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The Brain Norepinephrine System, Stress and Cardiovascular Vulnerability

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Highlights

- Social stress exposure is a risk factor for cardiovascular disease.
- The anatomical and physiological characteristics of the locus coeruleus-norepinephrine system position it to contribute to stress-induced cardiovascular disease.
- Individual differences in coping strategies during social stress parallel unique plasticity in LC afferent regulation.
- Contrasting regulation of the LC would result in differences in cardiovascular regulation, likely contributing to individual differences in the cardiovascular consequences of stress.

Abstract

Chronic exposure to psychosocial stress has adverse effects on cardiovascular health, however the stress-sensitive neurocircuitry involved remains to be elucidated. The anatomical and physiological characteristics of the locus coeruleus (LC)-norepinephrine (NE) system position it to contribute to stress-induced cardiovascular disease. This review focuses on cardiovascular dysfunction produced by social stress and a major theme highlighted is that differences in coping strategy determine individual differences in social stress-induced cardiovascular vulnerability. The establishment of different coping strategies and cardiovascular vulnerability during repeated social stress has recently been shown to parallel a unique plasticity in LC afferent regulation, resulting in either excitatory or inhibitory input to the LC. This contrasting regulation of the LC would translate to differences in cardiovascular regulation and may serve as the basis for individual differences in the cardiopathological consequences of social stress. The advances described suggest new directions for developing treatments and/or strategies for decreasing stress-induced cardiovascular vulnerability.

Introduction

The cardiovascular response to psychosocial stress, resulting in transient increases in blood pressure and heart rate, is functional in the acute sense. However, there is overwhelming evidence that chronic stress has adverse effects on cardiovascular health. Epidemiological studies such as INTERHEART revealed that those who reported “permanent stress” at work or at home were more than two times more likely to suffer from a myocardial infarction (Rosengren et al., 2004). Furthermore, marital stress

was associated with a nearly 3-fold increased risk of coronary artery disease (Orth-Gomer et al., 2000). The association between stress and cardiovascular disease is unmistakable and can be replicated in preclinical animal studies, however the stress-sensitive neurocircuitry that contributes to increased cardiovascular disease risk remains to be elucidated.

One system that has been implicated in the association between stress and cardiovascular disease is the brain norepinephrine (NE) system that arises from the pontine nucleus, locus coeruleus (LC). This review begins with an introduction to the anatomical and physiological characteristics of the LC-NE system that position it to be a major stress response system. Evidence that the LC processes cardiovascular information and in turn can impact cardiovascular function are discussed. Because social stress is most relevant for humans, this review focuses on cardiovascular vulnerability produced by social stress as predicted by the rodent resident-intruder model and the role of the LC-NE system in cardiovascular vulnerability in that model. A major theme highlighted in this review is that individual differences in coping strategy determine individual differences in social stress-induced cardiovascular vulnerability. Moreover, the recent discovery that the establishment of different coping strategies and cardiovascular vulnerability during repeated stress is paralleled by a unique plasticity in LC afferent regulation is discussed. The advances described suggest new directions for developing treatments and/or strategies for decreasing stress-induced cardiovascular vulnerability.

The locus coeruleus-norepinephrine stress response system

The hallmark of stress is generally considered to be activation of the hypothalamic-pituitary-adrenal (HPA) axis, with plasma glucocorticoid levels serving as the endpoint of that activation. However, sustained activation of the brain NE system may also be considered a hallmark of stress in that it is initiated by many of the same challenges in parallel with the HPA axis. Given its physiological attributes and connectivity, activation of the LC-NE system during stress has been suggested to serve as an arousal and cognitive limb of the stress response. Evidence discussed below however, underscores cardiovascular consequences of stress-related activation of the brain norepinephrine system. The coordination of cardiovascular and cognitive responses to stress may underlie co-morbidity of cardiovascular and affective symptoms of stress-related disorders.

