Published in final edited form as:

Brain Res. 2014 May 2; 1561: 35–47. doi:10.1016/j.brainres.2014.02.048.

Effects of intracerebroventricular corticotropin releasing factor on sensory-evoked responses in the rat visual thalamus

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Abstract

Corticotropin releasing factor (CRF) coordinates the brain's responses to stress. Recent evidence suggests that CRF-mediated activation of the locus coeruleus-norepinephrine (LC-NE) system contributes to alterations in sensory signal processing during stress. However, it remains unclear whether these actions are dependent upon the degree of CRF release. Using intracerebroventricular (ICV) infusions, we examine the dose-dependent actions of CRF on sensory-evoked discharges of neurons in the dorsal lateral geniculate nucleus of the thalamus (dLGN). The LGN is the primary relay for visual signals from retina to cortex, receiving noradrenergic modulation from the LC. *In vivo* extracellular recording in anesthetized rats was used to monitor single dLGN neuron responses to light flashes at three different stimulus intensities before and after administration of CRF (0.1, 0.3, 1.0, 3.0 or 10.0 µg). CRF produced three main effects on dLGN stimulus evoked activity: (1) increased magnitude of sensory evoked discharges at moderate doses, (2) decreased response latency, and (3) dose-dependent increases in the number of cells responding to a previously sub-threshold (low intensity) stimulus. These modulatory actions were blocked or attenuated by intra-LC infusion of a CRF antagonist prior to ICV CRF administration. Moreover, intra-LC administration of CRF (10 ng) mimicked the facilitating effects of moderate doses of ICV CRF on dLGN neuron responsiveness to light stimuli. These findings suggest that stressor-induced changes in sensory signal processing cannot be defined in terms of a singular modulatory effect, but rather are multi-dimensional and dictated by variable degrees of activation of the CRF-LC-NE system.

Keywords

locus coeruleus; corticotropin releasing factor; stress; norepinephrine; thalamus; sensory

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1. Introduction

An integral component of the stress response is activation of the hypothalamic-pituitary-adrenal (HPA) axis. This system is coordinated by the release of the neuropeptide corticotropin releasing factor (CRF), which initiates pituitary adrenocorticotropin release and subsequent adrenal corticosteroid release (Vale et al., 1981). In addition, extra-hypophyseal CRF functions as a neuromodulator with several targets throughout the brain that coordinate the cognitive and behavioral attributes of stress (Bale and Vale, 2004; Valentino and Van Bockstaele, 2001).

One target of CRF transmission is the noradrenergic brainstem nucleus locus coeruleus (LC) (Valentino and Van Bockstaele, 2001; Van Bockstaele et al., 1996). Exposure to various stressors elicits CRF release and activation of LC neurons, which can be blocked by administration of a CRF antagonist (Curtis et al., 2001; Valentino and Wehby, 1988; Valentino et al., 1991). Stressor-induced activation of the LC-NE system is associated with increased arousal and scanning attention, both of which serve as adaptive responses to stressors, facilitating executive function and sensorimotor responses (Valentino and Van Bockstaele, 2008). Activation of the LC prompts a range of modulatory effects on single neuron and neural circuit responses to afferent synaptic inputs (Berridge and Waterhouse, 2003; Devilbiss and Waterhouse, 2004; Devilbiss et al., 2006). Because of the extensive projection of the LC-NE system (Grzanna and Molliver, 1980; Swanson and Hartman, 1976), stressor exposure and subsequent CRF release can simultaneously influence the function of diverse populations of neurons across the brain.

Acute stress has been shown to impair sensory information processing (Clark et al., 1986; Ermultlu et al., 2005; Grillon and Davis, 1997; Liu et al., 2011; Miyazato et al., 2000; Sutherland and Conti, 2011). Recent findings suggest that CRF-mediated activation of the LC-NE system is a contributing factor. Acute exposure to a physiological stressor alters single neuron responsiveness to sensory stimuli by a LC-CRF-dependent mechanism (Zitnik et al., 2013). Additionally, local application of CRF onto the LC was shown to modulate thalamic and cortical sensory evoked responses (Devilbiss et al., 2012). Although these studies reveal how stress may alter sensory signal processing, the specific relationship between modulation of sensory signals and the degree of CRF activation of LC is unclear.

Although stressor-induced activation of the LC-NE system has been linked to distractibility and labile attention (Aston-Jones and Bloom, 1981; Berridge and Foote, 1991), other evidence suggests that the stress response can enhance cognitive performance according to an inverted-U relationship (Beylin and Shors, 1998; de Kloet et al., 1999; Luine et al., 1996). CRF has been implicated in this effect (Snyder et al., 2012). Similarly, activation of the LC has been shown to augment single neuron or neural circuit responses to sensory stimuli according to the same inverted-U dose-response relationship (Devilbiss and Waterhouse, 2004; Devilbiss et al., 2006). The link between CRF and LC activation provides a means through which stressors can impact behavior and sensory signal processing across a dynamic range of environmental and physiological challenges.

