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Pituitary adenylate cyclase activating polypeptide (PACAP), stress and sex hormones

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Abstract

Stressor exposure is associated with the onset and severity of many psychopathologies that are more common in women. Moreover, the maladaptive expression and function of stress-related hormones have been implicated in these disorders. Evidence suggest that PACAP has a critical role in the stress circuits mediating stress-responding, and PACAP may interact with sex hormones to contribute to sex differences in stress-related disease. In this review, we describe the PACAP/PAC1 system in stress biology focusing on the role of stress-induced alterations in PACAP expression and signaling in the development of stress-induced behavioral change. Additionally, we present more recent data suggesting potential interactions between stress, PACAP and E/estrus in pathological states, including PTSD. These studies suggest that the level of stress and circulating E/estrus may differentially regulate the PACAPergic system in males and females to influence anxiety-like behavior and may be one mechanism underlying the discrepancies in human psychiatric disorders.

Keywords

estrus; estrogen; PAC1; sex difference; anxiety; bed nucleus of the stria terminalis; PTSD

Introduction

Stress-related disorders are now as prevalent as many global health pandemics (Hariri & Holmes, 2015), leading the World Health Organization to declare stress as the “health epidemic of the 21st century” (WHO). Furthermore, there are significant sex differences among psychiatric and neurobiological disorders related to or exacerbated by stress. For example, alcoholism, attention deficit hyperactivity disorder (ADHD) and psychiatric disorders are more prevalent among men, while women have higher rates of mood and anxiety disorders, including depression, anxiety and stress-/trauma-related disorders (i.e., post-traumatic stress disorder; PTSD;Dalla & Shors, 2009). Several factors are suggested to contribute to these discrepancies, including sex differences in social, societal and psychological factors; however, when these factors are controlled for, the discrepancy remains, suggesting additional causes contribute to the sex bias in stress-related disorders

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(Bangasser, 2013). Recently, research investigating sex differences in stress responses indicates that biology is a major contributing factor to the disparate prevalence of stress-related psychiatric disorders in men and women. Thus, a better understanding of how the sex of an animal and stress affect central nervous system (CNS) stress circuits and behavioral output; both independently and in conjunction, will lead to more effective prevention and treatment of these stress-related disorders.

Here we describe the pituitary adenylate cyclase activating polypeptide (PACAP)/PAC1 system, including our data implicating this system in stress biology. We suggest that stress-induced alterations in PACAP expression and signaling may produce psychopathologies. We review our work implicating intra-bed nucleus of the stria terminalis (BNST) PACAP signaling in mediating multiple consequences of repeated stressor exposure, as well as our more recent work examining potential interactions between stress, PACAP and estrogen (E)/estrus in pathological states. We propose that the level of stress and circulating E/estrus may differentially regulate the PACAPergic system in males and females to influence anxiety-like behavior and may be one mechanism driving the discrepancies in human psychiatric disorders. Furthermore, we argue that there is now enough evidence to support the possibility of these types of interactions in stress circuitry and sexually divergent behaviors and emphasize the critical need for further investigation of these interaction to understand the susceptibility to stress-related disorders, especially in women.

The PACAP System

In 1989, Arimura and colleagues identified the pleiotropic and phylogenetically ancient peptide, PACAP. Since its initial discovery, PACAP has been extensively studied in multiple body systems as a potent stimulator of intracellular cyclic adenosine monophosphate (cAMP) production and intracellular calcium (Vaudry et al., 2009). PACAP is highly conserved across species and is the ancestral molecule in the glucagon/secretin/vasoactive intestinal peptide (VIP) superfamily of peptides (reviewed in Vaudry et al., 2009). Within the CNS and periphery, two major biologically active forms of PACAP exist: the 38 amino acid polypeptide, PACAP-38 and the 27 amino acid polypeptide PACAP-27, with PACAP-38 being 10—100-fold more abundant (Arimura et al., 1991; Kormos & Gaszner, 2013).

Three seven transmembrane G-protein coupled receptors (GPCR) for PACAP have been identified—PAC1, VPAC1 and VPAC2; however, only PAC1Rs are selective for PACAP, while VPAC1 and VPAC2 show equal affinities for PACAP and VIP (Shen, Gehlert, & Collier, 2013). All three subtypes are highly expressed in central and peripheral tissues, but generally PAC1 is the most abundant (Joo et al., 2004). A few exceptions to this exist. For instance, VPAC1Rs are more abundant in hippocampal pyramidal cells while the CeA expresses VPAC2Rs primarily (reviewed in Hashimoto et al., 2011).

Uniquely, alternative splicing produces multiple PAC1R isoforms which can be differentially coupled to Gαs and/or Gαq to produce diverse downstream consequences, including enhanced neurotransmitter and neuropeptide synthesis/release and structural plasticity within stress- and anxiety-related circuits (see Hammack and May, 2015 and Vaudry et al., 2009 for reviews). In support of this, the BNST, amygdala, hippocampus and mPFC, brain

regions known to underlie stress- and anxiety-related process, show high levels of PACAP and PAC1 transcripts and PACAP activation of PAC1 within these structures regulates various stress-like neuroendocrine and behavioral responses (Table 1).

Biological Functions of the PACAP System

PACAP and PACAP-PAC1 signaling are involved in several biological actions, including both cellular function and behavioral responses. Stimulation of PAC1 via binding of PACAP activates the MEK/ERK and PI3K/Akt pathways (for review, see May & Parsons, 2017), suggesting a key role for PACAP as a trophic signal facilitating the development, function, survival, repair and regeneration of neurons during development, and/or following injury. PACAP can activate PAC1 receptors both presynaptically and postsynaptically and hence, acts both as a neuromodulator and neurotransmitter (Vaudry et al., 2009). In the following sections, we focus on the role of PACAP signalling in the response to stressor exposure.

PACAP in the Response to Stress

In response to stressor exposure, the release of corticotropin-releasing hormone (CRH) from hypophysiotropic neurons in the medial parvocellular subdivision of the PVN into hypophysial portal vessels stimulates the release of adrenocorticotropic hormone (ACTH) from pituitary corticotropes into the bloodstream (see Smith & Vale, 2006 for review). ACTH, in turn stimulates synthesis and release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal cortex; together this cascade of events is referred to as the HPA axis. The sustained activation of the stress system in response to chronic stressor exposure has been argued to produce maladaptive morphological and functional changes within stress-related brain regions such as the hippocampus, amygdala, PVN and BNST and result in psychiatric conditions like anxiety disorders, and depression (reviewed in Herman, 2013; Herman, Ostrander, Mueller, & Figueiredo, 2005; Radley, Morilak, Viau, & Campeau, 2015). Furthermore, the development of these maladaptive responses appears to involve PACAP (Mustafa, 2013). In rodents, PACAP is most abundant in hypothalamic regions, with the magnocellular region of the PVN expressing high levels of both PACAP and PAC1 (Hashimoto et al., 2011). Furthermore, PACAP has a modulatory role on multiple levels of the HPA axis (Mustafa, 2013). PACAPergic projections can form synapses with PVN CRH-expressing neurons (Legradi, Hannibal, & Lechan, 1998); and, PACAP can stimulate CRH gene expression (Hashimoto et al., 2011) and promote CRH production and secretion in the PVN (Agarwal, Halvorson, & Legradi, 2005). Intracerebroventricular (ICV) infusions of PACAP increase CRH levels (Grinevich, Fournier, & Pelletier, 1997), CREB phosphorylation in CRH neurons, and c-Fos immunoreactivity in the PVN (Agarwal et al., 2005; Dore et al., 2013). Furthermore, PACAP-deficient mice show attenuated CRH content in the PVN, and diminished corticosterone release to a sustained stressor (Stroth & Eiden, 2010), suggesting that PACAP release may be upstream of PVN CRH activity. Together, these findings suggest that PACAP is critically involved in the activation of PVN neurons to regulate the HPA axis.

