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The locus coeruleus: a key nucleus where stress and opioids intersect to mediate vulnerability to opiate abuse

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Abstract

The interaction between the stress axis and endogenous opioid systems has gained substantial clinical attention as it is increasingly recognized that stress predisposes to opiate abuse. For example, stress has been implicated as a risk factor in vulnerability to the initiation and maintenance of opiate abuse and is thought to play an important role in relapse in subjects with a history of abuse. Numerous reports indicating that stress alters individual sensitivity to opiates suggest that prior stress can influence the pharmacodynamics of opiates that are used in clinical settings. Conversely, the effects of opiates on different components of the stress axis can impact on individual responsivity to stressors and potentially predispose individuals to stress-related psychiatric disorders. One site at which opiates and stress substrates may interact to have global effects on behavior is within the locus coeruleus (LC), the major brain norepinephrine (NE)-containing nucleus. This review summarizes our current knowledge regarding the anatomical and neurochemical afferent regulation of the LC. It then presents physiological studies demonstrating opposing interactions between opioids and stress-related neuropeptides in the LC and summarizes results showing that chronic morphine exposure sensitizes the LC-NE system to corticotropin releasing factor and stress. Finally, new evidence for novel presynaptic actions of kappa-opioids on LC afferents is provided that adds another dimension to our model of how this central NE system is co-regulated by opioids and stress-related peptides.

Keywords

opioids; enkephalins; dynorphin; corticotropin-releasing hormone; norepinephrine; locus coeruleus

Stress and vulnerability to drug abuse

In the midst of a perceived threat to survival, the body undergoes a number of adaptive responses aimed at mobilizing energy resources and sustaining arousal (Selye, 1953). This “stress response” includes an endocrine limb (involving the hypothalamic-pituitary adrenal axis activation), an autonomic limb (involving cardiovascular and gastrointestinal mobilization), an immunological limb (involving immunosuppression) and a behavioral and cognitive limb (involving enhanced arousal and alterations in attention) (Selye and Horava, 1953). The machinery underlying the coordinated function of these limbs is indispensable in effectively adapting to physiological and emotional threats. For almost three decades,

multiple evidence demonstrates the role of the neuropeptide, corticotropin-releasing factor (CRF) in the coordination of the stress response. CRF acting as a neurohormone and a neurotransmitter was originally characterized as the hypothalamic neurohormone that initiates the release of adrenocorticotropin from the adenohypophysis or anterior pituitary gland and consequently directs the cascade of events culminating in corticosteroid secretion (Rivier et al., 1982; Vale et al., 1981). Following the discovery of the CRF as a neurohormone, several lines of evidence support a role of CRF as a brain neurotransmitter involved in coordinating the different limbs of the stress response (Dunn and Berridge 1990; Owens and Nemeroff 1991).

Stress has been implicated as a risk factor in the vulnerability to the initiation and maintenance of opiate abuse and is thought to play an important role in relapse in subjects with a history of abuse (Gaal and Molnar, 1990; Goeders, 1998; Goeders, 2003; Hyman et al., 2007; Ilgen et al., 2008; Piazza and Le Moal, 1997; Piazza and Le Moal, 1998; Shaham et al., 1998; Shaham et al., 1997; Sinha et al., 2006; Zhou et al., 1996). However, the impact of stress-opioid interactions extends beyond vulnerability to opiate abuse. Numerous reports indicate that stress alters individual sensitivity to opiates and suggest that prior stress can influence the pharmacodynamics of opiates that are used in clinical settings (Benedek and Szikszay, 1985; Christie and Chesher, 1982; Christie et al., 1982; Hyman et al., 2007; Sinha, 2001; Sinha et al., 1999; Sinha et al., 2007; Stohr et al., 1999; Sutton et al., 1997; Terman et al., 1986; Terman and Liebeskind, 1986). Conversely, the effects of opiates on different components of the stress axis can impact on individual responsivity to stressors and potentially predispose individuals to stress-related psychiatric disorders (Burnett et al., 1999; Calogero et al., 1996; Carey et al., 2009; Price et al., 2004; Yamauchi et al., 1997). Because these interactions have potentially widespread clinical consequences, it is important to identify substrates of the stress response and endogenous opioid systems that interact and the specific points at which stress circuits and endogenous opioid systems intersect.

