Published in final edited form as:

Life Sci. 2014 September 1; 112(0): 2–9. doi:10.1016/j.lfs.2014.07.017.

Using high resolution imaging to determine trafficking of corticotropin-releasing factor receptors in noradrenergic neurons of the rat locus coeruleus

B. A. S. Reyes¹, D.A. Bangasser², R. J. Valentino³, and E. J. Van Bockstaele¹

¹Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA 19102

²Psychology Department and Neuroscience Program, Temple University, Philadelphia, PA 19122

³Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA 19104

Abstract

Trafficking of G protein-coupled receptors (GPCRs) is a critical determinant of cellular sensitivity of neurons. To understand how endogenous or exogenous ligands impact cell surface expression of GPCRs, it is essential to employ approaches that achieve superior anatomical resolution at the synaptic level. In situations in which light and fluorescence microscopy techniques may provide only limited resolution, electron microscopy provides enhanced subcellular precision. Dual labeling immunohistochemistry employing visually distinct immunoperoxidase and immunogold markers has been an effective approach for elucidating complex receptor profiles at the synapse and to definitively establish the localization of individual receptors and neuromodulators to common cellular profiles. The immuno-electron microscopy approach offers the potential for determining membrane versus intracellular protein localization, as well as the association with various identifiable cellular organelles. Corticotropin-releasing factor (CRF) is an important regulator of endocrine, autonomic, immunological, behavioral and cognitive limbs of the stress response. Dysfunction of this neuropeptide system has been associated with several psychiatric disorders. This review summarizes findings from neuroanatomical studies, with superior spatial resolution, that indicate that the distribution of CRF receptors is a highly dynamic process that, in addition to being sexually dimorphic, involves complex regulation of receptor trafficking within extrasynaptic sites that have significant consequences for adaptations to stress, particularly within the locus coeruleus (LC), the major brain norepinephrine-containing nucleus.

© 2014 Elsevier Inc. All rights reserved.

Corresponding Author: Beverly Reyes, DVM, PhD, Department of Pharmacology and Physiology, Drexel University College of Medicine, 245N. 15th Street, M.S.488 Rm. 8814B, Philadelphia, PA 19102, Voice: (215) 762-4932, Beverly.Reyes@drexelmed.edu.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

corticotropin-releasing factor; locus coeruleus; norepinephrine; corticotropin-releasing factor receptor

Introduction

G protein-coupled receptors (GPCRs) (Perrin and Vale 1999; Hillhouse and Grammatopoulos 2006), also called seven-transmembrane receptors, are a large family of cell-surface proteins capable of binding a diverse array of molecules that mediate a myriad of physiological responses to hormones, neurotransmitters, and environmental stimulants (Rosenbaum et al. 2009). The importance of GPCR signaling in dictating cellular sensitivity has been extensively characterized (Ferguson 2001, 2007; Bhattacharya et al. 2004; Luttrell 2008; Thompson et al. 2008; Bockaert et al. 2010; Magalhaes et al. 2010). Dysregulation of GPCRs has been associated with a host of pathophysiological conditions (Thompson et al. 2008). Given the diverse modulatory functions attributed to GPCRs, this family of cell surface proteins has been an important target in the development of therapeutic tools for the treatment of various disease conditions (Overington et al. 2006).

Trafficking of GPCRs has been studied in in vitro (Westlund et al. 1984; Childs et al. 1986; Yu et al. 1993; Vieira et al. 1996; Koenig and Edwardson 1997; Segredo et al. 1997; Rasmussen et al. 2004; Perry et al. 2005; Grampp et al. 2008) and in vivo system (Reyes et al. 2006, 2008, 2010, 2012; Glass et al. 2008; Grampp et al. 2008; Scavone and Van Bockstaele 2009; Torres-Reveron et al. 2009; Bangasser et al. 2010, 2013). Elucidating GPCR trafficking is vital for developing pharmacotherapy for the treatment of disease, which is highlighted by the statistics indicating that at least 30–40% of prescription drugs target GPCRs (Overington et al. 2006). Here, we review neuroanatomical studies using high resolution immuno-electron microscopy demonstrating the dynamic GPCR trafficking within the locus coeruleus (LC), a nucleus containing the largest cluster of noradrenergic neurons and projecting to the entire neuraxis (Foote et al., 1983; Waterhouse et al., 1993) that is involved in global brain functions such as emotion, vigilance, arousal (Aston-Jones et al., 1984), memory and adaptive response to stress (Aston-Jones et al., 1991).

Using high resolution immuno-electron microscopy for examining trafficking of GPCRs

Neuroanatomical techniques used to discern the cellular distribution of GPCRs have revealed important sites of action that can inform experimental approaches using pharmacological manipulation to target these systems under different experimental conditions (Reyes et al. 2006, 2008; Jaferi et al. 2009; Treweek et al. 2009; Bangasser et al. 2010, 2013; Williams et al. 2011). However, light and fluorescence microscopy techniques may provide only limited resolution. The pre-embedding immunogold-silver technique combined with electron microscopy has several advantages. This approach maintains morphological preservation while preserving discrete subcellular localization of the antigen of interest (Leranth and Pickel 1989). Furthermore, pre-embedding immunogold-silver

labeling can be more appropriate than post-embedding immunogold labeling for localizing immunoreactivity at extra-synaptic sites and, therefore, is particularly useful for determining the regional distribution of peptidergic receptors (Lujan et al. 1996; Milner et al., 2011; Sesack et al., 2006). Post-embedding immunogold labeling, although useful for some antigens, can yield a high degree of non-specific adhesion of the gold particles and, therefore, specificity of the reaction deposit is difficult to discern. In combination with peroxidase detection of a second antigen, which appears as a dense homogeneous precipitate within cellular compartments, the dual labeling approach can unequivocally establish co-existence of distinct receptor proteins in common cellular elements. Although the pre-embedding immunogold-silver approach may produce lower estimates of receptor number than immunoperoxidase labeling due to differences in reagent penetration (Leranth and Pickel 1989), limitations of the experimental approach are lessened by only examining sections from the outer surface of the tissue for data analysis. Furthermore, whenever penetration is considered more important than preservation of fine structure of the neuropil, enhancement methods (such as increased detergents i.e. Triton X-100) can be considered (Leranth and Pickel 1989). Techniques used to discern the cellular distribution of GPCRs have highlighted the importance of trafficking of these receptors under different conditions (Reyes et al. 2006, 2008; Jaferi et al. 2009; Treweek et al. 2009; Bangasser et al. 2010, 2013; Williams et al., 2011). In summary, under carefully controlled conditions, dual peroxidase and gold immunocytochemistry can enable a quantitative evaluation of the co-localization of neurotransmitters and receptors within the neuropil.