PACAP is also involved in autonomic stress responses. For instance, PACAP application increases the synthesis and release of adrenal catecholamines in cultured adrenomedullary

cells (Isobe, Nakai, & Takuwa, 1993; Wakade, Guo, Strong, Arimura, & Haycock, 1992; Watanabe et al., 1992) and stress-induced upregulation of catecholamine-synthesizing enzymes is blocked in PACAP-deficient mice (Stroth et al., 2013). Autonomic sensory areas also express PACAP (Kormos & Gaszner, 2013) and PACAP can activate the autonomic nervous system, driving increased sympathetic activity, including blood pressure and heart rate, and suppressed parasympathetic activity (Tanida et al., 2010). These studies suggest that PACAP regulates autonomic function and is critical for maintaining peripheral homeostasis.

The activation of PACAP systems in extrahypothalamic brain regions may also play key roles in regulating the responses to stressors. For example, PACAP infusion into the BNST, or central nucleus of the amygdala (CeA), mimics many of the consequences of stressor exposure, including glucocorticoid release (reviewed in Hammack & May, 2015). PACAP systems may also be implicated in the function of other key HPA-regulating brain regions. For instance, in addition to their roles in inhibitory control and memory formation, the hippocampus and mPFC are also key HPA-regulating brain areas (Herman et al., 2003) and both densely express PACAP and PAC1 (Vaudry et al., 2009). Additionally, ICV PACAP infusion has been shown to alter the expression levels of proteins involved in synaptic plasticity, cellular differentiation, neuroprotection, neurodegeneration and apoptosis in the hippocampus and prefrontal cortex (Gasperini et al., 2012). Together, these data led several investigators to argue that PACAP systems play a critical role in regulating neuroendocrine and cellular stress responses (Stroth et al., 2011). In the following sections, we discuss our findings which extend this role by associating PACAP with the regulation of the behavioral responses to stressful stimuli.

PACAP in Stress- and Anxiety-related behavior: Evidence from Rodent Studies

Early studies from Hashimoto and colleagues reported irregular behavioral phenotypes in mice deficient for PACAP (PACAP−/−; Hashimoto et al., 2001). These PACAP null mice showed significantly enhanced locomotor activity as compared to PACAP+/+ controls and mice heterozygous for the PACAP gene (PACAP+/− mice). This increased activity was associated with increased rearing and jumping behavior that increased in frequency during open-field testing (Hashimoto et al., 2001). Increased activity in PACAP−/− mice was also associated with increased exploration in the open-field, elevated plus-maze, and novel-object tests (Hashimoto et al., 2001; see also Gaszner, Kormos, Kozicz, & Hashimoto, 2012). These results suggested that PACAP systems may be involved in psychotic behavior and/or anxiety.

In mice, PAC1 deficiencies produced deficits in long-term potentiation induced by mossy fiber stimulation in the hippocampus and attenuated contextual, but not cued, fear conditioning (Otto, 2001A, 2001B). These results were consistent with a previous report (Sauvage, Brabet, Holsboer, Brockaert, & Steckler, 2000), and in congruence with earlier studies in which mice deficient for PAC1 receptors exhibited an anxiolytic behavioral phenotype (Hashimoto et al., 2001). Consistent with these data, ICV infusions of PACAP

were reported to increase stereotyped behaviors, and produce postures consistent with freezing behavior (Agarwal et al., 2005), which was shown to be dependent on CRH receptor activation (Dore et al., 2013). Moreover, PACAP−/− mice exhibit reduced c-Fos expression in response to a forced-swim stressor in the basolateral nucleus of the amygdala (BLA), BNST, and PVN, as well as other stress-related brain regions (Gaszner et al., 2012).

The data described above guided our own work with Victor May's laboratory on the role of PACAP systems in stress and anxiety (reviewed in Hammack & May, 2015). We initially reported a substantial upregulation of PACAP and PAC1 receptor transcript in the dorsal aspect of the BNST following exposure to a chronic variate stress paradigm in which rats were exposed to one of several possible stressors each day for 7 days (Hammack et al., 2009). While we also saw smaller but significant PACAP transcript increases in the PVN, the substantial elevations appeared selective to the BNST (Hammack et al., 2009). Furthermore, we showed that the increased transcript levels were associated with increased immunostaining for PACAP protein, specifically in the BNST oval nucleus (Roman et al., 2014). These results were notable for several reasons: 1) BNST PACAP and PAC1 transcripts were increased at a time when anxiety-like behavior was also substantially elevated in chronically stressed rats, 2) dense PACAP staining had been previously described in this region of the BNST, where PACAP-positive fibers appear to make contacts with CRH-expressing neurons in the BNST oval nucleus, and 3) BNST activity and CRH expression have been heavily implicated in mediating behavioral states that resembled "anxiety" as distinct from "fear" and have been characterized as the sustained response to long-duration stimuli that predict threat (Waddell, Morris, & Bouton, 2006; Walker & Davis, 1997).

Subsequently, we showed that in the absence of stress infusion of PACAP directly into the BNST was also behaviorally relevant, driving anxiogenic responses on multiple measures of anxiety-like behavior, including the open-field, EPM (Roman et al., 2014) and the acoustic startle response (Hammack et al., 2009). Furthermore, intra-BNST PACAP infusion mimicked many other consequences of chronic stress exposure, including anorexia and weight loss (Kocho-Schellenberg et al., 2014) and increased circulating plasma corticosterone (Lezak et al., 2014; Roman et al., 2014). Moreover, we showed that these effects were not due to leakage into the adjacent lateral ventricles, because PACAP infusions at the same lower concentrations and volumes directly into the lateral ventricles was ineffective. In addition, we reported that chronic BNST PACAP receptor antagonism during the 7-day stress paradigm (delivered directly to the BNST via an osmotic minipump loaded with the PAC1 specific antagonist, PACAP6–38) could completely block the increased anxiety-like behavior, anorexia and weight loss, and sensitized corticosterone release normally observed following chronic stress (Roman et al., 2014). Recently, we corroborated and extended these findings, showing that chronic stress couldsensitize the BNST PACAP circuitry, enhancing both the behavioral and endocrine responses to a normally subthreshold infusion of intra-BNST PACAP in males, and that this effect was likely mediated via PAC1Rs in the BNST (King et al., 2017). Hence, chronic stress substantially elevated BNST PACAP expression and signalling in the BNST; moreover, BNST PACAP activation was both necessary and sufficient to produce the behavioral and endocrine consequences of chronic stress exposure (see Hammack & May, 2015).