I. The LC as a key nucleus where stress and opioids interact

While it is known that interactions of stress and opiate substrates occur in various brain regions that may play a role in drug dependence and withdrawal (Houshyar et al., 2003; Maj et al., 2003; McNally and Akil, 2002), one site at which opiates and stress substrates may interact to have global effects on behavior is within the LC, the major brain norepinephrine (NE)-containing nucleus. The LC is targeted by several endogenous opioidergic peptides (Kreibich et al., 2008; Reyes et al., 2009; Reyes et al., 2008; Reyes et al., 2006; Reyes et al., 2007b; Tjounmakaris et al., 2003; Van Bockstaele et al., 1995; Van Bockstaele et al., 1996d), stress-related peptides including corticotropin releasing factor (CRF) (Valentino et al., 1992; Valentino et al., 2001; Van Bockstaele et al., 1996e; Van Bockstaele et al., 1998b; Van Bockstaele et al., 1999c) excitatory amino acids (Barr and Van Bockstaele, 2005; Kreibich et al., 2008) and orexin (Baldo et al., 2003; Koob et al., 2008) (Fig. 1). The LC receives afferents from multiple brain nuclei including the bed nucleus of the stria terminalis (Van Bockstaele et al., 1999b), the central nucleus of the amygdala (CNA) (Van Bockstaele et al., 1996a), the paraventricular nucleus of the hypothalamus (Reyes et al., 2005), the nucleus of the solitary tract (Van Bockstaele et al., 1999a), the nucleus paragigantocellularis (Van Bockstaele et al., 1998a) and the nucleus prepositus hypoglossi (Aston-Jones et al., 1986). In addition, the LC has been implicated in both the stress response (Valentino and Foote, 1987; Valentino and Foote, 1988; Valentino et al., 1993) and opiate actions (Kreibich et al., 2008; Nestler et al., 1994; Nestler et al., 1999; Reyes et al., 2009; Reyes et al., 2008; Reyes et al., 2007a). Chronic stress (Cuadra et al., 1999; Curtis et al., 1995; Curtis et al., 1999), chronic CRF (Conti and Foote, 1995; Conti and Foote, 1996) and chronic exposure to exogenous opiates (Aghajanian, 1978; Duman et al., 1988; Fiorillo and Williams, 1996; Valentino and Wehby, 1989) have all been shown to induce changes in LC plasticity.

II. Anatomical and physiological attributes of the LC

The anatomical and physiological characteristics of the LC-NE system have been reviewed in detail (Aston-Jones et al., 1984; Aston-Jones et al., 1991b; Berridge and Waterhouse, 2003; Foote et al., 1983) and are only briefly summarized here. The LC is a compact, homogenous nucleus that innervates the entire neuraxis through a divergent efferent system. Single LC neurons collateralize to distant and functionally diverse brain regions. It is the sole source of NE in many forebrain regions that have been implicated in cognition (e.g., cortex and hippocampus). LC neurons are spontaneously active and discharge in a synchronous manner that is linked to oscillations in membrane potential (Aston-Jones and Bloom, 1981b; Aston-Jones et al., 1991a; Ishimatsu and Williams, 1996). In addition to their synchronous spontaneous activity, LC neurons are homogeneous in their polymodal response to stimuli (Aston-Jones and Bloom, 1981a; Foote et al., 1980). Coupled with a highly divergent, collateralized efferent system, this synchronous activity provides a mechanism for global NE release throughout the neuraxis in response to stimuli.

Tonic LC activity co-varies with behavioral and electroencephalographic (EEG) indices of arousal, such that activity is highest in the awake state, lower during slow wave sleep and silent during REM sleep (Aston-Jones and Bloom, 1981b). LC neurons respond phasically to environmental stimuli of many modalities and phasic activity is associated with enhanced NE release in target regions (Berridge and Abercrombie, 1999; Florin-Lechner et al., 1996). Excitatory amino acid afferents have been demonstrated to mediate phasic activation of LC neurons by certain somatosensory and auditory stimuli (Ennis et al., 1992). Certain physiological stimuli produce a more tonic activation of LC neurons and the stress neurohormone, CRF has been implicated in these responses (Kosoyan et al., 2005; Lechner et al., 1997; Valentino et al., 1991). Regardless of the stimulus, LC activation is sufficient to activate forebrain (e.g., cortex and hippocampus) EEG and selective LC inhibition has the opposite effect (Berridge and Foote, 1991; Berridge et al., 1993; Curtis et al., 1997; De Sarro et al., 1987; de Sarro et al., 1988; De Sarro et al., 1992; Page et al., 1992; Page et al., 1993). Moreover stress-elicited LC activation has been shown to be necessary for EEG activation by some stressors (Page et al., 1993). Thus, the LC-NE system provides a mechanism by which external and internal stimuli elicit arousal.