Anatomical features of the LC-NE system. The pontine nucleus, LC is a primary source of norepinephrine in brain (Grzanna and Molliver, 1980; Swanson and Hartman, 1976). Recent studies using novel, selective tract tracing tools have refined our knowledge of the topography of LC neurons with respect to their afferent inputs and efferent projections (Robertson et al., 2013; Schwarz and Luo, 2015). However, for the most part the nucleus is still characterized by its homogeneous expression of norepinephrine in all neurons and its widespread, highly collateralized projection system that is a major source of NE in the forebrain (Aston-Jones et al., 1995; Swanson and Hartman, 1976). Notably, the LC serves as the primary source of NE in forebrain regions such as the hippocampus and cortex that govern cognition, memory and complex behaviors. With regard to cardiovascular function, the LC exerts both indirect

(via the nucleus ambiguus (Amb)-rostromedial medulla (RVLM) circuit) (Jones and Yang, 1985; McKittrick and Calaresu, 1996) and direct projections to the preganglionic sympathetic neurons within the intermediolateral nucleus of the spinal cord, a common pathway for stress induced cardiovascular responses (Jones and Yang, 1985; Spyer, 1992). Furthermore, there is clear evidence for projections from the LC to the central nucleus of the amygdala (CNA), another stress-sensitive brain region with cardiovascular impact (Kravets et al., 2015; Mason and Fibiger, 1979). Tract tracing studies have also provided evidence for LC projections to the medial part of the rostral dorsal motor vagal nucleus (DMV) and lateral part of the intermediate DMV, the location of preganglionic parasympathetic cardiac neurons (Ter Horst et al., 1991). Consistent with this, LC neurons are transsynaptically labeled from the heart (Standish et al., 1995). Evidence discussed below support the idea that LC exerts an inhibitory regulation on cardiac parasympathetic neurons and excitatory regulation on preganglionic sympathetic neurons.

Sources of LC afferents have been debated as a result of differences in retrograde tracers and whether the injection was limited to the cell body region (Aston-Jones et al., 1991). Because the dendrites of LC neurons can extend for several hundred microns outside of the nuclear zone, neurons with axons terminating in pericoerulear areas can synaptically contact LC dendrites and impact on LC activity although they may not be labeled by injections into the nuclear LC (Shipley et al., 1996). As a result, studies that limit retrograde tracers to the LC nucleus reveal a very limited number of afferents that include the dorsal cap of the paraventricular hypothalamic nucleus, the nucleus prepositus hypoglossi and nucleus paragigantocellularis (PGi) in

the ventrolateral medulla (Aston-Jones et al., 1990). Because the PGI is a source of inputs to the preganglionic sympathetic neurons it is positioned to coordinate peripheral sympathetic activity with central noradrenergic activity (Van Bockstaele and Aston-Jones, 1995). The PGI is a major source of enkephalinergic innervation of the LC (Drolet et al., 1990). The importance of this input in stress-coping strategy and stress-related cardiovascular vulnerability is discussed below. In contrast to the restricted number of LC afferents identified by tracer injections limited to the nucleus LC, injections of retrograde tracers in pericoerulear regions where LC dendrites extend, combined with anterograde labeling from putative afferents and electron microscopy to verify synaptic connections reveal relatively numerous LC afferents (Luppi et al., 1995; Van Bockstaele et al., 2001; Van Bockstaele et al., 1998; Van Bockstaele et al., 1999). Of these, the central nucleus of the amygdala is relevant to cardiovascular function and as discussed below conveys cardiovascular-related information to the LC (Curtis et al., 2002). This is also a major source of the stress-related neuropeptide, corticotropin-releasing factor (CRF), afferents to the LC and a primary route through which stressors activate the LC-NE system (Curtis et al., 2002; Van Bockstaele et al., 1998).

