acquisition of fear-related conditioned behaviors in female mice. BLA somatostatin (SST)-expressing interneurons also regulate fear conditioning through their interactions with PV+ interneurons. While a footshock suppresses PV+ interneuron activity in male mice, a footshock-predictive cue activates these PV+ interneurons which then provide robust inhibition to SST+ interneurons (Wolff et al., 2014). PV + and SST+ interneurons both inhibit pyramidal neurons, but during cue presentation, the indirect disinhibition of pyramidal neurons involving both PV+ and SST+ interneurons outweighs the direct inhibition of pyramidal neurons by PV+ interneurons and thereby facilitates fear learning (Wolff et al., 2014). Thus, SST+ interneurons are critical to regulating cued responses during fear learning and may underlay sex-specific vulnerabilities to fear conditioning. For example, the relative abundance of SST+ interneurons depends on the sex chromosomes (Puralewski et al., 2016). In pre-pubertal FCG mice, decoupled XX sex chromosomes increase SST expression compared to decoupled XY sex chromosomes, regardless of the presence of the testes-determining gene (Puralewski et al., 2016). Decoupled XX sex chromosomes also increase SST expression compared to XY sex chromosomes in adult mice that were exposed to unpredictable chronic mild stress, but not stress-naïve adult mice. Although testosterone does not appear to have organizational effects during development, activational testosterone during adulthood counteracts the lower SST expression in gonadectomized XY mice exposed to unpredictable chronic mild stress. Given the role of SST+ interneurons in fear conditioning and female vulnerability to cued fear conditioning after chronic variable stress (Sanders et al., 2010), stress-induced increases SST expression in the BLA may be acting as a compensatory mechanism to reduce female vulnerability to fear conditioning.

Cellular Morphology

Baseline Sex Differences and the Estrous Cycle—Current literature on sex differences in BLA neuron morphology varies considerably across studies. For instance, dendritic length and branching are similar between male and female rats (Blume et al., 2017; Koss et al., 2014), but these differences may be strain-dependent (Guadagno et al., 2018). Sex differences in dendritic spine characteristics are similarly unclear but cannot easily be explained by stain effects (Blume et al., 2017; Guadagno et al., 2018; Koss et al., 2014; Rubinow et al., 2009). However, these inconsistencies could highlight the divergent influence of sex hormones on LA and BA neurons. Hormonal fluctuations across the rodent estrous cycle cause distinct, subdivision-dependent changes to dendrite and spine morphology. Sex differences in spine or dendrite morphology can be overlooked if different subdivisions are sampled simultaneously (Blume et al., 2017, 2019; Rubinow et al., 2009).

Sex Differences and Stress Interactions—Stress also causes dendritic remodeling in BLA neurons, but these effects depend upon the sex of the animal and the type of stress paradigm. Both limited bedding (Guadagno et al., 2018) and chronic immobilization stress (Vyas et al., 2002, 2006) increase dendritic length, dendritic branching, total spine number, and spine density in male rats. However, limited bedding decreases spine density in females (Guadagno et al., 2018). Chronic unpredictable stress, which does not induce adrenal hypertrophy or anxiety, has no effect on BLA pyramidal neuron morphology in male rats (Vyas et al., 2002). In females, restraint stress decreases the dendritic length in LA neurons and disrupts the modulation of BA neuron morphology by estrous cycle (Blume et al., 2019). In male rats, restraint stress increases dendritic length and total spine number in BA neurons only (Blume et al., 2019). Note that while some stress models induce dendritic hypertrophy in male rodents, females are more likely to experience estrous cycle-independent dendritic hypotrophy or the disruption of estrous cycle effects.

Sex Differences in BLA Neurotransmitter and Neuromodulator Systems

Glutamate, GABA, and Intrinsic Excitability

Baseline Sex Differences—Female rats have higher basal glutamatergic and GABAergic synaptic function in the BLA compared to males (Table 2). For glutamatergic function, female BLA neurons express a higher miniature excitatory postsynaptic current (mEPSC) frequency than males, indicating increased presynaptic function either through greater presynaptic release probability or greater numbers of active synapses (Blume et al., 2017, 2019). Female rats also have larger mEPSC amplitudes, indicating increased postysnapic AMPA receptor function or quantity, but this is only present in LA neurons (Blume et al., 2017). Moreover, female BLA neurons exhibit a more pronounced increase in firing rate following exogenous glutamate application compared to males, suggesting that this increased AMPA receptor function may drive greater excitability of female BLA neurons (Blume et al., 2017). Ehanced basal GABAergic function in female rats compared to males is mediated presynaptically either through greater presynaptic GABA release probability or greater number of active GABAergic synapses (Blume et al., 2017). Interestingly, the postsynaptic function of GABAergic synapses is similar between male and female rats, but the sensitivity to exogenously applied GABA is sex-dependent with opposite patterns in LA and BA neurons. That is, GABA suppresses the firing rate of BA neurons in females more than males and suppresses the firing rate of LA neurons in males more than females (Blume et al., 2017).

The Effects of the Estrous Cycle and Sex Hormones—In female rats, glutamate and GABA neurotransmission fluctuate with the estrous cycle, but once again LA and BA neurons are affected differently. During proestrus, LA pyramidal neurons decrease both their intrinsic firing rate and their excitatory response to exogenous glutamate application (Blume et al., 2017). In addition, GABAergic function, as represented by the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) and interneuron firing rates, is diminished during proestrus. LA neurons during proestrus also exhibit a greater inhibition of firing rate in response to exogenous GABA application. These cycle-dependent changes to glutamate and GABA function suggest an overall shift toward greater inhibition during

proestrus. These data together also suggest that female LA principal neurons are 'protected' from hyperactive states during proestrus, analogous to the wealth of literature documenting the anxiolytic properties of estrogen and progestogens.

In contrast to rat LA neurons, BA neurons experience enhanced GABAergic inhibition during diestrus (increased sIPSC and miniature IPSC or mIPSC frequency; Blume et al., 2017). Since diestrus does not alter interneuron firing rates, this increased GABAergic synaptic function likely arises from an increase in GABA release probability. Diestrus also enhances glutamate presynaptic function (mEPSC frequency). Furthermore, exogenous GABA more effectively suppresses BA neuron firing rates while exogenous glutamate is less effective at increasing firing rates (Blume et al., 2017). Thus, diestrus has distinct effects on glutamatergic and GABAergic pre- and postsynaptic function. These findings together suggest that GABAergic inhibition onto BA neurons increases during diestrus when estrogen levels are low and progesterone levels have a small, secondary peak peak. In support of this, estrogen synthesis inhibitors impair long-term potentiation (LTP) induction in BA neurons of female mice, but not male mice (Bender et al., 2017). Notably, progesterone is converted to the neuroactive metabolite allopregnanolone which facilitates $GABA_A$ receptor function by increasing the affinity of GABA for its receptor and, at higher concentrations, directly activating the $GABA_A$ receptor (Belelli & Lambert, 2005; Finn & Jimenez, 2018; Porcu et al., 2016). There are several excellent reviews on how neuroactive steroids like allopregnanolone impact $GABA_A$ receptor function and subsequently modify behavior (Belelli & Lambert, 2005; Finn & Jimenez, 2018; Porcu et al., 2016). Since allopregnanolone is anxiolytic and enhances GABAergic inhibition in several brain regions, it is highly likely that allopregnanolone enhances GABAergic inhibition onto BA neurons as well.

In addition to the classical nuclear estrogen receptors, there is also considerable evidence that estradiol influences GABAergic neurophysiology through GPR30. Acute application of 17β-estradiol decreases BLA evoked excitatory postsynaptic potentials (EPSPs; (Womble et al., 2002); and, estrogen withdrawal increases EPSP slope and duration in the rodent BLA (Yang et al., 2017). Estrogen withdrawal was induced by co-administering estradiol and progesterone for 16 consecutive days followed by 7 days of high-dose estradiol to create a hormone-stimulated pregnancy in ovariectomized mice, and then 3–4 days of withdrawal from all hormone treatment (Yang et al., 2017; Zhang et al., 2016). Estrogen withdrawal reduces $GABA_A$-mediated inhibition and ultimately impairs long-term depression (LTD), leaving glutamatergic transmission and LTP unaltered (Yang et al., 2017). Direct activation of GPR30, but not ERα or ERβ, increases GABAergic inhibition in the BLA, reverses the neurophysiological effects of estrogen withdrawal, and alleviates estrogen withdrawal-induced anxiety (Tian et al., 2013; Yang et al., 2017). This suggests that estradiol activation of GPR30 reduces anxiety by enhancing GABAergic inhibiton in the BLA.

Estradiol may also impact neurophysiology by influencing metabotropic glutamate receptors (mGluRs). In the BLA of male rats, LTD depends on mGluR1 activation (Chen et al., 2017), and female rats have higher mGluR1 expression in the amygdala compared to males (De Jesus-Burgos et al., 2016). These higher levels may accentuate mGluR1-mediated depression at glutamate synapses and thereby facilitate anxiolysis. Indeed,

mGluR1-dependent anxiolysis in the EPM is only observed in ovariectomized female rats treated with estradiol (De Jesus-Burgos et al., 2012). Estrogen receptors ERα or ERβ and mGluRs may act together to activate intracellular signaling cascades. For example, ERα interacts with mGluR1/mGluR5 to initiate the rapid phosphorylation of cAMP-response element binding protein (CREB; Meitzen & Mermelstein, 2011). Notably, this is brain region- and sex-dependent. ERα increases CREB phosphorylation via interaction with mGluR1 in the hippocampus of female rats but not males, whereas CREB phosphorylation is mediated solely by mGluR5 in striatal neurons (Meitzen & Mermelstein, 2011). If a similar mechanism is involved in the amygdala, estrogen receptor activation could help drive mGluR1-mediated LTD.

The Effects of Stress and Fear Conditioning—Stressors also produce a variety of sex-specific effects on glutamate and GABA transmission that are paradigm-dependent. Chronic stress models, such as social isolation and chronic restraint stress increase male pyramidal neuron excitability *ex vivo* and *in vivo* (Blume et al., 2019; Lin et al., 2018; Rau et al., 2015). The enhanced excitability induced by social isolation coincides with increased mGluR5 expression in the amygdala and increased anxiety-like behavior. The enhanced excitability and anxiety-like behavior are abolished by blocking mGluR5 in the BLA (Lin et al., 2018). Chronic restraint stress increases glutamate release from dorsal mPFC (dmPFC) inputs entering the BLA through the *stria terminalis.* Reducing glutamate release from dmPFC inputs using low frequency stimulation attenuates the increased anxiety-like behavior in male mice exposed to chronic restraint stress (Liu et al., 2020). There were no effects of chronic restraint on glutamate release from ventral PFC (vmPFC) inputs, on the AMPA/NMDA ratio, or on inhibitory transmission (Liu et al., 2020). In female rats, chronic restraint stress disrupts the effects of estrous cycle and suppresses BLA neuron firing rates (Blume et al., 2019). Other stressors like forced swim stress increase expression of GPR30, GluR1-containing AMPA receptors, and NR2A-containing NMDA receptors while decreasing expression of NR2B-containing NMDA receptors in ovariectomized female mice (Tian et al., 2013). Microinjecting estradiol or a GPR30 agonist into the BLA reverses all swim stress-induced changes in expression and reduces anxiety-like behavior in the EPM and OFT (Tian et al., 2013). NR2A- and NR2B-containing NMDA receptors may play distinct roles in synaptic plasticity. In the hippocampus, for example, NR2A-containing receptors are required for LTP whereas NR2B-containing receptors are required for LTD (Liu et al., 2004). If NMDA receptor isoforms behave similarly in the BLA, estradiol would prevent AMPA and NMDA receptor-mediated events like LTP. Finally, both estradiol treatment and GPR30 activation reverse swim stress-induced decrease in $GABA_A$-α2 and γ2 subunit expression and potentiates GABAergic inhibition in ovariectomized mice (Tian et al., 2013). These findings illustrate that stressors can elicit sex-dependent effects on excitability and glutamate/GABA transmission.