In addition to arousal, the LC-NE system is hypothesized to facilitate shifts in the mode of attention, from focused to scanning. This is partly based on LC recordings in monkeys during a focused attention task (Aston-Jones et al., 1999; Rajkowski et al., 1994; Usher et al., 1999). Low tonic LC discharge rate, coupled with low phasic responses to sensory stimuli, are associated with inattention, drowsiness and poor task performance. In contrast, relatively higher tonic LC discharge rates are coupled with robust phasic responses to stimuli and associated with focused attention and optimal behavioral performance. However, increases in tonic LC discharge that exceed the optimal rate are associated with a decrement in attention to the target stimuli and poor task performance. During this time, monkeys attend to task-irrelevant stimuli. In spite of the increase in tonic discharge at this time, phasic responses to stimuli are blunted. This has led to a hypothesized inverted U-shaped relationship between tonic LC activity and focused attention. Importantly, moderate tonic LC activity and robust phasic activity are associated with focused attention, whereas high tonic activity and blunted phasic activity are associated with scanning or labile attention. We will consider the different modes of LC activity as they relate to CRF and μ -opioid agonists below.

III. Opioids and the LC

Afferent regulation of the LC by endogenous opioids is well-supported by anatomical evidence and electrophysiological effects of opiates on LC activity. Enkephalin (ENK)

densely innervates the nuclear core of the LC and is robust in peri-coerulear dendritic zones, particularly at the level of the rostral LC (Van Bockstaele et al., 1995; Van Bockstaele and Chan, 1997; Van Bockstaele et al., 1996c; Van Bockstaele et al., 1996d). Small molecule co-transmitters in ENK-containing axon terminals include glutamate (Barr and Van Bockstaele, 2005; Van Bockstaele et al., 2000) and gamma-amino butyric acid (GABA) (Valentino et al., 2001) (Fig. 1).

The three classes of opioid receptors, mu (μ), delta (δ) and kappa (κ) are prominently distributed within the LC (Elde and Hokfelt, 1993; Van Bockstaele et al., 1995; Van Bockstaele et al., 1996b; Van Bockstaele et al., 1996c). The μ -opioid receptor (μ -OR) is prominently localized postsynaptically within noradrenergic somatodendritic processes (Van Bockstaele et al., 1996b; Van Bockstaele et al., 1996c) while δ -OR and κ -OR are mainly localized to axon terminals (Kreibich et al., 2008; Reyes et al., 2009; van Bockstaele et al., 1997) suggesting that δ -OR and κ -OR may play important roles in presynaptic modulation of neurotransmitter release (see below).

Two brainstem regions that have been shown to provide the major sources of opioid innervation to the LC include the nucleus paragigantocellularis (PGi) in the rostral ventrolateral medulla and the nucleus prepositus hypoglossus (PrH) in the dorsomedial rostral medulla (Aston-Jones et al., 1986; Aston-Jones et al., 1991b; Drolet et al., 1992).

Potent inhibitory effects of μ -OR activation on LC neurons *in vivo* and *in vitro* have been well documented (Aghajanian and Wang, 1987; Korf et al., 1974; Valentino and Wehby, 1988b; Williams et al., 1984; Williams et al., 1982). However, the impact of endogenous opioids on the LC system remained unknown until we provided evidence that endogenous opioids are released within the LC following the termination of hypotensive stress to produce a robust inhibition (Curtis et al., 2001). Thus, during hypotensive stress, LC neurons are activated and this is temporally correlated with, and necessary for, cortical EEG activation (Page et al., 1993; Valentino et al., 1991). Pharmacological analysis revealed that this activation is mediated by CRF release in the LC (see below). Upon stress termination, LC neurons are inhibited for a period of time and this effect is completely blocked by microinfusion of naloxone into the LC (Curtis et al., 2001). In the presence of naloxone, LC neuronal activity takes longer to return to baseline, suggesting that endogenous opioid release in the LC helps to restore basal activity. This presumably protects against adverse consequences of continued activation of the system (e.g., hyperarousal). Thus, opioid-mediated post-stress inhibition of the LC-NE system may serve as a counterregulatory mechanism to balance or limit this cognitive limb of the stress response, much in the same vein that glucocorticoids counter-regulate the hypothalamo-pituitary limb.