CRF effects on the LC-norepinephrine arousal system

Corticotropin-releasing factor (CRF), the neurohormone that initiates pituitary adrenocorticotrophic hormone release during stress (Vale et al. 1981), also serves as a neuromodulator to activate the LC-norepinephrine system in response to certain challenges (Valentino and Van Bockstaele 2002, 2005; Reyes et al. 2006, 2008). CRF axon terminals synapse with LC dendrites and direct administration of CRF onto LC neurons *in vivo* and *in vitro* produces a long-lasting tonic increase in LC discharge rate (Van Bockstaele et al. 1996; Curtis et al. 1997; Jedema and Grace, 2004). This is associated with elevated cortical norepinephrine efflux (Curtis et al. 1997; Page and Abercrombie 1999). Importantly, LC activation by CRF is translated to activation of cortical electroencephalographic (EEG) activity, indicative of increased arousal (Curtis et al. 1997). We have previously demonstrated that endogenous CRF is released within the LC to activate this system during hypotensive challenge (Valentino et al. 1991; Berridge et al. 1993; Curtis et al. 2001). Moreover, the IC₅₀ for two different CRF antagonists in blocking CRF were similar to their IC₅₀s for preventing an equieffective activation by hypotensive stress, underscoring the involvement of a common receptor (Curtis et al. 1994). CRF-elicited LC activation was necessary for cortical EEG desynchronization that was associated with the stress, suggesting that LC activation is necessary for arousal associated with this stressor (Page et al. 1993). In addition to tonically increasing LC discharge rate, CRF blunts LC phasic responses to somatosensory and auditory stimuli (Valentino and Foote 1987, 1988). This blunting effect is also observed during hypotensive stress that engages endogenous CRF release in the LC (Valentino and Foote 1988). The net effect of CRF on LC neurons is to shift the mode of LC discharge to a high tonic-low phasic state (Valentino and Foote 1987, 1988; Valentino and

Van Bockstaele 2008). This mode of firing has been associated with high arousal, decreased focused attention and increased behavioral flexibility or going off-task in a search for optimal outcomes (Aston-Jones and Cohen 2005).

CRF receptors (CRFRs) are GPCRs that belong to the class B subtype. Two types of CRFR, the CRF receptor type 1 (CRFR1) and CRF receptor type 2 (CRFR2) are produced from distinct genes (Hillhouse and Grammatopoulos 2006) and have several splice variants. Both CRFR1 and CRFR2 have been expressed in various central and peripheral tissues. The transmembrane and intracellular domains of the CRFRs share 70% amino acid sequence similarity (Dautzenberg and Hauger 2002a; Hauger et al. 2003). CRFRs can be activated by stress, CRF and CRF-related peptides that include urocortin 1, urocortin 2 (also known as stresscopin-related peptide), and urocortin 3 (also known as stresscopin). Binding of CRFR agonists to extracellular domains of CRFR1 and CRFR2 leads to conformational changes of these receptors and consequent activation of G proteins. Coupling to G_s leads to stimulation of adenylyl cyclase and activation of protein kinase A and other cyclic AMP dependent signaling elements (Hauger and Dautzenberg 1999; Dautzenberg et al. 2000). CRFR interacts transiently with β -arrestin and can be recycled rapidly (Perry et al. 2005; Oakley et al. 2007). Internalized CRFR can either be recycled back to the cell surface, resulting in re-sensitization of the receptor or further transported to lysosomes for eventual degradation (Tsao and von Zastrow 2000).

Although GPCRs are generally inactive, in the presence of ligand or agonist, GPCRs respond by activation of a G protein. CRF, as one of the ligands of CRFR1, is known to regulate the LC-norepinephrine system through CRFR1 (Reyes et al. 2006, 2008). As described above, CRF, acting on CRFR1, increases spontaneous discharge rates of LC neurons when locally administered either *in vivo* or *in vitro* (Curtis et al. 1997; Jedema and Grace 2004). CRF activation of LC neurons is associated with increased c-fos expression and norepinephrine release in terminal fields (Rassnick et al. 1988; Page and Abercrombie 1999). Using molecular and cellular approaches, CRFR1 has been localized to the LC-norepinephrine system (Reyes et al. 2006, 2007, 2008; Fan et al., 2009; Taneja et al. 2011). As CRF binds with its receptor, CRFR1 preferentially binds G_s, leading to activation of adenylyl cyclase, formation of adenosine monophosphate (cAMP) and protein kinase A activation (Chalmers et al. 1996; Grammatopoulos and Chrousos 2002). Phosphorylation of a potassium channel through a cAMP-dependent kinase is thought to mediate the CRF-induced increases in LC firing (Jedema and Grace 2004).

CRF receptor trafficking following agonist treatment or exposure to stress

Agonist induced-internalization of G protein-coupled receptor is a common mechanism for modulating cellular sensitivity to neurotransmitters (Segredo et al. 1997). Agonist/stress stimulation of GPCRs can result in internalization of the receptor protein into intracellular vesicles. Subsequently, receptors are recycled to the cell surface or further transported to lysosomes for eventual degradation (Tsao and von Zastrow 2000). Evidence of CRF-induced trafficking of CRFR1 has been demonstrated in cultured neurons (Westlund et al. 1984; Childs et al. 1986; Rasmussen et al. 2004; Perry et al. 2005; Holmes et al. 2006). Following CRF administration, CRFR1 internalization was demonstrated *in vivo* in LC

neurons (Figure 1; Reyes et al. 2006). This was produced by a CRF dose that is maximally effective in increasing LC discharge rate (Curtis et al. 1997). This is a physiological relevant dose of CRF because previous studies demonstrated behavioral activation and anxiogenic effects of this dose of CRF when microinjected into the LC (Butler et al. 1990). The magnitude of LC activation produced by this CRF dose was similar to that produced by hypotensive stress in unanesthetized rats, a physiological stressor that activates LC neurons via CRF release (Curtis et al. 1993). Dual immunolabeling verified that the CRFr1 internalization occurs in LC neurons expressing the catecholamine synthesizing enzyme, tyrosine-hydroxylase (Reyes et al. 2006, 2008). Similar to findings in cultured cells, internalization of CRF was rapid and maintained for a relatively long time (Rasmussen et al. 2004; Perry et al. 2005).

CRFr internalization was also reported in the bed nucleus of stria terminalis following chronic morphine treatment using high resolution electron microscopy (Jaferi et al. 2009). Compared to saline, mice that received chronic morphine showed a significantly lower plasmalemma and greater cytoplasmic density of the CRFr immunogold particles. Interestingly, within the cytoplasmic compartment of somata and dendrites, the proportion of CRFr immunogold particles associated with mitochondria was three times as great in mice receiving morphine compared with saline (Jaferi et al. 2009). This subcellular distribution is consistent with morphine-, and CRFr-associated modulation of intracellular calcium release or oxidative stress (Pickel et al. 1997; Trushina and McMurray 2007).