Cued fear conditioning has input-dependent effects on LA and BA neurons in male rodents (Table 3). In EC-LA synapses, cued fear conditioning occludes LTP expression by increasing glutamate release from EC inputs (Tsvetkov et al., 2002). In comparison, dmPFC-BA synapses exhibit an increase in postsynaptic glutamatergic function via increased AMPA/NMDA ratio but no change in glutamate release from dmPFC inputs onto BA

neurons (Arruda-Carvalho & Clem, 2014). Likewise, ST-LA synapses exhibit an increase in AMPA/NMDA ratio, mEPSC amplitudes, and accentuated NMDA and mGluR1-dependent LTD (Clem & Huganir, 2010). Moreover, fear conditioning reduces GABAergic function in LPC and 'local' interneuron synapses (Skelly et al., 2017). Similar experiments have not been conducted in female rodents to our knowledge.

The Effects of Acute and Chronic Alcohol Exposure—Acute and chronic ethanol exposure have opposing neurophysiological effects on GABA and glutamate neurotransmission in the BLA. In male rats, acute ethanol increases GABA release from interneurons (Silberman et al., 2008) and reduces postsynaptic function of kainate- and NMDA-type glutamate receptors (Läck et al., 2008). In contrast, chronic ethanol exposure and withdrawal downregulates GABA function (Diaz et al., 2011b), upregulates glutamatergic function (Christian et al., 2012, 2013; McGinnis et al., 2020a, 2020b; Morales et al., 2018; Sizer et al., 2021) and increases BLA principal neuron excitability (*unpublished observations* by M Price). These effects illustrate a shift towards greater excitation in the BLA that may ultimately facilitate alcohol withdrawal-induced anxiety (McGinnis et al., 2020a, 2020b; Morales et al., 2018). This section will describe the neurophysiological changes observed in the BLA following alcohol exposure, some of which are sex-dependent.

Chronic intermittent ethanol and withdrawal (CIE/WD), using a vapor inhalation paradigm, enhances glutamatergic function in both sexes through input-dependent mechanisms (Table 3). Glutamatergic 'medial inputs' enter the BLA via the *stria terminalis* (ST), a white matter tract coursing into the region along the dorsomedial border. The ST contains axons arising in regions like the mPFC and thalamic nuclei. A 10-day exposure to chronic intermittent ethanol (12 hrs/day) followed by 24 hours of withdrawal enhances glutamate release probability from ST-BLA synapses in male and female rats (Christian et al., 2013; McGinnis et al., 2020a; Morales et al., 2018; Sizer et al., 2021) via increased vesicle release, increased glutamate concentration in the synapse, and reduced presynaptic failure rates (Christian et al., 2013). There are no apparent effects on postsynaptic glutamate receptors at these synapses in male rats, although female rats have not been examined (Christian et al., 2013). Recent optogenetic approaches have also shown that individual projections arising from specific brain regions can express unique adaptations to chronic ethanol. In male rats for example, CIE/WD increases glutamate release at dmPFC inputs (McGinnis et al., 2020a) and polymodal sensory thalamic inputs (Morales et al., 2019) onto BLA neurons while CIE/WD decreases glutamate release from vmPFC inputs onto BLA neurons (McGinnis et al., 2020a). The dmPFC facilitates fear conditioning and drug-seeking while the vmPFC promotes the extinction of these behaviors (Peters et al., 2009), suggesting that chronic ethanol exposure may shift the balance between these two regions in favor of dmPFC function, thereby facilitating the acquisition of conditioned responses like fear and drug-seeking behaviors.

Glutmatergic inputs from lateral cortical areas such as the agranular insula cortex or secondary sensory cortices enter the BLA through the external capsule (EC), a white matter tract delineating the lateral boundary of this brain region, and are also altered by chronic ethanol exposure. Unlike ST synapses, CIE/WD enhances postsynaptic glutamatergic function at EC-BLA synapses in both male and female rats (Christian et al., 2012; Läck

et al., 2007, 2009; McGinnis et al., 2020b; Morales et al., 2018) by increasing the function of kainate (Läck et al., 2009), NMDA (Läck et al., 2007), and AMPA receptors (Christian et al., 2012). Notably, the facilitation of AMPA-receptor function is associated with increased phosphorylation of GluA1 and GluA2 and increased trafficking of GluA1- and GluA2/3-containing AMPA receptors to the cell surface in male rats (Christian et al., 2012). These effects are reminiscent of canonical signaling events associated with LTP in several brain regions. Recent optogenetic studies targeting agranular insula cortical inputs to the BA have recapitulated the enhanced postsynaptic function in male rats (McGinnis et al., 2020b). In contrast, CIE/WD has no effect on presynaptic function at EC inputs in either sex (Christian et al., 2012; Läck et al., 2009; McGinnis et al., 2020b; Morales et al., 2018). Pre- and postsynaptic facilitation of glutamatergic signaling are believed to accentuate anxiety-like behavior during withdrawal. AMPA receptor antagonists (Läck et al., 2007), chemogenetic inhibition of presynaptic glutamate release (McGinnis et al., 2020a), and acute ketamine treatment, which inhibits NMDA receptors and delays the development of postsynaptic faciliation (McGinnis et al., 2020b), can all reduce withdrawal-induced anxiety-like behavior in male rats.

Although chronic ethanol ultimately enhances pre- and postsynaptic glutamatergic function in both sexes, recent evidence has demonstrated that the development of these neurophysiological changes is sex-dependent. The effects on glutamatergic transmission are delayed in female rats compared to males, such that female rats require longer chronic alcohol exposures to induce the same neurophysiological changes (Morales et al., 2018). Moreover, these changes may be more plastic in female rats as they appear to return to ‘normal’ status more quickly (*unpublished observations* by M Price). These data indicate that female rats may be more resilient to the effects of chronic ethanol on BLA neurophysiology than males, and therefore may be more resilient to withdrawal-induced anxiety influenced by BLA neurophysiology. Preclinical studies have yielded mixed results regarding sex differences in withdrawal-induced anxiety-like behavior. Some studies have found that chronic ethanol does not induce anxiety-like behavior in female mice using the novelty-suppressed feeding test (Jury et al., 2017) or that female rats require longer alcohol exposures to increase anxiety-like behavior using the social interaction test (Overstreet et al., 2004), consistent with the delayed neurophysiological changes in the BLA. However, other studies have showed that rats of both sexes develop anxiety-like behavior (Morales et al., 2015, 2018). The timecourse for developing withdrawal-induced neurophysiological changes in the BLA and anxiety-like behavior may suggest that the delayed neurophysiology has a stronger impact on certain preclinical anxiety models or coping strategies compared to others or that activity in other circuits initially contribute more robustly to withdrawal-induced anxiety.

In male rats, chronic ethanol alters GABAergic function as well, but these effects are dependent on the subpopulation of BLA GABAergic interneurons (Table 3). CIE/WD decreases presynaptic GABA release probability and postsynaptic zolpidem sensitivity of LPC feedforward inhibitory synapses (Diaz et al., 2011b). While the mechanisms controlling presynaptic alterations are not currently known, the postsynaptic changes are driven by a reduction in total protein levels, as well as the surface expression of the zolpidem-sensitive $GABA_A$-α1 subunit. CIE/WD also decreases postsynaptic $GABA_A$

receptor function at 'local' feedback-type inhibitory synapses, as shown by reduced postsynaptic sensitivity to the benzodiazepine midazolam, but does not alter GABA release from these synapses (Diaz et al., 2011b). The postsynaptic effects appear to be mediated by increased trafficking of benzodiazepine-insensitive $GABA_A$ receptor isoforms containing the α4 subunit to the cell surface (Diaz et al., 2011b). A similar increase in hippocampal $GABA_A$-α4 subunit surface expression coincides with benzodiazepine-insensitivity, potentiated responses to Ro15-4513 (a positive allosteric modulator of $GABA_A$ receptors containing the α4 subunit with minimal effect on α1-containing $GABA_A$ receptors), and elevated binding of [^{3}H]Ro15-4513 to benzodiazepine-insensitive sites containing the $GABA_A$-α4 subunit in the hippocampus of CIE-exposed male rats (Cagetti et al., 2003; Olsen & Liang, 2017). Likewise, chronic ethanol reduces $GABA_A$-α1 subunit expression in the hippocampus of male rats (Cagetti et al., 2003; Olsen & Liang, 2017).

Experiments regarding pre- and postsynaptic function in LPC and 'local' interneuron synapses have not been completed in CIE-exposed female rats; however, some evidence suggests that CIE/WD could dysregulate GABAergic inhibition in a sex-dependent manner. As mentioned, CIE-exposed male and female rats ultimately exhibit the same input-dependent increase in glutamatergic function but females require longer alcohol exposures to induce the same effect (Morales et al., 2018). A similar mechanism could delay CIE-induced suppression of BLA GABAergic inhibition or entirely prevent dysregulation of the GABAergic system in female rats. Sex hormones would likely contribute to any sex differences in GABAergic function following alcohol exposure given that estradiol and progestogens directly regulate GABAergic inhibition (Belelli & Lambert, 2005; Finn & Jimenez, 2018; Porcu et al., 2016; Womble et al., 2002; Yang et al., 2017). Notably, ERβ is expressed within PV+ 'local' interneurons in the BLA (Blurton-Jones & Tuszynski, 2002) and the activity of these interneurons varies throughout the the estrous cycle (Blume et al., 2017). Thus, sex hormone regulation of PV+ interneurons could be a potential protective mechanism in CIE-exposed female rats.

Dopamine

Dopamine has an important role in regulating BLA-mediated behaviors like fear conditioning (Greba et al., 2001; Heath et al., 2015; Prager et al., 2016; Sharp, 2017). The BLA receives dopaminergic innervation from the ventral tegmental area and the substantia nigra, and these inputs form synapses onto both glutamatergic pyramidal neurons (Muller et al., 2009) and GABAergic neurons, including PV+ and CR+ interneurons (Pinard et al., 2008). Electrophysiological studies conducted in male rodents have illustrated that dopamine generally facilitates BLA excitability through a variety of mechanisms depending on which dopamine receptor and cell population is involved. For example, activation of dopamine D1 receptors increases the intrinsic excitability of BLA pyramidal neurons (Kröner et al., 2005) and reduces feedforward inhibition onto BLA pyramidal neurons by decreasing the intrinsic excitability of LPCs and decreasing GABA release from LPCs (Marowsky et al., 2005). Dopamine D2 receptors suppress GABAergic transmission from PV+ local interneurons onto BLA principal neurons presynaptically by reducing GABA release (Bissière et al., 2003; Chu et al., 2012). Dopamine D3 receptor activation reduces GABAergic inhibition in LPCs and local interneurons through a dynamin-depdendent

postsynaptic mechanism likely involving the internalization of $GABA_A$ receptors, and by decreasing GABA release from local interneurons (Diaz et al., 2011a). Altogether, dopamine ultimately enhances BLA pyramidal neuron excitability and facilitates BLA-mediated behaviors. Indeed, D1/D5 (Heath et al., 2015), D2 (Greba et al., 2001), or D3 (Diaz et al., 2011a) receptor inhibition in the BLA blocks fear conditioning or anxiety-like behaviors.

