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The contribution of orexins to sex differences in the stress response

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Abstract

Women are twice as likely as men to suffer from stress-related psychiatric disorders, such as post-traumatic stress disorder (PTSD) and Major Depressive Disorder (MDD), however, the biological basis of these sex differences is not fully understood. Interestingly, orexins are known to be dysregulated in these disorders. This review first discusses the important role of orexins regulating the response to stress. Next, we review the evidence for sex differences in the orexin system, in which the majority of both preclinical and clinical studies have reported higher orexin system expression in females. Finally, we discuss the functional consequences of these sex differences in orexin expression. Most importantly, the preclinical literature reveals that higher orexin system activity in females contributes to exaggerated neuroendocrine and behavioral responses to stress. In sum, the available data suggests that orexins may be important in the etiology of stress-related psychiatric disorders that present differently in men and women. Thus, targeting orexins could potentially ameliorate many phenotypes of stress-related illness in a sex-specific way.

Keywords

Orexins; Hypocretins; Stress; HPA axis; Habituation; Sex differences

1. Introduction

Women are twice as likely as men to suffer from stress-related psychiatric disorders, such as post-traumatic stress disorder (PTSD) and Major Depressive Disorder (MDD), however, the biological basis of these sex differences is not fully understood (Keane et al., 2006; Kessler et al., 1994; Nestler et al., 2002; Seeman, 1997; Sheikh et al., 2002). This review will first discuss the role of orexins in regulating the stress response and next describe sex differences in the orexin system that could contribute to sex differences in the stress response. This information, together with the evidence that orexins are known to be altered in stress-related psychiatric disorders such as PTSD and MDD (Brundin et al., 2007; Johnson et al., 2010), strongly support the idea that orexins significantly contribute to sex differences in the prevalence of stress-related psychiatric disorders.

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2. The role of orexins in the stress response

Beyond their integral role in mediating general arousal and wakefulness, orexins are important in the response to stressful stimuli, which requires the animal to shift from a basal to a reactive state (Berridge and España, 2005). Thus, orexins are important in regulating the neurobiological systems that respond to stressful stimuli. The literature indicates that orexins promote the acute stress response, while less is known about the role of these neuropeptides in adaptations to repeated stress. Below, we will discuss the current data concerning the involvement of orexins in responses to both acute and to repeated stress.

Acute stress activates orexin neurons. For example, acute psychological stressors such as forced swim stress (Chang et al., 2007) and restraint stress (Grafe et al., 2017b) produce increases in both the percentage of orexin neurons dual labeled with cFos (a measure of neural activation) and orexin A levels in the cerebrospinal fluid. Acute physical stressors such as intraperitoneal injections and exercise have also been shown to increase numbers of orexin cells activated (Messina et al., 2016; Panhelainen and Korpi, 2012). Lastly, direct administration of stress peptide Corticotropin Releasing Hormone (CRH) has been shown to increase orexin neuron activity (Winsky-Sommerer et al., 2004). Thus, a variety of stressful stimuli, whether psychological or physical, activate orexin neurons.

Reciprocally, orexins impact acute stress-induced behavioral responses (Chen et al., 2013; Johnson et al., 2010; Li et al., 2010; Sears et al., 2013; Staples and Cornish, 2014; Steiner et al., 2012) and promote the Hypothalamic-Pituitary-Adrenal (HPA) axis response to acute stress (Berridge et al., 2010; Johnson et al., 2012; Winsky-Sommerer et al., 2005, 2004). In terms of promoting stress-induced behaviors, stimulation of orexins by sodium lactate has been shown to promote panic anxiety in rodents, and this is reversed with orexin siRNA or a systemic orexin type 1 receptor (OX_1R) antagonist (Johnson et al., 2010). Both nonselective and selective orexin receptor antagonists, administered either systemically or into the locus coeruleus, reduce threat learning with footshock stress (Chen et al., 2013; Sears et al., 2013; Steiner et al., 2013). While a systemic OX_1R antagonist did not reduce anxiety in the elevated plus maze (Staples and Cornish, 2014), orexin administration into the PVT was found to induce anxiety-like behaviors in the elevated plus maze, and this was reduced with antagonists for both CRF and the Orexin type 2 receptor (OX_2R). With regards to promoting the neuroendocrine response to stress, optogenetic stimulation of orexin neurons increases cFos expression CRH neurons in the paraventricular hypothalamic nucleus (PVN), likely through OX_2R activation (Bonnavion et al., 2015; Trivedi et al., 1998). Central administration of orexins increases downstream HPA hormones Adrenocorticotropic Hormone (ACTH) and corticosterone, and this can be reversed with an *icv* administered CRH antagonist (Al-Barazanji et al., 2001; Jászberényi et al., 2000; Kuru et al., 2000; Spinazzi et al., 2006). Beyond stimulating ACTH and corticosterone by way of CRH, orexins may also promote release of ACTH directly through its actions on both OX_1R and OX_2R in the pituitary (Date et al., 2000), and studies in human and rat adrenocortical cells revealed that orexins can stimulate glucocorticoids directly via OX_1R in the adrenal gland (López et al., 1999; Mazzocchi et al., 2001; Ziolkowska et al., 2005).

