The impact of sex as a biological variable in the search for novel antidepressants

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ABSTRACT

A roadblock to successful treatment for anxiety and depression is the high proportion of individuals that do not respond to existing treatments. Different underlying neurobiological mechanisms may drive similar symptoms, so a more personalized approach to treatment could be more successful. There is increasing evidence that sex is an important biological variable modulating efficacy of antidepressants and anxiolytics. We review evidence for sex-specific effects of traditional monoamine based antidepressants and newer pharmaceuticals targeting kappa opioid receptors (KOR), oxytocin receptors (OTR), and N-methyl-D-aspartate receptors (ketamine). In some cases, similar behavioral effects are observed in both sexes while in other cases strong sex-specific effects are observed. Most intriguing are cases such as ketamine which has similar behavioral effects in males and females, perhaps through sex-specific neurobiological mechanisms. These results show how essential it is to include both males and females in both clinical and preclinical evaluations of novel antidepressants and anxiolytics.

1. Introduction

Depression and anxiety disorders place a large burden on society (Friedrich, 2017). Through a combination of serendipity and systematic research, a variety of pharmaceutical and counseling based therapies have been developed. For those who respond positively to these treatments, the results can lead to dramatically positive outcomes. However, over half of individuals that seek treatment do not respond positively to currently available pharmacotherapy treatment options (Culpepper, 2010). This heterogeneity in treatment outcomes has driven research for new therapeutics as well as understanding of the mechanisms that contribute to positive behavioral outcomes. There is growing awareness that there are important sex differences in the prevalence of depression and anxiety disorders (Fernandez-Guasti et al., 2012), and in the pharmacokinetics and pharmacodynamics of antidepressants (Wolbold et al., 2003; Cheung et al., 2006; Damoiseaux et al., 2014). Differences in pharmacokinetics may lead to sex differences in the efficacy of antidepressant treatments (Sramek et al., 2016). An important risk factor for anxiety and depression-related disorders is exposure to psychosocial stress, and there are important sex differences in physiological responses to stress (Goel and Bale, 2009; Bale and Epperson, 2015; Laman-Maharg and Trainor, 2017). Together, it is likely that there are important sex differences in the mechanisms contributing to the behavioral symptoms associated with anxiety and depression. Until recently most studies examining the neurobiological mechanisms of mood disorders and antidepressants have focused only on males. However, growing awareness of sex differences in neurobiology and behavior (Shansky and Woolley, 2016; McCarthy et al., 2012; Labonté et al., 2017; Jazin and Cahill, 2010; Cahill, 2015) and new research policies in the United States (Clayton and Collins, 2014) have increased focus on how sex as a biological variable can modulate behavioral responses to pharmaceuticals with antidepressant or anxiolytic properties. This shift should ultimately contribute to better treatments for mood and anxiety disorders.

Here we review the growing literature assessing how sex affects the efficacy and neurobiological mechanisms of pharmaceuticals with antidepressant and anxiolytic properties. While medications targeting monoamine systems are the most established, depression-like and anxiety-like behaviors can be induced independently of monoamine systems (Angoa-Pérez et al., 2014). Additionally, serotonin-based therapeutics can have important adverse effects (Jakobsen et al., 2017). Alternative therapeutic targets could provide a mechanism for treating individuals that do not respond well to standard monoamine-targeting treatments. Anxiety and depression are often comorbid (Kessler et al., 2007; Lamers et al., 2011), which suggests at least some neurobiological mechanisms contribute to both disorders. However, the extent of comorbidity is variable, suggesting that there is some degree of independence in mechanisms. Thus, as novel antidepressants and anxiolytics are identified and studied, it is critical to understand their mechanisms of action. Here we discuss compounds targeting opioid

receptors, neuropeptide receptors, and glutamate receptors, which have anxiolytic and/or antidepressant properties. A consistent theme across all of these classes of pharmaceuticals is that sex-specific behavioral or neurobiological effects have been reported. Below we review evidence for sex differences in the behavioral and neurobiological effects of these novel classes of therapeutics.

2. Selective serotonin reuptake inhibitors

The anti-depressant properties of the most commonly prescribed medications were in some ways discovered by accident (Ban, 2006; Hillhouse and Porter, 2015). The first selective-serotonin reuptake inhibitor (SSRI), fluoxetine, was discovered in the 1970s (Wong et al., 1975; Wong et al., 1974), and the development of SSRIs continued into the 1980s (Aberg-Wistedt, 1989; Doogan and Caillard, 1988; Charney et al., 1990). While these drugs have been the most prescribed medication for depression for decades, their mechanisms of action are only partially understood. Sex differences in mechanisms of antidepressant therapeutics have been reviewed previously (Dalla et al., 2010); so we briefly review two primary mechanisms: pharmacokinetics and pharmacodynamics, and sex hormones (Sramek et al., 2016).

It has been suggested that physiological and molecular differences in males and females affect the pharmacokinetics and pharmacodynamics of antidepressant drugs (Damoiseaux et al., 2014). One possible contributing factor may be enzymes that metabolize drugs, cytochrome P450s (CYP). Sex differences in the expression or activity of these enzymes (Wolbold et al., 2003; Cheung et al., 2006) can cause faster drug metabolism and affect drug clearance (but see (Schmucker et al., 1990)). For example, the SSRI sertraline has a longer half-life in women compared to men (Ronfeld et al., 1997), which suggests that a single dose of sertraline has a longer duration of action in women. Consistent with this, plasma concentrations of sertraline are at least 50% higher in women compared to men (Ronfeld et al., 1997; Hartter et al., 1993). Higher plasma concentrations in women could be clinically significant, and may be relevant to reports that behavioral responses to SSRIs are stronger in women compared to men (Khan et al., 2005). This finding suggests that women are more sensitive to lower doses of SSRIs than men. This sex differences has also been observed in animal models of depression and anxiety (Fernández-Guasti et al., 2017; Kokras et al., 2015; Greenberg et al., 2014). In additional to sex differences in drug metabolism, gonadal hormones are important modulators of SSRI action.