Physiological Characteristics of LC neurons. The rate and pattern of LC neuronal discharge have implicated the LC-NE system in the regulation of arousal and attention (Aston-Jones and Bloom, 1981a, b; Aston-Jones and Cohen, 2005; Foote et al., 1980; Williams and Marshall, 1987). LC neurons fire spontaneously and their rate of discharge correlates positively with the state of arousal (Aston-Jones and Bloom, 1981a; Foote et al., 1980). This relationship is more than correlation as selective chemical activation or

inhibition of LC neurons is sufficient to impact on cortical and hippocampal network activity, indicating a degree of causality between LC discharge rate and forebrain indices of arousal (Berridge and Foote, 1991; Berridge et al., 1993). Notably, LC activation has been demonstrated to be necessary for cortical activation by stimuli including hypotensive stress (Page et al., 1993) (Lechner et al., 1997) (see below). Thus, this is a route by which autonomic challenges can affect cortical functions.

In addition to rate, the pattern of LC neuronal firing is relevant to behavior. LC neurons fire in both a tonic and a phasic pattern with the latter characterized by synchronous bursting. Salient sensory stimuli elicit a burst of LC discharge that typically precedes orientation to the eliciting stimuli (Aston-Jones and Bloom, 1981b; Foote et al., 1980). This feature of LC neurons has suggested that sensory-evoked activation of the LC-NE system resets or redirects attention towards salient environmental stimuli.

Shifts in the pattern of LC discharge between phasic and high tonic discharge modes are thought to differentially bias behavior. Phasic LC discharge is associated with focused attention and staying on-task, whereas high tonic discharge is associated with labile attention and going off-task (see for reviews (Aston-Jones and Cohen, 2005; Bouret and Sara, 2005)) (Rajkowski et al., 1994; Usher et al., 1999). In contrast, high tonic LC discharge rate is thought to promote behavioral flexibility by disengaging animals from attention to specific stimuli and ongoing behaviors and favoring scanning for stimuli that promote alternate behaviors that would lead to more rewarding outcomes (Aston-Jones and Cohen, 2005). Shifts between phasic and tonic LC firing patterns would promote rapid adjustments in a dynamic environment.

LC-NE activation is a cognitive limb of the stress response that occurs in parallel with the HPA initiated endocrine response. The same stressors that initiate the HPA response to stress also activate the LC-NE system, including shock, auditory stress, immunological challenges, autonomic stressors, restraint and social stress. This has been determined using different endpoints of LC activity including NE turnover, NE release, LC neuronal activity, c-fos expression or tyrosine hydroxylase expression (Beck and Fibiger, 1995; Bonaz and Tache, 1994; Britton et al., 1992; Campeau and Watson, 1997; Cassens et al., 1981; Cassens et al., 1980; Chan and Sawchenko, 1995; Chang et al., 2000; Curtis et al., 2012; Dun et al., 1995; Duncan et al., 1993; Funk and Amir, 2000; Graham et al., 1995; Ishida et al., 2002; Kollack-Walker et al., 1997; Korf et al., 1973; Lacosta et al., 2000; Makino et al., 2002; Rusnak et al., 2001; Sabban and Kvetnansky, 2001; Smagin et al., 1994; Smith et al., 1992; Smith et al., 1991; Thierry et al., 1968; Valentino et al., 1991). The parallel activation of the HPA and LC-NE system would coordinate endocrine and cognitive limbs of the stress response (Valentino and Van Bockstaele, 2008). Electrophysiological recordings in anesthetized and unanesthetized rats have demonstrated that acute stress (including hypotensive stress, see below) and exposure to CRF shifts LC discharge towards a high tonic state that would favor heightened arousal, disengagement from ongoing behavior, labile attention and cognitive flexibility (Curtis et al., 2012; Valentino and Foote, 1987; Valentino and Foote, 1988; Valentino and Wehby, 1988; Zitnik et al., 2015). Consistent with this, CRF microinfused directly into the LC increases cognitive flexibility in an attentional set-shifting task (Snyder et al., 2012). These responses are adaptive in coping with an immediate threat. However, these same consequences of increased LC-NE activity

would appear pathological outside of the context of stress and would translate to hyperarousal, loss of concentration, restlessness and poor performance on tasks that require focused attention. Notably, these are characteristic symptoms of stress-related psychiatric disorders such as PTSD and major depression and hyperactivity of the LC-NE system has been implicated in these disorders (Southwick et al., 1999; Wong et al., 2000).