IV. Corticotropin-releasing factor and the LC

CRF, the neurohormone that initiates pituitary adrenocorticotrophic hormone (ACTH) release during stress (Vale et al., 1981), also serves as a neuromodulator to activate the LC-NE system in response to certain challenges (Valentino and Van Bockstaele, 2005). 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, (Curtis et al., 1997; Jedema and Grace, 2004; Van Bockstaele et al., 1996e). This is associated with elevated cortical NE efflux (Curtis et al., 1997; Page and Abercrombie, 1999), and cortical EEG activation (Curtis et al., 1997). Our laboratory demonstrated that endogenous CRF is released within the LC to activate this system during hypotensive challenge, providing the first evidence for a neurotransmitter role of CRF in a particular nucleus (Curtis et al., 2001; Page et al., 1993; Valentino et al., 1991). Thus, CRF antagonists selectively and completely prevented LC activation by hypotensive challenge when microinfused into the LC. Moreover, the IC 50 for two different CRF antagonists in antagonizing CRF were similar to

their IC 50s for preventing an ineffectual 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; Valentino and Foote, 1988). This blunting effect is also observed during hypotensive stress which engages endogenous CRF release in the LC (Valentino and Wehby, 1988a). Increased tonic LC activity accompanied by decreased phasic responses to discrete stimuli (as occurs with CRF) may facilitate a shift from focused to scanning or labile attention. This may be adaptive in the acute response to a stressor.

In studies using lesions and functional neuroanatomy, the CNA was identified as the source of CRF that activates the LC during hypotension (Rouzade-Dominguez et al., 2001). Lesions of the CNA, but not Barrington's nucleus (Rouzade-Dominguez et al., 2001) or the bed nucleus of the stria terminalis (other sources of CRF afferents to the LC) greatly attenuated LC activation by hypotensive challenge. Additionally, hypotensive challenge induced phospho-cyclic adenosine monophosphate (cAMP) response element-binding proteins (pCREB) in CNA-CRF neurons that were retrogradely labeled from the LC, but not in other sources of CRF afferents to the LC (Curtis et al., 2002). Interestingly, our anatomical studies argue against the CNA as a source of ENK in the LC, consistent with other evidence that CRF mediating stress-induced LC activation and endogenous opioids mediating post-stress LC inhibition originate from different sources (Tjounmakaris et al., 2003).

V. Co-regulation of the LC-NE system by CRF and opioids

Together, the electrophysiological studies discussed above suggested that activity of the LC-NE system is co-regulated by CRF and endogenous opioids, such that the onset of stress releases CRF within the LC to activate this system with the consequence of increased arousal and shift from focused to scanning attention. The termination of stress engages endogenous opioids to inhibit the system and return activity back to baseline (Curtis et al., 2001; Valentino et al., 1991). In addition to the conceptual relevance of these findings, this study indicated that hypotensive challenge can be used as a tool to probe release of either endogenous CRF or endogenous opioids in the LC.

The finding that CRF and opioids regulate the activity of the LC-NE system during stress in an opposite manner is further supported by anatomical data showing prominent co-existence of CRF and μ -ORs in LC neurons (Reyes et al., 2007a). The co-localization of CRF receptors and μ -ORs in noradrenergic somatodendritic processes suggests that these two receptors may function principally in a postsynaptic fashion. CRF and opioid peptides may be co-released from the same axon terminals to affect LC neuronal activity or released from separate axon terminals that converge onto common LC dendrites (Tjounmakaris et al., 2003). The integrity of the CRF-opioid balance in the LC is important and particularly relevant to opiate-seeking behavior for individuals that are chronically taking opiates (see below). Thus, the strategic co-localization of CRF and μ -OR in LC dendrites may underlie continued opiate-seeking behavior in an effort to attenuate the hypersensitivity of the LC-NE system to stress.

VI. Implications of a CRF/opioid imbalance in the LC

Given the opposing regulation of the LC-NE system by CRF and opioids, upsetting the CRF:opioid balance in the LC could influence the stress-sensitivity of this system and enhance vulnerability to stress or conversely, vulnerability to opiate abuse. Consistent with this, chronic morphine administration sensitized LC neurons to CRF (Xu et al., 2004). LC

sensitization to CRF was expressed as increased sensitivity of the neurons to hypotensive stress. Importantly, this neuronal plasticity translated to a change in the behavioral repertoire of the animal in response to stress. Thus, when exposed to swim stress, morphine-treated rats were unusual in that they exhibited a strikingly higher incidence of climbing (Xu et al., 2004), a behavior that has been attributed to central NE activation in this model (Detke et al., 1995). These findings predict that chronic opiate use, whether as a result of abuse or clinical use, predisposes individuals to certain stress-related pathology. In addition to increasing vulnerability to stress-related pathology, opiate-induced sensitization of the LC-NE system may facilitate the maintenance of opiate use in an effort to counteract the hypersensitivity of the LC-NE system.