While the role of orexins in acute stress appears to be straightforward (acute stress activates orexins neurons, and orexins promote the neuroendocrine response to stress), the role of the orexin system in regulating responses to repeated stress is not as clearly defined. Whether orexin function is increased or decreased with repeated stress may be contingent on the type, intensity, and duration of the stressor (James et al., 2017; Yeoh et al., 2014), and whether habituation takes place. Failure to habituate to a stressor is a hallmark of stress-related illnesses such as PTSD and panic disorder (Grissom and Bhatnagar, 2009; Johnson et al., 2012). Interestingly, patients with panic anxiety symptoms have higher levels of orexin in their cerebrospinal fluid (CSF) (Johnson et al., 2010).

In studies using repeated exposure to the prototypical stress of restraint, both hypoactivity and hyperactivity of the orexin system have been reported. For example, both orexin neuronal activation (measured by dual cFos and orexin immunohistochemistry) and orexin A levels in the cerebrospinal fluid decreased after HPA habituation to 5 consecutive days of 30-min restraint in male rats (Grafe et al., 2017b). However, another study found that orexins were upregulated in the basolateral amygdala (BLA) of male mice after 14 consecutive days of 2-hour restraint stress, but habituation was not assessed (Kim et al., 2015). It is important to note that this was likely upregulation of orexin expression in terminals, as cell bodies in the BLA are not known to contain orexin cells. Regardless, rats in the former study habituated, while it is possible that rats in the latter study did not, suggesting that orexin levels decrease with repeated restraint stress only if habituation takes place. Indeed, in a study of male and female repeated restraint, female rats did not habituate as fully as males, and thus, had higher levels of orexin neuronal activation (Grafe et al., 2017a). In this simplistic view of the orexin system's role in repeated stress, if habituation does not take place, orexin function is increased, whereas if habituation does take place, orexin function is decreased. The response of the adult orexin system to restraint when primed with early life stress may be even more complex. For example, early life stress (by maternal separation) dampened the response of orexin neurons to restraint and increased anxiety-like behavior (as measured by the open field test), but this could be reversed with exposure to exercise in adolescence in male rats only (James et al., 2014). These findings highlight the inherent plasticity of the orexin system and a need to better understand sex-specific changes in orexin circuits and stress-related pathology.

Both decreased and increased orexin function have also been observed in rodents exposed to repeated social defeat. Specifically, decreased prepro-orexin mRNA and orexin A and B peptides were observed in the hypothalamus after social defeat paradigms in both mice and rats (Lutter et al., 2008; Nocjar et al., 2012). Though HPA habituation was not assessed in these paradigms, it has been suggested that when a stressor is chronic, predictable, and inescapable, this leads to cessation of coping behaviors, orexin system hypoactivity, and may lead to depressive-like behavior (Mahler et al., 2014). However, previous literature describes naturally occurring differences in response to social defeat, where some rodents display a passive coping strategy while others display an active coping strategy (Chen et al., 2015; Pearson-Leary et al., 2017; Wood et al., 2010). The passive coping strategy, assessed by a short latency to defeat, is typically associated with delayed habituation to repeated defeat, anxiety- and depressive-like behaviors, whereas the active coping strategy is not, suggesting vulnerability and resiliency to social defeat, respectively (Finnell et al., 2017; Wood et al.,

2010). In a recent study, we found that active coping behaviors in social defeat are associated with lower orexin function in male rats. Moreover, we found that inhibiting orexins by DREADDs promotes resilience to repeated social defeat in previously passive coping rodents (Grafe et al., 2018). Thus, lowering orexins may underlie resilience and habituation to social defeat stress.