Cross-talk between the LC and cardiovascular system

LC responses to cardiovascular stimuli. The anatomy of the LC-NE system discussed above supports reciprocal communication with cardiovascular-related centers. LC neurons receive and process cardiovascular-related information and conversely changes in LC activity impact on cardiovascular function. The most well characterized cardiovascular response of LC neurons is its activation by hypotensive stress. Hypovolemia, as would occur with hemorrhage, was demonstrated to increase the discharge rates of rat LC neurons and this is reliably reproduced by the intravenous infusion of a dose of the hypotensive agent, sodium nitroprusside that decreases blood pressure by 30-50% (Elam et al., 1985; Svensson, 1987). The same hypotensive challenge also activates the HPA axis and so provides an example of how the same stressor activates both stress response systems in parallel (Plotsky and Vale, 1984). As has been seen with other acute stressors, during hypotensive stress, the pattern of LC discharge shifts towards a high tonic state that would support increased arousal, scanning attention and behavioral flexibility (Valentino and Wehby, 1988). LC neuronal responses to hypotensive challenge translate to increased forebrain NE release and cortical EEG activation, indicative of arousal. The LC response to hypotension is

necessary for the cortical response because silencing the LC bilaterally with local infusion of clonidine prevents this effect (Page et al., 1993). Activation of the LC-NE system by hypotension would be adaptive, for example, serving to maintain arousal and cognitive flexibility in animals that are injured and hemorrhaging.

The response of LC neurons to hypotension is mediated by local CRF release. Indeed, the initial studies to support a functional role of endogenous CRF in the LC were those that demonstrated that local microinfusion of CRF antagonists into the LC prevented LC neuronal activation by hypotensive stress (Valentino et al., 1991). The finding that this same manipulation prevented cortical activation by hypotensive challenge demonstrated that a function of CRF neurotransmission in the LC during hypotension was to increase arousal (Page et al., 1993).

A primary source of CRF that mediates LC activation during hypotensive stress is the central nucleus of the amygdala (CNA). This was demonstrated in lesion studies and through functional neuroanatomy (Curtis et al., 2002). However, the role of CRF neurons in the PGI in this response could not be ruled out, as it was not possible to determine the effects of lesioning this nucleus because this often results in death.

An important component of the LC response to hypotensive stress is an inhibition that occurs with stress termination that promotes recovery of LC neuronal activity back to pre-stress levels (Curtis et al., 2001; Valentino et al., 1991). This inhibition becomes exaggerated when the CRF excitatory influence is abrogated by the administration of a CRF antagonist into the LC (Curtis et al., 2001). Indeed the administration of some CRF antagonists reveals an inhibition that is apparent even during the hypotensive challenge (Valentino et al., 1991). Intra-LC administration of the opioid antagonist,

naloxone, prevents this inhibition and slows recovery of LC neurons from hypotensive stress-induced excitation, providing evidence that this is an opioid-mediated effect (Curtis et al., 2001). These findings suggested a model whereby hypotensive stress engages CNA-CRF afferents that shift LC activity to a high tonic state as well as endogenous opioid afferents, which serve to restrain this excitation, promoting recovery of LC activity to pre-stress levels when hypotension is terminated. Thus, during hypotension, LC activity is fine-tuned by a convergence of CRF and opioid afferents with opposing actions. Later studies demonstrated a similar dual component response of LC neurons to predator stress (Curtis et al., 2012). The most prominent opioid input to the LC that could account for this inhibition is a PGI-enkephalin (ENK) input that densely innervates the LC (Drolet et al., 1992). Notably, LC neurons have a high expression of μ -opioid receptor (MOR) to respond to ENK innervation (Mansour et al., 1994; Tempel and Zukin, 1987).