Similar to CRF, swim stress resulted in a shift in CRFr1 internalization from the plasma membrane to the cytoplasm (Figure 1; Reyes et al. 2006, 2008) consistent with the idea that swim stress releases CRF that then activates CRFr1 in LC neurons and supports the concept that CRF serves as a neuromodulator to regulate LC activity during stress. CRFr1 that becomes internalized in LC dendrites following either agonist stimulation or stress is also associated with endosomes (Reyes et al. 2006, 2008). Interestingly, we have observed a progression from associations with early endosomes to multivesicular bodies as stress increases (Reyes et al. 2008). Because multivesicular bodies often provide a route to the lysosomal system, this path may favor degradation/down-regulation of CRFr1. Such a cellular effect would be consistent with the relatively enduring electrophysiological changes in LC sensitivity to CRF that have been reported (Conti and Foote 1995, 1996; Curtis et al. 1995, 1999). Increased association of CRFr1 with the Golgi apparatus at a later time after stress could reflect synthesis of new receptor (Reyes et al. 2008). Alternatively, the associations with vesicular bodies and the Golgi may reflect communication between the two compartments that is involved in recycling (Reyes et al. 2008). This is consistent with other studies showing alterations of CRFr signaling in stress-related disorders, particularly anxiety and depression (Gold et al., 1996; Arborelius et al., 1999; Hauger and Dautzenberg, 1999; Holsboer, 1999). Post-mortem measurements of CRFr in suicide victims, for example, showed elevated levels of CRF and decreased CRFr1 and CRFr2 mRNA levels in the frontal cortical region (Merali et al., 2004). Furthermore, using in-situ hybridization and quantitative PCR, a shift in the ratio of the CRFr1 and CRFr2 in the pituitaries of suicide victims has been reported (Hiroi et al., 2001).

To determine whether differences in cell surface expression of CRFr1 in LC neurons generalize to other ethologically pertinent stressors, we demonstrated changes in intracellular trafficking of CRFr following social defeat using a resident-intruder model. The resident-intruder paradigm is designed in which a rat (the intruder) is attacked after being placed into the home cage of a larger, aggressive rat (the resident). Typically, the intruder assumes a subordinate supine posture signaling defeat. At this time, the defeated rats are separated from the intruder using a wire mesh barrier for a period of 30 min (Wood et al. 2010). It was observed that, following this interaction, rats cluster into two distinct subpopulations defined by the latency to assume the defeat posture. These subpopulations exhibit different endocrine and behavioral phenotypes and differ in their expression of CRF and CRFr in certain brain regions (Wood et al. 2010). We have recently shown that repeated social stress has differential effects on CRFr1 trafficking in LC neurons depending on the latency for rats to assume defeat (Chaijale et al. 2013). Specifically, CRFr1 internalization was greater in social stress characterized by a short latency to defeat as indicated by a greater cytoplasmic:total CRFr1 ratio. There was no significant difference observed in CRFr1 trafficking between control and long latency rats (Chaijale et al. 2013). Social stress produced long lasting changes in behaviors and CRF activity suggesting that differences in the trafficking of CRFr1 between rats that exhibited short latency vs. long latency could explain differences in CRF regulation that translate to differential behavioral responses and vulnerability following exposure to social stress.

Sex difference in CRFr trafficking

As detailed, both acute and chronic stressor exposure can induce CRFr internalization in LC neurons of male rats (Figure 2; Reyes et al. 2006, 2008; Bangasser et al. 2010). In addition, in CRF over-expressing male mice CRFr1 immunolabeling indicated internalization (Bangasser et al. 2010). However in female rats, CRFr1 distribution and stress-induced trafficking in LC neurons are markedly different (Figure 2; Bangasser et al. 2010, 2013; Bangasser and Valentino 2012; Valentino et al. 2012, 2013). Under basal conditions, females have a greater proportion of receptors located in the cytoplasm than their male counterparts (Figure 2; Bangasser et al. 2010, 2013; Bangasser and Valentino 2012). Following swim stress, a large proportion of CRFr1 are found on plasma membrane in females (Bangasser et al. 2010). These results suggest that in female LC neurons stress induces CRFr1 trafficking to the plasma membrane. Moreover, they indicate that the cellular adaptation of internalization is compromised in females. This lack of internalization is likely due to sex differences in CRFr binding to β -arrestin2, the protein required for CRFr1 desensitization and internalization (Hauger and Dautzenberg 1999; Holmes et al. 2006; Oakley et al. 2007). In cortical tissue, swim stress increased β -arrestin2 binding to the CRFr1 in male, but not female rats, suggesting the intriguing possibility of sex differences in the conformation of the CRFr (Bangasser et al. 2010, 2013; Bangasser and Valentino 2012; Valentino et al. 2012, 2013). Unlike the case for CRF or CRF binding protein, circulating gonadal hormones are not involved in sex differences in CRFr function, as indicated by molecular and electrophysiological findings (Bangasser et al. 2010; Curtis et al., 2006).

Sex differences in CRFr trafficking (Figure 2) are consistent with sex differences in electrophysiological responses of LC neurons to CRF. In males, the CRF-dose response

curve for LC activation plateaus early, an effect attributable to CRFr internalization (Curtis et al. 1995, 1999, 2006). However, this early plateau is not observed in females (Curtis et al. 2006). Functionally, the compromised CRFr internalization in females would result in increased activation of the LC-arousal system under conditions of CRF hypersecretion, as are thought to occur in depression and post-traumatic stress disorder (Nemeroff et al. 1984; Kaye et al. 1987; Raadsheer et al. 1994; Checkley 1996; Bremner et al. 1997; Baker et al. 1999). Because these psychiatric diseases occur more frequently in women and they share hyperarousal as a common feature, sex differences in CRF modulation of the LC system may account for the sex bias in these disorders (Kessler et al. 1994; Kendler et al. 1995; Koob and Heinrichs 1999; Southwick et al. 1999; Keane et al. 2006; Valentino et al. 2012).