Sex Differences and the Effects of Sex Hormones—The dopamine system in the BLA is vastly understudied in females, but initial evidence suggests that male rodents have higher basal dopamine levels than females due to the actions of testosterone (Table 2). Extracellular dopamine levels in the BLA are more than doubled in adult male rodents compared to females, but neonatal castration equalizes dopamine levels between males and females, revealing an important example of the organizational effects of hormones on the BLA dopamine circuits (Mitsushima et al., 2006; Siddiqui & Shah, 1997). Conversely, testosterone treatment increases dopamine levels in the female amygdala, raising it to male-like levels (Siddiqui & Shah, 1997). In addition, progesterone increases BLA dopamine levels in male rodents (de Souza Silva et al., 2008), suggesting that BLA dopaminergic function may be affected by the estrous cycle.

The Effects of Stress—Despite male rodents having higher basal dopamine levels, the BLA dopaminergic system in females is more sensitive to stress. Stress typically increases extracellular dopamine levels in the BLA; but, like other end-points, this is stressor-specific. Predator odor and tail pinch stress increase dopamine in both sexes (Sullivan et al., 2009b), whereas restraint stress doubles extracellular dopamine levels in female rats but has no effect in males (Mitsushima et al., 2006). Stress can also alter dopamine receptor expression. Unpredictable chronic mild stress affects BLA D5 expression in opposite directions across sex, increasing expression in female mice and decreasing expression in males (Barko et al., 2019). Similarly, parental separation increases D1 receptor density in female rodents (Ziabreva et al., 2003). These female-specific increases in D1/D5 expression could enhance D1/D5-mediated neuromodulation, increasing pyramidal neuron excitability or suppressing LPC interneuron excitability, and thus preferentially initiate dopamine-mediated stress responses in females.

Interestingly, the stress responses of BLA dopamine also have a lateralization bias that is sex-specific. In male rats, predator odor and tail pinch stress preferentially increase dopamine release in the right BLA compared to the left (Sullivan et al., 2009b). Conversely, dopamine depletion in the right amygdala is anxiolytic in male rats (Sullivan et al., 2009a). These findings are consistent with stress-responsive brain regions in the right hemisphere driving stress behaviors (Sullivan & Gratton, 1999) and aversive learning (Coleman-Mesches & McGaugh, 1995) more so than the left hemisphere in males. In contrast, in female rats, predator odor and tail pinch stress induce greater dopamine release in the left BLA compared to the right (Sullivan et al., 2009b), suggesting that stress-induced dopaminergic signaling in the left BLA may govern stress responses in females. Sex-specific lateralization biases are also observed in other brain regions. In the cortex, for example, gonadectomies can reverse right- and left-biased lateralizations characteristic of males and females, respectively (Wisniewski, 1998). This indicates that the organizational effects of

sex hormones are critical for establishing lateralization biases, and therefore could direct how stress modulates dopaminergic signaling in the BLA and its ultimate impact on behavior.

Serotonin

Serotonergic transmission in the BLA has been implicated in anxiety and fear conditioning (Inoue et al., 2004; Kitaichi et al., 2014; Li et al., 2006; Wang et al., 2019). Serotonergic inputs to the BLA originate primarily from the dorsal raphe nucleus. Released serotonin (5-HT) binds to a multitude of 5-HT receptor subtypes which are expressed within distinct cell types and differentially affect BLA neurophysiology. Altogether, serotonin signaling decreases BLA principal neuron excitability, corresponding to impaired fear conditioning (Inoue et al., 2004; Kitaichi et al., 2014; Li et al., 2006) and a suppression of alcohol-seeking but not consummatory behaviors (McCool et al., 2014) in male rats. 5-HT_{1A} receptors directly inhibit BA pyramidal neurons (Sengupta et al., 2017) and reduce presynaptic glutamate release from EC inputs in rodents of both sexes (Cheng et al., 1998; Wang et al., 2019). Presynaptic 5-HT_{1B} receptors also reduce excitatory transmission by reducing glutamate release from ST and EC inputs onto BLA pyramidal neurons in male rats (Guo et al., 2017). In addition, activation of 5-HT_{1B} receptors decreases inhibitory transmission by reducing GABA release from interneurons onto LA pyramidal neurons (Yamamoto et al., 2020). In contrast to 5-$HT_{1A/B}$ receptors, 5-HT_{2A} and 5-HT_{2C} receptors have opposing effects in the BLA. 5-HT_{2A} receptors depolarize (Rainnie, 1999) and excite BA interneurons (Sengupta et al., 2017), including PV+ interneurons (Bocchio et al., 2015), to increase inhibitory drive onto pyramidal neurons (Bocchio et al., 2015; Jiang et al., 2009) in rodents of both sexes. Activation of 5-$HT_{2A/C}$ receptors hyperpolarizes the membrane potential of pyramidal neurons (McCool et al., 2014; Rainnie, 1999), reduces pyramidal neuron excitability by increasing the action potential threshold (McCool et al., 2014), and reduces excitatory transmission (Yamamoto et al., 2012) in male rats. These effects are likely mediated by the 5-HT_{2A} receptors whereas 5-HT_{2C} receptors are responsible for depolarizing pyramidal cells specifically in the LA (Yamamoto et al., 2012, 2014).

Sex Differences and Stress Interactions—Few studies have explored sex differences in serotonergic system in the BLA, but there is evidence that basal and stress-induced serotonin levels differ between males and females (Table 2). Basal extracellular serotonin levels are 54% higher in male rats compared to females (Mitsushima et al., 2006). Restraint stress increases extracellular serotonin levels in both sexes, but the response in female rats is greater and remains elevated for 15 minutes after the restraint ceases (Mitsushima et al., 2006), suggesting that female rats are more susceptible to serotonin-mediated stress responses.

The Effects of Sex Hormones—Sex hormones like estradiol modulate 5-HT receptor expression and function in female mice. Estradiol facilitates serotonin synthesis in the dorsal raphe nucleus (Wang et al., 2019) and increases 5-HT_1 receptor expression in the amygdala (Biegon & McEwen, 1982) of female rodents, indicating that 5-HT_1 signaling may be sex-specific and regulated by the estrous cycle. A study using a perimenopause model induced by chronic exposure to 4-vinylcycloxene diepoxide explored how estradiol

levels alter serotonergic function in female mice (Wang et al., 2019). In this model, low levels of estradiol enhance glutamate release and facilitate NMDA receptor-dependent LTP in EC-BLA synapses by downregulating 5-HT_{1A} receptors (Wang et al., 2019). Interestingly, female mice do not experience the 5-HT_{1B}-mediated inhibition of glutamate or GABA release typical of males, regardless of hormonal status (Wang et al., 2019). Low estradiol also reduces GABAergic inhibition and impairs LTD by downregulating 5-HT_2 receptors. Chronic estradiol treatment prevents increased glutamate release and the facilitation of LTP, and restores LTD caused by the downregulation of 5-HT_{1A} and 5-HT_2 receptors. These data indicate that low levels of estradiol in a perimenopause model have profound effects on BLA synaptic plasticity through its effects on the serotonergic system. Importantly, without sufficient estradiol, both 5-HT_{1A} and 5-HT_2 receptors must be activated to ameliorate the anxiety-like behavior associated with perimenopause (Wang et al., 2019), indicating that the effects on BLA neurophysiology translate to changes in anxiety.

Conclusion

Sex differences in BLA structure and function highlight potential mechanisms involved in female vulnerability to stress/anxiety and male vulnerability to AUD. These differences arise from the complement of sex chromosomes, organizational hormone effects - ‘permanent’ differences in neuro-architecture occurring during sensitive developmental periods, and activational effects represented by more transient influences of sex hormones on neuronal subpopulations. Our review details current literature related to significant sex differences in BLA structure and function as they relate to anxiety/fear, stress responsiveness, and ethanol. While many preclinical studies have examined the effects of sex hormones on the BLA, these have largely focused on general mechanisms and in particular activational effects (e.g. estrous cycle). Additional experiments are sorely needed to fully differentiate the organizational mechanisms from activational influences of sex hormones. Additionally, there is still much to be learned about how activational mechanisms may differ between males and females, particularly in the context of preclinical anxiety and AUD models. For instance, male rodents exhibit social isolation stress-induced enhancement of contextual fear conditioning that is due to testosterone-dependent reduction in allopregnanolone synthesis within the amygdala (Pibiri et al., 2008; Pinna et al., 2005; Sanders et al., 2010). This suggests that enhancing allopregnanolone synthesis in the amygdala would be particularly effective at preventing stress-induced enhancement of contextual fear conditioning in males. Chronic ethanol also reduces allopregnanolone levels in the male BLA (Beattie et al., 2017; Maldonado-Devincci et al., 2014b), but the same experiments have not been conducted in females. If chronic ethanol exposure produces a similar testosterone-dependent reduction in allopregnanolone levels, higher allopregnanolone levels in the female BLA could explain their resistance to severe withdrawal symptoms. Altogether, the literature demands a closer look at these sex hormone-mediated mechanisms and how they might be manipulated to suppress alcohol withdrawal symptoms.

References

Acca GM, Mathew AS, Jin J, Maren S, & Nagaya N (2017). Allopregnanolone induces state-dependent fear via the bed nucleus of the stria terminalis. Hormones and Behavior, 89, 137–144. [PubMed: 28104355]

Agís-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, & Guidotti A (2006). Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proceedings of the National Academy of Sciences of the United States of America, 103(39), 14602–14607. [PubMed: 16984997]

Albrechet-Souza L, Schratz CL, & Gilpin NW (2020). Sex differences in traumatic stress reactivity in rats with and without a history of alcohol drinking. Biology of Sex Differences, 11(1), 27. [PubMed: 32393336]

Alele PE, & Devaud LL (2007). Sex differences in steroid modulation of ethanol withdrawal in male and female rats. Journal of Pharmacology and Experimental Therapeutics, 320(1), 427–436.

Almeida OF, Shoaib M, Deicke J, Fischer D, Darwish MH, & Patchev VK (1998). Gender differences in ethanol preference and ingestion in rats. The role of the gonadal steroid environment. The Journal of Clinical Investigation, 101(12), 2677–2685. [PubMed: 9637701]

Amodeo LR, Wills DN, Sanchez-Alavez M, Nguyen W, Conti B, & Ehlers CL (2018). Intermittent voluntary ethanol consumption combined with ethanol vapor exposure during adolescence increases drinking and alters other behaviors in adulthood in female and male rats. Alcohol, 73, 57–66. [PubMed: 30293056]

Anchan D, Clark S, Pollard K, & Vasudevan N (2014). GPR30 activation decreases anxiety in the open field test but not in the elevated plus maze test in female mice. Brain and Behavior, 4(1), 51–59. [PubMed: 24653954]

Arnold AP (2009). The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. Hormones and Behavior, 55(5), 570–578. [PubMed: 19446073]

Arruda-Carvalho M, & Clem RL (2014). Pathway-selective adjustment of prefrontal-amygdala transmission during fear encoding. Journal of Neuroscience, 34(47), 15601–15609. [PubMed: 25411488]

Baran SE, Armstrong CE, Niren DC, Hanna JJ, & Conrad CD (2009). Chronic stress and sex differences on the recall of fear conditioning and extinction. Neurobiology of Learning and Memory, 91(3), 323–332. [PubMed: 19073269]

Barker JM, Torregrossa MM, Arnold AP, & Taylor JR (2010). Dissociation of genetic and hormonal influences on sex differences in alcoholism-related behaviors. Journal of Neuroscience, 30(27), 9140–9144. [PubMed: 20610747]

Barko K, Paden W, Cahill KM, Seney ML, & Logan RW (2019). Sex-Specific Effects of Stress on Mood-Related Gene Expression. Molecular Neuropsychiatry, 5(3), 162–176. [PubMed: 31312637]

