Sex differences in anxiety and depression: circuits and mechanisms

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Abstract | Epidemiological sex differences in anxiety disorders and major depression are well characterized. Yet the circuits and mechanisms that contribute to these differences are understudied, because preclinical studies have historically excluded female rodents. This oversight is beginning to be addressed, and recent studies that include male and female rodents are identifying sex differences in neurobiological processes that underlie features of these disorders, including conflict anxiety, fear processing, arousal, social avoidance, learned helplessness and anhedonia. These findings allow us to conceptualize various types of sex differences in the brain, which in turn have broader implications for considering sex as a biological variable. Importantly, comparing the sexes could aid in the discovery of novel therapeutics.

Anhedonia
A loss of or inability to feel pleasure.

Anxiety disorders, including post-traumatic stress disorder (PTSD) and generalized anxiety disorder, and major depressive disorder (MDD) are more common in women than in men[1–3]. Although psychology and cultural factors can contribute to these sex differences, there is evidence that biological factors also play a key role. Biological factors likely contribute to the different presentation of these disorders in men and women. As one example, women with depression often have an earlier onset, lower quality of life and greater comorbidity with anxiety disorders than men with depression[4–6]. Sex differences are also present in treatment responses: several studies suggest women respond better to selective serotonin reuptake inhibitors and have higher plasma concentrations of tricyclics along with greater drop-out rates for use of tricyclics[4,7–9], although these sex differences are not consistently found[9,10]. A more thorough characterization of sex differences in biological mechanisms that contribute to anxiety disorders and MDD is crucial if we want to develop better treatments that work well for everyone.

Human neuroimaging approaches cannot fully assess neurobiological mechanisms that contribute to brain disease, so animal models using rodents are necessary[11]. Moreover, preclinical models are part of the drug development pipeline used to test the safety and efficacy of novel treatments[12]. Given the sex differences in anxiety disorders and MDD, one might assume that animal models for these disorders include and compare data across sex. However, rodent work used mainly males, such that only about 20% of the animal studies in neuroscience published in 2009 included both sexes and, shockingly, 42% failed to report the sex of their subjects[13]. Since then, efforts to improve women's health, along with growing concerns about the reproducibility of studies that omit the sex of their subjects, prompted funding agencies in the United States and Canada to implement policies to encourage animal researchers to include female subjects[14]. These policies are having an impact: 52% of neuroscience studies in 2017 included males and females[15]. Although an improvement, only 15% of studies disambiguate data by sex[15], which is a problematic practice that misses the opportunity to identify factors that promote risk and resilience to sex-biased diseases.

This Review focuses primarily on emerging preclinical research revealing sex differences in circuits and molecular mechanisms relevant to anxiety disorders and MDD. Given that rodents do not present with the full complement of symptoms of psychiatric disorders[11], we focus on features of anxiety and depression that can be assessed in animal models[12,16]. We discuss sex differences in tests of conflict anxiety, fear processing, social avoidance, stress coping (for example, learned helplessness) and anhedonia, as well as physiological measures of arousal. It is important to note that most of these tests were developed for male rodents, and in some cases are not optimized for females. As an example, in one common conflict test, the elevated plus maze, male rat behaviour is influenced by anxiety, but female rat behaviour is more influenced by their activity levels[17]. We highlight other examples of tests that require optimization in female rodents throughout the Review. Given the link between stress and the onset and severity of anxiety disorders and MDD, stressor exposure is often used in rodents to induce aspects of the dysregulation that

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occurs in these disorders[18–20]. Acute stress or acute stress hormone exposure can be used to mimic aspects of a single trauma exposure, such as the type that can precipitate PTSD, whereas chronic stressor or stress hormone exposure is often used to cause depression-like behaviour. Throughout the Review, we highlight whether stressor exposure is used and its duration, as this informs the interpretation of the results. Although we do discuss responses to stress, hypothalamic–pituitary–adrenal axis dysregulation and stressor exposure across the lifespan are not covered because they have recently been reviewed elsewhere[21–23].

Comparisons of male and female rodents are limited to assessing differences due to biological sex, which is driven by the sex chromosomes and typically assessed via the gonads[24]. In humans, differences result from a combination of biological sex and gender, a composite term for both self and societal perception of sex[25], which is influenced by psychological and cultural factors[24]. In this Review, we refer to sex differences in rodent and human studies, with the caveat that most human studies do not disentangle sex from gender. An additional consideration is that sex is typically analysed as a dichotomous variable, which can lead to the interpretation that the sexes are substantively different, when most sex differences are on a continuum with biological end points having overlapping distributions[26]. It is also important to consider that sex is often used as a proxy for factors on a continuum, such as gonadal hormones[26]. For more on the origin of biological sex differences, see BOX 1.

Given the historical bias of excluding female animals, our current understanding of the female brain is insufficient. Because females are rarely included in designs, some sex differences detailed in the Review are limited in that they only include one read-out, are based on one study or have yet to be extended from region-specific findings to circuit findings, so these caveats should be considered. However, on the basis of the limited data we do have, it is clear that sex differences in the brain are common. Many of these sex differences drive adaptive behaviours in certain circumstances but dysregulated behaviours in others, and these distinctions are highlighted. We also discuss how comparing the sexes can inspire novel treatments for psychiatric disorders.