The relationship between serotonin and estrogens has been noted in the context of affect and mood and cognition (Amin et al., 2005; Rubinow et al., 1998), as well as in the context of mood disorders (Young and Korszun, 2010). In particular, estrogen receptor β (ERβ) is typically associated with having both antidepressant (Donner and Handa, 2009) and anxiolytic effects on behavior (Trainor et al., 2007; Hiroi et al., 2006; Handa et al., 2012) in both male and female rodents. One study suggested that in female rodents, estrogens may facilitate the action of SSRIs (Estrada-Camarena et al., 2004). Although the mechanism of action through which this occurs is unknown, treatment with estradiol in the dorsal raphe nucleus (DRN) has been to shown to increase the expression of tryptophan hydroxylase 2 (*TPH2*) (Hiroi et al., 2006), the brain-specific serotonin synthesizing enzyme (Hasegawa et al., 2010). This finding suggested that estradiol may have an indirect effect of increasing serotonin synthesis in the DRN through its modulation of *TPH2* expression.

Given the established relationship between ERβ and depression- and anxiety-like behavior, ERβ modulation of *TPH2* expression may contribute to the effects of estrogens on these behaviors. One study showed that treatment with 17β-estradiol or an ERβ agonist in the DRN increased *TPH2* mRNA (Donner and Handa, 2009). Similarly, an in vitro study using cultured medullary raphe serotonin neurons shows that 17β-estradiol activation of ERβ increased transcription of *TPH2* (Hiroi and Handa, 2013). This same study also showed that an estrogen

response element (ERE) exists within the *TPH2* 5′-untranslated region. It is possible estrogen receptor binding to this ERE alters *TPH2* expression. Further study is needed to test whether this promoter modulates to the ability of estrogens to facilitate the action of SSRIs.

In humans, ovarian hormones may also modulate antidepressant efficacy. Clinical studies show that premenopausal women respond better to SSRIs than both men (Berlanga and Flores-Ramos, 2006), older women (Grigoriadis et al., 2003), and postmenopausal women (Pae et al., 2009). In a study comparing SSRI use in combination with hormone replacement therapy, postmenopausal women responded better to SSRI treatment while undergoing this therapy than postmenopausal women receiving an SSRI alone (Schneider et al., 1997). Taken together, these data support the hypothesis that ovarian hormones play a role in the efficacy of SSRI treatments. However, not every study has reported effects of sex (Parker et al., 2003) or of menopausal stage (Quitkin et al., 2002; Kornstein et al., 2014) on antidepressant response. An issue with many of these studies is a lack of a proper placebo control, small sample sizes, uneven age-matched groups, and a lack of randomly assigned groups. Additionally, the treatment periods observed between studies vary widely, so it is difficult to determine whether the inconsistencies in findings are because sex and menopausal stage do not actually effect drug response, or whether the lack of differences observed in these studies are an artifact of differences in methodology. Finally, a major weakness of many studies is the use of premarine as hormone replacement therapy, which is a complex preparation of estrogen and progestin metabolites. More studies examining this topic, using larger sample sizes and consistent methods (such as purer hormone treatments) are necessary to determine the extent to which estrogens modulate SSRI treatment efficacy.

3. Kappa opioid receptors

Kappa-opioid receptors (KOR) have generated strong interest as a potentially novel therapeutic target for the treatment of mood disorders and anxiety. KOR are located widely throughout the brain, including throughout areas in the limbic system as well as the prefrontal cortex, dorsal raphe, and the periaqueductal gray (Mansour et al., 1995). The activation of these receptors by agonists induces depression-like states in humans (Pfeiffer et al., 1986) as well as in rodents (Land et al., 2008). In contrast, preclinical data indicate that antagonists have the potential to block many behavioral phenotypes induced by stress. These KOR-induced responses to stress are likely mediated by dynorphin, the endogenous ligand for KOR (Chavkin et al., 1982). The role of the dynorphin/KOR system in stress-induced behaviors has been studied extensively (for review, see (Bruchas et al., 2010; Lalanne et al., 2014). Overall, there is strong evidence that KOR plays a role in the physical, psychological, and aversive experiences of stress. Despite these promising foundations, initial tests of KOR antagonists in clinical trials were unsuccessful due to the onset of short-term cardiac diseases, bradycardia and ventricular tachycardia, in some patients (Buda et al., 2015; Chavkin and Martinez, 2015). Although several factors may have contributed to this unsuccessful outcome, the prolonged inhibition of KOR characteristic of many antagonists is likely an important factor. Despite this initial setback, there are some exciting new directions.

The KOR antagonists JDTic and norBNI were considered promising pharmaceuticals based on their performance in behavior tests predictive of antidepressant efficacy, and their ability to reduce the impact of stress on behavior. A single infusion of the KOR antagonist norBNI reduced immobility in male C57Bl/6J mice and in male rats (McLaughlin et al., 2003; Carr et al., 2009). Consistent with these results, the KOR agonist U-69593 dose-dependently increased immobility while norBNI pretreatment blocked this effect in male rats (Mague et al., 2003), indicating a distinct role of KOR activity in stress-induced immobility. Further support for the therapeutic potential of KOR antagonists came from animal models of stress-induced depression and addiction relapse. For example, norBNI blocked the formation of

learned helplessness following inescapable shock in male rats (Newton et al., 2002), and reduced submissive behavior in male mice exposed to social defeat stress (Land et al., 2008; McLaughlin et al., 2006). In the reinstatement model of relapse, stressors such as forced swim can re-instate an extinguished place preference for cocaine, and KOR antagonists can prevent the reinstatement of this preference (McLaughlin et al., 2003; Carr et al., 2009; Mague et al., 2003; Newton et al., 2002; McLaughlin et al., 2006; Redila and Chavkin, 2008). Taken together, these results suggest that activation of KOR during stress facilitates depression- and anxiety-like behaviors. One question that has been difficult to answer is how the time course of KOR activation impacts behavioral responses to stress. The most common KOR antagonists, norBNI and JDTic, inhibit KOR for weeks after a single dose. Because of this long-lasting action it's been unclear whether the effects of KOR activity on behavior are due to the activity of KOR during stress ex-clusively, or if prolonged KOR activity is necessary for depression-like phenotypes. Recent work with new short-acting KOR antagonists have shed light on this important question.