Functional consequences

LC neurons, with a widely distributed network of axons, promote cognitive and behavioral limbs of the stress response through changes in their discharge rate and pattern in tune with dynamic environments. The mode of LC activity is finely tuned by a convergence of afferents, particularly excitatory amino acids (EAA), CRF and endogenous opioids. Tonic and phasic modes of LC discharge facilitate distinct and exclusive processes (Aston-Jones and Cohen 2005). Phasic activity facilitates ongoing behavior and optimizes performance in tasks requiring selective attention, whereas high tonic activity promotes attention to irrelevant stimuli, disengagement from ongoing tasks and searching for alternate tasks when present behavior is not optimal. By biasing LC activity towards a particular discharge mode, EAA, opioid, and CRF afferents can shape predominant behavioral strategies in these environments. EAA afferents would facilitate selective attention and optimal performance of ongoing tasks through enhancement of phasic discharge. CRF shifts LC activity towards a high tonic and lower phasic mode, an effect associated with hyperarousal, disengagement from ongoing behavior and scanning of environmental stimuli (Valentino and Foote 1987, 1988). In contrast, moderate levels of endogenous opioids acting at mu-opioid receptors bias activity toward the phasic mode, by selectively decreasing tonic activity (Valentino and Foote 1988). By decreasing the ability of stimuli to phasically activate LC neurons, ongoing behavior and performance in tasks requiring focused attention will be disrupted. At the same time, by not increasing tonic discharge, the impetus to seek alternate strategies will not be promoted. The net effect of these interactions is postulated to adjust the activity and reactivity of the LC-norepinephrine system such that the state of arousal and processing of sensory stimuli are modified to facilitate adaptive behavioral responses to stressors.

Elucidating CRFr trafficking and signaling events in monoaminergic nuclei may help elucidate the neurobiological underpinnings underlying the pathophysiology of depression. An attractive hypothesis had previously centered on the notion that severe depression is accompanied by CRF hypersecretion. However, current concepts regarding GPCR regulation argue against this (Lefkowitz and Shenoy 2005). Considering the evidence that ligand-activated CRFr1 receptors typically undergo rapid phosphorylation, desensitization, and internalization in response to high agonist concentrations (Reisine and Hoffman 1983; Hoffman et al. 1985; Dieterich et al. 1996; Hauger et al. 1997, 2000; Dautzenberg et al. 2001, 2002b; Roseboom et al. 2001; Teli et al. 2005; Kageyama et al. 2006), it is unlikely that CRF hypersecretion alone is sufficient to account for the enhancement of central CRF

neurotransmission seen in severe depression (Hauger et al. 2006). Rather, dysfunction in processes regulating to CRF₁ receptor dynamics (internalization, recycling), as well as alterations in intracellular signaling cascades may be more relevant.

Conclusions and future directions

In summary, sensitivity of LC neurons to CRF is not static, but can be influenced by a number of factors. For example, CRF effects in the LC are altered by prior stress and may depend on the duration and severity of the stressor. In support of this, the effect of CRF on LC neuronal firing rates is potentiated by two weeks of daily cold exposure (Jedema et al. 2001) or in animals subjected to a forced swim test (Curtis et al. 1999). Immediately following an acute (30 min) session of footshock, LC neurons become desensitized to CRF, as indicated by a shift to the right of the CRF dose-response curve for LC activation (Curtis et al. 1995; Conti and Foote 1996). The physiological impact of this change in sensitivity to CRF is apparent as a loss of LC responses to hypotensive challenge (Curtis et al. 1995) and colon distention (Lechner et al. 1997). This change in LC sensitivity is selective to CRF because LC spontaneous discharge and sensitivity to other inputs is not altered. In male rats with a history of stress, the stress-induced sensitization to low doses of CRF is functionally relevant because it allows low levels of CRF, that would typically have no effect, to activate the brain norepinephrine system by a magnitude that produces arousal (Curtis et al. 1995, 1999). The magnitude of the shift varies with different conditions and that observed after 24 h is the most dramatic. Moreover, association of internalized CRF₁ with distinct subcellular compartments suggests distinct trafficking patterns at these two time points following stress.

A relevant feature of stress-induced sensitization of the LC is that, in animals with a history of severe or repeated stress, the consequences of activation of the LC-norepinephrine system may be elicited inappropriately by stimuli that would not typically activate the system. This stress-related sensitization of LC neurons to CRF may play a role in the hypervigilance seen in post-traumatic stress disorder, a condition in which subjects exposed to a severe trauma become selectively sensitized to stimuli previously associated with the trauma. Subjects exhibit hyperarousal, hypervigilance and sleep disturbances, symptoms that are associated with a hyperactive LC system (Charney et al. 1993).

Continued investigations into the neurobiology of CRF₁ signaling and its influence on broadly projecting neural networks such as the LC will provide important insights into the complex cellular and molecular mechanisms underlying normal and pathological defense mechanisms, and their contribution to onset and relapse of anxiety, stress, and depression. The use of high resolution anatomical approaches, as described here, will continue to inform adaptations in CRF₁ dynamics and that will contribute to our understanding of receptor trafficking as a pivotal target by which stressors or pharmacological agents regulate the sensitivity of the LC-norepinephrine system to CRF and subsequent stressors.

Acknowledgments

Preparation of this review was supported in part by National Institutes of Health grants DA#09082, MH #40008 to E.V.B. NIDA had no further role in the writing of the review or in the decision to submit the manuscript for publication. We are also grateful to Mr. Nathan Heldt for his excellent help.