VII. Potential mechanisms underlying stress/opioid plasticity of LC neurons

Chronic opiate use results in tolerance at the level of the LC that is expressed as a decreased ability of opiates to hyperpolarize and inhibit LC neurons (Aghajanian, 1978; Christie et al., 1987; Rasmussen and Krystal, 1990). At an intracellular level, repeated opiate administration mimics the effects of chronic stress in increasing expression of components of the cAMP pathway (Guitart and Nestler, 1993; Nestler, 1993; Nestler et al., 1994; Nestler et al., 1999; Nestler et al., 1993; Nestler and Tallman, 1988). The site of opiate-induced plasticity appears to be downstream of adenylyl cyclase because the dose-response curve for LC activation by 8-Br-cAMP was also shifted in rats chronically administered morphine (Kogan et al., 1992). One site of opioid regulation along this pathway is at the level of cAMP-dependent protein kinase, which has been demonstrated to be elevated in the LC by chronic morphine treatment (Nestler and Tallman, 1988). Assuming that CRF activation of the LC requires the same intracellular cascade, the effects of CRF and stressors that release CRF into the LC would be predicted to be enhanced in opiate-tolerant subjects.

Like chronic stress, the development of opiate tolerance results in an imbalance of influence on the LC-NE system in favor of CRF-induced activation. Repeated opiate use could predispose to stress-related disorders that have been attributed to the LC-NE system. Examples of these are depression, anxiety, or post-traumatic stress disorder symptoms. In support of this is a high co-morbidity of psychiatric disorders in opiate dependence (Cottler et al., 1992; Markou et al., 1998). The need to reestablish a balance between the influences of these neuropeptides on the LC-NE system may play a role in the maintenance of self-administration of opiates. However, the clinical impact of a CRF-opioid imbalance goes beyond opiate abuse and extends to individuals who chronically use opiates for medical disorders.

Dynorphin and κ -ORs: Presynaptic modulator of LC activity

As described above, the most well studied opioid-mediated effect on LC neurons is the μ -OR mediated-inhibition characterized by a decrease in spontaneous discharge *in vivo* and hyperpolarization at the cellular level *in vitro*. Given the prominent expression of μ -OR in the LC and innervation by ENK peptides that can elicit this μ -OR-mediated response, our original working hypothesis proposed that the LC was co-regulated by CRF excitation during stress and μ -OR mediated inhibition following stress. More recently, our model has expanded to include an additional layer of regulation mediated by κ -OR activation. Evidence suggests that κ -OR regulation of the LC is distinct from that mediated by CRF or μ -ORs in that it appears to involve presynaptic regulation of afferent input.

The dynorphin (DYN)/ κ -OR system has been implicated in the mediation of stress and vulnerability to drug abuse. For example, stress, which promotes relapse and can facilitate place preference for drugs of abuse, increases prodynorphin gene expression in the limbic system (Shirayama et al., 2004). Genetic deletion of prodynorphin or pharmacological

antagonism of kappa receptors prevented stress-induced preference, implicating the DYN/ κ -OR system in stress-induced facilitation of drug abuse (Shirayama et al., 2004). Additionally, κ -OR antagonists prevent stress-elicited behaviors that are endpoints of depression such as immobility in the forced swim test and passive behavior in learned helplessness (Mague et al., 2003; McLaughlin et al., 2003; Shirayama et al., 2004). Our recent evidence for localization of DYN in the LC region (Reyes et al., 2007b) and co-localization of DYN with CRF in axon terminals in the LC (Reyes et al., 2008) implicate the LC as one site at which DYN modulates stress responses and the consequences of stress on behavior. Furthermore, the prominent localization of κ -ORs in axon terminals in the LC that contain CRF or the vesicular glutamate transporter, indicate that κ -ORs are poised to presynaptically inhibit diverse afferent signaling to the LC. This is a novel and potentially powerful means of regulating the LC-NE system that can impact on forebrain processing of stimuli and the organization of behavioral strategies in response to environmental stimuli. As described below, our results implicate κ -ORs as a novel target for alleviating symptoms of opiate withdrawal, stress-related disorders or disorders characterized by abnormal sensory responses, such as autism.

I. Anatomical considerations

DYN afferents prominently target the LC (Reyes et al., 2007b) and co-exist with CRF in common axon terminals (Fig. 2). By electron microscopy, we have reported that DYN directly targets noradrenergic somatodendritic processes (Reyes et al., 2007b) and that these form primarily asymmetric (excitatory) type synapses (Fig. 3A). When combined with immunogold-silver labeling for CRF, single axon terminals were found to contain both peptides (Fig. 3C) (Reyes et al., 2008). Our data also indicate that the endogenous opioids, DYN and ENK, more frequently converge on common postsynaptic targets (Fig. 3B) rather than being co-localized, a finding that is similar to the synaptic organization of CRF and ENK afferents in the LC (Tjouwakaris et al., 2003).