Previous work with long-lasting KOR antagonists have suggested that KOR antagonists have greater efficacy if administered before stress exposure. Interestingly, when the short-acting KOR antagonist, CERC-501, was administered after social defeat stress, social avoidance was unaffected (Browne et al., 2017). In contrast, short-acting KOR an-tagonists administered immediately before episodes of social defeat stress had strong effects on behavior. Male and female California mice treated with a short-acting KOR antagonist, AZ-MTAB, 2 h prior to episodes of social defeat stress blocked the development of anhedonia two weeks later (Fig. 1A) (Williams et al., 2018). In females, AZ-MTAB also prevented the development of social avoidance. Importantly, AZ-MTAB treatment that was administered not during stress, but im-mediately before anhedonia or social interaction testing 2 weeks later, had no effect on behavior. Together these results suggest that KOR activation during stressful events induces long lasting neurobiological changes that promote depression-like behaviors for at least two weeks. Interestingly, treatment with the long-acting KOR antagonist JDTic prior to 10 days of chronic social defeat stress did not prevent the on-set of social withdrawal behavior or anhedonia in male mice (Donahue et al., 2015). This suggests that there are important differences between acute and prolonged inhibition of KOR. In addition, there is growing evidence that stress may alter the behavioral effects of KOR.

While there is strong evidence that acute activation of KOR induces depression-like behavior and aversion, there is growing evidence that these effects become weaker after exposure to stress. Many of the early studies examining the ability of KOR antagonists to reverse the effects of stress on behavior conducted tests within 24 h of stress exposure. For example, experiments showing KOR-dependent social avoidance in

male mice assessed social interaction immediately after a single episode of social defeat (Bruchas et al., 2011). However, other studies with longer periods of stress exposure or a delay between stress exposure and behavioral testing have reported different outcomes. In male c57BI/6J mice that lost aggressive encounters over a period of 3 weeks, the KOR agonist U50488 actually increased social interaction behavior (Kudryavtseva et al., 2004; Kudryavtseva et al., 2006). Consistent with these results, infusion of U50488 in to the dorsal raphe of male Cali-fornia mice exposed to social defeat reduced freezing behavior in a resident-intruder test (Wright et al., 2018). In California mice (Steinman et al., 2015) and hamsters (Gray et al., 2015), social defeat increases freezing behavior in residents tested in the resident-intruder test. Other evidence suggests that different forms of stress can reduce the aversive properties of KOR. Both chronic mild stress and chronic social defeat stress weakened KOR-dependent reinstatement of cocaine and nicotine place preference in male c57BI/6 mice (Al-Hasani et al., 2013). Similar results were seen in female California mice, where control females but not defeated females formed a place aversion to a low (2.5 mg/kg) dose of U50488 (Laman-Maharg et al., 2017). Taken together, this suggests that over time, stress-induced neuroadaptations may change the behavioral effects of KOR.

A series of studies have identified molecular pathways that mediate the short-term effects of KOR on behavior. Activation of KOR results in coupling to inhibitory $G_{i/o}$ proteins (Law et al., 2000); which in turn can induce activation of mitogen activated protein kinase (MAPK) signaling pathways via phosphorylation (Bruchas et al., 2006, 2008; Bruchas and Chavkin, 2010). Interestingly, several MAPK can be activated by KOR and it's thought that selective activation of specific signaling cascades can mediate different behavioral outcomes (Knoll and Carlezon, 2010). For example, forced swim stress induces KOR-dependent phosphoryla-tion of p38 MAPK and extracellular signal-regulated (ERK 1/2) in male C57Bl/6J mice (Bruchas et al., 2007). Infusion of the p38 MAPK in-hibitor intracerebroventricularly (icv) prevented U50488-induced im-mobility in the FST. Similarly, KOR activated p38 MAPK in serotonin neurons in the dorsal raphe, and dopamine neurons in the ventral tegmental area, are necessary for KOR- and stress-induced conditioned place aversion (Land et al., 2009; Ehrich et al., 2015). Together these data suggest that p38 MAPK signaling following KOR activation is ne-cessary for acute stress-induced behavioral responses. The extent to which this mechanism generalizes is still unclear, since all of these studies were performed in male C57Bl/6J mice. Male and female Ca-lifornia mice treated with U50488 show dose dependent increases in phospho-p38 immunoreactive cells in the nucleus accumbens, but only in microglia (Robles et al., 2014). It has been proposed that the binding of different ligands to KOR may result in different signaling cascades being activated, and that these different signaling pathways may have
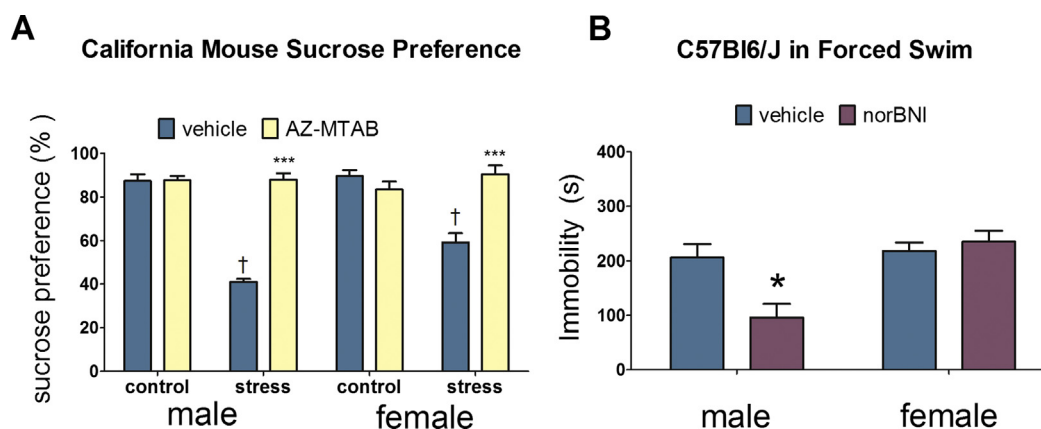