Cardiovascular responses to LC activation. The LC has a reciprocal relationship with the autonomic nervous system, so that in addition to cardiovascular stimuli activating the LC, LC-NE activation also produces a cardiovascular response. Noxious sensory stimuli produce a parallel and nearly identical increase in LC-NE activity and peripheral sympathetic nerve activity indicating a relationship between the central noradrenergic system that controls arousal and the peripheral sympathetic nervous system (Elam et al., 1986). For years, however, studies have been equivocal regarding the functional effect of LC activation on peripheral cardiovascular output. Electrical stimulation of LC neurons has been commonly reported to elicit cardiovascular and

noradrenergic changes consistent with increased sympathetic nervous system activity (Crawley et al., 1980). Furthermore, peptidergic activation of LC by corticotropin releasing factor or thyrotropin releasing hormone results in a significant pressor response (Brown, 1986; Paakkari et al., 1987). In contrast, chemical activation of LC neurons by the excitatory neurotransmitter, L-glutamate, has been reported to produce a depressor response and bradycardia in some studies (Sved and Felsten, 1987), while others report significant increases in blood pressure (Chen and Huang, 1997). Based on these contrasting findings, it was suggested that evidence obtained from electrical stimulation was the result of activating non-noradrenergic cells or fibers of passage within the LC (Crawley et al., 1980). We now understand these conflicting effects of LC activation on sympathetic output likely arose in part from differences in the effect of LC activation in awake versus anesthetized animals, the impact of different types of anesthesia, or different strains of rodents (Chen and Huang, 1997; Kawamura et al., 1978; Sved and Felsten, 1987).

More recent advances using optogenetic technology have further clarified the role of the LC in cardiovascular activation. For example, optogenetic stimulation of LC neurons revealed a direct influence on cardioinhibitory vagal neurons (Wang et al., 2014). In this study, photostimulation of LC neurons resulted in an increase in frequency of inhibitory postsynaptic currents in cardiac vagal neurons. In addition to the cardioinhibitory DMV, the nucleus ambiguus (Amb) also serves to decrease blood pressure and heart rate by suppressing the sympathoexcitatory rostroventrolateral medulla (RVLM) (McKittrick and Calaresu, 1996). Similar to the DMV, the LC also projects to the Amb and inhibits its activity (Jones and Yang, 1985; Samuels and

Szabadi, 2008a, b) thereby removing the inhibitory influence over the RVLM. Recent optogenetic studies revealed the cardiovascular response to LC activation. Optogenetic activation of astrocytes within the LC was used to promote the release of L-lactate into the LC, thereby activating LC neurons. Furthermore, this study revealed that, like L-glutamate, intra-LC L-lactate produced increased heart rate and blood pressure (Tang et al., 2014). Therefore, as functional evidence of the aggregate of the inhibitory influence on parasympathetic neurons and excitatory influence on sympathetic neurons, selective stimulation of the LC has been shown to result in peripheral catecholamine release and increased heart rate and blood pressure (Drolet and Gauthier, 1985; Gurtu et al., 1984; Kawamura et al., 1978).

Stress coping strategy and individual differences in stress vulnerability

For humans, stressors of a social nature are prevalent. The rodent resident-intruder stress has been used and modified by many laboratories to model human social stress. This stress involves placement of a rodent into the cage of an aggressive "resident" conspecific that will attack the intruder (Koolhaas et al., 1997). Repeated resident-intruder stress results in HPA dysfunction, decreased social interaction, anxiety-like behaviors, anhedonia, self-administration of drugs of abuse and decreased heart rate variability, alterations in circadian rhythm amplitudes and long lasting pro-arrhythmic effects (Carnevali et al., 2013b; Miczek et al., 2004; Rygula et al., 2005; Tornatzky and Miczek, 1994; Wood et al., 2012; Wood et al., 2010). Substantial individual variability exists in the consequences of resident-intruder stress. For mice this is best seen as differences in social interaction following the stress, such that