Previous anatomical studies have shown that the CNA is enriched with DYN cell bodies (Khachaturian et al., 1982; Watson et al., 1983). Likewise, retrograde and anterograde tract-tracing studies revealed that the CNA is the source of the CRF innervation in the LC (Sakanaka et al., 1986; Van Bockstaele et al., 1998b). Accordingly, the CNA is considered a potential source of DYN/CRF terminals in the LC. We have demonstrated that unilateral lesions of the CNA substantially decreased CRF-immunolabeling in the LC (Reyes et al., 2008), consistent with our previous reports (Tjouwakaris et al., 2003; Van Bockstaele et al., 1998b) as well as others (Sakanaka et al., 1986). Evidence of reduced DYN innervation in the same cases indicates that DYN innervation of this region and the dually labeled axon terminals are derived from common limbic sources. Our recent studies show that a common neuronal population in the CNA serves as a source for both DYN and CRF projecting to the LC (Van Bockstaele et al., 2009). Additional sources of potential afferent inputs to the LC that colocalize DYN and CRF include the bed nucleus of stria terminalis, the nucleus of the solitary tract and hypothalamic regions including the paraventricular nucleus of the hypothalamus (Reyes et al., 2005; Van Bockstaele et al., 1999b; Van Bockstaele et al., 1999b) and these remain to be investigated.

Anatomical studies revealed some evidence of modest co-localization of DYN- and ENK-labeled axon terminals in the LC (Reyes et al., 2008). However, the cellular interactions between individual DYN- and ENK-labeled axon terminals were more frequently convergence of separately labeled afferents on common LC dendrites (Fig. 3B), and also presynaptic interactions. This suggests that co-release of DYN and ENK in the LC by distinct afferents can impact common targets. While CNA provides CRF innervation to the LC (Reyes et al., 2008), it does not provide robust ENK innervation to the LC (Tjouwakaris et al., 2003). Therefore, while the CNA serves as a source of DYN in the LC, it is not the

source of axon terminals that co-localize DYN and ENK. A possible mechanism by which a common stimulus could result in DYN and ENK release to impact the same LC neuron is via parallel stimulation of separate populations of DYN and ENK neurons that converge on common targets in the noradrenergic LC. It has been established that the PGI provides a robust enkephalinergic projection to the core and peri-coerulear dendritic area (Drolet et al., 1992). Hence, it is likely that two important autonomic brain regions, the CNA and the PGI, converge on the LC to influence the activity of noradrenergic LC neurons under certain conditions.

As discussed above, κ -OR mRNA and protein have been identified in the LC (DePaoli et al., 1994; Mansour et al., 1994) and anatomical studies have shown that this receptor subtype is prominently distributed presynaptically in LC afferents (Kreibich et al., 2008; Reyes et al., 2009) (Fig. 3D). Presynaptic effects of κ -OR activation have been described in other brain regions (Ackley et al., 2001; Bie and Pan, 2003; Drake et al., 1997; Hjelmstad and Fields, 2001; Ogura and Kita, 2000; Simmons and Chavkin, 1996; Svingos et al., 2001; Svingos and Colago, 2002; Svingos et al., 1999; Wang et al., 2009; Weisskopf et al., 1993). In many of these regions, glutamate, glycine, and GABA neurotransmission were targets of κ -OR-mediated presynaptic effects. Indeed, κ -ORs have been identified in glutamatergic afferents to the LC (Barr and Van Bockstaele, 2005) (Fig. 3D) as well as in DYN (Reyes et al., 2009) and CRF-containing (Kreibich et al., 2008) axon terminals. Thus, κ -ORs can broadly impact LC function through presynaptic modulation of diverse neurotransmitters.