Fig. 1. Effects of KOR antagonists in the forced swim test and on anhedonia. Effects of AZ-MTAB on percent preference levels for sucrose water two weeks following social defeat stress in California mice (A, 7–14 per group). Effects of norBNI on time spent immobile in the forced swim test in C57Bl6/J mice (B, n = 5–10 per group). *p < 0.05 versus vehicle; ***p < 0.0001 versus same-sex stress/vehicle; †p < 0.01 vs. same-sex control/vehicle.

differing effects on behavior (Bruchas and Chavkin, 2010). The development of biased agonists could potentially provide novel therapeutic tools. It will be necessary to test the effects of new KOR targeting pharmaceuticals in both sexes because there is growing evidence for important sex differences in KOR function.

While the few studies examining KOR function in females cited above found similar behavioral effects of KOR in females and males, several lines of evidence indicate the potential for important sex differences. For example, sex differences exist in behavioral sensitivity of KOR agonists in males and females. Most evidence suggests that KOR activation has stronger effects on behavior in males than females (Chartoff and Mavrikaki, 2015). U50488 induces anhedonia for intracranial self-stimulation at lower doses in male rats compared to females (Russell et al., 2013) and female rats take longer to discriminate a KOR agonist from vehicle using a fixed ratio schedule of reinforcement (Craft et al., 1998). Consistent with these data, U50488 infusions in to the dorsal raphe have stronger effects on behavior in male California mice than in females (Wright et al., 2018). Finally, across a range of doses, norBNI does not reduce immobility in the FST in female California mice or c57Bl/6 mice as it does in males (Fig. 1B) (Laman-Maharg et al., 2018).

Across these studies KOR agonists and antagonists have stronger effects in males than in females. However, different results have been observed in place aversion studies. In California mice, females naïve to defeat form a CPA to a 2.5 mg/kg dose of U50488. However, at a higher 10 mg/kg dose, this aversion is no longer observed. In stressed females, two weeks following social defeat stress, CPA is not formed towards either dose of U50488 (Laman-Maharg et al., 2017). In contrast, male California mice naïve to defeat form a CPA to a high but not low dose of U50488, and following stress, males continue to show CPA to the high dose (Laman-Maharg et al., 2017; Robles et al., 2014). This suggests that before stress, females show a higher sensitivity to KOR agonists than males but that that following stress, KOR agonists no longer show aversive properties in females. In these studies, place aversion is induced after repeated exposure to U50488 and develops over several days, whereas most studies reporting stronger effects of KOR manipulations examine behavior over a shorter timeline. Further study is needed to determine whether there are consistent sex differences in the short- and long-term effects of KOR activation on behavior.

Moving forward, more data are needed on the mechanisms of action for KOR ligands in males and females. For example, while norBNI induces phosphorylation of JNK in the NAc of male C57Bl6/J, this effect is not observed in females (Laman-Maharg et al., 2018). Even less is known about the mechanism of action for short-acting KOR antagonists. To our knowledge this has not been studied in female animals or cells. Additional studies examining sex differences in behavioral responses to KOR activation or inhibition in different contexts, as well as in mechanisms of action for KOR, is needed in order to understand whether this line of potential therapeutics can be successful in both sexes. At present, it would appear that short-acting KOR antagonists may prove more valuable if used in a prophylactic approach before stress exposure (Van't Veer and Carlezon, 2013).

4. Oxytocin and oxytocin receptors

Converging lines of evidence suggest that pharmaceuticals targeting the oxytocin (OT) system could have important therapeutic uses. First, studies in nonhuman animals show that OT can reduce physiological stress response. Second, work in humans shows that intranasal OT is capable of having anxiolytic effects, at least in men. However, studies testing the efficacy of intranasal OT in clinical populations have been underwhelming (see MacDonald and Feifel, 2014) for review). One factor has been that most studies were small and focused on short term outcomes. A broader challenge is that the mechanisms through which OT modulates anxiety and social behavior are not understood. In rodents, oxytocin receptors (OTR) are expressed in hypothalamic and limbic circuits that modulate social behavior as well as stress responses (Dumais and Veenema, 2016; Insel et al., 1991). In the few primate species that have been examined, OTR is more widely expressed in new world primate species (Freeman et al., 2014) compared to old world primates such as rhesus monkey (Freeman et al., 2014). Studies in rodents have identified neural circuits in which OT can exert anxiolytic effects. However, the majority of these studies focus exclusively on males and there is growing evidence for strong sex differences in OT modulation of anxiety, especially in aversive social contexts. Furthermore, it is usually assumed that the behavioral effects of OT are mediated by OTR. Several lines of evidence indicate that behavioral effects of OT can be mediated through activation of the vasopressin V1a receptor (Song et al., 2014; Anacker et al., 2016; Song and Albers, 2018). Thus, it is critical to consider how any OT-related ligand interacts with both OTR and V1aR. Overall, while the OT system remains a promising target for novel therapeutics, many unanswered questions remain.

