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# Hypothalamic vasopressin systems are more sensitive to the long term effects of social defeat in males versus females

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**Summary** Vasopressin signaling has important effects on the regulation of social behaviors and stress responses, and is considered a promising pathway to target for new therapeutics of stress-induced psychiatric disorders. Although there is evidence for sex differences in the behavioral effects of arginine vasopressin (AVP), few data have directly compared the effects of stress on endogenous AVP signaling in males and females. We used California mice (*Peromyscus californicus*) to study the short and long term effects of social defeat stress on AVP immunoreactive cells in the paraventricular nucleus (PVN) and the posteromedial bed nucleus of the stria terminalis (BNSTmp). Acute exposure to defeat increased AVP/c-fos cells in the PVN and SON of both males and females. In contrast, there were sex differences in the long term effects of defeat. Males but not females exposed to defeat had less *avp* mRNA in the PVN, and in two experiments defeat reduced the number of AVP positive cells in the caudal PVN of males but not females. Interestingly, during relatively benign social encounters with a target mouse, there was a rapid decrease in AVP percent staining (including cell bodies and fibers) in the PVN of males but not females. Defeat reduced AVP percent staining in males, but did not block the socially induced decrease in percent staining. When mice were tested in resident—intruder tests, males exposed to defeat were no less aggressive than control males whereas aggression was abolished in females. However, bouts of aggression were positively correlated with the number

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of AVP neurons in the BNSTmp of control males but not stressed males, suggesting that different mechanisms mediate aggression in control and stressed males. These data show that while acute AVP responses to defeat are similar in males and females, the long term effects of defeat on AVP are stronger in males.


## 1. Introduction

Women are diagnosed with major depressive disorder at nearly twice the rate of men (Kessler et al., 1994). Interestingly, social stressors appear to be a more salient trigger for depression in women than men. Adolescent girls are more likely than boys to report interpersonal conflict before the onset of depression (Cyranowski et al., 2000) while a recent study demonstrated that in dizygotic opposite-sex twins, poor social support is a stronger risk factor for depression in sisters versus brothers (Kendler and Gardner, 2014). The neuropeptide arginine vasopressin (AVP) may play an important role in mediating sex differences in behavioral responses to stress. Intranasal administration of AVP enhances threat oriented neural responses to neutral faces in men but suppresses these responses in women (Thompson et al., 2006). Physiological responses to stressors (Wotjak et al., 1996) and social behaviors (Lim and Young, 2006) are regulated by AVP. Sex differences in the function of vasopressin V1a receptor (V1aR) have also been reported (Insel and Hulihan, 1995; Winslow et al., 1993). Although there is growing interest in targeting AVP signaling as a novel therapeutic approach (Hodgson et al., 2014; Lee et al., 2013), the impact of psychosocial stress on AVP signaling in males versus females is poorly understood. This is a critical question if AVP-based pharmaceuticals are to be used successfully in men and women.

Despite the strong female sex bias toward depression and anxiety in humans, only a few animal model-based studies employ female subjects (Beery and Zucker, 2011). The social defeat model is a widely used and ethologically valid method for examining depression-like behaviors in rodents (Krishnan et al., 2007). The paradigm relies on aggressive interactions, and the relatively low intra-female aggression levels of domestic mice have limited sex-based comparisons (Ter Horst et al., 2009) but see (Shimamoto et al., 2011; Solomon et al., 2007). The California mouse (*Peromyscus californicus*) is a monogamous species in which both males and females defend territories (Ribble and Salvioni, 1990). Unlike *Mus musculus*, female California mice are aggressive toward females in standard resident—intruder tests (Silva et al., 2010). It is also possible to titrate bouts of social defeat so that males and females are exposed to equivalent intensities of defeat stress (Trainor et al., 2013). One of the most widely reported effects of defeat stress is social aversion (Russo and Nestler, 2013). Three episodes of social defeat induce social aversion in female California mice to a greater extent than male California mice (Greenberg et al., 2014; Trainor et al., 2011), and this effect is independent of gonadal steroids (Trainor et al., 2013). Although male California mice may appear to be ''resilient'' to defeat stress in the social interaction test, behavior is strongly affected in other domains. In a spatial memory task, defeat stress induces deficits in a spatial reversal learning task in male but not female California mice (Laredo et al., 2014). Interestingly, the combination of social approach and inflexible behavior observed in stressed male California mice aligns well with previously described proactive coping strategies (Koolhaas et al., 1999). Proactive coping strategies in rodents and domestic animals have been linked to higher aggression levels (Koolhaas et al., 1999). Vasopressin is an important regulator of male aggression, and defeat stress impacts AVP signaling in males (Litvin et al., 2011; Wood et al., 2010). However, to our knowledge the effects of defeat on AVP systems have not been examined in females.

Here we took a multifaceted approach to examine how defeat stress impacts AVP signaling systems in male and female California mice. First, we used c-fos immunohistochemistry to examine how AVP neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) responded acutely to episodes of defeat stress. Vasopressin cells in these nuclei can release AVP both peripherally and within the brain (Landgraf and Neumann, 2004). Next we focused on the long term effects (2—10 weeks) of defeat stress on AVP signaling in different social contexts. An important property of AVP signaling is that the behavioral effects depend on whether the environment is familiar or unfamiliar (Marler et al., 2003). We examined the effects of defeat on AVP function in an unfamiliar environment. Focal mice were exposed to either an unfamiliar stimulus mouse confined to a wire cage or an empty wire cage. This allowed us to gain more insights into how defeat stress altered the response of AVP in social versus nonsocial contexts. We also examined the posteromedial bed nucleus of the stria terminalis (BNSTmp) and the anterior hypothalamus (AH). Vasopressin neurons in the BNSTmp are highly sensitive to social experience (Bester-Meredith and Marler, 2001; Ho et al., 2010) and AVP acting in the AH facilitates male aggression (Ferris and Potegal, 1988). Next we examined the impact of defeat stress on brain and behavior in a familiar environment using resident—intruder tests. The effects of defeat stress on male (McCann et al., 2014) and female (Solomon et al., 2007) behavior in the resident—intruder test are well described in Syrian hamsters. However, the effects of defeat on AVP signaling in males and females have never been directly compared. In this study we used a triple-label immunochemistry protocol to examine both AVP and oxytocin (OT) neurons in light of recent reports that OT signaling inhibits male aggression (Calcagnoli et al., 2013). We also distinguished between rostral and caudal portions of the PVN because recent work in male *Mus* reported that AVP neurons in the caudal PVN are more reactive to social cues than rostral AVP neurons (Ho et al., 2010). Overall we found that short term responses of AVP-ir neurons to defeat were similar in males and females whereas over the long term, more changes in AVP-ir were observed in males. These results