In short, it is clear that the neuropeptides orexins are important regulators of the stress response. The role orexins play in acute stress is relatively straightforward, with numerous studies providing evidence of orexins increasing HPA activity, while acute stress produces reliable increases in orexin actions, thus, this relationship between orexins and acute stress is reciprocal. While orexins modulate the response to repeated stress, less data is available on this topic. Currently, it appears that the involvement of the orexin system in repeated stress depends on the type, intensity, and duration of the stressor (James et al., 2017; Yeoh et al., 2014), and whether one habituates to this repeated stress. There are significant gaps in the literature that need to be addressed. First, more research should be done on the particular brain regions that orexins modulate during repeated stress. Some likely brain regions include the PVN, amygdala, paraventricular thalamus, and locus coeruleus (Grafe et al., 2017b). Additionally, it would be helpful to measure orexin function in the same individuals before, during, and after stress in order to determine exactly how this system changes in response to repeated stress. Currently, dependable measures of central orexins (mRNA, protein in brain and csf) require terminal procedures in rodent studies. Though we are able to take repeated measures of plasma orexin in rat, this may not be a reliable indication of central orexin activity (Dalal et al., 2001). Therefore, we cannot directly determine whether differences in orexin expression pre-dates exposure to stress. Future studies that assess central orexin activity before, during, and after stress (perhaps by calcium imaging) would be ideal. Lastly, more studies examining the effects of early life stress on later orexin function such as (Campbell et al., 2015; James et al., 2014) may shed more light on long term plasticity exhibited by the orexin system.

3. Sex differences in orexin expression

Most studies examining the orexin system, whether preclinical or clinical, have only used male subjects. However, a limited number of studies examining both male and female subjects have reported sex differences in orexin expression. Below, we will first discuss what has been shown in the preclinical literature regarding these apparent sex differences in orexins, and next discuss the role of gonadal hormones in contributing to the differences observed. We will then review clinical studies examining sex differences in orexin expression. For a summary of sex differences in orexin measures, please see Table 1.

3.1. Preclinical studies

The first studies to reveal sex differences in prepro-orexin and orexin receptor mRNA both peripherally and centrally were published over 15 years ago (Jöhren et al., 2002, 2001; Taheri et al., 1999). Importantly, qPCR analysis revealed that prepro-orexin mRNA in the hypothalamus was two times higher in female rats compared with male rats (Jöhren et al., 2002). A detailed analysis of the distribution of orexin A content indicated that it is in the

lateral and posterior portions of the hypothalamus where these sex differences are apparent (Taheri et al., 1999). More recent studies have confirmed the higher mRNA expression of prepro-orexin the in the lateral hypothalamus of female rats, and extended these findings to show increased activation of orexin neurons in female rats (by cFos and orexin dual immunohistochemistry), as well as higher levels of Orexin A in the cerebrospinal fluid compared with male rats (Grafe et al., 2017a). Earlier studies examining orexin receptor expression found that OX_1R were more highly expressed in female hypothalamic tissue compared to that of males, while OX_2R expression was not sexually dimorphic (Jöhren et al., 2002). However, a more recent study found that OX_2R expression specifically in the paraventricular nucleus of the hypothalamus (PVN) was in higher female rats compared with male rats (Loewen et al., 2017). Increased PVN OX_2R expression might suggest increased CRH activity in response to orexins, exacerbating the response to stress in female rats. While at much lower levels than in the brain, sexually dimorphic expression of both orexin receptors were also observed in the pituitary and adrenal glands, suggesting sex-specific roles of orexins in the control of endocrine functions at multiple levels of the HPA and potentially other neuroendocrine axes (Jöhren et al., 2001).