vulnerable mice exhibit low levels of social interaction relative to resilient mice that show levels that are comparable to controls (Krishnan et al., 2007). For rats, individual differences in the consequences of resident-intruder stress for HPA function, anhedonia, drug self-administration and heart rate variability align with individual differences in coping strategies (Chaijale et al., 2013b; Wood et al., 2012; Wood et al., 2010; Wood et al., 2015). Repeated resident-intruder stress exposure results in the emergence of two distinct populations defined by their latency to assume the subordinate defeat posture (Wood et al., 2010). Notably, this latency is distributed bimodally rather than normally, underscoring that these are distinct populations and not extremes of a single population. Analysis of defeat latency over days of resident-intruder stress indicates that rats typically start off with relatively short latencies and that the resistance to defeat becomes established in certain rats after repeated exposures. Furthermore, this increase in defeat latency over subsequent stress exposures parallels the emergence of active coping upright postures in the intruder rat. Rats that exhibit short defeat latencies (SL rats) show significantly more anhedonia in sucrose preference tests, immobility in the forced swim test, propensity for self-administration of drugs of abuse and decreased heart rate variability. They also exhibit HPA dysregulation that is comparable to that reported in depression, compared to rats that resist or that have a long latency to defeat (LL rats) (Wood et al., 2012; Wood et al., 2010; Wood et al., 2015).

The neurobiological mechanisms underlying the development of the two distinct coping strategies have yet to be elucidated. However, recent functional neuroanatomical studies implicate a unique plasticity of LC afferent regulation that

becomes established with the development of these styles (Reyes et al., 2015b). The initial exposure to resident-intruder stress, when rats generally exhibit a short latency to defeat, activates LC neurons and CRF-CNA afferents, as well as ENK-PGi afferents to the LC as determined by *c-fos* expression in these neurons. This is consistent with the electrophysiological evidence discussed above that acute stress increases LC discharge and engages both CRF and opioid afferents to the LC. However, by the fifth stressor exposure, when the SL and LL phenotypes have developed, *c-fos* expression in LC neurons and CRF-CNA afferents is maintained only in SL rats. In this subordinate population, activation of ENK-PGi neurons that project to the LC is lost with repeated resident-intruder stress. Opposing effects occur for LL rats, where activation of ENK-PGi afferents to the LC is maintained and activation of CRF-CNA inputs is lost. As a result, LC neurons of LL rats are not activated (as indicated by *c-fos* expression) above control levels following the fifth exposure to resident-intruder stress. Postsynaptic changes in CRF1 receptors and MOR in LC neurons are consistent with these presynaptic effects (Chaijale et al., 2013a). For example Western blots reveal higher levels of MOR and lower CRF1 receptor levels in LC tissue punches of LL rats compared to SL or control rats. These convergent findings suggest that the development of the LL coping strategy is associated with a shift in the balance of LC afferent regulation to favor the ENK-MOR inhibitory influence. By contrast, the SL strategy is maintained through a loss of ENK-MOR inhibition. Whether the stress-induced afferent plasticity is causal to the development of different coping strategies and vulnerability phenotypes requires further characterization. However, the findings that administration of CRF antagonists prior to resident-intruder stress bias populations

to the LL coping strategy and attenuate some of the consequences, including cardiovascular consequences supports this idea (see below) (Wood et al., 2012).

Role of the LC in individual differences in cardiovascular responses to social defeat.

The robust, ethologically relevant nature of social stress is evident by the vast and long-lasting repercussions that occur following social defeat exposure. In addition to the endocrine and behavioral deficits that are common following social stress, this robust sympathomimetic stressor also elicits cardiovascular consequences. Intense sympathetic activation elicits 30 times the number of arrhythmias (ventricular premature beats) during social defeat as compared to other non-social stressors (i.e. restraint or foot shock) (Sgoifo et al., 1999). The long-term consequences of social defeat clearly define the ability of this naturalistic stressor to promote cardiovascular dysfunction (for a review see (Sgoifo et al., 2014)). For example, repeated social stress disrupts the circadian rhythm of heart rate (Carnevali et al., 2013b; Meerlo et al., 1999; Sgoifo et al., 2002) and is capable of promoting atherosclerosis (Black and Garbutt, 2002). Social stress also decreases resting heart rate variability (HRV) (Wood et al., 2012), an indication of decreased parasympathetic and increased sympathetic drive that is indicative of enhanced cardiovascular disease risk (Novak et al., 1997; Tsuji et al., 1996). As evidence of the pathological consequences of this autonomic imbalance, social defeat produces maladaptive cardiac hypertrophy (Carnevali et al., 2013b; Gelsema et al., 1994). Furthermore, a recent study conducted a comprehensive analysis of cardiac electrophysiological variables in socially defeated rats where high