Box 1 | **Origins of sex differences in the brain**

Sex differences originate from the different complement of genes on the XY (male) and XX (female) chromosomes. Some genes are expressed only on the Y chromosome, whereas a portion of X chromosome genes escape X inactivation resulting in a higher dosage in females than males[144–146]. Also, females are a mosaic of X chromosome genes from their father and mother, which reduces both deleterious and beneficial effects of X chromosome genes[147]. The role of sex chromosome genes in establishing sex differences in the circuits and mechanisms contributing to anxiety disorders and major depressive disorder is underexplored. However, in a mouse model, sex chromosome complement has been shown to influence the size of cortical and limbic regions implicated in these disorders[148,149] and the expression of genes involved in mood regulation[150].

One gene found only on the Y chromosome is *SRY*, the testis-determining gene, which encodes the SRY transcription factor that causes testis formation[144]. Ovaries are formed in the absence of *SRY*. Gonads produce different levels of hormones in males versus females that sexually differentiate the brain[151]. In males, a prenatal testosterone surge organizes the brain, causing the permanent masculinization of certain regions[151]. The pubertal rise in gonadal hormones (oestrogens and progesterone in females, and testosterone in males) can also affect the brain, sometimes causing permanent changes, whereas other times transiently activating circuits such that levels of hormones correlate with function[152]. Circulating levels of adult gonadal hormones are relatively easy to assess, so are most often linked to sex differences[27]. However, not all reported sex differences result from circulating gonadal hormones. To better understand the origin of sex differences in anxiety disorders and major depressive disorder, more research is needed to assess the effects of hormones and sex chromosome genes.

Types of sex differences in circuits

The studies discussed in this Review exemplify different patterns of sex differences in circuits[27,28] (FIG. 1a), which are relevant for thinking more broadly about sex differences. In some cases, the same circuit mediates a behaviour in males and females but is more sensitive to perturbations in one sex than the other, causing a difference in the magnitude or duration of the response (FIG. 1b). There are other examples of only one sex responding to an environmental manipulation, leading to sex-specific circuit activation (FIG. 1c). Emerging evidence also suggests that the same circuitry can mediate completely different behaviours in males versus females (FIG. 1d). Finally, it is possible that males and females engage different circuits to achieve the same behavioural outcome, known as a convergent sex difference[29] (FIG. 1e). For more on convergent sex differences in anxiety and depression, see BOX 2. These different types of sex differences are noted throughout the Review as a way to conceptualize and compare male and female data.

Sex differences in preclinical models

Anxiety disorders and MDD are distinguished by their symptoms[30]. However, many symptoms are shared across these disorders. For example, social avoidance can be observed in certain anxiety disorders as well as MDD, whereas altered fear processing is a feature of PTSD and phobias[30–33]. This section presents data from animal models on the neurobiological basis for sex differences in anxiety-like and depression-like behaviour. We note when behaviours are relevant to multiple disorders.

Sex differences in conflict anxiety. One feature of certain anxiety disorders, such as generalized anxiety disorder, is an inhibited approach in ambiguous situations[34]. In rodents, this feature is modelled with conflict tests, where avoidance of a novel (and thus anxiogenic) environment competes with exploratory drive. The literature within the field is inconsistent regarding stable sex differences in conflict tests due to variables such as species, strain, age, oestrous stage and reproductive status, as well as testing conditions such as time of day, luminosity and habituation to the testing environment[34–37]. Despite these considerations, conflict tests remain a common tool used to understand circuits and mechanisms contributing to anxiety. For a thorough review on sex differences in conflict tests, see REF.[34]. Here, we focus on how, using conflict tests, new data are revealing a role for arginine vasopressin (AVP) and the medial prefrontal cortex (mPFC) oxytocin systems in driving male-specific anxiogenic behaviour[38,39].

AVP manipulations appear to affect social behaviour more in females and anxiety-related behaviour more in males. In a novel environment, AVP administration in