References

- Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol.* 1999; 160:1–12. [PubMed: 9854171]
- Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci.* 2005; 28:403–50. [PubMed: 16022602]
- Aston-Jones G, Foote SL, Bloom FE. Anatomy and physiology of locus coeruleus neurons: functional implications. In: Ziegler, M.; Lake, CR., editors. *Norepinephrine (Frontiers of Clinical Neuroscience)*. Vol. 2. Baltimore: Williams and Wilkins; 1984. p. 92–116.
- Aston-Jones G, Shipley MT, Chouvet G, Ennis M, Van Bockstaele E, Pieribone V, Shiekhata R, Akaoka H, Drolet G, Astier B. Afferent regulation of locus coeruleus neurons: anatomy, physiology and pharmacology. *Prog Brain Res.* 1991; 88:47–75. [PubMed: 1687622]
- Baker DG, West SA, Nicholson WE, Ekhtor NN, Kasckow JW, Hill KK, et al. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiatry.* 1999; 156:585–8. [PubMed: 10200738]
- Bangasser DA, Curtis A, Reyes BAS, Bethea TT, Parastatidis I, Ischiropoulos H, et al. Sex differences in corticotropin-releasing factor receptor signaling and trafficking: potential role in female vulnerability to stress-related psychopathology. *Mol Psychiatry.* 2010; 15:877, 896–904. [PubMed: 20548297]
- Bangasser DA, Valentino RJ. Sex differences in molecular and cellular substrates of stress. *Cell Mol Neurobiol.* 2012; 32:709–23. [PubMed: 22488525]
- Bangasser DA, Reyes BA, Piel D, Garachh V, Zhang XY, Plona ZM, et al. Increased vulnerability of the brain norepinephrine system of females to corticotropin-releasing factor overexpression. *Mol Psychiatry.* 2013; 18:166–73. [PubMed: 22508464]
- Berridge CW, Page ME, Valentino RJ, Foote SL. Effects of locus coeruleus inactivation on electroencephalographic activity in neocortex and hippocampus. *Neuroscience.* 1993; 55:381–93. [PubMed: 8104319]
- Bhattacharya M, Babwah AV, Ferguson SS. Small GTP-binding protein-coupled receptors. *Biochem Soc Trans.* 2004; 32:1040–4. [PubMed: 15506958]
- Bockaert J, Perroy J, Becamel C, Marin P, Fagni L. GPCR interacting proteins (GIPs) in the nervous system: Roles in physiology and pathologies. *Annu Rev Pharmacol Toxicol.* 2010; 50:89–109. [PubMed: 20055699]
- Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, et al. Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. *Am J Psychiatry.* 1997; 154:624–9. [PubMed: 9137116]
- Butler PD, Weiss JM, Stout JC, Nemeroff CB. Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J Neurosci.* 1990; 10:176–83. [PubMed: 2299391]
- Chaijale NN, Curtis AL, Wood SK, Zhang XY, Bhatnagar S, Reyes BA, Van Bockstaele EJ, Valentino RJ. Social stress engages opioid regulation of locus coeruleus norepinephrine neurons and induces a state of cellular and physical opiate dependence. *Neuropsychopharmacology.* 2013; 38:1833–43. [PubMed: 23660707]
- Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotrophin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci.* 1996; 17:166–72. [PubMed: 8984745]
- Charney DS, Deutch AY, Krystal JH, Southwick SM, Davis M. Psychobiologic mechanisms of posttraumatic stress disorder. *Arch Gen Psychiatry.* 1993; 50:295–305. [PubMed: 8466391]
- Checkley S. The neuroendocrinology of depression and chronic stress. *Br Med Bull.* 1996; 52:597–617. [PubMed: 8949260]
- Childs GV, Morell JL, Niendorf A, Aguilera G. Cytochemical studies of corticotropin-releasing factor (CRF) receptors in anterior lobe corticotropes: binding, glucocorticoid regulation, and endocytosis of [biotinyl-Ser1]CRF. *Endocrinology.* 1986; 119:2129–42. [PubMed: 2876885]

- Conti LH, Foote SL. Effects of pretreatment with corticotropin-releasing factor on the electrophysiological responsivity of the locus coeruleus to subsequent corticotropin-releasing factor challenge. *Neuroscience*. 1995; 69:209–19. [PubMed: 8637619]
- Conti LH, Foote SL. Reciprocal cross-desensitization of locus coeruleus electrophysiological responsivity to corticotropin-releasing factor and stress. *Brain Res*. 1996; 722:19–29. [PubMed: 8813346]
- Curtis AL, Drolet G, Valentino RJ. Hemodynamic stress activates locus coeruleus neurons of unanesthetized rats. *Brain Res Bull*. 1993; 31:737–44. [PubMed: 8518964]
- Curtis AL, Grigoriadis DE, Page ME, Rivier J, Valentino RJ. Pharmacological comparison of two corticotropin-releasing factor antagonists: in vivo and in vitro studies. *J Pharmacol Exp Ther*. 1994; 268:359–65. [PubMed: 8301577]
- Curtis AL, Pavcovich LA, Grigoriadis DE, Valentino RJ. Previous stress alters corticotropin-releasing factor neurotransmission in the locus coeruleus. *Neuroscience*. 1995; 65:541–50. [PubMed: 7777167]
- Curtis AL, Lechner SM, Pavcovich LA, Valentino RJ. Activation of the locus coeruleus noradrenergic system by intracoeular microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. *J Pharmacol Exp Ther*. 1997; 281:163–72. [PubMed: 9103494]
- Curtis AL, Pavcovich LA, Valentino RJ. Long term regulation of locus coeruleus sensitivity to corticotropin-releasing factor by swim stress. *J Pharmacol Exp Ther*. 1999; 289:1211–9. [PubMed: 10336508]
- Curtis AL, Bello NT, Valentino RJ. Evidence for functional release of endogenous opioids in the locus coeruleus during stress termination. *J Neurosci*. 2001; 21:1–5.
- Curtis AL, Bethea T, Valentino RJ. Sexually dimorphic responses of the brain norepinephrine system to stress and corticotropin-releasing factor. *Neuropsychopharmacology*. 2006; 31:544–54. [PubMed: 16123744]
- Dautzenberg FM, Higelin J, Teichert U. Functional characterization of corticotropin-releasing factor type 1 receptor endogenously expressed in human embryonic kidney 293 cells. *Eur J Pharmacol*. 2000; 390:51–9. [PubMed: 10708706]
- Dautzenberg FM, Braun S, Hauger RL. GRK3 mediates desensitization of CRF1 receptors: a potential mechanism regulating stress adaptation. *Am J Physiol Regul Integr Comp Physiol*. 2001; 280:R935–46. [PubMed: 11247813]
- Dautzenberg FM, Hauger RL. The CRF peptide family and their receptors: yet more partners discovered. *Trends Pharmacol Sci*. 2002a; 23:71–7. [PubMed: 11830263]
- Dautzenberg FM, Wille S, Braun S, Hauger RL. GRK3 regulation during CRF- and urocortin-induced CRF1 receptor desensitization. *Biochem Biophys Res Commun*. 2002b; 298:303–8. [PubMed: 12413940]
- Dieterich KD, Grigoriadis DE, De Souza EB. Homologous desensitization of human corticotropin-releasing factor1 receptor in stable transfected mouse fibroblast cells. *Brain Res*. 1996; 710:287–92. [PubMed: 8963673]
- Fan JM, Chen XQ, Jin H, Du JZ. Gestational hypoxia alone or combined with restraint sensitizes the hypothalamic-pituitary-adrenal axis and induces anxiety-like behavior in adult male rat offspring. *Neuroscience*. 2009; 159:1363–73. [PubMed: 19409200]
- Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev*. 2001; 53:1–24. [PubMed: 11171937]
- Ferguson SS. Phosphorylation-independent attenuation of GPCR signalling. *Trends Pharmacol Sci*. 2007; 28:173–9. [PubMed: 17350109]
- Foote SL, Bloom FE, Aston-Jones G. Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. *Physiol Rev*. 1983; 63:844–914. [PubMed: 6308694]
- Glass MJ, Lane DA, Colago EE, Chan J, Schlussman SD, Zhou Y, et al. Chronic administration of morphine is associated with a decrease in surface AMPA GluR1 receptor subunit in dopamine D1 receptor expressing neurons in the shell and non-D1 receptor expressing neurons in the core of the rat nucleus accumbens. *Exp Neurol*. 2008; 210:750–761. [PubMed: 18294632]