density epicardial mapping analysis revealed a shortening of the effective refractory period, a decreased transversal conduction velocity of the wavefront and increased ventricular myocardial excitability in defeated rats compared with controls (Carnevali et al., 2013b). These cardiac electrophysiological variables are important determinants of arrhythmias and were attributed to increased cardiac sympathetic drive in rats with a history of social stress exposure (Carnevali et al., 2013b). In addition, social defeat has been shown to produce cardiac fibrosis, another contributing factor to cardiac arrhythmias (Costoli et al., 2004).

Studies assessing individual differences revealed that rodents are not uniformly sensitive to the cardiovascular consequences of stress, but rather susceptibility is related to coping strategy. The reduction in resting HRV 24-48 hours after social stress was exaggerated in rats displaying passive coping responses (Wood et al., 2012). Furthermore, chronic recordings revealed long-lasting elevations in resting systolic pressure following defeat selectively in rats displaying passive coping responses (Lombard et al., unpublished manuscript). In a related study, active coping determined by countering the resident's attacks was related to a reduced and shorter lasting disruption of heart rate circadian rhythms compared to rats adopting a passive coping response (Meerlo et al., 1999). Furthermore, when coping style was determined prior to chronic intermittent stress there was an association between passive coping and hypertension (Hawley et al., 2010). Alternatively, the cardiovascular responsivity of rats exhibiting either high or low aggression is also distinctly different; with high aggression rats displaying reduced vagal antagonism and increased tachyarrhythmias compared with non-aggressive rats (Carnevali et al., 2013a) Taken together, these studies indicate

that the behavioral coping response is associated with cardiovascular susceptibility to stress.

The LC plays a principal role in stress-related behaviors and cardiovascular function; therefore distinctions within the LC may provide insight into susceptibility to stress-related cardiac pathology. As discussed above, LC efferents target several cardiovascular centers of the brain, inhibiting parasympathetic centers such as the DMV and Amb and activating preganglionic sympathetic neurons (PSN). In active coping rats, the afferent regulation of LC that is biased towards PGi-ENK would translate to decreased activity of the LC's cardiovascular efferents (Figure 1). This would reduce inhibition of the parasympathetic DMV and Amb and suppress excitation of PSN, promoting resilience to stress-related cardiovascular pathology. Furthermore, restraint stress has been shown to increase ENK expression in the ventrolateral medulla, the region containing PGi neurons (Boone and McMillen, 1994). Within this region, ENK serves to inhibit sympathetic nerve activity, resulting in hypotension and bradycardia (Punnen et al., 1984). Therefore, beyond the cardioprotective effects of ENK within the LC, if ENK levels were also increased within the PGi/ventrolateral medulla in the active coping rats, this would contribute to a cardioprotective decrease in SNS activity. In contrast, the passive coping phenotype is characterized by exaggerated CNA-CRF afferent regulation and thus hyperactivity of LC neurons. This distinction in LC regulation would translate to exaggerated inhibition of parasympathetic centers and enhanced sympathetic drive, thereby increasing cardiovascular vulnerability. In support of this, daily administration of a CRF antagonist before social stress not only shifted the population of rats towards the LL, active coping strategy, but also attenuated the

reduction in HRV (Wood et al., 2012). While Reyes and colleagues (Reyes et al., 2015a) provided evidence of the CNA afferents as a distinct regulator of the LC in SL passive rats, the LC also projects to the CNA, which contributes to the cardiovascular response to stress (Kravets et al., 2015; Mason and Fibiger, 1979). For example, stimulation of the CNA elicits increased blood pressure in conscious rats (Chiou et al., 2009) and lesions of the CNA attenuate the pressor response to acute stress (Sanders et al., 1994). Therefore, the increased excitatory drive from the LC to the CNA that would be anticipated in passive coping rats may likely contribute to the exaggerated sympathetic activation in this susceptible phenotype. Taken together, these studies suggest that exaggerated LC activation may, in part, drive the enhanced cardiovascular vulnerability. In support of this, recent findings using intracerebroventricular administration of DSP-4 to partially lesion LC-NE projections prior to social defeat identified that an intact LC is necessary for the chronic increases in resting blood pressure to develop in the passive coping SL rats (Lombard et al., unpublished manuscript). In sum, these findings support a central role for LC regulation of cardiovascular vulnerability.