The activation of PVN and BNST PACAP systems has now been heavily implicated in modulating the physiological and behavioral responses to stressor exposure; however as mentioned above, other stress- and emotion-related brain regions are also likely key sites of PACAP action. For example, subregions of the amygdala have been heavily implicated in the acquisition, expression and extinction of learned fear (see Walker & Davis, 2008), and converging evidence suggests that these regions are also regulated by PACAP (Cho et al., 2012; Legradi et al., 2007). High levels of both CRH and PACAP expression are observed in the CeA, particularly in the lateral capsular division (CeLC; Missig et al., 2014), and PACAP has been shown to enhance excitatory synaptic input in CeLC neurons in a VPAC1-dependent fashion (Cho et al., 2012). Behaviorally, CeA PACAP infusion has been shown to increase passive avoidance in the shock-probe defensive burying test (Legradi et al., 2007). In the BLA, a region critical for the acquisition of learned fear responses, PAC1 receptor transcripts were found to be elevated following a standard fear conditioning procedure, and PAC1 transcript levels were significantly correlated with freezing behavior (Andero, Dias, & Ressler, 2014). BLA PACAP receptor antagonism with PACAP6–38 immediately after contextual fear conditioning significantly attenuated the expression of freezing to the context, implicating BLA PACAP release in the consolidation of contextual fear (Schmidt et al., 2015).

Taken together, the data implicate key functions for PACAP in several principal stress-related brain regions and suggest that PACAP signalling within these CNS regions mediates fear- and anxiety-like behaviors in rodents. Furthermore, PACAP dysregulation in these regions is implicated in animal models of anxiety and fear. In humans, dysregulated PACAP signaling has been linked to a number of stress-related psychiatric disorders (Hammack & May, 2015; Lebois and Ressler, 2016) and several single nucleotide polymorphisms (SNPs) in PACAP-related genes have been reportedly linked to schizophrenia (Hashimoto et al., 2007) and major depression disorder (MDD; Hashimoto et al., 2010).

Estrogen in the Response to Stress

In addition to their well-known reproductive actions, E and estrogen receptors (ER) have been shown to function as critical mediators of several neurobiological stress systems. For instance, animal studies have shown that E can modulate several neurotransmitter systems, including the serotonergic, dopaminergic, adrenergic, and cholinergic systems (reviewed in ter Horst, Wichmann, Gerrits, Westenbroek, & Lin, 2009). Additionally, E can induce differential limbic activity and have profound and dynamic influence on both HPA axis (Carey, Deterd, de Koning, Helmerhorst, & De Kloet, 1995) and behavioral stress responses (reviewed in ter Horst, de Kloet, Schachinger, & Oitzl, 2012). For instance, E can both inhibit and enhance ACTH and corticosterone responses to perceived threats or stressors; likely due to the opposing effects of its two ER’s (reviewed in Weiser, Foradori, & Handa, 2008). A full review of the effects of E and sex differences on stress circuits and behaviors is beyond the scope of this review (but see Bangasser & Wicks, 2017; McEwen & Alves, 1999; Solomon & Herman, 2009; ter Horst et al., 2012 for reviews); however, we briefly discuss the evidence for sex differences in stress responses and subsequent behavioral effects.

In many species, endogenous glucocorticoid levels show a prominent sex difference with higher levels in females under both basal and stressed conditions (reviewed in Bangasser & Valentino, 2014). For example, female rodents have a higher basal (Carey et al., 1995) and diurnal level of corticosterone compared to males (Seale et al., 2004) and in response to stress, females show a greater and longer elevation in levels of ACTH and corticosterone compared to males (Figueiredo, Dolgas, & Herman, 2002). These sex differences in the neuroendocrine response are mediated, at least in part, by circulating ovarian hormones as females in proestrus (i.e., high E and progesterone) have high corticosterone levels compared to females in diestrus (i.e., low E and progesterone levels) or males (Bangasser & Valentino, 2014). Overall, studies indicate that females exhibit stronger activation of the HPA axis by CRH compared to males (reviewed in Bangasser & Valentino, 2012).

Studies also report sex differences in glucocorticoid negative feedback of the HPA axis in rats, with females showing a longer latency to return to baseline levels, an effect that can be prolonged by E (reviewed in Bangasser & Valentino, 2012; Weiser & Handa, 2009). Glucocorticoid negative feedback is mediated via GR receptors, and females have lower GR expression and binding in the hypothalamus and pituitary, likely induced by the effect of E in theses brain regions (Bangasser & Valentino, 2012). Taken together, these results suggest a number of sex differences at multiple levels of the endocrine stress response

Estrogen in Anxiety-related Behavior

Tests designed to provoke anxiety-like responses in rodents typically use novel, innately threatening environmental stimuli such as the elevated plus maze, open-field test, social interaction tests, and the acoustic startle response. Using these tests, studies have reported that proestrus females (high E), relative to diestrus (low E) females and males show less anxiety-like behaviors, spend more time in the open arm of the EPM, enhanced exploratory behavior in the open-field, and increased social interaction with conspecifics (Frye, Petralia, & Rhodes, 2000; Frye & Walf, 2002; Marcondes et al., 2001; Walf et al., 2009). Similar results have been obtained in ovariectomized (OVX) females with E replacement. For instance, Frye and Walf (2004) report that OVX females that receive subcutaneous or intra-amygdala E show increased center entries in the open-field and more time in the open arm of the EPM compared to vehicle treated OVX females. Additional studies have reported similar decreases in anxiety-like behavior in the open-field, EPM and social interaction in OVX females treated with E compared to vehicle (Bowman, Ferguson, & Luine, 2002; McCarthy, Schwartz-Giblin, & Wang, 1997). However, it should be noted that not all studies are consistent with this interpretation as several studies have reported increased anxiogenic behavior in proestrus or in sexually receptive female rats and mice (i.e., high endogenous levels of E) compared to other phases with lower levels of E (Mora, Dussaubat, & Diaz-Veliz, 1996; Morgan & Pfaff, 2001). Still other studies have shown no difference between OVX females treated with E or with vehicle on anxiety-related behaviors. Indeed, we have previously reported no differences in plasma corticosterone responses to intra-BNST PACAP in OVX female rats treated with E2 compared to cholesterol (Lezak et al., 2014).

A number of factors likely contribute to the paradoxical effects of E on anxiety-like behavior, including species differences, time of day tested, phase of cycle when tested,

hormonal dosage, and method of administration. Importantly, previous exposure to other behavioral tasks likely play a critical role, as repeated exposure would decrease novelty and thus anxiety-like behaviors. It is also important to keep in mind that other hormones in addition to E fluctuate over the course of the estrus cycle and with OVX, including progesterone and corticosterone, both of which are known to influence anxiety-like behavior in rodents. Of these potential factors, stress is a major contributor as will be further discussed in the subsequent sections.

Estrogen effects during chronic stress and PTSD may involve PACAP

ERs (alpha and beta) are densely expressed in CNS structures shown to be critical for anxiety-like responding, including the pituitary, PVN and extra-hypothalamic HPA regulatory sites (i.e., hippocampus, BNST); and E likely mediates HPA axis activity at multiple levels (Solomon & Herman, 2009). Additionally, E has been implicated in the regulation of PACAP and PAC1 expression and function (Mercer et al., 2016; Ressler et al., 2011) and recent evidence from our lab, as well as our colleagues suggest that E may interact with and/or modulate PACAP/PAC1 signaling in central stress- and anxiety-associated brain regions and circuits.