Beattie MC, Madlonado-Devincci AM, Porcu P, O'Buckley TK, Dauanis JB, Grant KA, & Morrow AL (2017). Voluntary ethanol consumption reduces GABAergic neuroactive steroid (3alpha,5alpha)3-hydroxypregnan-20-one (3alpha,5alpha-THP) in the amygdala of the cynomolgus monkey. Addiction Biology, 22(2), 318–330. [PubMed: 26625954]

Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, & Young E (2005). Strategies and methods for research on sex differences in brain and behavior. Endocrinology, 146(4), 1650–1673. [PubMed: 15618360]

Belelli D, & Lambert JJ (2005). Neurosteroids: Endogenous regulators of the GABAA receptor. Nature Reviews Neuroscience, 6(7), 565–575. [PubMed: 15959466]

Bender RA, Zhou L, Vierk R, Brandt N, Keller A, Gee CE, Schäfer MKE, & Rune GM (2017). Sex-dependent regulation of aromatase-mediated synaptic plasticity in the basolateral amygdala. Journal of Neuroscience, 37(6), 1532–1545. [PubMed: 28028198]

Beyeler A, Namburi P, Glober GF, Simonnet C, Gwendolyn G, Conyers GF, Luck R, Wildes CP, & Tye KM (2016). Divergent routing of positive and negative information from the amygdala during memory retrieval. Neuron, 90(2), 348–361. [PubMed: 27041499]

Biegon A, & McEwen BS (1982). Modulation by estradiol of serotonin receptors in brain. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 2(2), 199–205. [PubMed: 7199565]

Bissière S, Humeau Y, & Lüthi A (2003). Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. Nature Neuroscience, 6(6), 587–592. [PubMed: 12740581]

Bitran D, & Dowd JA (1996). Ovarian steroids modify the behavioral and neurochemical responses of the central benzodiazepine receptor. Psychopharmacology, 125(1), 65–73. [PubMed: 8724450]

Bitran D, Shiekh M, & McLeod M (1995). Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. Journal of Neuroendocrinology, 7(3), 171–177. [PubMed: 7606242]

Bitran Daniel, Kellogg CK, & Hilvers RJ (1993). Treatment with an anabolic-androgenic steroid affects anxiety-like related behavior and alters the sensitivity of cortical GABAA receptors in the rat. Hormones and Behavior, 27(4), 568–583. [PubMed: 8294123]

Blume SR, Freedberg M, Vantrease JE, Chan R, Padival M, Record MJ, De Joseph MR, Urban JH, & Rosenkranz JA (2017). Sex- And estrus-dependent differences in rat basolateral amygdala. Journal of Neuroscience, 37(44), 10567–10586. [PubMed: 28954870]

Blume SR, Padival M, Urban JH, & Rosenkranz JA (2019). Disruptive effects of repeated stress on basolateral amygdala neurons and fear behavior across the estrous cycle in rats. Scientific Reports, 9(12292), 1–18. [PubMed: 30626917]

Blurton-Jones M, & Tuszynski MH (2002). Estrogen receptor-beta colocalizes extensively with parvalbumin-labeled inhibitory neurons in the cortex, amygdala, basal forebrain, and hippocampal formation of intact and ovariectomized adult rats. The Journal of Comparative Neurology, 452(3), 276–287. [PubMed: 12353223]

Bocchio M, Fucsina G, Oikonomidis L, McHugh SB, Bannerman DM, Sharp T, & Capogna M (2015). Increased Serotonin Transporter Expression Reduces Fear and Recruitment of Parvalbumin Interneurons of the Amygdala. Neuropsychopharmacology, 40(13), 3015–3026. [PubMed: 26052039]

Breslau N, Davis GC, Peterson EL, & Schultz L (1997). Psychiatric sequelae of posttraumatic stress disorder in women. Archives of General Psychiatry, 54(1), 81–87. [PubMed: 9006404]

Breslau N, Davis GC, & Schultz LR (2003). Posttraumatic stress disorder and the incidence of nicotine, alcohol, and other drug disorders in persons who have experienced trauma. Archives of General Psychiatry, 60(3), 289–294. [PubMed: 12622662]

Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, & Bonci A (2012). Synaptic and behavioral profile of multiple glutamatergic inputs to the Nucleus Accumbens. Neuron, 76(4), 790–803. [PubMed: 23177963]

Brunton PJ, & Russell JA (2010). Prenatal social stress in the rat programmes neuroendocrine and behavioural responses to stress in the adult offspring: sex-specific effects. Journal of Neuroendocrinology, 22(4), 258–271. [PubMed: 20136688]

Butcher RL, Collins WE, & Fugo NW (1974). Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17beta throughout the 4-day estrous cycle of the rat. Endocrinology, 94(6), 1704–1708. [PubMed: 4857496]

Cagetti E, Liang J, Spigelman I, & Olsen RW (2003). Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function and decreases behavioral responses to positive allosteric modulators of GABAA receptors. Molecular Pharmacology, 63(1), 53–64. [PubMed: 12488536]

Cagetti E, Pinna G, Guidotti A, Baicy K, & Olsen RW (2004). Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. Neuropharmacology, 46(4), 570–579. [PubMed: 14975681]

Carrier N, & Kabbaj M (2012). Sex differences in social interaction behaviors in rats are mediated by extracellular signal-regulated kinase 2 expression in the medial prefrontal cortex. Neuroscience, 212, 86–92. [PubMed: 22521590]

Carrier Nicole, Saland SK, Duclot F, He H, Mercer R, & Kabbaj M (2015). The anxiolytic and antidepressant-like effects of testosterone and estrogen in gonadectomized male rats. Biological Psychiatry, 78(4), 259–269. [PubMed: 25683735]

Chen A, Hu WW, Jiang XL, Potegal M, & Li H (2017). Molecular mechanisms of group I metabotropic glutamate receptor mediated LTP and LTD in basolateral amygdala in vitro. Psychopharmacology, 234, 681–694. [PubMed: 28028604]

Cheng LL, Wang SJ, & Gean PW (1998). Serotonin depresses excitatory synaptic transmission and depolarization-evoked Ca2+ influx in rat basolateral amygdala via 5-HT1A receptors. The European Journal of Neuroscience, 10(6), 2163–2172. [PubMed: 9753102]

Christian DT, Alexander NJ, Diaz MR, & McCool BA (2013). Thalamic glutamatergic afferents into the rat basolateral amygdala exhibit increased presynaptic glutamate function Following Withdrawal from Chronic Intermittent Ethanol. Neuropharmacology, 65, 134–142. [PubMed: 22982568]

Christian DT, Alexander NJ, Diaz MR, Robinson S, & McCool BA (2012). Chronic intermittent ethanol and withdrawal differentially modulate basolateral amygdala AMPA-type glutamate receptor function and trafficking. Neuropharmacology, 62(7), 2429–2438.

Chu H-Y, Ito W, Li J, & Morozov A (2012). Target-specific suppression of GABA release from parvalbumin interneurons in the basolateral amygdala by dopamine. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 32(42), 14815–14820. [PubMed: 23077066]

Clem RL, & Huganir RL (2010). Calcium-permeable AMPA receptor dyanmics mediate fear memory erasure. Science, 330(6007), 1108–1112. [PubMed: 21030604]

Coleman-Mesches K, & McGaugh JL (1995). Differential involvement of the right and left amygdalae in expression of memory for aversively motivated training. Brain Research, 670(1), 75–81. [PubMed: 7719727]

Cook JB, Dumitru AMG, O'Buckley To. K., & Morrow AL (2014). Ethanol administration produces divergent changes in GABAergic neuroactive steroid immunohistochemistry in the rat brain. Alcohol Clin Exp Res, 38(1), 90–99. [PubMed: 23906006]

Daviu N, Andero R, Armario A, & Nadal R (2014). Sex differences in the behavioural and hypothalamic-pituitary-adrenal response to contextual fear conditioning in rats. Hormones and Behavior, 66, 713–723. [PubMed: 25311689]

De Jesus-Burgos MI, Stephanie G-G, Yanira C-S, & Perez-Acevedo NL (2016). Amygdalar activation of group I metabotropic glutamate receptors produces anti- and pro-conflict effects depending upon animal sex in a sexually dimorphic conditioned conflict-based anxiety model. Behavioural Brain Research, 302, 200–212. [PubMed: 26777900]

De Jesus-Burgos MI, Torres-Llenza V, & Perez-Acevedo NL (2012). Activation of amygdalar metabotropic glutamate receptors modulates anxiety, and risk assessment behaviors in ovariectomized estradiol-treated female rats. Pharmacology Biochemistry and Behavior, 101(3), 369–378.

de Souza Silva MA, Topic B, Huston JP, & Mattern C (2008). Intranasal administration of progesterone increases dopaminergic activity in amygdala and neostriatum of male rats. Neuroscience, 157(1), 196–203. [PubMed: 18824215]

Deshmukh A, Rosenbloom MJ, Sassoon S, O'Reilly A, Pfefferbaum A, & Sullivan EV (2003). Alcoholic men endorse more DSM-IV withdrawal symptoms than alcoholic women matched in drinking history. Journal of Studies on Alcohol, 64(3), 375–379. [PubMed: 12817826]

Devaud LL, & Chadda R (2001). Sex differences in rats in the development of and recovery from ethanol dependence assessed by changes in seizure susceptibility. Alcoholism, Clinical and Experimental Research, 25(11), 1689–1696.

Devaud LL, Purdy RH, Finn DA, & Morrow AL (1996). Sensitization of γ-aminobutyric acidA receptors to neuroactive steroids in rats during ethanol withdrawal. Journal of Pharmacology and Experimental Therapeutics, 278(2), 510–517.

Devaud LL, Purdy RH, & Morrow AL (1995). The Neurosteroid, 3α-Hydroxy-5α-pregnan-20-one, Protects against Bicuculline-Induced Seizures during Ethanol Withdrawal in Rats. Alcoholism: Clinical and Experimental Research, 19(2), 350–355.

Devaud LL, Risinger FO, & Selvage D (2006). Impact of the hormonal milieu on the neurobiology of alcohol dependence and withdrawal. The Journal of General Psychology, 133(4), 337–356. [PubMed: 17128955]

Diatroptov ME (2011). Infradian fluctuations in serum testosterone levels in male laboratory rats. Bulletin of Experimental Biology and Medicine, 151(5), 638–641. [PubMed: 22462066]

Diatroptov ME, Slesarev SM, & Slesareva EV (2017). Characteristics of 4-Day Infradian Biorhythms in Mature Male Wistar Rats after Pinealectomy. Bulletin of Experimental Biology and Medicine, 163, 109–113. [PubMed: 28577091]

Diaz MR, Chappell AM, Christian DT, Anderson NJ, & McCool BA (2011a). Dopamine D3-like receptors modulate anxiety-like behavior and regulate gabaergic transmission in the rat lateral/basolateral amygdala. Neuropsychopharmacology, 36, 1090–1103. [PubMed: 21270771]

Diaz MR, Christian DT, Anderson NJ, & McCool BA (2011b). Chronic ethanol and withdrawal differentially modulate lateral/ basolateral amygdala paracapsular and local GABAergic synapses. Journal of Pharmacology and Experimental Therapeutics, 337(1), 162–170.