The idea that OT might act as a damper of physiological responses to stress grew in part out of findings in rodents that various forms of stressors increased OT levels in the blood (Wotjak et al., 1998; Lang et al., 1983; Neumann et al., 2001). When an OTR antagonist was infused in to the lateral ventricle, adrenocorticotropin hormone and corticosterone levels in both male and female rats were increased (Neumann et al., 2000). This effect was observed under stressful conditions like forced swim as well as under baseline conditions. Similarly, chronic systemic (Detillion et al., 2004) or central (Windle et al., 1997) administration of OT reduced stress-induced corticosterone in female rodents. While the HPA axis is frequently disrupted in disorders such as depression and PTSD, roughly half of patients do not exhibit disrupted cortisol secretion (Dossat et al., 2018). Nonetheless, the inhibitory effects of OT and OTR on the HPA axis sparked investigations of oxytocin modulation of anxiety-like behavior.

Overall, most evidence suggest that in both males and females OT and OTR activation reduce anxiety behaviors in novel environments. While an initial study reported no effect of icv infusion of an OTR antagonist in the elevated plus maze (Neumann et al., 2000), protocols that increased the novelty of the testing environment observed anxiolytic effects of chronic central infusion of OT in female rats (Windle et al., 1997). In male mice a single icv infusion of OT had anxiolytic effects in the elevated zero maze (Law et al., 2000) while in male rats, a single infusion of OT into the paraventricular nucleus (PVN) had anxiolytic effects in the elevated plus maze (EPM) (Blume et al., 2008). These effects appear to be mediated by OTR, as icv infusion of the OTR-specific agonist carbetocin also had anxiolytic effects in male rats tested in the EPM (Loyd and Murphy, 2014). Site-specific infusion of OT in to the central nucleus of the amygdala (CEA) also had anxiolytic effects in non-social contexts in female rats (Bale et al., 2001; Knobloch et al., 2012). While these studies generally report similar results in males and females, sex differences emerge in social contexts, especially aversive contexts.

In males, most data point towards anxiolytic effects of OT and OTR in social contexts. Male rats that were exposed to social defeat stress showed reduced social avoidance after receiving an icv infusion of OT (Lukas et al., 2011). Consistent with this effect, intranasal OT also decreased social avoidance behavior in male California mice that were exposed to social defeat (Steinman et al., 2016) while icv OTR antagonist treatment reduced social interaction in unstressed male rats (Lukas et al., 2011). A related approach pairs foot shocks with unfamiliar social interactions to induce a conditioned avoidance of novel social contexts (Toth et al., 2013). Infusion of a selective OTR agonist in to the lateral ventricle reduced this conditioned social-fear response in male rats (Craft et al., 1998). In addition, OT infusions in to the lateral septum facilitated extinction of conditioned social fear trials conducted in a familiar home cage (Zoicas et al., 2014). In human studies, OT is most often administered intranasally. Although OT is clearly behaviorally active, it's still uncertain whether OT crosses the blood brain

barrier and if so, the extent to which OT diffuses into different brain structures (Bale and Epperson, 2015). These uncertainties aside, intranasal OT was found to induce "calmness" (Heinrichs et al., 2003) and reduce negative affect (Kubzansky et al., 2012) in men following the Trier Social Stress Test (TSST) in which the audience consisted of two women. Imaging experiments showed that in men, intranasal OT reduced amygdala responses to emotional faces of both positive and negative valences (Domes et al., 2007), including angry faces (Kirsch et al., 2005; Radke et al., 2017). Similar inhibitory effects of intranasal OT on amygdala activity were observed in men with generalized anxiety disorder, although there was no effect of OT on subjective mood (Labuschagne et al., 2010). Together, these results suggest OT and OTR are anxiolytic in social contexts, at least for males. However, analyses of OT function during stressful social contexts indicate that OT can have anxiogenic effects.

Social defeat increases c-fos expression in OT neurons within the PVN (Steinman et al., 2016; Nasanbuyan et al., 2018) in both males and females, suggesting that these neurons are activated by social defeat. Consistent with these results, social defeat is associated with increased OT release in the lateral septum (Ebner et al., 2005). Interestingly, activation of OTR in the lateral septum enhances contextual fear conditioning six hours later (Guzman et al., 2013). The enhancement of adverse memories by OTR may alter behavior in future social encounters, as OTR knockout mice showed reduced submissive behaviors during a second episode of defeat (Nasanbuyan et al., 2018). In humans, men treated with intranasal OT reported higher levels of perceived stress while receiving negative evaluative feedback during a mental arithmetic task (Eckstein et al., 2014). Intranasal OT also had an anxiogenic effect in men diagnosed with depression who were instructed to discuss a distressful personal incident with a therapist (MacDonald et al., 2013). Together, these results suggest that in the short term, activation of OT or OTR during aversive social contexts amplify the effects of this experience on behavior in males. This is consistent with the social salience hypothesis of OT, which posits that OT enhances the salience of both positive and negative social experiences (Shamay-Tsoory and Abu-Akel, 2016). Less is known about how defeat affects OT and OTR systems in females. Recent evidence indicates that the long term effects of defeat on OT and OTR systems show important sex differences (Steinman et al., 2016).

While OT and OTR are generally anxiolytic in social contexts for males, experiments across multiple species have failed to detect anxiolytic effects in females. Systemic OT treatment did not reduce anxiety-inducing effects of social isolation (Grippo et al., 2012) while icv infusion of OT did not reduce social avoidance in stressed female rats (Lukas and Neumann, 2014). Furthermore, the same dose of intranasal OT that reduced social avoidance in male California mice exposed to social defeat induced social avoidance in female California mice that were naïve to defeat (Steinman et al., 2016). Interestingly, analyses of OT neurons in the PVN and anteroventral bed nucleus of the stria terminalis (BSTav) showed that social defeat increases the reactivity of these neurons (as estimated by OT/c-fos immunohistochemistry) in female but not male California mice (Steinman et al., 2016). This effect was seen up to 10 weeks after the last episode of social defeat, indicating that these changes are persistent. Immediate early gene analyses suggested the nucleus accumbens (NAc) and anteromedial BST (BSTam) as possible sites mediating OT-induced social avoidance in females. Site specific infusions of an OTR antagonist in stressed females showed that OTR inactivation in the BSTam but not NAc reduced social avoidance responses. Together these results suggest that OTR antagonists might have potential for reducing social anxiety. Supporting this hypothesis, a single i.p. treatment of 5 mg/kg the brain-accessible OTR antagonist L-368899 reduced social avoidance in stressed females (Duque-Wilckens et al., 2018). This is an exciting result because 4 weeks of daily treatment are necessary to obtain the same response with sertraline (Greenberg et al., 2014). Women are generally underrepresented in intranasal OT studies, even though depression and