Functional MRI (fMRI) studies have shown changes in brain activity in areas associated with emotional processing across the menstrual cycle in women (Goldstein et al., 2005). For instance, emotion centers like the amygdala show increased activity when women are scanned during the early follicular phase (low E) and attenuated activity during phases marked by high E levels (i.e., ovulation; Goldstein et al., 2005; Goldstein, Jerram, Abbs, Whitfield-Gabrieli, & Makris, 2010). Additionally, women show differences in behavioral biomarkers such as acoustic startle, across the menstrual cycle (i.e., more startle during phases marked by low E; Jovanovic et al., 2004). These studies suggest a neuromodulatory role of E on emotional processing and suggest that the cyclical release of E may play a critical role in women's increased propensity to stress- and anxiety-related disorders. Furthermore, these findings emphasize the need for continued examination of the role of E, and other cyclical ovarian hormones in psychopathologies, like PTSD, that particularly affect women (Dalla & Shors, 2009).

Evidence from rodent models of stress and anxiety support a neuromodulatory role for E in PACAPergic BNST stress circuits. For instance, we examined BNST PACAP-mediated stress effects in OVX female rats with or without E replacement and found that acute BNST PACAP infusion dramatically reduced food intake and caused weight loss over 24 hours in OVX females with E replacement (Kocho-Schellenberg et al., 2014), effects which were comparable with what we have observed in male rats (Kocho-Schellenberg et al., 2014; Lezak et al., 2014; Roman et al., 2014). Surprisingly, although E was required for the effect of PACAP on weight-loss, significant PACAP interactions with E were only observed on food intake, suggesting that the interaction effect may be subtle. BNST PACAP infusions also produced an increase in circulating plasma corticosterone levels 30 minutes after infusion in OVX female rats, but this effect was independent of E replacement and similar to the increase seen in males (Lezak et al., 2014). These nuanced findings could be due to a number of factors. For instance, PACAP treatment regimens could have obscured the effects

of E. Additionally, the OVX surgery could have influenced the effects of PACAP in these studies as PACAP levels in multiple stress-related regions, including the hypothalamohypophyseal system, pituitary and brainstem have been shown to be decreased in females following OVX (Nemeth et al., 2006). Finally, it seems likely that the interaction between these two systems may depend on the natural cyclical nature of E across the estrous cycle.

With this last point in mind, we recently examined the effects of prior stress on anxiety-like startle responding to a subthreshold dose of intra-BNST PACAP in intact, cycling females. Unlike what we showed in males in the same study, stress did not enhance the acoustic startle response in intact, cycling females, insteadstartle amplitude appeared attenuated following the subthreshold PACAP infusion (King et al., 2017). A number of factors could account for the opposing results in males and females. It seems likely that the cyclical nature of gonadal hormones (i.e., E and progesterone) across the phase of the female's cycle imparted some sort of protection to the stressed females. For instance, studies have shown that females in proestrus (high levels of E and progesterone) are less anxious compared diestrus (low E) females and males (Frye & Walf, 2002; Marcondes et al., 2001). However, E has been shown to upregulate BNST PACAP and PAC1 transcripts in rodents (Ressler et al., 2011), so the exact mechanism of this protection remains unclear.

Interestingly, King et al. (2017) also found that startle amplitude following PACAP infusion was a function of both stress and estrous. Proestrus females who were stressed showed attenuated startle following PACAP infusion, while those who were in proestrus, but were not stressed showed an enhanced startle response that was similar to males (King et al., 2017). Females in other phases showed basal levels of startle responding following PACAP infusion, regardless of prior stress, suggesting that the level of stress and phase of estrus differentially mediates the response to intra-BNST PACAP in females (King et al., 2017).

Following chronic stress, the Es that act to potentiate acute stress responses are thought to mitigate the effects of chronic stress in females, but the mechanism for this is unknown (Mosca et al., 2015). The results from King et al. (2017) suggest that PACAP may be acting as one of the mechanism through which E mitigates the effects of chronic stress in females as stress, proestrus (when E is high) females had attenuated startle following PACAP infusion. Proestrus females who were not stressed showed an enhanced startle response that was similar to males (King et al., 2017), suggesting that the PACAP infusions may have been functioning as an acute stressor in these females.

Our findings suggest that stress may modulate the BNST PACAP system differently in males and females. Supporting this assumption, studies have shown that males and females show sex differences in PACAP expression in stress-related tissues, including the adrenals and superior cervical ganglia (SCG) under basal conditions (Mosca et al., 2015). This difference likely influences sex-dependent stress signaling and responses to stress in males and females. Moreover, stress (i.e., food/water deprivation) has been shown to sex-dependently alter PACAP expression in the hypothalamus and brain stem, resulting in decreased PACAP levels in females, and increased levels in males (Kiss et al., 2007). Thus, the PACAP system may be sexually dimorphic in stress-related regions, and stress may result in further sexual

divergences in the PACAP system and subsequent responses to stressors. It seems likely that PACAP levels and signaling may be similarly altered in the BNST by stress.

Additionally, similar sexual divergences have been observed in the CRH system following stress. Bangasser and colleagues (2013) have shown robust sex difference in neuronal responses to CRH. Furthermore, these sex differences were linked to differences in CRH1 receptor function such that following stress, females showed decreased coupling of β-arrestin to CRH1 receptors and enhanced coupling to Gs, rendering females more responsive to acute stress and less able to adapt to chronic stress (Bangasser et al., 2010). Thus, at the cellular level, there are robust sex difference in CRH receptors, and additional research suggests analogous sex difference may occur with other seven transmembrane G proteincoupled receptor systems (reviewed in Valentino, Bangasser, & Van Bockstaele, 2013). Furthermore, sex-biased signaling may result in divergent behavior at a systems level. For example, under normal, unstressed conditions males and females show similar performance on a standard sustained attention task (SAT); however, following central administration of CRH, task performance decreases in both sexes, but females are more impaired than males, and this was dependent on estrous stage (Cole et al., 2016). Hence, stress may result in sexual divergences in multiple neuropeptide/transmitter systems, including the PACAP system, which are then further influenced by E/estrus cycle. These sex differences may underlie the increased prevalence of stress-related disorders in females. These findings also suggest that compounds that could shift the bias of CRH1R signaling away from Gs could make females more resilient. In the present scenario, PACAP may be acting in this fashion. The various PACAP receptor isoforms can be coupled to both Gs and Gq (reviewed in Hammack & May, 2015) and it could be that following stress, PACAP signals more through Gq coupled receptors as a sort of compensatory response to the already enhanced CRH1 Gs signaling in cycling females, resulting in the sex difference in anxiety-like behavioral responses to PACAP in males and females. Additionally, as mentioned above, activation of the different PAC1 signaling pathways may lead to opposing responses (i.e., excitability versus neuroplasticity). These hypotheses are currently under investigation in our laboratory.

There is evidence that PACAP's interaction with E in the response to chronic stress is involved in the etiology of PTSD in women. It was reported that a SNP (rs2267735) within a putative estrogen response element (ERE) within the PAC1 receptor promoter region was associated with PTSD symptoms and diagnosis in women, but not men (Ressler et al., 2011). Women with a C-C genotype exhibited higher levels of PTSD-associated hyperarousal than G-carrying women. Moreover, blood levels of PACAP38 were similarly correlated with PTSD symptoms in these women (Ressler et al., 2011). Notably, the sex differences in PAC1 SNP effects on anxiety-like responses were not observed in pre-pubertal children, suggesting the presence of ovarian hormones are responsible for the SNP-induced vulnerability towards PTSD in post-pubertal women (Jovanovic et al., 2012). The same SNP is also associated with impaired fear discrimination and dark-enhanced startle in women, (Almli et al., 2013) and with amygdala and hippocampal activity in response to threat (Stevens et al., 2014), as well as hippocampal activity during contextual (but not cued) fear conditioning (Pohlack et al., 2015). Recently, Ressler and colleagues provided evidence for a functional role of rs2267735 in the dysregulation of ERα/ERE transcriptional activation of the PAC1R gene.