Domonkos E, Borbélyová V, Csongová M, Bosý M, Kačmárová M, Ostatníková D, Hodosy J, & Celec P (2017). Sex differences and sex hormones in anxiety-like behavior of aging rats. Hormones and Behavior, 93, 159–165. [PubMed: 28576648]

Dozier BL, Stull CA, Baker EJ, Ford MM, Jensen JP, Finn DA, & Grant KA (2019). Chronic ethanol drinking increases during the luteal menstrual cycle phase in rhesus monkeys: implication of progesterone and related neurosteroids. Psychopharmacology (Berl), 236(6), 1817–1828. [PubMed: 30645681]

Egashira N, Iba H, Kuwano H, Kawanaka R, Nagao M, Moriyama H, Watanabe T, Kubota K, Katsurabayashi S, & Iwasaki K (2016). Kamishoyosan reduces conditioned fear-induced freezing behavior in socially isolated ovariectomized rats. Journal of Pharmacological Sciences, 131, 279–283. [PubMed: 27558587]

Erol A, & Karpyak VM (2015). Sex and gender-related differences in alcohol use and its consequences: Contemporary knowledge and future research considerations. Drug and Alcohol Dependence, 156, 1–13. [PubMed: 26371405]

Fernandes C, González MI, Wilson CA, & File SE (1999). Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. Pharmacology Biochemistry and Behavior, 64(4), 731–736.

Fernandes PR, Almeida FB, da Cunha MMMV, Feddern CF, Freese L, & Barros HMT (2020). The effects of caffeine on alcohol oral self-administration behavior in rats. Physiology and Behavior, 223, 112966. [PubMed: 32442583]

Fernández-Guasti A, & Martínez-Mota L (2005). Anxiolytic-like actions of testosterone in the burying behavior test: Role of androgen and GABA-benzodiazepine receptors. Psychoneuroendocrinology, 30, 762–770. [PubMed: 15919582]

Finn DA (2020). The Endocrine System and Alcohol Drinking in Females. Alcohol Research : Current Reviews, 40(2), 2.

Finn DA, & Jimenez VA (2018). Dynamic adaptation in neurosteroid networks in response to alcohol. Handbook of Experimental Pharmacology, 248, 55–78. [PubMed: 29242992]

Ford MM, Eldridge JC, & Samson HH (2002). Microanalysis of ethanol self-administration: Estrous cycle phase-related changes in consumption patterns. Alcoholism: Clinical and Experimental Research, 26(5), 635–643.

Frye CA, Petralia SM, & Rhodes ME (2000). Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3α,5α-THP. Pharmacology Biochemistry and Behavior, 67, 587–596.

Fulenwider HD, Nennig SE, Price ME, Hafeez H, & Schank JR (2019). Sex Differences in Aversion-Resistant Ethanol Intake in Mice. Alcohol and Alcoholism, 54(4), 345–352. [PubMed: 30888414]

Giacometti L, & Barker J (2020). Sex Differences in the Glutamate System: Implications for Addiction. Neuroscience & Biobehavioral Reviews, 113, 157–168. [PubMed: 32173404]

Graham BM, & Scott E (2018). Estradiol-induced enhancement of fear extinction in female rats: The role of NMDA receptor activation. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 86, 1–9. [PubMed: 29763633]

Greba Q, Gifkins A, & Kokkinidis L (2001). Inhibition of amygdaloid dopamine D2 receptors impairs emotional learning measured with fear-potentiated startle. Brain Research, 899(1–2), 218–226. [PubMed: 11311883]

Gruene TM, Flick K, Stefano A, Shea SD, & Shansky RM (2015). Sexually divergent expression of active and passive conditioned fear responses in rats. ELife, 4, e11352. [PubMed: 26568307]

Guadagno A, Wong TP, & Walker CD (2018). Morphological and functional changes in the preweaning basolateral amygdala induced by early chronic stress associate with anxiety and fear behavior in adult male, but not female rats. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 81, 25–37. [PubMed: 28963066]

Guo J-D, O'Flaherty BM, & Rainnie DG (2017). Serotonin gating of cortical and thalamic glutamate inputs onto principal neurons of the basolateral amygdala. Physiology & Behavior, 126, 224–232.

Gupta RR, Sen S, Diepenhorst LL, Rudick CN, & Maren S (2001). Estrogen modulates sexually dimorphic contextual fear conditioning and hippocampal long-term potentiation (LTP) in rats(1). Brain Research, 888(2), 356–365. [PubMed: 11150498]

Hamson DK, Jones BA, Csupity AS, Ali FM, & Watson NV (2014). Androgen insensitive male rats display increased anxiety-like behavior on the elevated plus maze. Behavioural Brain Research, 259, 158–163. [PubMed: 24269497]

Heath FC, Jurkus R, Bast T, Pezze MA, Lee JLC, Voigt JP, & Stevenson CW (2015). Dopamine D1-like receptor signalling in the hippocampus and amygdala modulates the acquisition of contextual fear conditioning. Psychopharmacology, 232(14), 2619–2629. [PubMed: 25743759]

Henricks AM, Berger AL, Lugo JM, Baxter-Potter LN, Bieniasz KV, Petrie G, Sticht MA, Hill MN, & McLaughlin RJ (2017). Sex- and hormone-dependent alterations in alcohol withdrawal-induced anxiety and corticolimbic endocannabinoid signaling. Neuropharmacology, 124, 121–133. [PubMed: 28554848]

Hilakivi-Clarke L (1996). Role of estradiol in alcohol intake and alcohol-related behaviors. Journal of Studies on Alcohol, 57(2), 162–170. [PubMed: 8683965]

Hilderbrand ER, & Lasek AW (2018). Estradiol enhances ethanol reward in female mice through activation of ERα and ERβ. Hormones and Behavior, 98, 159–164. [PubMed: 29305887]

Hoffman AN, Armstrong CE, Hanna JJ, & Conrad CD (2010). Chronic stress, cyclic 17β-estradiol, and daily handling influences on fear conditioning in the female rat. Neurobiology of Learning and Memory, 94, 422–433. [PubMed: 20807583]

Imwalle DB, Gustafsson JÅ, & Rissman EF (2005). Lack of functional estrogen receptor β influences anxiety behavior and serotonin content in female mice. Physiology and Behavior, 84, 157–163. [PubMed: 15642619]

Inoue T, Li XB, Abekawa T, Kitaichi Y, Izumi T, Nakagawa S, & Koyama T (2004). Selective serotonin reuptake inhibitor reduces conditioned fear through its effect in the amygdala. European Journal of Pharmacology, 497(3), 311–316. [PubMed: 15336949]

Jiang X, Xing G, Yang C, Verma A, Zhang L, & Li H (2009). Stress impairs 5-HT2A receptor-mediated serotonergic facilitation of GABA release in juvenile rat basolateral amygdala. Neuropsychopharmacology, 34(2), 410–423. [PubMed: 18536707]

Johnston AL, & File SE (1991). Sex differences in animal tests of anxiety. Physiology & Behavior, 49(2), 245–250. [PubMed: 2062894]

Jury NJ, DiBerto JF, Kash TL, & Holmes A (2017). Sex differences in the behavioral sequelae of chronic ethanol exposure. Alcohol, 58, 53–60. [PubMed: 27624846]

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, & Kendler KS (1994). Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Archives of General Psychiatry, 51(1), 8–19. [PubMed: 8279933]

Kessler Ronald C, Berglund P, Demler O, Jin R, Merikangas KR, & Walters EE (2005). Lifetime Prevalence and Age-of-Onset Distributions of. Arch Gen Psychiatry, 62(June), 593–602. 10.1001/archpsyc.62.6.593 [PubMed: 15939837]

Kessler Ronald C, Petukhova M, Sampson NA, Zaslavsky AM, & Wittchen H-U (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in

the United States. International Journal of Methods in Psychiatric Research, 21(3), 169–184. [PubMed: 22865617]

Kitaichi Y, Inoue T, Nakagawa S, Omiya Y, Song N, An Y, Chen C, Kusumi I, & Koyama T (2014). Local infusion of citalopram into the basolateral amygdala decreased conditioned fear of rats through increasing extracellular serotonin levels. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 54, 216–222. [PubMed: 24928686]

Knight P, Chellian R, Wilson R, Behnood-Rod A, Panunzio S, & Bruijnzeel AW (2021). Sex differences in the elevated plus-maze test and large open field test in adult Wistar rats. Pharmacology Biochemistry and Behavior, 204, 173168.

Koss WA, Belden CE, Hristov AD, & Juraska JM (2014). Dendritic remodeling in the adolescent medial prefrontal cortex and the basolateral amygdala of male and female rats. Synapse, 68, 61–72. [PubMed: 24105875]

Koss WA, Gehlert DR, & Shekhar A (2004). Different effects of subchronic doses of 17-β estradiol in two ethologically based models of anxiety utilizing female rats. Hormones and Behavior, 46, 158–164. [PubMed: 15256305]

Kosten TA, Lee HJ, & Kim JJ (2006). Early life stress impairs fear conditioning in adult male and female rats. Brain Research, 1087, 142–150. [PubMed: 16626646]

Kröner S, Rosenkranz JA, Grace AA, & Barrionuevo G (2005). Dopamine modulates excitability of basolateral amygdala neurons in vitro. Journal of Neurophysiology, 93(3), 1598–1610. [PubMed: 15537813]

Läck AK, Ariwodola OJ, Chappell AM, Weiner JL, & McCool BA (2008). Ethanol inhibition of kainate recpetor-mediated excitatory neurotransmission in the rat basolateral nucleus of the amygdala. Neuropharmacology, 55(5), 661–668. [PubMed: 18617194]

Läck AK, Christian DT, Diaz MR, & McCool BA (2009). Chronic ethanol and withdrawal effects on kainate receptor-mediated excitatory neurotransmission in the rat basolateral amygdala. Alcohol, 43(1), 25–33. [PubMed: 19185207]

Läck AK, Diaz MR, Chappell A, DuBois DW, & McCool BA (2007). Chronic ethanol and withdrawal differentially modulate pre- and postsynaptic function at glutamatergic synapses in rat basolateral amygdala. Journal of Neurophysiology, 98(6), 3185–3196. [PubMed: 17898152]

Leichnetz GR, & Astruc J (1977). The course of some prefrontal corticofugals to the pallidum, substantia innominata, and amygdaloid complex in monkeys. Experimental Neurology, 54, 104–109. [PubMed: 401742]

Li XB, Inoue T, Abekawa T, Weng SM, Nakagawa S, Izumi T, & Koyama T (2006). 5-HT1A receptor agonist affects fear conditioning through stimulations of the postsynaptic 5-HT1A receptors in the hippocampus and amygdala. European Journal of Pharmacology, 532(1–2), 74–80. [PubMed: 16460727]

Lin S, Li X, Chen YH, Gao F, Chen H, Hu NY, Huang L, Luo ZY, Liu JH, You QL, Yin YN, Li ZL, Li XW, Du ZJ, Yang JM, & Gao TM (2018). Social Isolation During Adolescence Induces Anxiety Behaviors and Enhances Firing Activity in BLA Pyramidal Neurons via mGluR5 Upregulation. Molecular Neurobiology, 55(6), 5310–5320. [PubMed: 28914419]

Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, & Wang YT (2004). Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science (New York, N.Y.), 304(5673), 1021–1024.

Liu WZ, Zhang WH, Zheng ZH, Zou JX, Liu XX, Huang SH, You WJ, He Y, Zhang JY, Wang XD, & Pan BX (2020). Identification of a prefrontal cortex-to-amygdala pathway for chronic stress-induced anxiety. Nature Communications, 11, 2221.

Logrip ML, & Gainey SC (2020). Sex differences in the long-term effects of past stress on alcohol self-administration, glucocorticoid sensitivity and phosphodiesterase 10A expression. Neuropharmacology, 164, 107857. [PubMed: 31756338]

Lorrai I, Contini A, Gessa GL, Mugnaini C, Corelli F, Colombo G, & Maccioni P (2019). Operant, oral alcohol self-administration: Sex differences in Sardinian alcohol-preferring rats. Alcohol, 79, 147–162. [PubMed: 31029630]

Lund TD, Rovis T, Chung WCJ, & Handa RJ (2005). Novel actions of estrogen receptor-beta on anxiety-related behaviors. Endocrinology, 146(2), 797–807. [PubMed: 15514081]

Maeng LY, & Milad MR (2015). Sex differences in anxiety disorders: interactions between fear, stress, and gonadal hormones. Physiology & Behavior, 76, 106–117.