anxiety disorders are more common in women. What little evidence is available suggests that the effects on intranasal OT are sex dependent in humans. While intranasal OT reduced negative affect following the TSST in men, the same dose of OT increased negative affect in women (Kubzansky et al., 2012). Consistent with these data, imaging studies show that intranasal OT enhances amygdala (Chartoff and Mavrikaki, 2015; Domes et al., 2010; Lischke et al., 2012) and anterior cingulate responses (Luo et al., 2017; Sarton et al., 2000) to fearful faces and scenes. Curiously, one study reported that while intranasal OT increased amygdala reactivity to emotional faces in healthy trauma-exposed women, women diagnosed with PTSD showed reduced responses (Koch et al., 2016). Although the mechanism for these inconsistent results is unknown, one report showed that social defeat stress reduced sex differences in DNA methyltransferase 1 mRNA within the central nucleus of the amygdala (Wright et al., 2017). If this result generalizes to other species and stressors, a change in DNA methylation could potentially have important effects on how amygdala nuclei respond to neuropeptides such as OT. In summary, it is clear that there are important sex differences in the behavioral effects of OT and OTR in aversive social contexts but the mechanisms underlying these differences have not yet been identified.

Observations that increased OT activity can have anxiogenic effects suggests alternate interpretations for some clinical findings. Women diagnosed with depression (Holt-Lunstad et al., 2011; Cryanowski et al., 2008) or PTSD (Pierrehumber et al., 2010) have been found to have elevated OT levels in plasma, and this result is usually interpreted as a homeostatic response to cope with stressful experiences. Mechanistic experiments with OTR ligands suggest that enhanced activation of OTR might actually have anxiogenic effects, at least in aversive social contexts. Familiar social contexts may be different. Female California mice exposed to social defeat showed reduced freezing behavior if treated with intranasal OT in the home cage (Steinman et al., 2016). Activation of OTR also had anxiolytic effects in female prairie voles while recovering from restraint stress in a familiar home cage (Smith and Wang, 2014). One possible explanation for these diverse behavioral effects is that different neural circuits may mediate anxiogenic and anxiolytic effects of OT Fig. 2. While inhibition of OTR in the BST increased social interaction in stressed female California mice, infusions of OT into prelimbic cortex had anxiolytic effects in female rats (Sabihi et al., 2014). While the endogenous source of OT may differ between the
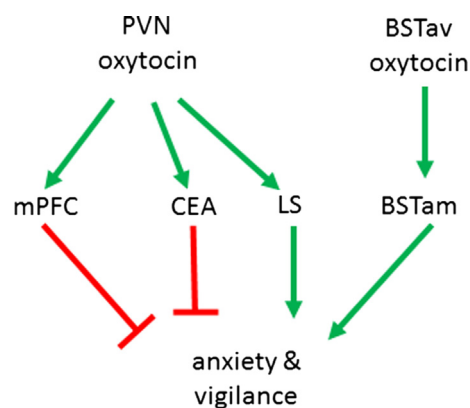

Fig. 2. Proposed oxytocin circuit in female California mice. Neural circuits mediating anxiolytic and anxiogenic effects of OT. Oxytocin neurons within the PVN send projections to the mPFC and CEA where activation of OTR exerts anxiolytic effects. Oxytocin neurons in the BSTav are activated by stress and OTR in the adjacent BSTam induces social anxiety responses. The LS also received OT projections from the PVN, but activation of OT in LS can promote social fear. PVN: paraventricular nucleus, mPFC: medial prefrontal cortex, CEA: central nucleus of the amygdala, LS: lateral septum, BSTav: bed nucleus of the stria terminalis anteroventral, BSTam: bed nucleus of the stria terminalis anteromedial.

mPFC and BSTam, the CEA and LS both receive OT fibers from the PVN. Currently, it is not clear whether different OT neurons within the PVN innervate the CEA and LS or whether the behavioral effects of OTR activation are context dependent. Further characterization and manipulation of these circuits is needed to understand how OT exert such different effects on behavior. Overall it appears that OTR agonists and antagonists have potential to reduce social anxiety, as well as other disorders with social deficits (Andari et al., 2017; Suzuki et al., 2017). The continued development of novel OTR ligands also holds great promise for more selective approaches for modulating OTR function (Muttenthaler et al., 2017). The preclinical data suggest that successful use of OTR ligands to treat social anxiety will require a personalized approach as opposed to a one-size fits all strategy.