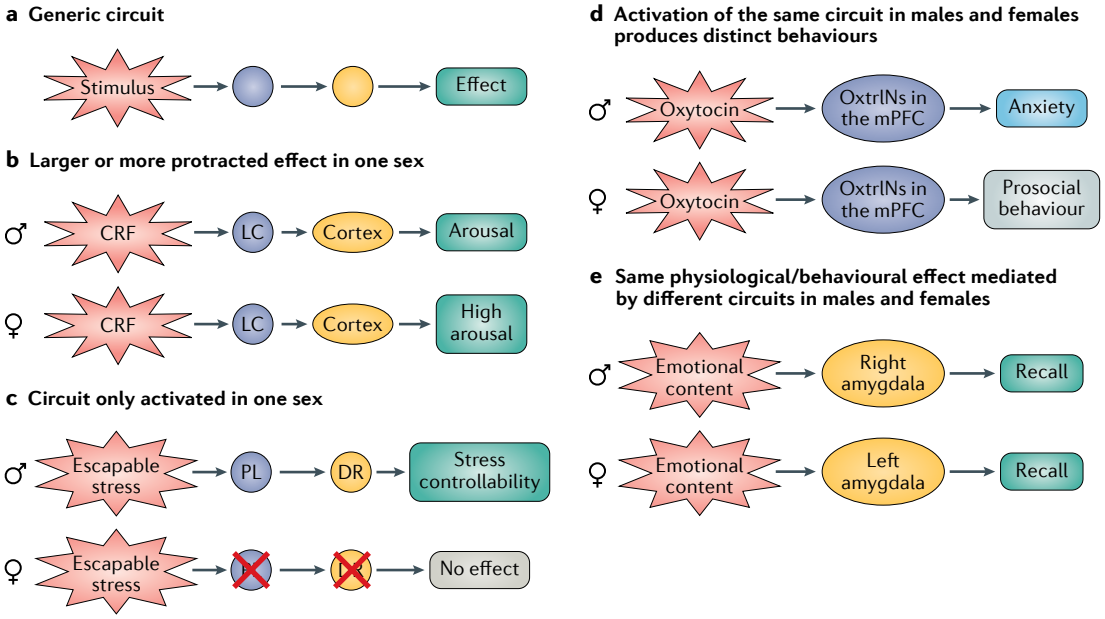

Fig. 1 | **Different types of sex differences in brain circuits. a** | Generic behavioural circuit where a stimulus triggers activation of a circuit that leads to a behavioural effect or physiological response. **b** | When sexes are compared, there are instances where the same circuit is engaged, but the response is larger or longer lasting in one sex than the other. For example, corticotropin-releasing factor (CRF) causes greater activation of the locus coeruleus (LC) arousal system in females than males. **c** | Sometimes a circuit is only activated in one sex. For example, escapable stress activates the prelimbic (PL) projection to the dorsal raphe (DR) to mediate controllable stress in males but not females. **d** | Sometimes a circuit subserves completely different behaviours in males versus females. For example, oxytocin activation of oxytocin receptor-containing interneurons (OxtrINs) in the medial prefrontal cortex (mPFC) mediates distinct behavioural response in males and females. **e** | Physiological and/or behavioural effects are the same in both sexes but the circuits and/or mechanisms by which these effects are achieved differ between males and females. For example, recall of the emotional content activates the right amygdala in men but the left amygdala in women. Note for examples in parts **b** and **c** that the converse effect (for example, larger in males than females) can also be true.

the lateral septum (LS) reduces social play behaviour in juvenile female but not male rats[39]. By contrast, AVP administration in the LS has a strong anxiogenic effect on the elevated plus maze conflict test only in juvenile males[39]. This sex difference may result from the fact that the AVP system is sexually dimorphic. Compared with females, males have enhanced AVP projections to limbic regions, including the LS, which could result in differences in basal activity in the system[40]. There are also differential effects of AVP in the paraventricular nucleus of the hypothalamus. Deleting AVP-expressing cells in the paraventricular nucleus of adult mice increases social investigation only in females and anxiety-related behaviours in the elevated plus maze only in males[41]. AVP signalling in the LS and paraventricular nucleus under normal conditions likely mediates adaptive behaviours: it may be useful for males to avoid risky environments, as an example. However, if these systems become dysregulated, maladaptive behaviours may occur, such as avoidance in situations where it would be useful to investigate. Given that the behavioural end points affected by AVP are distinct across sex, there are likely sex-specific underlying mechanisms. Much more work is needed to identify such mechanisms and determine whether AVP in other regions can drive sex differences in behaviour.

Oxytocin signalling in the mPFC also mediates sexually divergent behaviour. A specific class of oxytocin receptor-containing interneurons (OxtrINs) in the mouse mPFC activates in response to oxytocin[42]. OxtrINs in the mPFC mediate female social interactions with male mice during oestrus, the sexually receptive phase of the cycle, but not during dioestrus[42]. Activation of these neurons in male mice does not alter their social preference for a female[38]. Instead, activating these mPFC OxtrINs increases anxiety-like behaviour in conflict tests in male, but not in female, mice[38]. Thus, mPFC OxtrINs mediate prosocial behaviours in females and anxiety-related behaviour in male mice, highlighting sex-specific functions of the same circuitry[38] (FIG. 1d).

The mechanism underlying OxtrIN-mediated anxiety-like behaviour in males involves regulation of the stress neuropeptide corticotropin-releasing factor (CRF). mPFC OxtrINs produce CRF-binding protein (CRFBP)[38], which binds and limits the availability of CRF, diminishing the activity of CRF receptors[43,44]. CRFBP in mPFC OxtrINs mediates anxiety-like behaviour only in males but not social behaviour in either sex[38]. These results indicate that a different molecular mechanism must be involved in regulating the social behaviour controlled by this circuit in females.

Collectively, the studies on AVP and oxytocin in conflict anxiety highlight an interesting phenomenon: activation of the same neuropeptide system within a circuit can drive completely different behaviours in males versus females (FIG. 1d). Thus, we cannot assume that the function of a circuit delineated in males will be the same in females.

Dioestrus
A period of the oestrous cycle immediately preceding pro-oestrus in which female subjects are not sexually receptive and there are relatively low levels of oestrogens and progesterone.

Box 2 | **Convergent sex differences in mechanisms that contribute to anxiety disorders and MDD**

Epidemiological sex differences in anxiety disorders and major depressive disorder (MDD) prompt most investigators to focus on factors that drive divergent phenotypes in males versus females. There are fewer reports of convergent (also known as latent) sex differences where a similar phenotype occurs via sex-specific mechanisms[27,29,153]. Moreover, many studies fail to disambiguate data by sex, obfuscating reports of convergent sex differences. Despite the limited data, there are a couple relevant examples of this type of sex difference.

Sustained attention, the ability to monitor a situation for intermittent and unpredictable events, is disrupted in MDD[154]. A preclinical study found comparable attention deficits in male and female rats following a 6-day variable stress procedure[155]. Across sex, this stressor induced hypertrophy of dendrites of cholinergic neurons in the basal forebrain, which mediate sustained attention[155]. However, this stressor caused divergent transcriptional changes in the basal forebrain of males and females[155]. These findings suggest that sex-specific mechanisms underlie the stress-induced alterations in structural plasticity and attention.