Previously, female rats were shown to have a stress-induced increase in both PACAP and PAC1 transcript in the BNST (reviewed in Hammack et al., 2010) and E treatment resulted in higher levels of PAC1 mRNA in the BNST (Ressler et al., 2011). Mercer et al. (2016) extends this to female mice and provides further evidence that E can increase the expression of the PAC1 gene through ligand activation of ER alpha and binding to an ERE within the gene. The authors argue that carriers of the risk allele ('C') have compromised binding of E/ERα, resulting in less transcription and expression of PAC1, which is associated with PTSD (Mercer et al., 2016). Lower mRNA likely results in lower PAC1 protein, further complicating and disrupting cellular PACAP/PAC1 mechanisms, which are normal processes of the stress response. Thus, the E mediated adaptive response to stress (i.e., E/ER alpha binding to the ERE to induce PAC1 upregulation) is inhibited, resulting in less PAC1 in carriers of the risk allele. This dysregulation may be further disrupted by chronic stress and/or extreme trauma, leading to increased PTSD symptoms.

Corroborating this, there may be differences in BNST PACAP and PAC1 transcript levels across the estrus cycle, which suggest that the effects of PACAP may be strongest during proestrus (King, Brumbaugh, Hammack and May, unpublished). These results are in line with other studies showing that PACAP expression within the PVN also varies across the estrus cycle (Moore et al., 2005). These potential interactions represent an important area of future research.

Conclusion and Future Directions

The studies described in this review illustrate how convergence from animal and human studies can inform potential treatments for stress related disorders. Stress produces maladaptive consequences on multiple levels of responding including HPA axis activity, plasticity within stress responsive regions, and enhanced anxiety-like behavioral responding. Furthermore, dysregulation within these stress systems has been argued to result in stress-related psychopathologies like anxiety disorders and PTSD. Here, we reviewed studies that suggest that PACAP is a critical mediator for both the adaptive and maladaptive stress response. These studies further suggest that stress-induced dysregulation of the PACAP system results in psychopathologies. Moreover, the results describe an E/estrus-PACAP interaction that may underlie the disproportionate occurrence of anxiety disorders and PTSD in women. However, it is important to emphasize the continued exploration and characterization of E modulation of PACAP/PAC1 signaling in stress-related psychopathologies as these mediators may provide novel therapeutic targets and treatments for psychopathologies more prevalent in women.

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References

Agarwal A, Halvorson LM, & Legradi G (2005). Pituitary adenylate cyclase activating polypeptide (PACAP) mimics neuroendocrine and behavioral manifestations of stress: Evidence for PKA-

mediated expression of the corticotropin-releasing hormone (CRH) gene. Brain research. Molecular brain research, 138(1), 45–57. doi:10.1016/j.molbrainres.2005.03.016 [PubMed: 15882914]

Almli LM, Mercer KB, Kerley K, & Feng H (2013). ADCYAP1R1 genotype associates with post-traumatic stress symptoms in highly traumatized African-American females. American Journal of …. doi:10.1002/ajmg.b.32145

Andero R, Dias BG, & Ressler KJ. (2014). A role for Tac2, NkB, and Nk3 receptor in normal and dysregulated fear memory consolidation. Neuron. Retrieved from http://www.sciencedirect.com/science/article/pii/S0896627314004437

Bangasser, Curtis, Reyes, Bethea, Parastatidis, Ischiropoulos, … Valentino. (2010). Sex differences in corticotropin-releasing factor signaling and trafficking. Molecular Psychiatry, 15(9), 877–877. doi:10.1038/mp.2010.89 [PubMed: 20548297]

Bangasser DA (2013). Sex differences in stress-related receptors: ″micro″ differences with ″macro″ implications for mood and anxiety disorders. Biology of sex differences, 4(1), 2. doi:10.1186/2042-6410-4-2 [PubMed: 23336736]

Bangasser DA, & Valentino RJ (2012). Sex differences in molecular and cellular substrates of stress. Cellular and molecular neurobiology, 32(5), 709–23. doi:10.1007/s10571-012-9824-4 [PubMed: 22488525]

Bangasser DA, & Valentino RJ (2014). Sex differences in stress-related psychiatric disorders: neurobiological perspectives. Frontiers in neuroendocrinology, 35(3), 303–19. doi:10.1016/j.yfrne.2014.03.008 [PubMed: 24726661]

Bangasser D, & Wicks B (2017). Sex-specific mechanisms for responding to stress. Journal of Neuroscience Research, 95(1–2), 75–82. doi:10.1002/jnr.23812 [PubMed: 27870416]

Bowman RE, Ferguson D, & Luine VN. (2002). Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. Neuroscience. Retrieved from http://www.sciencedirect.com/science/article/pii/S0306452202001562

Carey MP, Deterd CH, de Koning J, Helmerhorst F, & de Kloet ER (1995). The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. J Endocrinol, 144(2), 311–321. [PubMed: 7706984]

Cho JH, Zushida K, & Shumyatsky GP, Carlezon WA Jr, Meloni EG, & Bolshakov VY (2012). Pituitary adenylate cyclase-activating polypeptide induces postsynaptically expressed potentiation in the intra-amygdala circuit. The Journal of …. doi:10.1523/JNEUROSCI.1402-12.2012

Cole RD, Kawasumi Y, Parikh V, & Bangasser DA. (2016). Corticotropin releasing factor impairs sustained attention in male and female rats. Behavioural brain …. Retrieved from http://www.sciencedirect.com/science/article/pii/S0166432815301571

Dalla C, & Shors TJ (2009). Sex differences in learning processes of classical and operant conditioning. Physiology & behavior, 97(2), 229–38. doi:10.1016/j.physbeh.2009.02.035 [PubMed: 19272397]

Dore R, Iemolo A, Smith K, Wang X, Cottone P, & Sabino V (2013). CRF Mediates the Anxiogenic and Anti-Rewarding, But Not the Anorectic Effects of PACAP. Neuropsychopharmacology, 38(11). doi:10.1038/npp.2013.113

Figueiredo HF, Dolgas CM, & Herman JP (2002). Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. Endocrinology, 143(7), 2534–40. doi:10.1210/endo.143.7.8888 [PubMed: 12072385]

Frye CA, & Walf AA. (2002). Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Hormones and Behavior. Retrieved from http://www.sciencedirect.com/science/article/pii/S0018506X02917630

Frye CA, & Walf AA. (2004). Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. Behavioral neuroscience. Retrieved from http://psycnet.apa.org/journals/bne/118/2/306/

Frye CA, Petralia SM, & Rhodes ME (2000). Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha,5alpha-THP. Pharmacology, biochemistry, and behavior, 67(3), 587–96.