5. Ketamine

In the mid-1990's several studies reported that antagonists for the *N*-methyl-D-aspartate (NMDA) receptor had antidepressant-like effects in animal model systems such as learned helplessness (Meloni et al., 1993) and forced swim (Layer et al., 1995; Moryl et al., 1993). One of the first small clinical trials on men and women diagnosed with treatment-resistant depression showed that a single infusion of ketamine (0.5 mg/kg) improved depression ratings up to 72 h later (Berman et al., 2000). A subsequent randomized clinical trial reported that 0.5 mg/kg improved depression ratings in 71% of patients (12 women, 6 men) with treatment resistant depression (Zarate et al., 2006). Similar antidepressant effects of ketamine were observed in a clinical trial of patients (12 women, 6 men) diagnosed with treatment resistant bipolar depression (Diazgranados et al., 2010). One of the first larger clinical trials reported that ketamine reduced depression ratings in patients (35 men, 37 women) with treatment resistant depression (Murrough et al., 2013). Recent evidence also suggests that ketamine treatment (0.5 mg/kg) reduced suicidal thoughts in patients (48 women, 32 men) diagnosed with depression (Grunebaum et al., 2017). An important feature of these studies is that participants were diagnosed with treatment resistant depression, meaning that they did not respond to standard antidepressant medications. Thus, the fact that positive responses to ketamine were observed is of high clinical significance. None of these studies conducted analyses to determine whether effects of ketamine differed between men and women. However, emerging evidence in rodents suggest that there could be important sex differences in dose-response curves as well as mechanism of action. The doses tested in these rodent studies are generally 10 times higher than doses used in humans based in part on higher metabolic rates of smaller animals (see (Boxenbaum and DiLea, 1995) for discussion).

One of the first studies to consider sex differences in the antidepressant effects of ketamine reported that female Sprague-Dawley rats showed reduced immobility in the forced swim test and reduced latency to feed in the novelty suppressed feeding test following 2.5 mg/kg i.p. of ketamine (Carrier and Kabbaj, 2013). This dose had no effect on behavior in male rats. Female C57Bl6/J mice also showed reduced immobility in the forced swim test following treatment with lower doses of ketamine (3 mg/kg) that were not behaviorally active in males (Franceschelli et al., 2015; Zanos et al., 2016). In female rats, effects of low doses of ketamine on sucrose preference were dependent on gonadal hormones (Saland et al., 2016). Ovariectomized females showed no change in response to ketamine treatment while ovariectomized females treated with implants containing estradiol and progesterone showed increased sucrose consumption following ketamine treatment. In the forced swim test, effects of ketamine are enhanced by estrogen receptors. During diestrus, when endogenous estrogen levels are at lower levels, estrogen receptor α or β agonists increased sensitivity to low doses of ketamine in the forced swim test (Dossat et al., 2018). An important aspect of these studies is that behavioral results come from assays that are predictive of antidepressant action, such as forced swim (Cryan et al., 2005). One disadvantage of some of these assays is that

behavioral effects may rely on different mechanisms than is required for clinical efficacy. For example, acute SSRI treatment reduces immobility in the forced swim test while chronic SSRI treatment is required for a clinical response in humans. Complementary approaches like chronic mild stress (Hodes et al., 2015) and social defeat stress (Golden et al., 2011) can induce depression- and anxiety-like behavioral responses that allow for the assessment of antidepressant action.

Only a few studies have tested the effects of ketamine in rodent models of stress-induced psychiatric illness. In male rats exposed to chronic mild stress (CMS), acute ketamine (10 mg/kg) increased sucrose preferences and decreased latency to feed in a novel environment (Li et al., 2011). However, acute ketamine treatment (15 mg/kg) also increased sucrose consumption in unstressed males, suggesting that these responses may not be specific to stress (Garcia et al., 2009). Similar ketamine-induced decreases in immobility were observed in male C57Bl6/J mice exposed to control or CMS, although stronger effects were observed in stressed males (Franceschelli et al., 2015). Acute ketamine treatment (20 mg/kg) also blocked social defeat-induced social avoidance, but this effect was not sustained one week later (Donahue et al., 2014). To achieve this same response with SSRI, four weeks of chronic treatment is required (Berton et al., 2006). Subsequent studies suggested that individual differences in behavioral responses to defeat moderate the impact of ketamine. In male C57Bl6/J mice exposed to social defeat, some mice exhibit a susceptible phenotype defined as reduced interaction with an unfamiliar mouse while mice presenting with an unsusceptible maintain high levels of social interaction (Krishnan et al., 2007). Mice showing a susceptible phenotype showed increased social interaction following a single treatment of ketamine (Zanos et al., 2016). Ketamine treatment had no effects on social interaction in stressed male mice exhibiting an unsusceptible phenotype, although this may have been due to a ceiling effect. In susceptible males acute ketamine treatment (10 mg/kg) was also found to ameliorate defeat-induced sucrose anhedonia and increased immobility in the forced swim test for up to 3 days (Zhang et al., 2018; Qu et al., 2017). In these studies, mice exhibiting an unsusceptible phenotype based on the social interaction test were excluded. Other studies of male C57Bl/6NTac mice showed that ketamine (30 mg/kg) administered before social defeat or inescapable shock prevented stress-induced social avoidance and learned helplessness respectively (Brachman et al., 2016). Curiously, ketamine administered after defeat stress did not reduce stress-induced immobility, although social avoidance was not examined. The lack of efficacy of post-stress ketamine treatment on immobility would appear to conflict with other studies in male mice (Zhang et al., 2018; Qu et al., 2017). Furthermore, another study of male C57Bl/6J mice reported that pre-defeat ketamine did not block the development of social avoidance (Donahue et al., 2014). One important factor contributing to these variable results is that different strains of mice were used across the different studies. There are many genetic differences between different C57Bl6 lines (Keane et al., 2011), including entire deletions of exons (Mekada et al., 2009). Currently it is not clear how genetic differences between inbred mouse lines may impact responses to ketamine. One possibility is that higher corticosterone levels in C57Bl/6NTac mice compared to C57Bl/6J are required for the prophylactic effects of ketamine. While this hypothesis has not been tested directly, further study of sex differences in behavioral and neural responses to ketamine could be extremely informative on this question. In most species of rodents corticosterone levels are higher in females than males (Trainor et al., 2010; Handa and Weiser, 2014).