Similarly, transcriptomic studies on post-mortem MDD tissue find sex-specific transcriptional profiles in cortical and limbic regions[136,156]. In some cases, these differences appear to converge on the same physiological mechanisms. Seney et al. propose a model where MDD results in similar changes in cortical microglia and spines on pyramidal neurons of men and women via different mechanisms[157]. Control male subjects have low numbers of activated microglia but very dense spines, whereas the reverse is true of females[156,158–161]. MDD shifts both sexes into an intermediate phenotype with moderate microglia activation and spine density[157]. This shift is thought to occur through different transcriptional regulation and has important treatment implications[156,157]. Reducing microglia activation may ameliorate symptoms in men with MDD but exacerbate symptoms in women with MDD. This research underscores how understanding convergent sex differences is crucial to developing effective treatments.

Sex differences in fear processing. In PTSD, cues associated with the trauma can often trigger negative memories. This associative process can be modelled with the fear conditioning procedure, where a neutral cue is paired with an aversive stimulus until the neutral cue elicits a fear response. If the neutral cue is subsequently presented without the aversive stimulus, the fear response will subside via a process called extinction, and the efficacy of extinction can be tested with a recall test. Enhanced fear conditioning and impaired extinction and extinction recall can contribute to PTSD[32,33,45,46]. Extinction is also a component of the exposure therapy used to treat many anxiety disorders, including phobias and PTSD[47]. Given the epidemiological sex differences in these disorders, fear learning has been assessed across sex and thoroughly reviewed elsewhere[48–50]. Here, we just highlight several interesting mechanisms.

Circuits involved in fear conditioning are well delineated and involve the PFC and amygdala (reviewed in REFS[51,52]). In healthy adult humans, fronto-limbic circuits show greater connectivity in women than in men[53]. Additionally, within the amygdala, there is a sex-dependent hemispheric lateralization of memory for emotional content[54]. There is a stronger relationship between recall of emotionally arousing content and activity of the right amygdala in men, but the left amygdala in women[54]. This finding is another example of sex convergence (FIG. 1e). These sex differences in fear circuits within healthy people likely promote adaptive responses to fear across sex in most circumstances, but trauma may cause a further divergence in their function that contributes to sex differences observed in PTSD.

Extinction
A weakening of a conditioned response that yields a decrease in behaviour.

Rodent fear conditioning studies are revealing molecular mechanisms that cause altered fear processing. Most of these studies quantify freezing behaviour as their index of a learned fear response[48]. Yet females display lower levels of freezing than males and are more likely to display darting behaviour: a rapid, forward movement resembling an active escape-like response[48,55]. The darting is conditioned and represents an alternative fear response strategy[56]. These findings are another example of the issue that most animal tests of anxiety and depression were designed for male rodents[48,57,58].

There is a role for the endocannabinoid system in fear conditioning[59,60]. There are two main endogenous endocannabinoids: anandamide and 2-arachidonoyl glycerol (2-AG). Anandamide can bind to cannabinoid receptors, such as CB_1, and the transient receptor potential vanilloid type 1 (TRPV1)[60]. Increasing anandamide signalling via CB_1 receptors facilitates fear extinction, as assessed with freezing behaviour in males (females were not tested)[61]. Yet increasing anandamide signalling via TRPV1 receptors impairs fear extinction as measured with freezing females, but has no effect in males[59]. There are also sex differences in the effects of 2-AG. Elevated 2-AG signalling via activation of CB_1 receptors increases freezing in males but decreases freezing in females, and instead shifts the female response to active darting behaviour[59,62]. Taken together, these studies highlight a role for the endocannabinoid system in fear extinction across sex, but the effects exerted by different endocannabinoids result in sexually divergent fear responses. This work taken together with early evidence for sex differences in the endocannabinoid system in people with PTSD[63] underscores the critical need to include sex as a variable in developing endocannabinoid-related therapeutics.

The aforementioned data reveal sex differences in the mechanisms underlying fear conditioning, but reports on whether there are sex differences in the degree of fear learning and extinction at the behavioural level are mixed[64–66]. This inconsistency can be partially explained by a failure to consider gonadal hormonal status. Women and rats with low oestrogen levels exhibit impaired extinction retrieval[67,68]. A decrease in oestradiol levels in females due to cycle fluctuations or oral contraception reduces the extinction of a fearful memory by altering the activity in the fear extinction circuit[67]. Altered brain activity levels are dependent on the phase of fear conditioning. Specifically, women in the high oestradiol group had increased activity within the insular and cingulate cortices across all phases of fear conditioning, whereas increased activity within the amygdala and hypothalamus was observed in the conditioning phase only[67]. Rodent data suggest that the deficit in extinction memory due to low oestradiol levels can be overcome by activating the dopaminergic system[68]. Treatment with a dopamine D1 receptor agonist improved extinction retrieval in females in the low oestrogen phases of their cycle, bringing their performance to pro-oestrus (high-oestrogen phase) levels[68]. This result suggests that women with low oestradiol levels going through exposure therapy for anxiety disorders may benefit from drugs that increase dopamine.

Collectively, these studies underscore the need to consider sex when designing therapeutics for PTSD. Also, fear learning is one process that is clearly linked to cycling ovarian hormones. Considering the oestrous or menstrual cycle should not be a requirement for studying females generally, just as assessing testosterone levels is not a requirement to study males[69]. However, in cases where there is evidence that gonadal hormones regulate an end point, such as in fear processing, assessing gonadal hormones will further our understanding of factors that contribute to vulnerability to psychiatric disease.