To determine whether gonadal hormones might regulate these sex differences in central orexin expression, various labs examined orexin expression in the lateral hypothalamus over the estrous cycle. While two studies report that expression of orexins and their receptors (both mRNA and protein) are increased in the lateral hypothalamus in proestrus compared to diestrus (Porkka-Heiskanen et al., 2004; Silveyra et al., 2010), another study found that there is no change in hypothalamic orexin mRNA levels throughout the estrous cycle (Wang et al., 2003). Our lab has examined the relationship between estrogen and testosterone levels in the plasma with measures of orexin expression and activation in the lateral hypothalamus. In support of the former two studies, we found that estrogen plasma levels positively correlate with orexin neural activation (unpublished). Specifically, as estrogen concentrations increased (100 pg/ml and up) to levels characteristic of proestrous (Dupon and Kim, 1973; Faccio et al., 2013; Zhu et al., 2013), the numbers of activated orexin neurons increased in females to above male levels. However, we found no correlation between testosterone and activated orexin cells in male rats. Moreover, we did not observe this correlation in estrogen or testosterone levels with orexin mRNA expression in the lateral hypothalamus. A separate gonadectomy study found that replacement with estrogen or testosterone did not affect prepro-orexin or orexin receptor mRNA levels in the hypothalamus (Jöhren et al., 2003). Thus, while there are indeed sex differences in orexin activation and expression in the brain, the current data fail to elucidate whether activational effects of estrogen and testosterone regulate these differences. It should be noted that mRNA and protein levels may not always positively correlate, as transcriptional and translational regulation mechanisms differ (Tian et al., 2004). Thus, studies evaluating gonadal regulation of orexins at the mRNA level versus protein level may differ due to this fact.

Other studies have focused on the relationship between orexins and higher levels of the hypothalamic-pituitary-gonadal axis, e.g., at the level of the hypothalamus, where gonadotropin releasing hormone (GnRH) is secreted (Greep, 1973), or at the level of the pituitary, where luteinizing hormone (LH) is released, triggering ovulation in females and production of testosterone in males (McCann and Ramirez, 1964). Several studies have

demonstrated that GnRH neurons are innervated by orexins, express OX_1R, and release GnRH in response to orexins (Campbell et al., 2003; Iqbal et al., 2001; Small et al., 2003). Moreover, orexins have been found to modulate LH release (Pu et al., 1998). A more recent study has demonstrated that increased expression of orexins and their receptors in the lateral hypothalamus is associated with the LH surge in proestrus rats and is abolished in neonatally androgenized female rats (Cataldi et al., 2017). Furthermore, perinatally demasculinized male rats that were treated with estrogen and progesterone in adulthood showed increased expression of orexins and their receptors (Cataldi et al., 2017; Muschamp et al., 2007). Thus, it appears that organizational effects of gonadal hormones may be responsible for the sex differences observed in orexin expression.

Other studies explored how hormones like prolactin and oxytocin might regulate orexin expression by studying lactating female rats. Interestingly, in lactating females, where prolactin and oxytocin levels are very high (Higuchi and Okere, 2002; Sinha, 1995), increased prepro-orexin mRNA levels have been observed compared with pregnant females (Wang et al., 2003). Another study found that while the number of orexin neurons was not increased in lactating females, orexin neural activation was significantly increased (Espña et al., 2004). Thus, besides sex differences in orexin expression and activation, there may be further regulation of orexin expression within female rats depending on their reproductive status. Indeed, a more recent study has indicated that orexin A administration into the medial preoptic area promotes maternal behavior (namely, pup licking) in lactating rats, and this is reversed with an orexin 1 receptor antagonist (Rivas et al., 2016).

In summary, preclinical data highlight a definite sex difference in lateral hypothalamic expression of orexins (Jöhren et al., 2002; Taheri et al., 1999), with females expressing more orexin mRNA and showing more activation of orexin neurons as well as a higher concentration of the orexin A peptide in cerebrospinal fluid (Grafe et al., 2017a). There is a lot of variability in results regarding gonadal regulation of orexin expression, which may be due to differences in age or strain of rat amongst other things, as these factors varied between labs. However, on the whole, gonadal hormones have been shown to play an important role in sex-specific orexin expression (Cataldi et al., 2017; Porkka-Heiskanen et al., 2004; Silveyra et al., 2007), with possible organizational effects, though the exact mechanism by which this occurs has not yet been determined. In addition, it should be noted that organizational effects potentially occurring during adolescence have not been addressed nor has the orexin system been studied with any depth in the adolescent animal.