Gaszner B, Kormos V, Kozicz T, & Hashimoto H (2012). The behavioral phenotype of pituitary adenylate-cyclase activating polypeptide-deficient mice in anxiety and depression tests is accompanied by blunted c-Fos …. Neuroscience. Retrieved from http://www.sciencedirect.com/science/article/pii/S0306452211013339

Goldstein JM, Jerram M, Poldrack R, Ahern T, Kennedy DN, Seidman LJ, & Makris N (2005). Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. The journal of neuroscience, 25(40), 9309–9316. doi:10.1523/JNEUROSCI.2239-05.2005 [PubMed: 16207891]

Goldstein JM, Jerram M, Abbs B, Whitfield-Gabrieli S, & Makris N (2010) Sex differences in stress response circuitry activation dependent on female hormonal cycle. J Neurosci, 30,431–438. [PubMed: 20071507]

Grinevich V, Fournier A, & Pelletier G (1997). Effects of pituitary adenylate cyclase activating polypeptide (PACAP) on corticotropin-releasing hormone (CRH) gene expression in the rat hypothalamic …. Brain research. Retrieved from http://www.sciencedirect.com/science/article/pii/S0006899397010111

Hammack SE. & May V (2015). Pituitary adenylate cyclase activating polypeptide in stress related disorders: data convergence from animal and human studies. Biological Psychiatry. doi:10.1016/j.biopsych.2014.12.003

Hammack SE, Cheung J, Rhodes KM, Schutz KC, Falls WA, Braas KM, & May V (2009). Chronic stress increases pituitary adenylate cyclase-activating peptide (PACAP) and brain-derived neurotrophic factor (BDNF) mRNA expression in the bed nucleus of the stria terminalis (BNST): roles for PACAP in anxiety-like behavior. Psychoneuroendocrinology, 34(6), 833–43. doi:10.1016/j.psyneuen.2008.12.013 [PubMed: 19181454]

Hammack SE, Roman CW, Lezak KR, Kocho-Shellenberg M, Grimmig B, Falls WA, … May V (2010). Roles for pituitary adenylate cyclase-activating peptide (PACAP) expression and signaling in the bed nucleus of the stria terminalis (BNST) in mediating the behavioral consequences of chronic stress. Journal of molecular neuroscience: MN, 42(3), 327–40. doi:10.1007/s12031-010-9364-7 [PubMed: 20405238]

Hariri AR, & Holmes A (2015). Finding translation in stress research. Nature Neuroscience, 18(10), 1347–1352. doi:10.1038/nn.4111 [PubMed: 26404709]

Hashimoto H, Shintani N, & Tanaka K (2001). Altered psychomotor behaviors in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP). Proceedings of the …. Retrieved from http://www.pnas.org/content/98/23/13355.short

Hashimoto H, Shintani N, & Tanida M (2011). PACAP is implicated in the stress axes. Curr Pharm Des, 17,985–989. doi:10.2174/138161211795589382 [PubMed: 21524255]

Hashimoto R, Hashimoto H, Shintani N, Chiba S, Hattori S, Okada T, … Nemoto K (2007). Pituitary adenylate cyclase-activating polypeptide is associated with schizophrenia. Molecular psychiatry, 12(11), 1026–1032. doi:10.1038/sj.mp.4001982 [PubMed: 17387318]

Hashimoto R, Hashimoto H, Shintani N, Ohi K, & Hori H (2010). Possible association between the pituitary adenylate cyclase-activating polypeptide (PACAP) gene and major depressive disorder. Neuroscience …. Retrieved from http://www.sciencedirect.com/science/article/pii/S0304394009014852

Herman JP, Ostrander MM, & Mueller NK. (2005). Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Progress in Neuro- …. doi:10.1016/j.pnpbp.2005.08.006

Herman JP (2013). Neural control of chronic stress adaptation. Frontiers in behavioral neuroscience, 7(61). doi:10.3389/fnbeh.2013.00061

Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, & Cullinan WE (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Frontiers in neuroendocrinology, 24(3), 151–80. [PubMed: 14596810]

Horst TG, Wichmann R, & Gerrits M (2009). Sex differences in stress responses: focus on ovarian hormones. Physiology & …. Retrieved from http://www.sciencedirect.com/science/article/pii/S0031938409000791

Horst TJ, Kloet DE, Schächinger H, & Oitzl MS. (2012). Relevance of stress and female sex hormones for emotion and cognition. Cellular and molecular neurobiology, 32(5), 725–735. doi:10.1007/s10571-011-9774-2 [PubMed: 22113371]

Isobe K, Nakai Y, & Takuwa Y (1993). Ca^{2+}-dependent stimulatory effect of pituitary adenylate cyclase-activating polypeptide on catecholamine secretion from cultured porcine adrenal medullary chromaffin cells. Endocrinology, 132(4), 1757–1765. doi: 10.1210/endo.132.4.8384995 [PubMed: 8384995]

Jovanovic T, Norrholm SD, Davis J, Mercer KB, Almli L, Nelson A, … Bradley B (2013). PAC1 receptor (ADCYAP1R1) genotype is associated with dark-enhanced startle in children. Molecular psychiatry, 18(7), 742 Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3750970/ [PubMed: 22776899]

Jovanovic T, Szilagyi S, Chakravorty S, Fiallos AM, Lewison BJ, Parwani A, … Duncan EJ (2004). Menstrual cycle phase effects on prepulse inhibition of acoustic startle. Psychophysiology, 41(3), 401–6. doi:10.1111/1469-8986.2004.00166.x [PubMed: 15102125]

King SB, Lezak KR, O'Reilly M, Toufexis DJ, Falls WA, Braas K, May V, & Hammack SE (in press). The effects of prior stress on anxiety-like responding to intra-BNST pituitary adenylate cyclase activating polypeptide (PACAP) in male and female Rats. Neuropsychopharmacology.

Kocho-Schellenberg M, Lezak KR, Harris OM, Roelke E, Gick N, Choi I, … Hammack SE (2014). PACAP in the BNST produces anorexia and weight loss in male and female rats. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology, 39(7), 1614–23. doi:10.1038/npp.2014.8 [PubMed: 24434744]

Kormos V, & Gaszner B (2013). Role of neuropeptides in anxiety, stress, and depression: from animals to humans. Neuropeptides. Retrieved from http://www.sciencedirect.com/science/article/pii/S0143417913000802

Legradi G, Das M, Giunta B, Hirani K, & Mitchell EA. (2007). Microinfusion of pituitary adenylate cyclase-activating polypeptide into the central nucleus of amygdala of the rat produces a shift from an active to passive mode of …. Neural …. doi:10.1155/2007/79102

Lezak KR, Roman CW, Braas KM, Schutz KC, Falls WA, Schulkin J, … Hammack SE (2014). Regulation of bed nucleus of the stria terminalis PACAP expression by stress and corticosterone. Journal of molecular neuroscience: MN, 54(3), 477–84. doi:10.1007/s12031-014-0269-8 [PubMed: 24614974]

Légrádi G, Hannibal J, & Lechan RM. (1998). Pituitary adenylate cyclase-activating polypeptide-nerve terminals densely innervate corticotropin-releasing hormone-neurons in the hypothalamic paraventricular …. Neuroscience letters. Retrieved from http://www.sciencedirect.com/science/article/pii/S0304394098002559

Marcondes FK, Miguel KJ, Melo LL, & Spadari-Bratfisch RC (2001). Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiology & behavior, 74(4–5), 435–40. doi:10.1016/S0031-9384(01)00593-5 [PubMed: 11790402]

May V, & Parsons R (2017). G Protein-Coupled Receptor Endosomal Signaling and Regulation of Neuronal Excitability and Stress Responses: Signaling Options and Lessons From the PAC1 Receptor. Journal of Cellular Physiology, 232(4), 698–706. doi:10.1002/jcp.25615 [PubMed: 27661062]