Sex differences in arousal. Hyperarousal, the disruptive feeling of being on edge, is a key feature of PTSD and contributes to some symptoms of MDD, such as a lack of concentration, rumination, restlessness and sleep disturbance[30]. Compared with men, women with PTSD and MDD have more hyperarousal symptoms[70–74]. There is also evidence that people with these disorders hypersecrete CRF[75–79]. One target of CRF is the locus coeruleus (LC) arousal system[80,81]. The LC is a major source of noradrenaline for the brain, and the release of noradrenaline increases levels of arousal[82–85]. During a stressful event, CRF is released into the LC, which increases the tonic firing rate of LC neurons, increasing noradrenaline levels[86–90]. This activation is typically an adaptive response because it is important to be alert during a stressful event. However, if this system is pushed, as might occur under conditions of CRF hypersecretion, then hyperarousal symptoms can emerge.

There are sex differences in CRF regulation of the LC that can increase levels of arousal in females compared with males (sex difference type shown in FIG. 1b). Female LC neurons are more sensitive to an acute dose of CRF, such that a low dose of CRF that fails to increase tonic LC neuronal firing in male rats causes neuronal firing in female rats[91,92]. This heightened female sensitivity is due to a sex difference in the CRF_1 receptor, a G-protein-coupled receptor (GPCR) that mediates the effect of CRF on LC neurons[87,93]. The CRF_1 receptor is more highly coupled to the GTP-binding protein G_s in female than male rats, which causes greater signalling through the cyclic AMP–PKA pathway in females[92]. This pathway increases LC neuronal firing[94], and this greater activation of cyclic AMP–PKA signalling in females underlies their increased neuronal sensitivity to CRF[92]. In CRF-overexpressing mice, which model the chronic hypersecretion of CRF observed in PTSD and MDD[95], there is evidence of a similar increase in the cyclic AMP–PKA pathway in cortical tissue of females but not males[96]. In male rodents, the CRF_1 receptor binds more readily to a different protein called β-arrestin[92]. Activation of β-arrestin also causes signalling but via distinct pathways from those activated by G_s (REF.[97]). CRF overexpression in male mice results in signalling through β-arrestin-mediated pathways in the cortex[96,98]. Taken together, these findings reveal that CRF_1 activation in the LC and cortex results in G_s-mediated signalling in females and β-arrestin-mediated signalling in males, providing the first evidence of stress-induced sex-specific signalling[99–101] (FIG. 2). Different signalling pathways result in distinct cellular events, so sex-specific signalling can drive sexually divergent responses to stress.

In addition to initiating signalling pathways, β-arrestin can also induce receptor internalization[102], the movement of the receptor from the plasma membrane into the cytosol. Because the internalized receptors cannot be activated, internalization is thought to mitigate against excess CRF release. Acute stressor exposure and chronic CRF hypersecretion cause CRF_1 receptor internalization in LC neurons of male rodents[92,103,104]. However, these manipulations do not cause CRF_1 receptor internalization in female LC neurons, which is consistent with their reduced binding of the CRF_1 receptor to β-arrestin[92,104]. Under most circumstances, this sex difference in internalization may not be physiologically or behaviourally consequential. However, under conditions of CRF hypersecretion, LC neurons in males, but not females, could reduce their response to CRF, protecting against high levels of arousal. Consistent with this idea, the tonic firing rate of LC neurons in male CRF-overexpressing mice, which have excessive CRF in their LC, is maintained at wild-type levels[104]. By contrast, LC neurons of female CRF-overexpressing mice fire roughly three times faster than wild-type controls[104]. Thus, if similar mechanisms occur in humans, under conditions of excessive CRF release, women would be more likely to experience hyperarousal symptoms than men (FIG. 3). This prediction is consistent with the epidemiological data revealing that women have greater hyperarousal symptoms in disorders characterized by CRF hypersecretion[70–74].

Several CRF_1 antagonists were developed for MDD and other disorders but failed clinical testing[105]. One issue is that preclinical testing of CRF_1 antagonists occurred in male rodents, whereas most clinical studies recruited exclusively or predominantly women without disambiguating data by sex (reviewed elsewhere[22]). The one successful trial, with the CRF_1 antagonist NBI-34041, tested efficacy only in men with MDD[106]. Given what is now known about sex differences in CRF_1, the discrepancy in the sex of the preclinical and clinical subjects likely contributes to the failure of traditional CRF_1 antagonists. However, this failure does not mean CRF_1 receptors should not be considered as a therapeutic target. Instead, the knowledge of receptor sex differences can be leveraged into ideas for novel treatments, such as biased ligands for CRF_1 receptors[107]. Biased ligands bind to GPCRs to direct signalling through β-arrestin-mediated pathways instead of G protein signalling pathways and are being developed for other receptors[97,108]. β-Arrestin-initiated signalling and receptor internalization found in male rodents render their LC arousal neurons less sensitive to low levels of CRF and more adaptable to CRF hypersecretion. Thus, a biased ligand for the CRF_1 receptor could be useful in reducing hyperarousal symptoms, especially in women[99–101]. Critically, an idea for such a compound would never have come about if only males were used in studies of CRF_1 receptor function. Given the male bias in preclinical research, there is a strong possibility that the field has missed out on many ideas for innovative therapeutics by not comparing the sexes.