3.2. Clinical studies

Sex differences in orexin expression have recently been reported in the clinical literature as well. For example, orexin A immunoreactivity in postmortem anterior cingulate cortex and dorsolateral prefrontal cortex was reported to be increased in female patients with MDD compared with male patients with MDD (Lu et al., 2017). While they did not observe sex differences between control subjects, it is important to note that all of the samples were from postmenopausal women, and thus, if gonadal hormone regulation of orexins is important (beyond perinatal or pubertal organizational effects), we may not expect to see differences in this population. Interestingly, orexin immunoreactivity showed diurnal regulation in control

subjects and this was absent in MDD patients. These findings demonstrate the well-established role that orexins have in sleep-wake regulation, and the absence of diurnal fluctuation observed in MDD patients may contribute to comorbid sleep disorders observed in depression (Salomon et al., 2003). Another study examining control and Alzheimer's patients found that females had higher orexin levels in the cerebrospinal fluid than males, regardless of the diagnosis (Schmidt et al., 2013). This differs slightly from a previous study in which higher orexin levels were found in female patients with dementia with Lewy bodies compared to males with this diagnosis, as well as both control males and females (Wennström et al., 2012). As Alzheimer's has a higher incidence in women (Gao et al., 1998), with pronounced impairments in sleep and cognition (of which orexins are known to regulate), this clinical sex difference in orexin expression is not surprising. Interestingly, narcolepsy, due to hypofunction of orexins, is more common in men, whereas insomnia, possibly due to overactive orexin action, is more common in women (Mallampalli and Carter, 2014; Nakamura et al., 2011; Prober et al., 2006), further supporting the clinical observation of higher orexin expression in women.

There are some data from the clinical literature that do not support definite sex differences in orexin expression. For example, one earlier study found that there are no differences in plasma orexin levels between healthy male and female subjects (Arihara et al., 2001). However, orexin levels in plasma are close to the resolving limit of radioimmunoassay, and thus, may not be accurate (Chen et al., 2015). Moreover, measures of orexin in plasma may not be physiologically meaningful as they do not represent central orexin system activity. For example, plasma levels of orexins do not differ between narcoleptic patients and normal controls, whereas central levels of these neuropeptides are the hallmark of this disorder (Dalal et al., 2001). Another recent clinical trial found that similar doses of the dual orexin antagonist suvorexant were equally effective in sleep onset and maintenance in male and female patients (Herring et al., 2017). However, this study did not account for menstrual cycle or menopausal status in women. Moreover, the effect of an antagonist on both types of orexin receptors at many potential binding sites (both central and peripheral), may not provide a clear evaluation of potential existing sex differences in the orexin system.

In short, there are a limited number of clinical studies examining sex differences in orexin expression. However, the available data indicate that female subjects may express higher levels of central orexin measures (orexin in the CSF and orexin immunoreactivity in specific brain regions) than males (Schmidt et al., 2013), especially in patients who have been diagnosed with MDD, Alzheimer's, or dementia with Lewy bodies (Lu et al., 2017; Schmidt et al., 2013; Wennström et al., 2012). As these disorders are more common in women, orexins may be an important substrate in contributing to the development of these disorders. While other studies have not found sex differences in orexin expression, these data may include peripheral measures and actions of orexins, which may not be as physiologically meaningful. If females express higher orexin system activity, it would be pertinent to consider sex-specific guidelines in the dosing of FDA approved orexin antagonist suvorexant (Belsomra) for treatment of insomnia. However, studies would also have to take into account the metabolization of suvorexant in both sexes; Zolpidem (Ambien), which is also used to treat insomnia (exerting sedative effects by binding to the GABA receptor), is known to be metabolized more slowly in females, and thus, is one of the only FDA approved drugs to

have sex-specific dosing (Mallampalli and Carter, 2014). It is clear that more research needs to be done in evaluating sex differences in orexin system function so that treatments targeting this system are equally effective in both males and females.