II. Effect of the κ -OR-agonists on LC activity

Our findings of co-localization of DYN with vesicular glutamate transporters (Barr and Van Bockstaele, 2005) or CRF in axon terminals in the LC suggested that this unique endogenous opioid system contributes to the regulation the LC-NE system during stress (Reyes et al., 2008). In support of this, the κ -OR agonists, DYN A or U50488, microinfused directly into the LC had effects on single unit LC activity in halothane-anesthetized rats that were distinct from the μ -agonists, morphine and DAMGO. Without altering LC spontaneous discharge, κ -OR-agonists attenuated LC activation by sciatic nerve stimulation, a sensory-evoked response mediated by excitatory amino acid neurotransmission in the LC (Kreibich et al., 2008). Similar effects were seen in unanesthetized rats where intracerebroventricular administration of U50488 decreased LC activation by auditory stimuli with no effect on spontaneous discharge (Kreibich et al., 2008). These *in vivo* findings are consistent with *in vitro* studies in LC slice preparations demonstrating that κ -OR activation depresses excitatory synaptic potentials without affecting passive membrane properties or voltage-sensitive potassium currents (Pinnock, 1992) and suggest that this is a presynaptic effect on glutamate release. The co-localization of DYN with vesicular glutamate transporters in axon terminals within the LC (Barr and Van Bockstaele, 2005) is consistent with presynaptic modulation of glutamate release. Interestingly, LC neuronal activation during opiate withdrawal is mediated in part by excitatory amino acid inputs to the LC (Han et al., 2006; Maldonado et al., 1992; Rasmussen et al., 1990; Redmond and Huang, 1982) and this was also attenuated by U50488 microinfusion into the LC. This is particularly relevant for opiate addiction because activation of the LC-norepinephrine system during opiate withdrawal is thought to underlie some of the aversive aspects of opiate withdrawal and contribute to negative reinforcing properties of morphine. These results suggest that this aspect of withdrawal may be attenuated by engaging kappa opiate receptors in the LC. Nevertheless, it has been shown that κ -OR agonists such as U50-488 cause place aversion in animals (Land et al., 2008; Shippenberg and Herz 1986).

To determine whether κ -OR could modulate CRF afferents to LC, the effects of U50488 on LC activation elicited by hypotensive stress were examined. Pretreatment with U50488 prevented LC activation by hypotensive challenge, a stressor known to selectively engage

CRF afferents to the LC (Kreibich et al., 2008). These data underscore the general nature of presynaptic inhibition by κ -OR in the LC.

III. Implications for κ -OR-mediated presynaptic inhibition for LC function

Based on the aforementioned studies, κ -OR modulation of LC phasic sensory responses should translate to diminished reactions to sensory stimuli and decreased ability of these stimuli to alter the course of ongoing behaviors. The absence of an effect on tonic activity implies that this would occur in the absence of alterations in general arousal. Interestingly, kappa agonists have been shown to disrupt performance in the 5-choice serial reaction time task by increasing number of omissions and latency to respond (Paine et al., 2007; Shannon et al., 2007). These effects could also be expressed as a blunting of affect. Consistent with this, the dynorphin- κ -OR system has been implicated in depression and supports the notion that κ -OR antagonists may be useful antidepressants (Mague et al., 2003; McLaughlin et al., 2006; McLaughlin et al., 2003; Pliakas et al., 2001; Shirayama et al., 2004).

In other clinical conditions that are characterized by excessive responses to sensory stimuli, the ability of κ -OR agonists to attenuate responses without altering the general state of arousal might be a useful therapeutic approach. Examples include attentional disorders, where sensory stimuli are significant distracters, or autism, which can present as unusually heightened sensory responses (Gomot et al., 2002; Tomchek and Dunn, 2007). The demonstration that κ -OR activation in the LC also attenuates the excitation of LC neurons by opiate withdrawal is consistent with presynaptic inhibition of glutamate afferents that mediate the effect (Han et al., 2006; Maldonado et al., 1992; Rasmussen and Krystal, 1990; Redmond Jr, 1982) and suggests that κ -OR agonists might be useful in alleviating symptoms of opiate withdrawal (Akaoka and Aston-Jones, 1991; Rasmussen and Aghajanian, 1989; Rasmussen et al., 1991).

Conclusions

LC neurons, with their 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, CRF and endogenous opioids. Aston-Jones and colleagues proposed a model whereby tonic and phasic modes of LC discharge facilitate distinct and exclusive processes (Aston-Jones and Cohen, 2005). In this model, phasic activity facilitates ongoing behavior and optimizes performance in tasks requiring selective attention, whereas high tonic activity promotes attention to extraneous 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; Valentino and Foote, 1988). In contrast, moderate levels of endogenous opioids acting at μ -OR receptors bias activity toward the phasic mode, by selectively decreasing tonic activity (Valentino and Foote, 1988). In opposition to CRF, engaging μ -OR should promote focused attention and maintenance of ongoing behavior. Consequences of κ -OR presynaptic inhibition contrast all of these and suggest a novel level of regulation that takes the LC "offline". 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. Because

spontaneous activity is unaffected, this unresponsive state should be present in the absence of sedation.

In summary, elucidating the circuitry by which particular afferents target LC neurons to influence its activity that may ultimately influence behavioral repertoires in an attempt to adapt to specific situations or environments is important in understanding and formulating therapeutic approaches in the treatment of diverse stress-related psychiatric disorders as well as relapse and vulnerability to opiate abuse.