- Gold PW, Wong ML, Chrousos GP, Licinio J. Stress system abnormalities in melancholic and atypical depression: molecular, pathophysiological, and therapeutic implications. *Mol Psychiatry*. 1996; 1:257–64. [PubMed: 9118349]
- Grammatopoulos DK, Chrousos GP. Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists. *Trends Endocrinol Metab*. 2002; 13:436–44. [PubMed: 12431840]
- Grampp T, Notz V, Broll I, Fischer N, Benke D. Constitutive, agonist-accelerated, recycling and lysosomal degradation of GABA(B) receptors in cortical neurons. *Mol Cell Neurosci*. 2008; 39:628–37. [PubMed: 18948198]
- Hauger RL, Dautzenberg FM, Flaccus A, Liepold T, Spiess J. Regulation of corticotropin-releasing factor receptor function in human Y-79 retinoblastoma cells: rapid and reversible homologous desensitization but prolonged recovery. *J Neurochem*. 1997; 68:2308–16. [PubMed: 9166723]
- Hauger, RL.; Dautzenberg, FM. Regulation of the stress response by corticotropin-releasing factor receptors. In: Conn, PM.; Freedman, ME., editors. *Neuroendocrinology in Physiology and Medicine*. New York: Humana Press; 1999. p. 261–86.
- Hauger RL, Smith RD, Braun S, Dautzenberg FM, Catt KJ. Rapid agonist-induced phosphorylation of the human CRF receptor, type 1: a potential mechanism for homologous desensitization. *Biochem Biophys Res Commun*. 2000; 268:572–6. [PubMed: 10679245]
- Hauger RL, Grigoriadis DE, Dallman MF, Plotsky PM, Vale WW, Dautzenberg FM. International Union of Pharmacology. XXXVI Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. *Pharmacol Rev*. 2003; 55:21–6. [PubMed: 12615952]
- Hauger RL, Risbrough V, Brauns O, Dautzenberg FM. Corticotropin releasing factor (CRF) receptor signaling in the central nervous system: new molecular targets. *CNS Neurol Disord Drug Targets*. 2006; 5:453–79. [PubMed: 16918397]
- Hillhouse EW, Grammatopoulos DK. The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. *Endocr Rev*. 2006; 27:260–86. [PubMed: 16484629]
- Hiroi N, Wong ML, Licinio J, Park C, Young M, Gold PW, et al. Expression of corticotropin releasing hormone receptors type I and type II mRNA in suicide victims and controls. *Mol Psychiatry*. 2001; 6:540–6. [PubMed: 11526468]
- Hoffman AR, Ceda G, Reisine TD. Corticotropin-releasing factor desensitization of adrenocorticotrophic hormone release is augmented by arginine vasopressin. *J Neurosci*. 1985; 5:234–42. [PubMed: 2981299]
- Holmes KD, Babwah AV, Dale LB, Poulter MO, Ferguson SS. Differential regulation of corticotropin releasing factor 1alpha receptor endocytosis and trafficking by beta-arrestins and Rab GTPases. *J Neurochem*. 2006; 96:934–49. [PubMed: 16412099]
- Holsboer F. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J Psychiatr Res*. 1999; 33:181–214. [PubMed: 10367986]
- Jaferi A, Lane DA, Pickel VM. Subcellular plasticity of the corticotropin-releasing factor receptor in dendrites of the mouse bed nucleus of the stria terminalis following chronic opiate exposure. *Neuroscience*. 2009; 163:143–54. [PubMed: 19539724]
- Jedema HP, Finlay JM, Sved AF, Grace AA. Chronic cold exposure potentiates CRH-evoked increases in electrophysiologic activity of locus coeruleus neurons. *Biol Psychiatry*. 2001; 49:351–9. [PubMed: 11239906]
- Jedema HP, Grace AA. Corticotropin-releasing hormone directly activates noradrenergic neurons of the locus ceruleus recorded in vitro. *J Neurosci*. 2004; 24:9703–13. [PubMed: 15509759]
- Kageyama K, Hanada K, Moriyama T, Nigawara T, Sakihara S, Suda T. G protein-coupled receptor kinase 2 involvement in desensitization of corticotropin-releasing factor (CRF) receptor type 1 by CRF in murine corticotrophs. *Endocrinology*. 2006; 147:441–50. [PubMed: 16195412]
- Kaye WH, Gwirtsman HE, George DT, Ebert MH, Jimerson DC, Tomai TP, et al. Elevated cerebrospinal fluid levels of immunoreactive corticotropin-releasing hormone in anorexia nervosa: relation to state of nutrition, adrenal function, and intensity of depression. *J Clin Endocrinol Metab*. 1987; 64:203–8. [PubMed: 3491830]