4. The contribution of orexins to sex differences in the stress response

While sex differences in orexin expression and activation have been previously reported (Cataldi et al., 2017; Grafe et al., 2017a; Jöhren et al., 2002, 2001; Porkka-Heiskanen et al., 2004; Schmidt et al., 2013; Silveyra et al., 2007; Taheri et al., 1999), the functional consequences of this dimorphism remained unclear. As orexins regulate stress, sleep, cognitive function, food intake, autonomic responses, and emotional memory, (Berridge et al., 2010; Hungs and Mignot, 2001; Johnson et al., 2012; Kilduff and Peyron, 2000; Sakurai, 2014; Winsky-Sommerer et al., 2005; Yamashita and Yamanaka, 2017), sex differences in the orexin system may have widespread functional effects. Below, we will discuss the current literature exploring how sex differences in the orexin system contribute to sex differences in the stress response, as well as other stress-related behaviors.

We recently evaluated the role of orexins in mediating sex differences in the HPA response to repeated stress and in subsequent cognitive flexibility (Grafe et al., 2017a). Using inhibitory DREADDs specifically targeted to orexin neurons, we determined that elevated orexins in female rats are responsible for heightened HPA responses to repeated restraint stress and stress-induced cognitive impairments (as assessed by the Attentional Set Shifting Test). For the first time, this data suggested that orexins mediate sex differences in functions that are altered in stress-related psychiatric disorders such as MDD and PTSD. We next used chromatin immunoprecipitation (ChIP) to determine that glucocorticoid receptors act directly on the orexin promoter to increase prepro-orexin expression in females, providing a novel regulatory mechanism for the control of orexin system activity. Additional studies from our lab have shown that the orexin 1 receptor antagonist SB334867 differentially affects arousal-related behaviors in an open field test in males versus female rats; moreover, these effects are age-dependent (Blume et al., 2018). Thus, while it is important to evaluate how orexins mediate sex differences in stress-related behaviors in adults, it may also be critical to investigate the role that these neuropeptides play in the stress response throughout development.

Two recent studies have examined the role orexins play in stress exposure both early in life and in adolescence, and how that affects responsivity to stress in both sexes as adults. For example, early life stress (by maternal separation) decreases the response of orexin neurons to restraint in adults (consistent with a depressive-like phenotype), but this is reversed with exposure to exercise in adolescence in male rats only (James et al., 2014). On the other hand, social isolation during adolescence (day 30–50) increases the female adrenal response to restraint, but decreases the male adrenal response to restraint in adulthood. Interestingly, socially isolated males have lower levels of orexin mRNA compared to group housed males (Weintraub et al., 2010). Thus, there are sex-specific effects on orexin system function and responsivity to stress in adulthood depending on the type of stressor used in critical periods of development. Further research must be done to evaluate if the orexin system is directly regulating these changes in stress reactivity later in life.

Other preclinical studies have also noted that orexins may contribute to sex differences in stress-related behaviors. For example, a recent study found that altered orexin signaling underlies stress-induced abrupt awakening in female rats, which can be observed even 21 days after the stress has ended, when rats are re-exposed to the original footshock context (Yu et al., 2016). The abrupt awakening was present in both female rats that had undergone footshock stress and also those that had witnessed cage mates undergoing footshock stress, demonstrating that observing a stressful event is sufficient to cause these orexin-mediated disruptions in arousal. This sex-specific, orexin-mediated behavior is extremely clinically relevant, as increased arousability, including an exaggerated startle response, sleep disturbances, and hypervigilance, is a key phenotype of stress-related psychiatric disorders such as PTSD, often predating a formal diagnosis of these disorders (Benca, 1996; Jorge, 2015). Importantly, a novel orexin 1 receptor antagonist has recently been discovered that will reduce panic-like responses in rodents with no apparent sedative effects, and consequently, may be a good candidate for treatment of anxiety disorders (Bonaventure et al., 2017).

Another recent study found that chronic unpredictable mild stress resulted in depressive-like and anxiety-like behaviors (as measured by sucrose preference and open field tests) in female but not male rats (Lu et al., 2017). Interestingly, there was a significant positive correlation between CRH mRNA and prepro-orexin mRNA and a significant increase in OX_1R in the frontal cortex of female but not male rats. This suggests a sex-specific interaction between the HPA axis and the orexin system that leads to these sex-specific stress-induced behavioral consequences. The involvement of orexins in regulating the response to chronic unpredictable mild stress is further supported by earlier studies that have shown that a dual orexin receptor antagonist (almorexant) prevents HPA axis dysregulation produced by this stress model (Nollet et al., 2012).