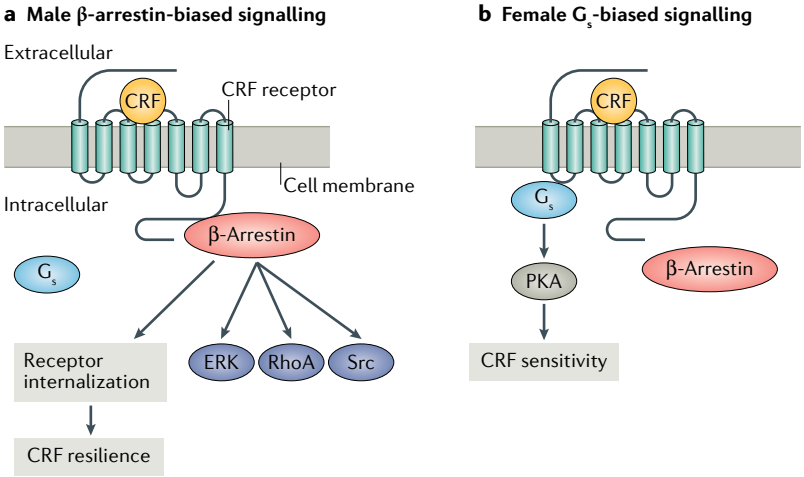

Fig. 2 | **Sex-biased receptor signalling. a** | In males, the CRF_1 receptor associates with β-arrestin, which biases signalling towards β-arrestin-mediated pathways and causes receptor internalization. **b** | In females, the CRF_1 receptor signals more through G_s-mediated pathways, which increases the sensitivity of their locus coeruleus neurons to corticotropin-releasing factor (CRF). Given reduced β-arrestin binding in females, their CRF_1 receptors do not internalize following acute stress or CRF overexpression. Adapted with permission from REF.[165], Elsevier.

Sex differences in social avoidance. Social avoidance is a feature of MDD and several anxiety disorders, most notably social anxiety disorder (SAD)[30,31]. Women tend to have more social avoidance in MDD and greater fear of social situations in SAD[109,110]. In addition to avoidance, SAD is characterized by social vigilance, the exacerbated monitoring of social cues in the environment[111]. Both social avoidance and social vigilance can be modelled in rodents by inducing social stress. Given the social structure of rats (*Rattus norvegicus*) and mice (*Mus musculus*) — the most common rodents used in research — social stress caused by territorial aggression is difficult to induce in females. Although housing certain strains of female mice with males can increase female aggression[112], another approach is to use a species in which females regularly engage in territorial aggression similar to males, such as California mice (*Peromyscus californicus*)[113]. In California mice, males and females can be readily used in the social defeat procedure, during which an intruder mouse is placed in the cage of an aggressive resident to induce stress in the intruder[114].

One modulator of social behaviour is oxytocin. Oxytocin can promote prosocial behaviour, but antisocial effects are also observed in certain contexts[115]. These conflicting findings can be reconciled by interpretation of oxytocin's role as enhancing the salience of social cues[116]. Within this framework, it may not be surprising that oxytocin has a role in promoting social avoidance and social vigilance in California mice. Three days of social defeat stress in California mice has the immediate effect of activating oxytocin neurons in the medioventral bed nucleus of the stria terminalis (BNSTmv) across sexes[117]. However, activation of oxytocin neurons in the BNSTmv persists for 10 weeks in females but not males, revealing an enduring effect of social stress on neuronal sensitivity only in females (sex difference type shown in FIG. 1b). Mirroring the sex difference in the time course of social defeat on oxytocin neuronal activation in the BNSTmv, reduced social approach and increased social vigilance are observed after 3 days of social defeat in males and weeks after this stressor only in females[118]. This persistent effect of social defeat on social behaviour in female California mice is prevented by knocking down oxytocin in the BNSTmv, revealing that oxytocin in this region causes the change in social behaviour[118].

BNSTmv oxytocin neurons project to the anteromedial bed nucleus of the stria terminalis (BNSTam)[118]. In female California mice, blocking oxytocin receptors in the BNSTam with direct administration of the antagonist L-368,899 increases social approach and reduces social vigilance, reversing the negative effect of social stress on these behaviours[118,119] (FIG. 3). This effect is also replicated with systemic administration of the oxytocin receptor antagonist in females[119]. Surprisingly, in unstressed males this same systemic administration decreased social approach, revealing sex differences in oxytocin receptor blockade on social behaviour. Activating oxytocin receptors by administering intranasal oxytocin reduces social interaction in unstressed females, mimicking social defeat. By contrast, systemic oxytocin infusion increases social interaction in males exposed to social defeat[117,120]. Similarly, in humans, a single dose of intranasal oxytocin increases distress and anger in women, but reduces distress in men following a social stress test[121].

Collectively, these findings have important implications. Although in California mice it may be adaptive in some situations for oxytocin signalling in females to promote persistent avoidance of potential social threats, similar oxytocin signalling in humans, if unchecked, may lead to inappropriate social avoidance. Indeed, women with high MDD symptoms have elevated peripheral oxytocin[122,123], although it is unclear whether peripheral oxytocin levels are reflective of central oxytocin release. The rodent work suggests that internasal oxytocin, a putative treatment for social deficits, may not ameliorate social avoidance and social vigilance in stressed females. Thus, internasal oxytocin may be an unsuitable treatment for women with SAD and MDD. By contrast, the preclinical data indicate that blocking oxytocin receptors could promote social behaviour in stressed females. It is possible that women experiencing social stress with high levels of peripheral oxytocin would benefit most from an oxytocin antagonist. If so, peripheral oxytocin levels could be used as a screening tool to distinguish those most likely to benefit from this treatment. More research is needed, but these data underscore that when developing and testing oxytocin-based pharmacotherapies in humans, data need to be compared by sex.