Maldonado-Devincci AM, Beattie MC, Morrow DH, McKinley RE, Cook JB, O'Buckley TK, & Morrow AL (2014a). Reduction of circulating and selective limbic brain levels of (3α,5α)-3-hydroxy-pregnan-20-one (3α,5α-THP) following forced swim stress in C57BL/6J mice. Psychopharmacology, 231(17), 3281–3292. [PubMed: 24744202]

Maldonado-Devincci AM, Cook JB, O'Buckley TK, Morrow DH, Mckinley RE, Lopez MF, Becker HC, & Morrow AL (2014b). Chronic Intermittent Ethanol Exposure and Withdrawal Alters (3α,5α)-3-Hydroxy-Pregnan-20-One Immunostaining in Cortical and Limbic Brain Regions of C57BL/6J Mice. Alcoholism: Clinical and Experimental Research, 38(10), 2561–2571.

Marcondes FK, Miguel KJ, Melo LL, & Spadari-Bratfisch RC (2001). Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiology & Behavior, 74(4–5), 435–440. [PubMed: 11790402]

Maren S, De Oca B, & Fanselow MS (1994). Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning. Brain Research, 661(1–2), 25–34. [PubMed: 7834376]

Marowsky A, Yanagawa Y, Obata K, & Vogt KE (2005). A specialized subclass of interneurons mediates dopaminergic facilitation of amygdala function. Neuron, 48(6), 1025–1037. [PubMed: 16364905]

Martin AL, & Brown RE (2010). The lonely mouse: Verification of a separation-induced model of depression in female mice. Behavioural Brain Research, 207, 196–207. [PubMed: 19819265]

Mascagni F, Muly EC, Rainnie DG, & McDonald AJ (2009). Immunohistochemical characterization of parvalbumin-containing interneurons in the monkey basolateral amygdala. Neuroscience, 158(4), 1541–1550. [PubMed: 19059310]

McCool BA, Christian DT, Fetzer JA, & Chappell AM (2014). Lateral/basolateral amygdala serotonin type-2 receptors modulate operant self-administration of a sweetened ethanol solution via inhibition of principal neuron activity. Frontiers in Integrative Neuroscience, 8, 1–13. [PubMed: 24474908]

McDonald AJ (1998). Cortical pathways to the mammalian amygdala. Progress in Neurobiology, 55, 257–332. [PubMed: 9643556]

McDonald AJ, & Betette RL (2001). Parvalbumin-containing neurons in the rat basolateral amygdala: morphology and co-localization of Calbindin-D(28k). Neuroscience, 102(2), 413–425. [PubMed: 11166127]

McDonald AJ, & Mascagni F (2001). Colocalization of calcium-binding proteins and GABA in neurons of the rat basolateral amygdala. Neuroscience, 105(3), 681–693. [PubMed: 11516833]

McDonald AJ, Mascagni F, & Guo L (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: A phaseolus vulgaris leucoagglutinin study in the rat. Neuroscience, 71(1), 55–75. [PubMed: 8834392]

McDonald AJ, Mascagni F, Mania I, & Rainnie DG (2005). Evidence for a perisomatic innervation of parvalbumin-containing interneurons by individual pyramidal cells in the basolateral amygdala. Brain Research, 1035(1), 32–40. [PubMed: 15713274]

McDonald AJ, & Pearson JC (1989). Coexistence of GABA and peptide immunoreactivity in non-pyramidal neurons of the basolateral amygdala. Neuroscience Letters, 100(1–3), 53–58. [PubMed: 2569703]

McGinnis MM, Parrish BC, Chappell AM, Alexander NJ, & McCool BA (2020a). Chronic ethanol differentially modulates glutamate release from dorsal and ventral prefrontal cortical inputs onto rat basolateral amygdala principal neurons. ENeuro, 7(2), 1–17.

McGinnis MM, Parrish BC, & McCool BA (2020b). Withdrawal from chronic ethanol exposure increases postsynaptic glutamate function of insular cortex projections to the rat basolateral amygdala. Neuropharmacology, 172, 108129. [PubMed: 32418906]

McGuire J, Herman JP, Horn PS, Sallee FR, & Sah R (2010). Enhanced fear recall and emotional arousal in rats recovering from chronic variable stress. Physiological Behavior, 101(4), 474–482.

Meitzen J, & Mermelstein PG (2011). Estrogen receptors stimulate brain region specific metabotropic glutamate receptors to rapidly initiate signal transduction pathways. J Chem Neuroanat, 42(4), 236–241. [PubMed: 21458561]

Milad MR, Igoe SA, Lebron-Milad K, & Novales JE (2009). Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience, 164(3), 887–895. [PubMed: 19761818]

Mitsushima D, Yamada K, Takase K, Funabashi T, & Kimura F (2006). Sex differences in the basolateral amygdala: The extracellular levels of serotonin and dopamine, and their responses to restraint stress in rats. European Journal of Neuroscience, 24, 3245–3254.

Morales M, McGinnis MM, Chappell AM, Parrish BC, & McCool BA (2019). Polymodal Sensory Thalamic Inputs to the Rat Lateral Amygdala are Facilitated by Chronic Ethanol Exposure and Regulate Withdrawal-associated Anxiety. BioRxiv

Morales M, McGinnis MM, & McCool BA (2015). Chronic ethanol exposure increases voluntary home cage intake in adult male, but not female, Long-Evans rats. Pharmacology, Biochemistry and Behavior, 139, 67–76.

Morales M, McGinnis MM, Robinson SL, Chappell AM, & McCool BA (2018). Chronic intermittent ethanol exposure modulation of glutamatergic neurotransmission in rat lateral/basolateral amygdala is duration-, input-, and sex-dependent. Neuroscience, 371, 277–287. [PubMed: 29237566]

Muller JF, Mascagni F, & McDonald AJ (2006). Pyramidal cells of the rat basolateral amygdala: synaptology and innervation by parvalbumin-immunoreactive interneurons. The Journal of Comparative Neurology, 494(4), 635–650. [PubMed: 16374802]

Muller JF, Mascagni F, & McDonald AJ (2009). Dopaminergic innervation of pyramidal cells in the rat basolateral amygdala. Brain Structure & Function, 213(3), 275–288. [PubMed: 18839210]

Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichman R, Holden SS, Mertens KL, Anahtar M, Felix-Ortiz AC, Wickersham IR, Gray JM, & Tye KM (2015). A circuit mechanism for differentiating positive and negative associations. Nature, 50(7549), 675–678.

Negrón-Oyarzo I, Pérez MÁ, Terreros G, Muñoz P, & Dagnino-Subiabre A (2014). Effects of chronic stress in adolescence on learned fear, anxiety, and synaptic transmission in the rat prelimbic cortex. Behavioural Brain Research, 259, 342–353. [PubMed: 24216268]

Nuss P (2015). Anxiety disorders and GABA neurotransmission: a disturbance of modulation. Neuropsychiatric Disease and Treatment, 11, 165–175. [PubMed: 25653526]

Olsen RW, & Liang J (2017). Role of GABAA receptors in alcohol use disorders suggested by chronic intermittent ethanol (CIE) rodent model. Molecular Brain, 10(1), 45. [PubMed: 28931433]

Osterlund M, Kuiper GG, Gustafsson JA, & Hurd YL (1998). Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. Brain Research. Molecular Brain Research, 54(1), 175–180. [PubMed: 9526077]

Overstreet DH, Knapp DJ, & Breese GR (2004). Similar anxiety-like responses in male and female rats exposed to repeated withdrawals from ethanol. Pharmacology, Biochemistry, and Behavior, 78(3), 459–464.

Pereda-Pérez I, Popović N, Otalora BB, Popović M, Madrid JA, Rol MA, & Venero C (2013). Long-term social isolation in the adulthood results in CA1 shrinkage and cognitive impairment. Neurobiology of Learning and Memory, 106, 31–39. [PubMed: 23867635]

Peters J, Kalivas PW, & Quirk GJ (2009). Extinction circuits for fear and addiction overlap in the prefrontal cortex. Learning and Memory, 16, 279–288. [PubMed: 19380710]

Phoenix HC, Goy RW, Gerall AA, & Young WC (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology, 65, 369–382. [PubMed: 14432658]

Pibiri F, Nelson M, Guidotti A, Costa E, & Pinna G (2008). Decreased corticolimbic allopregnanolone expression during social isolation enhances contextual fear: A model relevant for posttraumatic stress disorder. Proceedings of the National Academy of Sciences of the United States of America, 105(14), 5567–5572. [PubMed: 18391192]

Picazo O, & Fernández-Guasti A (1995). Anti-anxiety effects of progesterone and some of its reduced metabolites: an evaluation using the burying behavior test. Brain Research, 680(1–2), 135–141. [PubMed: 7663969]

Pinard CR, Muller JF, Mascagni F, & McDonald AJ (2008). Dopaminergic innervation of interneurons in the rat basolateral amygdala. Neuroscience, 157(4), 850–863. [PubMed: 18948174]

Pinna G, Costa E, & Guidotti A (2005). Changes in brain testosterone and allopregnanolone biosynthesis elicit aggressive behavior. Proceedings of the National Academy of Sciences of the United States of America, 102(6), 2135–2140. [PubMed: 15677716]

Porcu P, Barron AM, Frye CA, Walf AA, Yang S-Y, He X-Y, Morrow AL, Panzica GC, & Melcangi RC (2016). Neurosteroidogenesis today: Novel targets for neuroactive steroid synthesis and action and their relevance for translational research. In Journal of Neuroendocrinology (Vol. 28, Issue 2).

Prager EM, Bergstrom HC, Wynn GH, & Braga MFM (2016). The Basolateral Amygdala GABAergic System in Health and Disease. J Neurosci Res, 94(6), 548–567. [PubMed: 26586374]

Priddy BM, Carmack SA, Thomas LC, Vendruscolo JCM, Koob GF, & Vendruscolo LF (2017). Sex, strain, and estrous cycle influences on alcohol drinking in rats. Pharmacology, Biochemistry and Behavior, 152, 61–67.

Puralewski R, Vasilakis G, & Seney ML (2016). Sex-related factors influence expression of mood-related genes in the basolateral amygdala differentially depending on age and stress exposure. Biology of Sex Differences, 7, 50. [PubMed: 27660699]

Rainnie DG (1999). Serotonergic modulation of neurotransmission in the rat basolateral amygdala. Journal of Neurophysiology, 82(1), 69–85. [PubMed: 10400936]

Randall PA, Stewart RT, & Besheer J (2017). Sex differences in alcohol self-administration and relapse-like behavior in Long-Evans rats. Pharmacology, Biochemistry and Behavior, 156, 1–9.