Together, these data support the idea that females demonstrate higher orexin system activity compared with males, and this contributes to sex differences in the response to stress. The majority of these studies providing evidence for functional consequences of sex differences in the orexin system have only been published within the last couple of years. While the studies discussed in this section focus specifically on stress-related functional consequences, others have reported that sex differences in orexins contribute to differences in reward behaviors (Cason and Aston-Jones, 2014) and feeding behaviors (Fukushima et al., 2015). However, it has been suggested the orexins only regulate feeding when the hunger is accompanied by stress, such as in the case of fasting (Fukushima et al., 2014). Future studies may reveal more about the exact mechanism by which orexins mediate these sex differences in stress-related behaviors, as well as pinpoint other functional consequences mediated by sex-specific orexin expression, such as differences in sleep, autonomic function, and emotional memory.

5. Conclusions

The orexin system is critical for our response to stress (Berridge et al., 2010), and modulates many behaviors relevant to stress-related mental illness such as cognitive function, sleep, food intake, and emotional memory (Sakurai, 2014). Interestingly, dysfunction of the orexin

system has been reported in stress-related psychiatric disorders such as MDD and PTSD (Brundin et al., 2007; Johnson et al., 2010), which are known to be twice as common in women (Keane et al., 2006; Kessler et al., 1994; Nestler et al., 2002; Seeman, 1997; Sheikh et al., 2002). This review highlighted the role of orexins in regulating the stress response and described sex differences in orexin expression. Specifically, the literature provides evidence that females express increased orexin mRNA and protein, higher levels of orexin neuronal activation (as measured by cFos and orexin immunohistochemistry), and augmented orexin release in the cerebrospinal fluid. Moreover, sexual dimorphism of orexin receptor expression in various brain regions has been reported. These sex differences in orexin function have been shown to contribute to sex differences in the stress response and subsequent behaviors. An orexin receptor antagonist is currently FDA approved to treat insomnia (Winrow and Renger, 2014), thus, the previously discussed sex differences in orexin activity may signify a need to develop sex-specific guidelines in dosing. Moreover, given that orexins mediate sex differences in behavioral and neuroendocrine adaptations to repeated stress and in the cognitive consequences of repeated stress, this orexin antagonist could potentially ameliorate many phenotypes of stress-related illness in a sex-specific way. Regardless, current evidence supports the idea that orexins are important in the etiology of stress-related psychiatric disorders that present differently in men and women.

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HIGHLIGHTS

- Orexins promote the acute stress response; their role in repeated stress depends on habituation.
- Females have higher orexin system function; organizational gonadal hormones may be responsible.
- Sex differences in orexin function contribute to sex differences in the response to stress.

Table 1

Summary of sex differences in orexin measures. Summary of the literature reporting sex differences in orexin measures in both preclinical and clinical studies. Details concerning the exact orexin measure and analysis method, whether it was increased in females compared to males, and citation for the study are included.

Orexin measure and method	Sex difference	Citation
Preclinical studies		
Orexin A in the lateral and posterior portion of the hypothalamus measured by radioimmunoassay	Higher in female rats compared to male rats	Taheri et al. (1999)
Prepro-orexin, OX_1R, and OX_3R mRNA in hypothalamus measured by QPCR	Prepro-orexin and OX_1R higher in female rats compared to male rats; OX_2R no sexual dimorphism	Jöhren et al. (2002)
OrexinA/cFos dual stain in the lateral hypothalamus measured by immunohistochemistry and orexinAlevels in the cerebrospinal fluid measured by radioimmunoassay	Higher in female compared to male rats	Grafe et al. (2017)
OX_2R gene in the paraventricular nucleus of the hypothalamus measured by transcriptomics	Higher in female compared to male rats	Loewen et al. (2017)
Clinical studies		
Orexin A in the plasma measured by radioimmunoassay	No differences between healthy men and women	Arihara et al. (2001)
Orexin A in the cerebrospinal fluid measured by fluorescence immunoassay	Higher in women compared with men (with or without Alzheimer's diagnosis)	Schmidt et al. (2013)
Orexin A in anterior cingulate cortex and dorsolateral prefrontal cortex measured by immunohistochemistry	Higher in women with MDD compared with men with MDD	Lu et al. (2017)