Sex differences in learned helplessness. One feature of MDD is a feeling of helplessness. Laboratory manipulations using repeated shock can induce 'learned helplessness', a failure to control aversive events, in both humans and rodents[124]. For details on the learned helplessness and behavioural immunization procedures, see BOX 3. The rodent learned helplessness test is used to elucidate circuits relevant to MDD. Inescapable stress activates serotonin neurons in the dorsal raphe (DR)

Monetary incentive delay task
An imaging paradigm used to study the neural activity of anticipatory incentive processing.

nucleus, causing a greater release of serotonin in target regions to drive the negative consequences of inescapable stress[125]. Behavioural immunization results from strengthened prelimbic (PL) inputs to the DR that exert top-down inhibitory control to reduce the negative stress outcomes[126]. One major limitation to this circuitry work is that, for many years, these studies were exclusively conducted in male rats, despite evidence that the learned helplessness procedure was ineffective in females[127]. This omission is now being addressed, and new circuitry studies are including females and finding different results. Unlike males, females exposed to escapable stress neither are protected from negative stress-induced behaviours nor are behaviourally immunized against subsequent inescapable stress[128,129]. In females, escapable stress fails to activate the PL projection to the DR and does not induce the structural plasticity in these neurons thought to suppress DR function[128,129]. Thus, engagement of this circuit is sex-specific (sex difference type shown in FIG. 1c). However, this projection does appear functional in females, as activating their PL region prevents the stress-induced reduction in social exploration[129].

This research suggests that stressor controllability does not promote resilience in females. More work is needed to determine whether this effect is specific to certain experimental conditions or is a broader phenomenon. If, under many circumstances, stressor controllability only buffers males, this could explain their lower rates of disorders such as MDD. In terms of treatments, although activation of the PL to DR circuit does not occur naturally following escapable stress in females, as it does in males, pharmacological activation of the PL region promotes stress resilience in females. It is therefore possible that activating this circuit in humans would buffer against the negative effects of stress, regardless of sex.

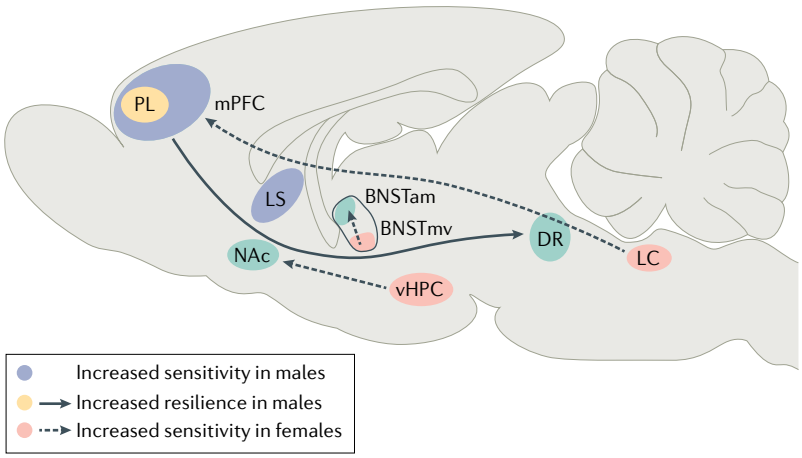

Fig. 3 | **Brain regions and circuits that bias male or female anxiety-like and/or depression-like behaviour.** All circuits are present in both sexes but for simplicity are illustrated for the sex where they are a more potent regulator of behaviour. The regions in purple indicate areas where certain neuropeptides increase sensitivity to anxiety-like behaviour in males but not females. Regions in red illustrate areas of increased sensitivity and/or activity in females. The region in yellow is part of a projection that promotes resilience to depression-like behaviour in males. Target structures are in green. BNSTam, anteromedial bed nucleus of the stria terminalis; BNSTmv, medioventral bed nucleus of the stria terminalis; DR, dorsal raphe; LC, locus coeruleus; LS, lateral septum; mPFC, medial prefrontal cortex; PL, prelimbic; NAc, nucleus accumbens; vHPC, ventral hippocampus.

Sex differences in anhedonia. In patients with MDD, the symptom of anhedonia presents as a difficulty using rewards to modulate behaviour[130,131]. Anhedonia is mediated by reward circuitry, which includes the nucleus accumbens (NAc). The NAc receives dopaminergic inputs from the ventral tegmental area (VTA) that signal motivational salience, as well as inputs from cortical, thalamic and limbic regions that carry cognitive and emotional information[132]. The NAc then outputs to basal ganglia circuits to drive motivated actions. Unlike other symptoms of depression and anxiety, anhedonia, as assessed with the monetary incentive delay task, is associated with reduced NAc volume and reduced NAc responses to rewards[133]. In humans, these anhedonia-related structural and functional NAc changes (assessed by functional MRI) do not differ by sex[133]. However, in rodents, stress-induced molecular sex differences in NAc circuitry have been identified[134,135], revealing mechanisms that may contribute to sex differences in this aspect of MDD.