- Keane TM, Marshall AD, Taft CT. Posttraumatic stress disorder: etiology, epidemiology, and treatment outcome. *Annu Rev Clin Psychol.* 2006; 2:161–97. [PubMed: 17716068]
- Kendler KS, Kessler RC, Walters EE, MacLean C, Neale MC, Heath AC, et al. Stressful life events, genetic liability, and onset of an episode of major depression in women. *Am J Psychiatry.* 1995; 152:833–42. [PubMed: 7755111]
- Kessler RC, McGonagle KA, Nelson CB, Hughes M, Swartz M, Blazer DG. Sex and depression in the National Comorbidity Survey. II: Cohort effects. *J Affect Disord.* 1994; 30:15–26. [PubMed: 8151045]
- Koenig JA, Edwardson JM. Endocytosis and recycling of G protein-coupled receptors. *Trends Pharmacol Sci.* 1997; 18:276–87. [PubMed: 9277131]
- Koob GF, Heinrichs SC. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. *Brain Res.* 1999; 848:141–52. [PubMed: 10612706]
- Lechner SM, Curtis AL, Brons R, Valentino RJ. Locus coeruleus activation by colon distention: role of corticotropin-releasing factor and excitatory amino acids. *Brain Res.* 1997; 756:114–24. [PubMed: 9187321]
- Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. *Science.* 2005; 308:512–7. [PubMed: 15845844]
- Leranth, C.; Pickel, VM. Electron microscopic preembedding double-labeling methods. In: Heimer, L.; Zaborszky, L., editors. *Neuroanatomical tracing methods 2 (I)*. New York: Plenum Press; 1989. p. 129–72.
- Lujan R, Nusser Z, Roberts JD, Shigemoto R, Somogyi P. Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *Eur J Neurosci.* 1996; 8:1488–500. [PubMed: 8758956]
- Luttrell LM. Reviews in molecular biology and biotechnology: transmembrane signaling by G protein-coupled receptors. *Mol Biotechnol.* 2008; 39:239–64. [PubMed: 18240029]
- Magalhaes AC, Holmes KD, Dale LB, Combs-Agrar L, Lee D, Yadav PN, et al. CRF receptor 1 regulates anxiety behavior via sensitization of 5-HT2 receptor signaling. *Nat Neurosci.* 2010; 13:622–9. [PubMed: 20383137]
- Merali Z, Du L, Hrdina P, Palkovits M, Faludi G, Poulter MO, Anisman H. Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. *J Neurosci.* 2004; 24:1478–85. [PubMed: 14960621]
- Milner, TA.; Waters, EM.; Robinson, DC.; Pierce, JP. Degenerating processes identified by electron microscopic immunocytochemical methods. In: Manfredi, Kawamata, editors. *Neurodegeneration: Methods and Protocols*. Vol. Chapter 3. New York: Springer; 2006. p. 23–59.
- Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, et al. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science.* 1984; 226:1342–4. [PubMed: 6334362]
- Oakley RH, Olivares-Reyes JA, Hudson CC, Flores-Vega F, Dautzenberg FM, Hauger RL. Carboxyl-terminal and intracellular loop sites for CRF1 receptor phosphorylation and beta-arrestin-2 recruitment: a mechanism regulating stress and anxiety responses. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293:R209–22. [PubMed: 17363685]
- Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? *Nat Rev Drug Discov.* 2006; 5:993–6. [PubMed: 17139284]
- Page ME, Berridge CW, Foote SL, Valentino RJ. Corticotropin-releasing factor in the locus coeruleus mediates EEG activation associated with hypotensive stress. *Neurosci Lett.* 1993; 164:81–4. [PubMed: 8152620]
- Page ME, Abercrombie ED. Discrete local application of corticotropin-releasing factor increases locus coeruleus discharge and extracellular norepinephrine in rat hippocampus. *Synapse.* 1999; 33:304–13. [PubMed: 10421711]
- Perrin MH, Vale WW. Corticotropin releasing factor receptors and their ligand family. *Ann N Y Acad Sci.* 1999; 885:312–28. [PubMed: 10816663]

- Perry SJ, Junger S, Kohout TA, Hoare SR, Struthers RS, Grigoriadis DE, et al. Distinct conformations of the corticotropin releasing factor type 1 receptor adopted following agonist and antagonist binding are differentially regulated. *J Biol Chem.* 2005; 280:11560–8. [PubMed: 15653688]
- Pickel VM, Clarke CL, Meyers MB. Ultrastructural localization of sorcin, a 22 kDa calcium binding protein, in the rat caudate-putamen nucleus: association with ryanodine receptors and intracellular calcium release. *J Comp Neurol.* 1997; 386:625–34. [PubMed: 9378856]
- Raadsheer FC, Hoogendijk WJ, Stam FC, Tilders FJ, Swaab DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology.* 1994; 60:436–44. [PubMed: 7824085]
- Rasmussen TN, Novak I, Nielsen SM. Internalization of the human CRF receptor 1 is dependent of classical phosphorylation sites and of beta-arrestin 1 recruitment. *Eur J Biochem.* 2004; 271:4366–74. [PubMed: 15560778]
- Rassnick S, Hoffman GE, Rabin BS, Sved AF. Injection of corticotropin-releasing hormone into the locus coeruleus or foot shock increases neuronal Fos expression. *Neuroscience.* 1988; 85:259–268. [PubMed: 9607717]
- Reisine T, Hoffman A. Desensitization of corticotropin-releasing factor receptors. *Biochem Biophys Res Commun.* 1983; 111:919–25. [PubMed: 6301492]
- Reyes BAS, Fox K, Valentino RJ, Van Bockstaele EJ. Agonist-induced internalization of corticotropin-releasing factor receptors in noradrenergic neurons of the rat locus coeruleus. *Eur J Neurosci.* 2006; 23:2991–8. [PubMed: 16819988]
- Reyes BAS, Glaser JD, Van Bockstaele EJ. Ultrastructural evidence for co-localization of corticotropin-releasing factor receptor and mu-opioid receptor in the rat nucleus locus coeruleus. *Neurosci Lett.* 2007; 413:216–21. [PubMed: 17194545]
- Reyes BAS, Valentino RJ, Van Bockstaele EJ. Stress-induced intracellular trafficking of corticotropin-releasing factor receptors in rat locus coeruleus neurons. *Endocrinology.* 2008; 149:122–30. [PubMed: 17947354]
- Reyes BAS, Chavkin C, Van Bockstaele EJ. Agonist-induced internalization of kappa-opioid receptors in noradrenergic neurons of the rat locus coeruleus. *J Chem Neuroanat.* 2010; 40:301–9. [PubMed: 20884346]
- Reyes BAS, Vakharia K, Ferraro TN, Levenson R, Berrettini WH, Van Bockstaele EJ. Opiate agonist-induced re-distribution of Wntless, a mu-opioid receptor interacting protein, in rat striatal neurons. *Exp Neurol.* 2012; 233:205–13. [PubMed: 22001156]
- Roseboom PH, Urben CM, Kalin NH. Persistent corticotropin-releasing factor(1) receptor desensitization and downregulation in the human neuroblastoma cell line IMR-32. *Brain Res Mol Brain Res.* 2001; 92:115–27. [PubMed: 11483248]
- Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. *Nature.* 2009; 459:356–63. [PubMed: 19458711]
- Scavone JL, Van Bockstaele EJ. Mu-opioid receptor redistribution in the locus coeruleus upon precipitation of withdrawal in opiate-dependent rats. *Anat Rec.* 2009; 292:401–11.
- Segredo V, Burford NT, Lameh J, Sadee W. A constitutively internalizing and recycling mutant of the mu-opioid receptor. *J Neurochem.* 1997; 68:2395–404. [PubMed: 9166733]
- Sesack, SR.; Miner, LH.; Omelchenko, N. *Neuroanatomical Tract-Tracing 3: Molecules, Neurons, Systems.* Zaborsky, et al., editors. New York: Springer; 2006. p. 6-71.
- Southwick SM, Bremner JD, Rasmusson A, Morgan CA III, Arnsten A, Charney DS. Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry.* 1999; 46:1192–204. [PubMed: 10560025]
- Taneja M, Salim S, Saha K, Happe HK, Qutna N, Petty F, et al. Differential effects of inescapable stress on locus coeruleus GRK3, alpha2-adrenoceptor and CRF1 receptor levels in learned helpless and non-helpless rats: a potential link to stress resilience. *Behav Brain Res.* 2011; 221:25–33. [PubMed: 21333691]
- Teli T, Markovic D, Levine MA, Hillhouse EW, Grammatopoulos DK. Regulation of corticotropin-releasing hormone receptor type 1 alpha signaling: structural determinants for G protein-coupled receptor kinase-mediated phosphorylation and agonist-mediated desensitization. *Mol Endocrinol.* 2005; 19:474–90. [PubMed: 15498832]