Rankov Petrovic B, Hrncic D, Mladenovic D, Simic T, Suvakov S, Jovanovic D, Puskas N, Zaletel I, Velimirovic M, Cirkovic V, Macut D, Stanojlovic O, & Rasic-Markovic A (2019). Prenatal Androgenization Induces Anxiety-Like Behavior in Female Rats, Associated with Reduction of Inhibitory Interneurons and Increased BDNF in Hippocampus and Cortex. BioMed Research International, 2019, 3426092. [PubMed: 31281833]

Rau AR, Chappell AM, Butler TR, Ariwodola OJ, & Weiner JL (2015). Increased basolateral amygdala pyramidal cell excitability may contribute to the anxiogenic phenotype induced by chronic early-life stress. Journal of Neuroscience, 35(26), 9730–9740. [PubMed: 26134655]

Renczés E, Borbélyová V, Steinhardt M, Höpfner T, Stehle T, Ostatníková D, & Celec P (2020). The Role of Estrogen in Anxiety-Like Behavior and Memory of Middle-Aged Female Rats. Frontiers in Endocrinology, 11, 570560. [PubMed: 33117285]

Ribeiro AM, Barbosa FF, Godinho MR, Fernandes VS, Munguba H, Melo TG, Barbosa MT, Eufrasio RA, Cabral A, Izídio GS, & Silva RH (2010). Sex differences in aversive memory in rats: Possible role of extinction and reactive emotional factors. Brain and Cognition, 74, 145–151. [PubMed: 20727653]

Równiak M, Bogus-Nowakowska K, & Robak A (2015). The densities of calbindin and parvalbumin, but not calretinin neurons, are sexually dimorphic in the amygdala of the guinea pig. Brain Research, 1604, 84–97. [PubMed: 25660848]

Rubinow MJ, Drogos LL, & Juraska JM (2009). Age-related dendritic hypertrophy and sexual dimorphism in rat basolateral amygdala. Neurobiol Aging, 30(1), 137–146. [PubMed: 17570563]

Sah P, Faber ESL, Lopez De Armentia M, & Power J (2003). The amygdaloid complex: anatomy and physiology. Physiological Reviews, 83(3), 803–834. [PubMed: 12843409]

Sanders MJ, Stevens S, & Boeh H (2010). Stress enhancement of fear learning in mice is dependent upon stressor type: Effects of sex and ovarian hormones. Neurobiology of Learning and Memory, 94(2), 254–262. [PubMed: 20601029]

Scholl JL, Afzal A, Fox LC, Watt MJ, & Forster GL (2019). Sex differences in anxiety-like behaviors in rats. Physiology and Behavior, 211, 112670. [PubMed: 31487491]

Schulz KM, & Sisk CL (2016). The organizing actions of adolescent gonadal steroid hormones on brain and behavioral development. Neuroscience and Biobehavioral Reviews, 70, 148–158. [PubMed: 27497718]

Scott H, Tjernström N, & Roman E (2020). Effects of pair housing on voluntary alcohol intake in male and female Wistar rats. Alcohol, 86, 121–128. [PubMed: 31978460]

Seedat S, Scott KM, Angermeyer MC, Berglund P, Bromet EJ, Brugha TS, Demyttenaere K, de Girolamo G, Haro JM, Jin R, Karam EG, Kovess-Masfety V, Levinson D, Medina Mora ME, Ono Y, Ormel J, Pennell B-E, Posada-Villa J, Sampson NA, … Kessler RC (2009). Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys. Archives of General Psychiatry, 66(7), 785–795. [PubMed: 19581570]

Sengupta A, Bocchio M, Bannerman DM, Sharp T, & Capogna M (2017). Control of amygdala circuits by 5-HT neurons via 5-HT and glutamate cotransmission. Journal of Neuroscience, 37(7), 1785–1796. [PubMed: 28087766]

Sharp BM (2017). Basolateral amygdala and stress-induced hyperexcitability affect motivated behaviors and addiction. Translational Psychiatry, 7(8), e1194. [PubMed: 28786979]

Siddiqui A, & Shah BH (1997). Neonatal androgen manipulation differentially affects the development of monoamine systems in rat cerebral cortex, amygdala and hypothalamus. Brain Research. Developmental Brain Research, 98(2), 247–252. [PubMed: 9051266]

Silberman Y, Shi L, Brunso-Bechtold JK, & Weiner JL (2008). Distinct mechanisms of ethanol potentiation of local and paracapsular GABAergic synapses in the rat basolateral amygdala. The Journal of Pharmacology and Experimental Therapeutics, 324(1), 251–260. [PubMed: 17921186]

Simerly RB, Swanson LW, Chang C, & Muramatsu M (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. Journal of Comparative Neurology, 294(1), 76–95.

Sizer SE, Parrish BC, & McCool BA (2021). Chronic Ethanol Exposure Potentiates Cholinergic Neurotransmission in the Basolateral Amygdala. Neuroscience, 455, 165–176. [PubMed: 33385490]

Skelly MJ, Ariwodola OJ, & Weiner JL (2017). Fear conditioning selectively disrupts noradrenergic facilitation of GABAergic inhibition in the basolateral amygdala. Neuropharmacology, 113, 231–240. [PubMed: 27720769]

Skelly MJ, Chappell AE, Carter E, & Weiner JL (2015). Adolescent social isolation increases anxiety-like behavior and ethanol intake and impairs fear extinction in adulthood: possible role of disrupted noradrenergic signaling. Neuropharmacology, 97, 149–159. [PubMed: 26044636]

Smith SS, Gong QH, Li X, Moran MH, Bitran D, Frye CA, & Hsu FC (1998). Withdrawal from 3α-OH-5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal GABA(A)-gated current and increases the GABA(A) receptor α4 subunit in association with increased anxiety. Journal of Neuroscience, 18(14), 5275–5284. [PubMed: 9651210]

Smith Y, Paré JF, & Paré D (2000). Differential innervation of parvalbumin-immunoreactive interneurons of the basolateral amygdaloid complex by cortical and intrinsic inputs. The Journal of Comparative Neurology, 416(4), 496–508. [PubMed: 10660880]

Sorvari H, Miettinen R, Soininen H, Paljärvi L, Karkola K, & Pitkänen A (1998). Calretinin-immunoreactive terminals make synapses on calbindin D28k-immunoreactive neurons in the lateral nucleus of the human amygdala. Brain Research, 783(2), 355–358. [PubMed: 9507193]

Spampanato J, Polepalli J, & Sah P (2011). Interneurons in the basolateral amygdala. Neuropharmacology, 60(5), 765–773. [PubMed: 21093462]

Stack A, Carrier N, Dietz D, Hollis F, Sorenson J, & Kabbaj M (2010). Sex differences in social interaction in rats: Role of the immediate-early gene zif268. Neuropsychopharmacology, 35, 570–580. [PubMed: 19847159]

Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, Zhang F, Deisseroth K, & Bonci A (2011). Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature, 475(7356), 377–380. [PubMed: 21716290]

Substance Abuse and Mental Health Services Administration. (2019). Results from the 2018 National Survey on Drug Use and Health: Detailed tables. https://www.samhsa.gov/data/

Sullivan RM, Duchesne A, Hussain D, Waldron J, & Laplante F (2009). Effects of unilateral amygdala dopamine depletion on behaviour in the elevated plus maze: Role of sex, hemisphere and retesting. Behavioural Brain Research, 205, 115–122. [PubMed: 19643146]

Sullivan RM, Dufresne MM, & Waldron J (2009). Lateralized sex differences in stress-induced dopamine release in the rat. Neurochemistry, 20(3), 229–232.

Sullivan RM, & Gratton A (1999). Lateralized effects of medial prefrontal cortex lesions on neuroendocrine and autonomic stress responses in rats. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 19(7), 2834–2840. [PubMed: 10087094]

Takuma K, Mizoguchi H, Funatsu Y, Hoshina Y, Himeno Y, Fukuzaki E, Kitahara Y, Arai S, Ibi D, Kamei H, Matsuda T, Koike K, Inoue M, Nagai T, & Yamada K (2012). Combination of chronic stress and ovariectomy causes conditioned fear memory deficits and hippocampal cholinergic neuronal loss in mice. Neuroscience, 207, 261–273. [PubMed: 22314316]

Tavares ER, Silva-Gotay A, Riad WV, Bengston L, & Richardson HN (2019). Sex differences in the effect of alcohol drinking on myelinated axons in the anterior cingulate cortex of adolescent rats. Brain Sciences, 9(7), 167.

Tian Z, Wang Y, Zhang N, Guo Y. yan, Feng B, Liu S. bing, & Zhao M. gao. (2013). Estrogen receptor GPR30 exerts anxiolytic effects by maintaining the balance between GABAergic and glutamatergic transmission in the basolateral amygdala of ovariectomized mice after stress. Psychoneuroendocrinology, 38(10), 2218–2233. [PubMed: 23669322]

Tsvetkov E, Carlezon WA, Benes FM, Kandel ER, & Bolshakov VY (2002). Fear conditioning occludes LTP-induced presynaptic enhancement of synaptic transmission in the cortical pathway to the lateral amygdala. Neuron, 34(2), 289–300. [PubMed: 11970870]

van Veen JF, Jonker BW, van Vliet IM, & Zitman FG (2009). The effects of female reproductive hormones in generalized social anxiety disorder. International Journal of Psychiatry in Medicine, 39(3), 283–295. [PubMed: 19967900]

Vetter-O'Hagen CS, & Spear LP (2011). The effects of gonadectomy on age- and sex-typical patterns of ethanol consumption in Sprague-Dawley rats. Alcoholism: Clinical and Experimental Research, 35(11), 2039–2049.

Vetter-O'Hagen CS, & Spear LP (2012). Hormonal and physical markers of puberty and their relationship to adolescent-typical novelty-directed behavior. Dev Psychobiol, 54(5), 523–535. [PubMed: 21953609]

Vetter-O'Hagen CS, Varlinskaya E, & Spear L (2009). Sex differences in ethanol intake and sensitivity to aversive effects during adolescence and adulthood. Alcohol and Alcoholism, 44(6), 547–554. [PubMed: 19767625]

Vyas A, Jadhav S, & Chattarji S (2006). Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. Neuroscience, 143(2), 387–393. [PubMed: 16962717]

Vyas A, Mitra R, Rao BSS, & Chattarji S (2002). Chronic Stress Induces Contrasting Patterns of Dendritic Remodeling in Hippocampal and Amygdaloid Neurons 22(15), 6810–6818.

Waite E, Kershaw Y, Spiga F, & Lightman SL (2009). A glucocorticoid sensitive biphasic rhythm of testosterone secretion. Journal of Neuroendocrinology, 21(9), 737–741. [PubMed: 19602101]

Walf AA, & Frye CA (2005a). Antianxiety and antidepressive behavior produced by physiological estradiol regimen may be modulated by hypothalamic-pituitary-adrenal axis activity. Neuropsychopharmacology, 30, 1288–1301. [PubMed: 15756306]

Walf AA, & Frye CA (2005b). ERbeta-selective estrogen receptor modulators produce antianxiety behavior when administered systemically to ovariectomized rats. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 30(9), 1598–1609. [PubMed: 15798780]

Wallen K (2009). The Organizational Hypothesis: Reflections on the 50th anniversary of the publication of Phoenix, Goy, Gerall, and Young (1959). In Hormones and behavior (Vol. 55, Issue 5, pp. 561–565). [PubMed: 19446072]

Wang Y, Liu Y, Xiong J, Di T, Yuan Z, Wu J, & Chen L (2019). Reduced serotonin impairs long-term depression in basolateral amygdala complex and causes anxiety-like behaviors in a mouse model of perimenopause. Experimental Neurology, 321, 113030. [PubMed: 31377402]

Wisniewski AB (1998). Sexually-dimorphic patterns of cortical asymmetry, and the role for sex steroid hormones in determining cortical patterns of lateralization. Psychoneuroendocrinology, 23(5), 519–547. [PubMed: 9802126]

Wolff SBE, Gründemann J, Tovote P, Krabbe S, Jacobson GA, Müller C, Herry C, Ehrlich I, Friedrich RW, Letzkus JJ, & Lüthi A (2014). Amygdala interneuron subtypes control fear learning through disinhibition. Nature, 509(7501), 453–458. [PubMed: 24814341]