Conclusion

Identifying adaptations within the brain that are related to coping strategy will be critical to understanding the etiology of susceptibility to stress-induced cardiovascular disease. In particular, evidence of distinct regulation of LC afferents in passive versus active coping rats (Reyes et al., 2015a) may provide a window into the neurobiological changes driving susceptibility to cardiopathological conditions. In agreement with this, a

study in humans combined measurements of HRV with fMRI and demonstrated that the high frequency index of HRV, a reflection of cardiovagal output (Novak et al., 1997), was negatively correlated with activity of the LC (Napadow et al., 2008), underscoring the inhibitory effect that the LC has on parasympathetic tone.

Hyperactivity of the LC has also been implicated in stress-related psychiatric disorders such as depression and PTSD (Southwick et al., 1997; Wong et al., 2000). Preclinical studies in rodents reveal that social stress is capable of producing depressive-like or anxiety-like behaviors comorbid with cardiovascular disturbances and together suggest that LC dysregulation may be involved in this comorbid condition (Carnevali et al., 2013b; Wood et al., 2012). In patients suffering from PTSD, HRV is reduced compared with non-PTSD controls (Agorastos et al., 2013; Shah et al., 2013), contributing to the increased risk of developing cardiovascular disease in these psychiatric disorders (Wood, 2014; Wood and Bhatnagar, 2015). A recent study expanded on these findings by comparing predeployment HRV in active-duty marines with postdeployment development of PTSD (Minassian et al., 2014). This study showed that marines are significantly more likely to develop PTSD if they start off with lower HRV. Therefore, if mechanisms capable of reducing HRV are engaged before stress, such as exaggerated LC activity, this may increase susceptibility to stress-related disorders. Studies evaluating the permissive role that the LC may play in enhancing vulnerability to stress-related disorders will shed light on neurobiological mechanisms driving stress-induced cardiovascular disease and the high rate of comorbidity with psychiatric disorders.

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Figure 1. Schematic depicting distinct engagement of CRF and ENK afferents to the LC and the resulting adaptations in receptors that are associated with different coping strategies (top). This contrasting regulation of the LC in passive versus active coping rats would translate to differences in cardiovascular (CV) regulation (bottom). Top: The LC is regulated by CRF-excitatory afferents from the CNA and enkephalinergic-inhibitory afferents from the PGI. LC efferents inhibit parasympathetic centers (DMV and Amb) and excite preganglionic sympathetic neurons (PSN). In response to repeated social defeat stress, an active coping strategy is associated with a bias towards PGI-ENK afferents to the LC resulting in internalization and upregulation of MORs. In addition, the CRF influence from the CNA is decreased in LL rats. In passive coping (SL) rats, the CNA-CRF afferents are engaged, resulting in CRF1 internalization in LC dendrites and the PGI-ENK influence is reduced. Bottom: Distinctions in afferent regulation of the LC during social stress would determine how the LC regulates CV efferents. In the active coping phenotype, diminished LC activity would translate to reduced inhibition of the parasympathetic DMV and Amb and reduced excitation of the PSN. This would promote resilience to stress-related cardiovascular vulnerability. In the passive coping phenotype the hyperactive LC would inhibit parasympathetic tone and enhance sympathetic drive, resulting in increased cardiovascular vulnerability. The selective bias towards either an excitatory or inhibitory afferent to LC may serve as the basis for individual differences in the cardiopathological consequences of social defeat.

Dotted lines indicate reduced activity of neuronal projections; thick lines indicate increased activity of neuronal projection. Excitatory and inhibitory projections are denoted by a + or a solid line perpendicular to the projection (-).