- Thompson MD, Cole DE, Jose PA. Pharmacogenomics of G protein-coupled receptor signaling: insights from health and disease. *Methods Mol Biol.* 2008; 448:77–107. [PubMed: 18370232]
- Torres-Reveron A, Williams TJ, Chapleau JD, Waters EM, McEwen BS, Drake CT, et al. Ovarian steroids alter mu opioid receptor trafficking in hippocampal parvalbumin GABAergic interneurons. *Exp Neurol.* 2009; 219:319–27. [PubMed: 19505458]
- Treweek JB, Jaferi A, Colago EE, Zhou P, Pickel VM. Electron microscopic localization of corticotropin-releasing factor (CRF) and CRF receptor in rat and mouse central nucleus of the amygdala. *J Comp Neurol* 2009. 2009; 512:323–35.
- Trushina E, McMurray CT. Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. *Neuroscience.* 2007; 145:1233–48. [PubMed: 17303344]
- Tsao P, von Zastrow M. Downregulation of G protein-coupled receptors. *Curr Opin Neurobiol.* 2010; 10:365–9. [PubMed: 10851176]
- Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science.* 1981; 213:1394–7. [PubMed: 6267699]
- Valentino RJ, Foote SL. Corticotropin-releasing factor disrupts sensory responses of brain noradrenergic neurons. *Neuroendocrinology.* 1987; 45:28–36. [PubMed: 3492683]
- Valentino RJ, Foote SL. Corticotropin-releasing hormone increases tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats. *J Neurosci.* 1988; 8:1016–25. [PubMed: 3258021]
- Valentino RJ, Page ME, Curtis AL. Activation of noradrenergic locus coeruleus neurons by hemodynamic stress is due to local release of corticotropin-releasing factor. *Brain Res.* 1991; 555:25–34. [PubMed: 1933327]
- Valentino, RJ.; Van Bockstaele, EJ. Corticotropin-releasing factor: putative neurotransmitter actions of a neurohormone. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior (I).* San Diego: Academic Press; 2002. p. 81–102.
- Valentino, RJ.; Van Bockstaele, EJ. Functional interactions between stress neuromediators and the locus-coeruleus noradrenaline system. In: Steckler, TK.; Kalin, NH.; Reul, JMHM., editors. *Handbook of stress and the brain Part 1: The Neurobiology of Stress.* Amsterdam: Elsevier; 2005. p. 465–86.
- Valentino RJ, Van Bockstaele E. Convergent regulation of locus coeruleus activity as an adaptive response to stress. *Eur J Pharmacol.* 2008; 583:194–203. [PubMed: 18255055]
- Valentino RJ, Reyes B, Van Bockstaele E, Bangasser D. Molecular and cellular sex differences at the intersection of stress and arousal. *Neuropharmacology.* 2012; 62:13–20. [PubMed: 21712048]
- Valentino RJ, Bangasser D, Van Bockstaele EJ. Sex-biased stress signaling: the corticotropin-releasing factor receptor as a model. *Mol Pharmacol.* 2013; 83:737–45. [PubMed: 23239826]
- Van Bockstaele EJ, Chan J, Pickel VM. Input from central nucleus of the amygdala efferents to pericoerulear dendrites, some of which contain tyrosine hydroxylase immunoreactivity. *J Neurosci Res.* 1996; 45:289–302. [PubMed: 8841990]
- Vieira AV, Lamaze C, Schmid SL. Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science.* 1996; 274:2086–9. [PubMed: 8953040]
- Waterhouse BD, Border B, Wahl L, Mihailoff GA. Topographic organization of rat locus coeruleus and dorsal raphe nuclei: distribution of cells projecting to visual system structures. *J Comp Neurol.* 1993; 336:345–361. [PubMed: 8263226]
- Westlund KN, Wynn PC, Chmielowiec S, Collins TJ, Childs GV. Characterization of a potent biotin-conjugated CRF analog and the response of anterior pituitary corticotropes. *Peptides.* 1984; 5:627–34. [PubMed: 6089148]
- Williams TJ, Akama KT, Knudsen MG, McEwen BS, Milner TA. Ovarian hormones influence corticotropin releasing factor receptor colocalization with delta opioid receptors in CA1 pyramidal cell dendrites. *Exp Neurol.* 2011; 230:186–96. [PubMed: 21549703]
- Wood SK, Walker HE, Valentino RJ, Bhatnagar S. Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: role of corticotropin-releasing factor. *Endocrinology.* 2010; 151:1795–805. [PubMed: 20160137]

Yu SS, Lefkowitz RJ, Hausdorff WP. Beta-adrenergic receptor sequestration. A potential mechanism of receptor resensitization. *J Biol Chem.* 1993; 268:337–41. [PubMed: 8380158]

Figure 1.

Electron microscopic visualization of CRFr internalization following agonist exposure and swim stress. Examples of CRFr localization in LC dendrites of males and females is also depicted. **A–B.** Sections from rats perfused 5 min after artificial cerebrospinal fluid (A) or CRF (B) microinfusion. Panel A shows a dendritic profile containing gold-silver labeling for CRFr (arrowheads) along the plasmalemma. The dendrite receives synaptic contacts from axon terminals (t). Panel B shows that receptor labeling shifts from the plasmalemma to the cytoplasm following CRF microinfusion. Arrows point to gold-silver labeling in the cytoplasm. **C–D.** Sections from a control male rat (C) and a male rat 24 h following 15-min swim stress (D). Immunogold-silver labeling for CRFr (arrowheads) can be seen along the plasmalemma in a dendritic profile (C). CRFr labeling shifts from the plasmalemma to the cytoplasm 24 (D) following 15-min swim stress. The dendrites receive synaptic contacts from axon terminals (t). Arrows point to immunogold-silver labeling in the cytoplasm. **E–F.** Sections from a handled female rat (E) and a female rat 24 h following 15-min swim stress (F). Immunogold-silver labeling for CRFr (arrows) can be seen within the cytoplasm of a handled female rat (E). Immunogold-silver labeling for CRFr can be seen along the plasmalemma in a dendritic profile of a female rat 24 h following 15-min swim stress (F). The dendrites receive synaptic contacts from axon terminals (t). m, mitochondria. Scale bar = 0.5 μ m.

Figure 2.

The schematic depicts CRFr internalization induced by agonist treatment or swim stress in male LC neurons. It also depicts sex differences in CRFr trafficking following swim stress.