In mice, a procedure that uses alternating stressors across a 6-day period induces depression-like behaviour in females[134]. This 6-day manipulation is referred to as subchronic variable stress (SCVS), to contrast it with their other 'chronic' variable stress manipulations that last longer than 21 days. One of several behaviours altered by SCVS in females is a rodent test of anhedonia, the sucrose preference test, such that consumption of a palatable sucrose solution is reduced in SCVS versus control female mice. Male mouse behaviour is unaffected by SCVS, but if the alternating stressors continue for 21 days, both sexes demonstrate similar reductions in sucrose preference[135,136]. Thus, this stressor exposure more rapidly affects females than males, and may represent a maladaptive mechanism increasing female vulnerability to depression-like behaviour. Epigenetic changes in the NAc account for female sensitivity to SCVS[134]. In females relative to males, SCVS increases the expression of DNA methyltransferase 3a (*Dnmt3a*) in the NAc, which codes for an enzyme that promotes DNA methylation to regulate gene expression[134]. SCVS did not cause a sex difference in *Dnmt3a* in the mPFC, suggesting this stressor does not globally alter this gene[134]. Overexpressing DNMT3A in the NAc of males makes them susceptible to SCVS. By contrast, knocking down DNMT3A in the NAc of females makes them resilient to SCVS and alters gene transcription in the NAc to make transcriptional signatures more similar to those of males[134].

Given that psychiatric disorders are caused by a dysregulation in circuits, it is likely that connections with the NAc contribute to sex differences in stress-induced anhedonia. One candidate input is the dopaminergic input from the VTA. Chronic mild stress attenuates VTA dopamine neuronal activity more in female than in male rats[137]. The effect of chronic stress on the VTA might be expected to cause a sex difference in dopamine neurotransmission in the NAc, but the data do not support this idea[138,139]. Instead, chronic mild stress reduces

Box 3 | **Learned helplessness procedure**

In a typical rodent learned helplessness procedure, there is an initial phase when one group of rats is exposed to escapable shock (where the rat can control the shock termination), whereas a second group is exposed to inescapable shock (where no response can terminate the shock)[124]. Each subject in the inescapable group is yoked to a subject in the escapable group, which equates shock exposure such that the only difference between the two manipulations is shock controllability. The next day, both groups are tested in a different environment where all rats can escape an aversive event. Subjects in the inescapable group typically fail to escape the aversive event in this new environment, even though escape is now possible. The interpretation is that they learned to be helpless. Exposure to the inescapable stressor also results in other negative outcomes, such as decreased social exploration and exaggerated fear responses, which are not observed following the escapable stressor[162,163]. Instead of learning to be helpless, rodents in the escapable group learn that they can control aversive events. In fact, escapable stress can even buffer against the negative outcomes of future inescapable stressor exposure, an effect known as 'behavioural immunization'[164]. This research, which was established in male rodents, reveals that their prior experience with the ability to control stress can affect future behaviour.

dopaminergic activity in another VTA target region, the PFC, only in females[139]. This PFC effect was not associated with a sex difference in anhedonia. Collectively, these studies suggest that stress-induced sex differences in VTA dopamine regulation do not contribute to sex differences in anhedonia, but could contribute to sex differences in other depression-related behaviours mediated by the PFC.

In addition to the dopaminergic input, the NAc is regulated by glutamatergic inputs from the mPFC, thalamus, basolateral amygdala and hippocampus. SCVS alters presynaptic glutamatergic inputs to the NAc by affecting vesicular glutamate transporters in females[140]. One glutamate input to the NAc studied in the context of sex differences in stress-induced anhedonia is the ventral hippocampus (vHPC) to NAc input[135]. SCVS increases excitability of the vHPC to NAc input in female relative to male mice, but this effect was not observed with the vHPC to basolateral amygdala projection. Moreover, the vHPC–NAc is required for SCVS-induced anhedonia in females (FIG. 3). Male resilience to SCVS-induced anhedonia and vHPC–NAc hyperexcitability is due to testosterone[135]. Treating females with testosterone promotes resilience to SCVS. In addition to mediating sex differences in SCVS-induced anhedonia, the vHPA–NAc circuit also plays a role in other disease-relevant behaviour. In males and females, individual differences in excitability of this circuit predict anxiety-like behaviour, such that male and female mice with high vHPC–NAc activity appear more anxious[141]. Thus, reducing activation of this circuit may have widespread therapeutic implications.

Data investigating sex differences in the mechanisms and circuits underlying sex differences in anhedonia are just beginning to emerge. There are many unanswered questions, including whether there are sex differences in the outputs of the NAc that influence anhedonia differently in males versus females. However, the current findings do suggest that targeting reward circuitry may be a useful approach to mitigate symptoms of anhedonia, especially in women.

Conclusions

Emerging data that include males and females in studies of anxiety and depression-like end points are finally being published. These studies make it clear that we cannot assume that a female brain is the same as a male brain. They also reveal several mechanisms that can contribute to female vulnerability to anxiety and MDD. Convergent sex differences are also crucial to consider for optimizing treatments across sex. Unfortunately, they have not been a focus of the field and are often under-reported due to the practice of collapsing data across sex. To address this, researchers, funding agencies and journal editors need to encourage the practice of disambiguating data by sex[142]. As noted throughout, there is also a need for animal tests for anxiety and depression-related end points that are well validated in females. In short, researchers need to design tasks for female subjects, test female subjects and present data on female subjects, just as they have for male subjects for decades.

In the twenty-first century, neuroscience has made huge technological advances allowing for the detailed mapping of neural circuits and their precise manipulation in behaving animals[143]. What has lagged behind is the conceptual advance that the field must study males and females to understand and adequately treat disorders across sex. Hopefully, we are on the verge of the next advance in neuroscience: inclusive data collection. If so, the field will finally fill a huge gap in knowledge about female brains and develop better treatments for anxiety and MDD that work for all.

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