List of abbreviations

ACTH	adrenocorticotrophic hormone
cAMP	cyclic adenosine monophosphate
CNA	central nucleus of the amygdala
CRF	corticotropin releasing factor
δ-OR	δ-opioid receptor
DYN	dynorphin
EEG	electroencephalographic
ENK	enkephalin
GABA	gamma-amino butyric acid
κ-OR	κ-opioid receptor
LC	locus coeruleus
μ-OR	μ-opioid receptor
NE	norepinephrine
PGI	nucleus paragigantocellularis
PrH	nucleus prepositus hypoglossus
pCREB	phospho-cAMP response element-binding proteins

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Co-transmitters in LC afferents

x/y	CRF	ENK	Glutamate	GABA	POMC	Dynorphin
CRF	-	12% [a]	30% [b]	5% [b]	33% [c]	35% [f]
ENK	16% [a]	-	28% [d]	38% [e]	ne	<10% [f]
Glutamate	13% [b]	43% [d]	-	ne	ne	48% [d]
GABA	6% [b]	nc [e]	ne	-	ne	ne
POMC	27% [c]	ne	ne	ne	-	ne

[a] Tjouwakis et al. (2003) *J Comp Neurol* **466**, 445-56.
[b] Valentino et al. (2001) *Neurosci* **106**, 375-84.
[c] Reyes et al. (2006) *Eur J Neurosci* **23**, 2991-8.
[d] Barr and Van Bockstaele (2005) *Anat Rec A Discov Mol Cell Evol Biol* **284**, 466-74.
[e] Van Bockstaele et al. (2000) *J Comp Neurol* **417**, 103-114.
[f] Reyes et al. (2008) *J Comp Neurol* **508**, 663-675

Figure 1. Our previous studies indicate that NE activity is regulated via distinct CRF and ENK afferents targeting postsynaptically distributed CRF and μ -opioid receptors (μ -OR). The circuitry that links DYN to the LC-NE system and the conditions that engage this circuitry are steadily emerging (see text). Our working model now includes presynaptic modulation of NE activity via dynorphin- κ -OR regulation of afferent inputs that is posited to differentially affect behavior. **Left schematic:** Schematic diagram of an LC neuron containing μ -OR (symbol: fleur de lis), CRF-R (symbol: ink spot) and glutamate receptors (Glu-R, symbol: circle) targeted by axon terminals containing CRF, ENK, Glu or GABA. Anatomical studies support the localization of κ -OR on terminals containing glutamate, CRF and dynorphin. **Right schematic:** Schematic depicting selected afferents to the LC that are known to differentially modulate LC activity. CRF afferents from the amygdala are engaged to increase LC neuronal activity following hypotensive stress. Upon termination of the stress, opioid modulation of LC neurons (most likely arising from medullary sources) results in inhibition for a period of time, an effect that is completely blocked by microinfusion of naloxone. Glutamatergic afferents from the nucleus paragigantocellularis (PGi) convey sciatic nerve stimulation that is completely abolished by excitatory amino acid antagonists. Both stimuli are temporally correlated with, and necessary for, cortical EEG activation via LC efferent projections to forebrain structures. Table summarizing percentages of co-transmitters in LC afferents. Percentages are shown with respect to total number of profiles for neurotransmitters in the vertical column (e.g., ENK/total CRF sampled = 12%). Abbreviations: nc: not counted; ne: not examined

Figure 2.

A. Darkfield photomicrograph showing immunoperoxidase labeling of DYN in the core of the LC (straight black arrow) and peri-LC area (arrowheads). Cb: cerebellum; IV: 4th ventricle; scp: superior cerebellar peduncle. **B-F.** Processes exhibiting DYN (red), CRF (green) or both DYN/CRF (yellow) overlap TH neurons (blue) in the LC. White arrows indicate DYN/CRF processes in close proximity to TH somatodendritic profiles. Panels D and E are taken through the core of the LC while panels B, C and F are in the peri-LC. Scale bar = 250 μ m.

Figure 3.

A. Immunoperoxidase labeled DYN axon terminal (DYN-t) forms an asymmetric-type (excitatory) synapse with a dendrite containing TH (TH-d). **B.** Immunoperoxidase labeling for DYN (that is particularly enriched in dense core vesicles, dcv) in an axon terminal (DYN-t) that forms a synapse (curved arrow) with an unlabeled dendrite (ud) that receives convergent input from an axon terminal containing gold-silver labeling for ENK. **C.** An axon terminal contains immunogold-silver labeling for CRF (arrowheads) and immunoperoxidase labeling for DYN. CRF-DYN-t contains several dense core vesicles (dcv) and forms a synapse (black arrow) with an unlabeled dendrite (ud). **D.** An axon terminal containing immunoperoxidase labeling for VGLUT also exhibits immunogold-silver labeling for κ -OR in the LC. Scale bars: 0.5 μ m.