McCarthy MM, & Schwartz-Giblin S (1997). Does estrogen facilitate social behavior by reducing anxiety? Annals of the New …. doi:10.1111/j.1749-6632.1997.tb51962.x

McEwen BS, & Alves SE (1999). Estrogen actions in the central nervous system. Endocrine reviews, 20(3), 279–307. doi:10.1210/edrv.20.3.0365 [PubMed: 10368772]

Mercer KB, Dias B, Shafer D, Maddox SA, Mulle JG, Hu P, … Ressler KJ (2016). Functional evaluation of a PTSD-associated genetic variant: estradiol regulation and ADCYAP1R1. Translational psychiatry, 6(12), e978. doi:10.1038/tp.2016.241 [PubMed: 27959335]

Missig G, Roman CW, Vizzard MA, Braas KM, Hammack SE, & May V (2014). Parabrachial nucleus (PBn) pituitary adenylate cyclase activating polypeptide (PACAP) signaling in the amygdala: implication for the sensory and behavioral effects of pain. Neuropharmacology, 86, 38–48. doi:10.1016/j.neuropharm.2014.06.022 [PubMed: 24998751]

Mora S, Dussaubat N, & Díaz-Véliz G (1996). Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology. Retrieved from http://www.sciencedirect.com/science/article/pii/S0306453096000157

Morgan MA, & Pfaff DW. (2001). Effects of estrogen on activity and fear-related behaviors in mice. Hormones and behavior. Retrieved from http://www.sciencedirect.com/science/article/pii/S0018506X01917167

Mustafa T (2013). Pituitary adenylate cyclase-activating polypeptide (PACAP): a master regulator in central and peripheral stress responses. Advances in pharmacology (San Diego, Calif.), 68, 445–57. doi:10.1016/B978-0-12-411512-5.00021-X

Otto C, Martin M, Wolfer DP, & Lipp HP. (2001). Altered emotional behavior in PACAP type-I-receptor-deficient mice. Molecular brain …. doi:10.1016/S0169-328X(01)00153-X

Otto C, Kovalchuk Y, Wolfer DP, Gass P, Martin M, Zuschratter W, … Schütz G (2001). Impairment of mossy fiber long-term potentiation and associative learning in pituitary adenylate cyclase activating polypeptide type I receptor-deficient mice. The Journal of neuroscience: the official journal of the Society for Neuroscience, 21(15), 5520–7. [PubMed: 11466423]

Pohlack ST, Nees F, Ruttorf M, & Cacciaglia R (2015). Neural mechanism of a sex specific risk variant for posttraumatic stress disorder in the type I receptor of the pituitary adenylate cyclase activating polypeptide. Biological …. Retrieved from http://www.sciencedirect.com/science/article/pii/S0006322315000025

Radley J, Morilak D, Viau V, & Campeau S (2015). Chronic stress and brain plasticity: Mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders. Neuroscience and biobehavioral reviews, 58, 79–91. doi:10.1016/j.neubiorev.2015.06.018 [PubMed: 26116544]

Ressler KJ, Mercer KB, Bradley B, Jovanovic T, Mahan A, Kerley K, … May V (2011). Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature, 470(7335), 492–7. doi:10.1038/nature09856 [PubMed: 21350482]

Roman CW, Lezak KR, Hartsock MJ, Falls WA, Braas KM, Howard AB, … May V (2014). PAC1 receptor antagonism in the bed nucleus of the stria terminalis (BNST) attenuates the endocrine and behavioral consequences of chronic stress. Psychoneuroendocrinology, 47, 151–65. doi:10.1016/j.psyneuen.2014.05.014 [PubMed: 25001965]

Sauvage M, Brabet P, Holsboer F, & Bockaert J (2000). Mild deficits in mice lacking pituitary adenylate cyclase-activating polypeptide receptor type 1 (PAC1) performing on memory tasks. Molecular brain …. doi:10.1016/S0169-328X(00)00219-9

Schmidt SD, Myskiw JC, Furini C, & Schmidt BE. (2015). PACAP modulates the consolidation and extinction of the contextual fear conditioning through NMDA receptors. Neurobiology of learning …. Retrieved from http://www.sciencedirect.com/science/article/pii/S1074742714002123

Shen S, Gehlert DR, & Collier DA (2013). PACAP and PAC1 receptor in brain development and behavior. Neuropeptides, 47(6), 421–30. doi:10.1016/j.npep.2013.10.005 [PubMed: 24220567]

Smith CB, & Eiden LE (2012). Is PACAP the major neurotransmitter for stress transduction at the adrenomedullary synapse? Journal of Molecular Neuroscience. Retrieved from http://link.springer.com/article/10.1007/s12031-012-9749-x

Smith SM, & Vale WW (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues in clinical neuroscience, 8(4), 383–95. [PubMed: 17290797]

Solomon MB, & Herman JP (2009). Sex differences in psychopathology: of gonads, adrenals and mental illness. Physiology & behavior, 97(2), 250–8. doi:10.1016/j.physbeh.2009.02.033 [PubMed: 19275906]

Stevens JS, Almli LM, Fani N, Gutman DA, Bradley B, Norrholm SD…(2014). PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus. Proc Natl Acad Sci USA, 111, 3158–3163. [PubMed: 24516127]

Stroth N, Kuri BA, Mustafa T, Chan SA, & Smith CB. (2013). PACAP controls adrenomedullary catecholamine secretion and expression of catecholamine biosynthetic enzymes at high splanchnic nerve firing rates characteristic …. …. doi:10.1210/en.2012-1829

Stroth N, & Eiden LE (2010). Stress hormone synthesis in mouse hypothalamus and adrenal gland triggered by restraint is dependent on pituitary adenylate cyclase-activating polypeptide signaling. Neuroscience, 165(4), 1025–30. doi:10.1016/j.neuroscience.2009.11.023 [PubMed: 19931358]

Stroth N, Holighaus Y, Ait-Ali D, & Eiden L (2011). PACAP: a master regulator of neuroendocrine stress circuits and the cellular stress response. Annals of the New York Academy of Sciences, 1220(1), 49–59. doi:10.1111/j.1749-6632.2011.05904.x [PubMed: 21388403]

Tanida M, Shintani N, Morita Y, Tsukiyama N, Hatanaka M, Hashimoto H, … Nagai K (2010). Regulation of autonomic nerve activities by central pituitary adenylate cyclase-activating polypeptide. Regulatory peptides, 161(1–3), 73–80. doi:10.1016/j.regpep.2010.02.002 [PubMed: 20171991]

Valentino R, Bangasser D, & Bockstaele E (2013). Sex-Biased Stress Signaling: The Corticotropin-Releasing Factor Receptor as a Model. Molecular Pharmacology, 83(4), 737–745. doi:10.1124/mol.112.083550 [PubMed: 23239826]

Vaudry D, Falluel-Morel A, Bourgault S, & Basille M (2009). Pituitary adenylate cyclas activating polypeptide and its receptors: 20 years after the discovery. Pharmacological …. Retrieved from http://pharmrev.aspetjournals.org/content/61/3/283.short

Waddell J, Morris RW, & Bouton ME (2006). Effects of bed nucleus of the stria terminalis lesions on conditioned anxiety: aversive conditioning with long-duration conditional stimuli and reinstatement of extinguished fear. Behavioral neuroscience, 120(2), 324–36. doi:10.1037/0735-7044.120.2.324 [PubMed: 16719697]

Wakade AR, Guo X, Strong R, Arimura A, & Haycock JW (1992) Pituitary adenylate cyclase-activating polypeptide (PACAP) as a neurotransmitter in rat adrenal medulla. Reg. Peptides, 37, 331.