For females, ketamine also exerts antidepressant effects in model systems of depression or anxiety, but the effects are usually weaker than in males. In rats, social isolation stress increased immobility in the forced swim test for both males and females, and a moderate dose of ketamine (5 mg/kg) reduced immobility for both sexes (Sarkar and Kabbaj, 2016). However, while the effect of ketamine reduced immobility in stressed males to the levels of controls, the effect was weaker in females. Similar weaker effects of ketamine in females during

the forced swim test were also observed in C57Bl6/J mice exposed to chronic mild stress (Franceschelli et al., 2015). In a witness defeat approach, female C57Bl6/J mice treated with a single dose of ketamine (20 mg/kg) showed higher levels of social interaction with an unfamiliar female than saline treated females (Iñiguez et al., 2018). While the dose of ketamine used here was higher than other studies, there were no differences in locomotor behavior. So far, no study has tested whether pre-stress ketamine treatment has the same prophylactic effects that have been observed in male C57Bl/6NTac mice. A potentially concerning outcome was observed when repeated ketamine administration (10 mg/kg daily for 3 weeks) was tested in C57Bl/6J mice (Thelen et al., 2016). For males, this dosing regimen reduced immobility in the forced swim test and had anxiolytic effects in the open field test. However, for females this dosing regimen increased immobility in the forced swim test. It's not clear whether lower doses would have the same effect in females, or whether females exposed to CMS or witness defeat would show a different response. Still, these observations highlight the relative lack of knowledge on the anti-depressive effects of ketamine in females. Even less is known about the mechanisms underlying the effects of ketamine in females.

One of the first studies to examine molecular mechanisms of the antidepressant effects of ketamine focused on neuroplasticity in the hippocampus (Autry et al., 2011). Ketamine induces rapid and sustained increases in brain derived neurotrophin factor (BDNF) protein in the hippocampus. This response was blocked with the translation inhibitor anisomycin but not the transcriptional inhibitor actinomycin D, suggesting that ketamine modulates synthesis of BDNF protein rather than increasing *Bdnf* gene expression. Consistent with this translational mechanism, ketamine also decreases phosphorylation of eukaryotic elongation factor 2 (eEF2). Dephosphorylation of eEF2 leads to enhanced ribosomal translocation and protein synthesis. A subsequent report presented evidence that the ketamine metabolite (2R,6R)-hydroxynorketamine (HNK) could induce antidepressant effects in forced swim, novelty suppressed feeding as well as defeat-induced social avoidance (Zanos et al., 2016). This is a potentially very important finding since (2R;6 R)-HNK does not have many of the adverse side effects associated with ketamine. As reported for ketamine, (2R,6R)-HNK was also found in decrease phosphorylation of eEF2 and increase BDNF protein in the hippocampus. However, in this case changes were only observed 24 hr after treatment and not 1 hr after treatment. There is disagreement over the precise mechanism of action for (2R,6R)-HNK. One the one hand, (2R,6R)-HNK did not displace tritiated MK-801 from NMDA receptors in vitro and was found to not functionally inhibit NMDA receptors in hippocampal slices. These findings have been interpreted as evidence that (2R,6R)-HNK may work independently of NMDA receptors. On the other hand higher doses of (2R,6R)-HNK were found to reduce NMDA receptor currents in cultured hippocampal neurons (Suzuki et al., 2017). Further study of these intracellular mechanisms is needed to sort out the precise role of ketamine and its metabolites. An additional blind spot in this literature is that it's not clear whether electrophysiological studies have been conducted on neurons from both males and females. Some initial analyses suggest that there could be sex differences in the mechanism of ketamine action.

In female C57Bl6/J mice, 3 mg/kg of ketamine increased BDNF in the hippocampus during diestrus but not proestrus even though ketamine reduced immobility during both phases of the estrus cycle (Dossat et al., 2018). This suggests the possibility that there could be complementary mechanisms of ketamine actions. In addition, systemic ketamine treatment results in higher brain concentrations of (2R,6R)-HNK in females than males (Zanos et al., 2016). Despite evidence for important sex differences in sensitivity to and metabolism of ketamine, it is not clear whether any electrophysiological studies of ketamine have considered sex as a biological variable. Sex differences in signal transduction of G-protein receptors have been observed in cortical neurons (Bangasser et al., 2010), so future studies should include neurons from

Table 1
Summary of findings of sex differences for the effects of different classes of pharmaceuticals with antidepressant and anxiolytic properties. X = observed effect, ? = unknown effect.

Target	Administration timing for efficacy	Males more sensitive	Females more sensitive	Behavior test where targets show efficacy
SSRI	Chronic		X	Forced Swim Test Elevated Plus Maze Open Field
KOR	Acute (before stress)	X		Forced Swim Test (males) Social Interaction Test Sucrose Preference Test Autogrooming Assays Inescapable Shock
OTR	Acute	X (OT)	X (OTA)	Elevated Plus Maze Elevated Zero Maze Social Interaction Test
Ketamine	Acute		?	Forced Swim Test Novelty-Suppressed Feeding Sucrose Preference Test Social Interaction Test

both males and females. Thus, although ketamine has similar behavioral effects in male and female rodents, effects on brain function are quite different. At this time, most clinical trials have not detected strong differences in antidepressant responses to ketamine between men and women. However, these trials may not have been sufficiently powered to detect sex differences and preclinical data suggest that the mechanisms underlying antidepressant responses to ketamine could be quite different in men and women.

6. Conclusions

After decades of heavy reliance on monoamine-based therapies, emerging data suggest that pharmaceuticals targeting opioid, oxytocin, or NMDA receptors could have exciting applications for treating stress-related disorders including anxiety and depression. In some cases, compounds with anxiolytic or antidepressant properties have similar effects in males and females (Table 1). In other cases striking differences in the behavioral effects of these compounds are observed between males and females. Sufficiently powered studies or metanalyses should assess whether clinical and neurobiological responses to novel antidepressants differ between men and women. Overall, these findings highlight how essential it is to include both sexes in preclinical and clinical assessment of novel pharmaceuticals. As the field moves towards more personalized approaches for treating mental illness, consideration of sex as a biological variable should be a key piece in solving this puzzle.

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