Womble MD, Andrew JA, & Crook JJ (2002). 17β-estradiol reduces excitatory postsynaptic potential (EPSP) amplitude in rat basolateral amygdala neurons. Neuroscience Letters, 331(2), 83–86. [PubMed: 12361846]

Xiang X, Huang W, Haile CN, & Kosten TA (2011). Hippocampal GluR1 associates with behavior in the elevated plus maze and shows sex differences. Behavioural Brain Research, 222, 326–331. [PubMed: 21497621]

Yamamoto R, Furuyama T, Sugai T, Ono M, Pare D, & Kato N (2020). Serotonergic control of GABAergic inhibition in the lateral amygdala. Journal of Neurophysiology, 123(2), 670–681. [PubMed: 31875487]

Yamamoto R, Hatano N, Sugai T, & Kato N (2014). Serotonin induces depolarization in lateral amygdala neurons by activation of TRPC-like current and inhibition of GIRK current depending on 5-HT 2C receptor. Neuropharmacology, 82, 49–58. [PubMed: 24662600]

Yamamoto R, Ueta Y, Sugai T, & Kato N (2012). A serotonergic discrimination favoring synaptic inputs that accompany robust spike firing in lateral amygdala neurons. Neuroscience, 220, 119–130. [PubMed: 22698688]

Yang R, Zhang B, Chen T, Zhang S, & Chen L (2017). Postpartum estrogen withdrawal impairs GABAergic inhibition and LTD induction in basolateral amygdala complex via down-regulation of GPR30. European Neuropsychopharmacology, 27, 759–772. [PubMed: 28619359]

Zeidan MA, Igoe SA, Linnman C, Vitalo A, Levine JB, Klibanski A, Goldstein JM, & Milad MR (2011). Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. Biological Psychiatry, 70, 920–927. [PubMed: 21762880]

Zhang W, & Rosenkranz JA (2013). Repeated restraint stress enhances cue-elicited conditioned freezing and impairs acquisition of extinction in an age-dependent manner. Behavioural Brain Research, 248, 12–24. [PubMed: 23538069]

Zhang Z, Hong J, Zhang S, Zhang T, Sha S, Yang R, Qian Y, & Chen L (2016). Postpartum estrogen withdrawal impairs hippocampal neurogenesis and causes depression- and anxiety-like behaviors in mice. Psychoneuroendocrinology, 66, 138–149. [PubMed: 26803529]

Ziabreva I, Poeggel G, Schnabel R, & Braun K (2003). Separation-induced receptor changes in the hippocampus and amygdala of Octodon degus: influence of maternal vocalizations. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 23(12), 5329–5336. [PubMed: 12832558]

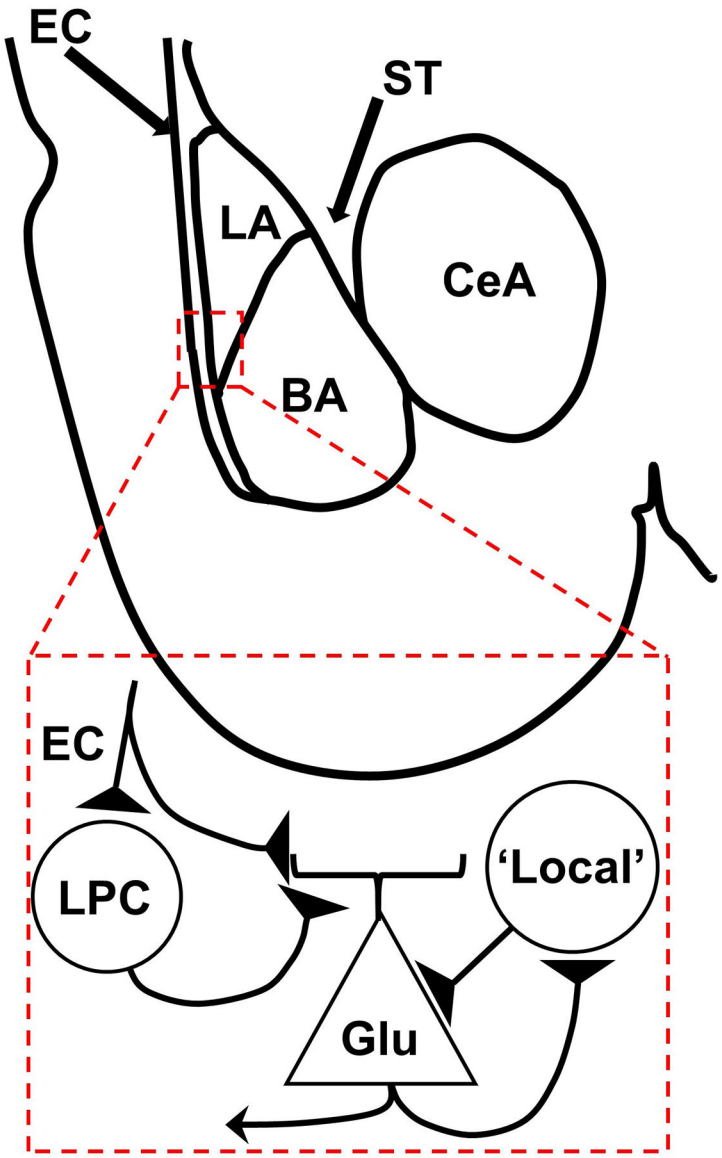

Figure 1. Circuitry in the BLA.

The lateral amygdala (LA) and basolateral amygdala (BA) together form the BLA. Glutamatergic inputs arising from the medial prefrontal cortex and thalamus enter the BLA through the *stria terminalis* (ST) located along the dorsomedial side of the BLA just anterior and lateral to the central amygdala (CeA). The BLA also receives glutamatergic inputs from lateral cortical areas, including the agranular insula cortex, through the external capsule (EC) located along the lateral border of the BLA. The red box illustrates local circuit relationships between BLA glutamatergic principal neurons (Glu) and distinct populations of inhibitory GABAergic interneurons. These include lateral paracapsular cell groups (LPC) clustered in/near the EC and 'local' interneurons scattered throughout these regions. LPCs provide feedforward inhibition whereas 'local' interneurons are primarily involved in feedback inhibition.

Table 1.

Sex Differences and the Effects of Sex Hormones on Anxiety, Fear Conditioning, and Ethanol-Related Behaviors in Rodents

	Sex Differences	Estrous Cycle	Estrogens	Progestogens	Androgens
Anxiety Models: Elevated Plus Maze (EPM) Open Field Test (OFT) Vogel Conflict Test (VCT) Social Interaction Test (SIT)	Females ≤ Males (EPM, OFT) Females > Males (VCT, SIT)	**Proestrus:** ↓ Anxiety (EPM)	**Females:** ↓ Anxiety through ERβ or GPR30 activation, estrogen withdrawal ↑ anxiety (EPM, OFT)	**Females:** ↓ Anxiety through allopregnanolone potentiation of $GABA_A$ receptor function, progestogen withdrawal ↑ anxiety (EPM)	**Males:** ↓ Anxiety (EPM and OFT), **Females:** Neonatal testosterone ↑ anxiety (EPM)
Fear Conditioning: Cued and Contextual Conditioning, Extinction	Conditioned freezing: Females ≤ Males Conditioned darting: Females > Males Cued Extinction: Males > Females Contextual Extinction: Males < Females	**Proestrus:** ↓ Freezing decreases; no change in "darting" ↑ Cued Extinction	**Females:** ↓ Freezing during Contextual Conditioning	**Males:** BLA allopregnanolone ↓ freezing during fear conditioning	
Alcohol Intake (Non-Dependent): 2-bottle choice (2BC) Operant self-administration (SA)	Typically Females > Males (2BC, SA)	**Proestrus:** No change in intake (2BC, SA)	**Males:** ↑ Intake (2BC) **Females:** ↓ Intake (2BC)	**Females:** No effect on intake (2BC)	**Males:** ↓ Intake (2BC) **Females:** Neonatal exposure ↓ intake (2BC)
Alcohol Withdrawal	Increased seizure susceptibility: delayed and recovers more quickly in females Withdrawal-induced anxiety: Females ≤ Males (EPM, OFT, novelty-suppressed feeding task)	Chronic ethanol disrupts estrous cycle	**Females:** No effect on withdrawal-induced anxiety (EPM)	**Males and Females:** ↓ seizure susceptibility (greater effect in females) **Males:** ↓ withdrawal-induced anxiety (EPM)	

Alcohol. Author manuscript; available in PMC 2022 February 01.

Table 2.

Sex Differences and the Effects of Sex Hormones on BLA Structure and Function in Rodents

	Sex Differences	Estrous Cycle	Estrogens	Progestogens	Androgens
BLA Structure Calbindin (CB) Parvalbumin (PV) Somatostatin (SST)	CB+ interneurons density: Females > Males Stress: Dendritic hypertrophy in males and dendritic hypotrophy in females	**Proestrus:** ↓ PV+ interneurons	ERβ and PV co-express in the LA		**Males:** ↑ SST expression in stressed adults
Glutamate/GABA Function Long-term depression (LTD)	Basal glutamate and GABA function: Females > Males	↓ firing rate and shift towards greater inhibition during proestrus in LA neurons and diestrus in BA neurons	**Females:** Estrogen withdrawal ↓ GABAergic inhibition and impairs LTD. Reversed by GPR30 activation	Allopregnanolone ↑ $GABA_A$ receptor function	
Dopamine System Dopamine D1 receptor (D1R) Dopamine D5 receptor (D5R)	Basal levels: Males > Females Stress: Preferentially ↑ dopamine levels and D1R/D5R expression in females			**Males:** ↑ dopamine levels	**Males:** Castration ↓ dopamine levels **Females:** ↑ dopamine levels
Serotonin System Serotonin (5-HT) 5-HT receptors (5-HT_{1A}R, 5-HT_{1B}R, 5-HT_2R) Long-term potentiation (LTP)	Basal levels: Males > Females Stress-induced ↑ in 5-HT levels: Females > Males 5-HT_{1B}R activation: ↓ glutamate and GABA release only in males		**Females:** ↑ 5-HT_1 expression, low estrogen levels ↑ glutamate release and NMDA-dependent LTP via 5-HT_{1A}R downregulation & ↓ GABAergic inhibition (↓ LTD) via 5-HT_2R downregulation		

Alcohol. Author manuscript; available in PMC 2022 February 01.

Table 3.

Sex, Stress, Fear Conditioning, and Chronic Ethanol in BLA Circuits

	EC-BLA synapses	ST-BLA synapses	GABAergic synapses
Stress		Restraint stress: Males: ↑ firing rate of pyramidal neurons ↑ glutamate release from dmPFC inputs Females: ↓ firing rate of pyramidal neurons Disrupts estrous cycle effects on LA neuron firing rates	
Cued fear conditioning	Only tested in males: ↑ glutamate release Occludes LTP expression	Only tested in males: ↑ postsynaptic function in dmPFC-BA and ST-LA synapses ↑ NMDA- and mGluR1-dependent LTD in ST-LA synapses	Only tested in males: ↓ GABAergic inhibition in LPC and 'local' interneuron synapses
Chronic ethanol	Males and females: ↑ postsynaptic function (delayed in females) Recapitulated ↑ postsynaptic function in agranular insula cortical synapses with male BA neurons	Males and females: ↑ glutamate release (delayed in females) Recapitulated ↑ glutamate release from dmPFC and polymodal sensory thalamic inputs, as well as ↓ glutamate release from vmPFC inputs onto male BLA neurons	Only tested in males: LPCs ↓ GABA release and postsynaptic function 'Local' interneurons ↓ postsynaptic function

Alcohol. Author manuscript; available in PMC 2022 February 01.