Walf AA, Koonce C, Manley K, & Frye CA (2009). Proestrous compared to diestrous wildtype, but not estrogen receptor beta knockout, mice have better performance in the spontaneous alternation and object recognition tasks and reduced anxiety-like behavior in the elevated plus and mirror maze. Behavioural brain research, 196(2), 254–60. doi:10.1016/j.bbr.2008.09.016 [PubMed: 18926853]

Walf AA, Paris JJ, & Frye CA (2009). Chronic estradiol replacement to aged female rats reduces anxiety-like and depression-like behavior and enhances cognitive performance. Psychoneuroendocrinology, 34(6), 909–16. doi:10.1016/j.psyneuen.2009.01.004 [PubMed: 19216030]

Walker DL, & Davis M (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by …. The Journal of neuroscience. Retrieved from http://www.jneurosci.org/content/17/23/9375.short

Walker DL, & Davis M (2008). Role of the extended amygdala in short-duration versus sustained fear: a tribute to Dr. Lennart Heimer. Brain Structure and Function. doi:10.1007/s00429-008-0183-3

Watanabe T, Masuo Y, Matsumoto H, Suzuki N, Ohtaki T, Masuda Y, Kitada C, Tsuda M, & Fujino M (1992). Pituitary adenylate cyclase activating polypeptide provokes cultured rat chromaffin cells to secrete adrenaline. Biochem Biophys Res Commun, 182 403–411. [PubMed: 1310018]

Weiser MJ, Foradori CD, & Handa RJ. (2008). Estrogen receptor beta in the brain: from form to function. Brain research reviews, 54 doi:10.1016/j.brainresrev.2007.05.013.

Weiser MJ, & Handa RJ. (2009). Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic–pituitary–adrenal axis via estrogen receptor alpha within the hypothalamus. Neuroscience. Retrieved from http://www.sciencedirect.com/science/article/pii/S0306452208018484

Table 1.

PACAP in Stress-related Structures and Circuits

BNST	
PACAP and PAC1R transcripts are densely expressed at high levels in the BNST	(Hannibal, 2002; Hashimoto et al., 1996; Joo et al., 2004)
BNST PACAP and PAC1R transcripts are upregulated following CVS	(Hammack et al., 2009)
BNST PACAP and PAC1R transcripts are upregulated by chronic E2 treatment in OVX females	(Ressler et al., 2011)
Anxiety-like behaviors in the open-field, EPM, and acoustic startle paradigms are increased by intra-BNST PACAP infusion	(Hammack et al., 2009; King et al., 2017; Roman et al., 2014)
Circulating plasma corticosterone and release is increased by intra-BNST PACAP infusion	(Lezak et al., 2014; Roman et al., 2014)
Chronic stress effects on behavioral and endocrine responses are blocked by intra-BNST PAC1R antagonism	(Roman et al., 2014)
BNST of male rats is sensitized to subsequent PACAP release and signaling following CVS	(King et al., 2017)
c-Fos activation in the BNST is blunted in PACAP KO mice	(Gaszner et al., 2012)
HPA axis	
PACAP and PAC1R transcripts are expressed endogenously in the PVN	(Condro et al., 2016; Hannibal, 2002; Hashimoto et al., 1996; Joo et al., 2004)
PVN PACAP transcripts are upregulated by CVS	(Hammack et al., 2009)
PVN PACAP expression is mediated by estrous cycle and is correlated with gonadotropin synthesis and secretion	(Moore et al., 2005)
c-Fos and vasopressin are elevated in the PVN by central PACAP infusion	(Meloni et al., 2016; Nomura et al., 1999)
PACAPergic projections form synapses with PVN CRH neurons	(Legradi et al., 1998)
CRH expression, production and secretion in the PVN is stimulated by PACAP	(Agarwal et al., 2005; Hashimoto et al., 2010)
PVN CRH levels are attenuated and stress-induced corticosterone release is impaired in PACAP KO mice	(Stroth & Eiden, 2010)
Corticosterone levels are increased by ICV PACAP	(Agarwal et al., 2005; Meloni et al., 2016)
Amygdala	
CeA	
PACAP (dense fiber staining) is expressed at high levels in CeA	(Condro et al., 2016; Hannibal, 2002; Hashimoto et al., 1996; Joo et al., 2004; Missig et al., 2014)
CeA PACAP and PAC1R expression is not upregulated by CVS	(Hammack et al., 2009)
Fear-like avoidant behaviors and anxiety-like behaviors are increased by intra-CeA PACAP infusion	(Legradi et al., 2007; Missig et al., 2014)
Intra-CeA PACAP effects on fear/anxiety are likely mediated via VPACRs (not PAC1Rs)	(Cho et al., 2012)
c-Fos is increased in the CeA following ICV PACAP	(Meloni et al., 2016)
CRH expression in the CeA is enhanced by ICV PACAP	(Dore et al., 2013)
BLA	
PAC1Rs and low levels of PACAP are expressed in the BLA	(Hannibal, 2002; Hashimoto et al., 1996; Joo et al., 2004)

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PAC1R transcripts are upregulated following fear conditioning	(Andero et al., 2014)
BLA PACAP or PAC1R expression is not upregulated by CVS	(Hammack et al., 2009)
ANS	
PACAP is expressed in autonomic sensory areas	(Kormos & Gaszner, 2013)
Sympathetic outflow is stimulated by PACAP	(Tanida et al., 2010)
Epinephrine secretion is stimulated by PACAP, and compensatory catecholamine synthesis is enhanced by PACAP during prolonged stress to sustain epinephrine secretion	(Stroth & Eiden, 2010; Watanabe et al., 1992)
PACAP is involved in peripheral homeostatic balance under conditions of stress	(Tanida et al., 2010)
PACAP is expressed at higher levels in the adrenals of males under basal conditions	(Mosca et al., 2015)
Hippocampus	
PACAP and PAC1Rs are expressed throughout the hippocampal formation, including entorhinal cortex, CA1, CA2, CA3, and DG.	(Condro et al., 2016; Hannibal, 2002; Hashimoto et al., 1996; Joo et al., 2004; Kozicz et al., 1997; Otto et al., 1998; Vaudry et al., 2009)
PAC1R expression is primarily limited to mossy fibers in the hippocampus	(Otto et al., 2001)
PAC1Rs are expressed by newly proliferated NSCs in the SGZ	(Matsumoto et al., 2016)
Structural plasticity within the hippocampus is enhanced by PACAP (i.e., increased number of newly proliferated cells in the SVZ, SGZ and DG, and enhancesd neurite length, number, and increases soma size)	(Matsumoto et al., 2016; Mercer et al., 2004; Ogata et al., 2015; Ohta et al., 2006)
mPFC	
PACAP and PAC1Rs are expressed in the mPFC	(Condro et al., 2016; Hashimoto et al., 1996; Koves et al., 1991; Kozicz et al., 1997; Vaudry et al., 2009)
PAC1Rs are upregulated in the mPFC following fear conditioning	(Ressler et al., 2011)
PACAP transcripts are down regulated in the mPFC following CVS	(Makinson et al., 2015)

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