Several stressors generate CRF-mediated increases in LC output, but the degree of activation varies according to the stressor (Curtis et al., 2012; Lechner et al., 1997; Page et al., 1992; Valentino, 1989). One way to mimic stressor-induced activation of the LC-NE system is by direct infusion of CRF into the ventricular system of the brain. Intracerebroventricular (ICV) infusions of CRF cause dose-dependent increases in tonic LC output, thereby elevating NE levels throughout the forebrain (Curtis et al., 1997; Valentino and Foote, 1988; Page and Abercrombie, 1999; Palamarchouk et al., 2000; Zhang et al., 1998). ICV administration of CRF induces changes that mimic many of the behavioral responses observed during stress (Buwalda et al., 1997; Sherman and Kalin, 1988), including disruption of sensory processing (Conti et al., 2000; Risbrough et al., 2004).

The goal of the current study was to examine the effect of varying ICV doses of CRF on sensory-driven responses in the visual thalamus. The responses of dorsal lateral geniculate (dLGN) neurons to light stimuli were recorded before and after ICV CRF in the anesthetized rat. The results show that ICV CRF decreases the latency, increases the magnitude of light evoked responses according to an inverted-U dose-response relationship, and causes a dose-dependent increase in the number of dLGN cells responding to a previously sub-threshold light stimulus. A separate group of animals was pretreated with the CRF antagonist DpHeCRF, via direct application onto the LC ipsilateral to the monitored dLGN prior to administration of 3.0 μ g CRF ICV. This prevented or attenuated many of the modulatory actions normally observed after administration of CRF. These results, together with those obtained with intra-LC application of CRF, indicate that the observed changes in dLGN neuron responsiveness to light stimuli are likely due to CRF-mediated activation of the LC-NE system.

2. Results

Action potential waveforms from individual cells were recorded from the dLGN thalamus in 36 anesthetized animals. ICV drug treatments in these animals were as follows: 0.1 μ g ($n = 5$), 0.3 μ g ($n = 7$), 1.0 μ g ($n = 6$), 3.0 μ g ($n = 6$), 10.0 μ g CRF ($n = 6$), and intra-LC CRF antagonist treatment prior to administration of 3.0 μ g CRF: DpHeCRF + 3.0 μ g ($n = 6$). A sample of 156 cells at various drug doses [0.1 μ g ($n = 26$), 0.3 μ g ($n = 28$), 1.0 μ g ($n = 25$), 3.0 μ g ($n = 25$), 10.0 μ g ($n = 26$), DpHeCRF + 3.0 μ g ($n = 26$)] was deemed suitable for subsequent analysis, i.e. having well discriminated waveforms recorded for the duration of experimental protocols (described in methods). Another set of experiments was performed using multiple doses of CRF (3, 10, 100 ng in 100 nL aCSF) infused locally into the LC ipsilateral to the dLGN recording site. A detailed analysis was conducted on the cells recorded from animals receiving 10 ng CRF ($n = 6$, 25 cells), a dose that has been shown in prior experiments to increase LC output similar to moderate doses of CRF ICV (Curtis et al., 1997). Placement of dLGN recording electrodes (Fig. 1) and location of LC cannulae (Fig. 2) were confirmed post-mortem.

2.1 Dose-dependent effects of ICV CRF on the magnitude of light evoked dLGN responses

Figure 3 shows records from a typical dLGN neuron whose response to light stimuli was facilitated after CRF ICV. Figure 4A summarizes the time course and dose-dependent

effects of CRF ICV on the magnitude of dLGN responses to light flashes, displayed as percent control. Repeated measures ANOVA revealed that dLGN neurons recorded from animals administered 0.1 and 10.0 μg CRF ICV exhibited no significant change in response magnitude over time ($F(3, 75) = .813, p > .05$) and ($F(3, 75) = 1.82, p > .05$), respectively (Table 1A). In contrast, there was a significant change in response magnitude over time in animals treated with most doses greater than 0.1 μg , including 0.3 μg ($F(3, 81) = 4.183, p < .01$), 1.0 μg ($F(3, 72) = 3.72, p < .05$), and 3.0 μg CRF ICV ($F(3, 72) = 24.204, p < .01$). Follow up tests confirmed that these increases in magnitude of evoked responses observed post-CRF were significant vs. control ($p < .05$) (Table 1A). In dLGN neurons recorded from animals pretreated with an intra-LC infusion of DPheCRF prior to administration of 3.0 μg CRF, no significant effect was observed in response magnitude over time ($F(3, 75) = .574, p > .05$) (Table 1A).

The magnitude of dLGN evoked responses post-CRF was significantly different between treatment groups (Treatment*Time, $F(1, 5) = 4.889, p < .01$). Follow up tests confirmed that the increase in magnitude occurring 10-20 mins after administration of 0.3 μg ($30 \pm 3\%, p < .05$) and 1.0 μg ($42 \pm 12\%, p < .01$) was significantly different from 0.1 μg ($5 \pm 5\%$) and 10.0 μg CRF ($10 \pm 13\%, p > .05$). 3.0 μg ($32 \pm 15\%, p < .05$) was significant different from 0.1 μg , but not 10.0 μg CRF. Figure 4B summarizes these findings. 10.0 μg and DpHeCRF + 3.0 μg ($4 \pm 5\%, p > .05$) were not different from 0.1 μg CRF. There was no significant difference between 0.3, 1.0, and 3.0 μg CRF ($p > .05$). The magnitude of evoked responses was significantly different between DpHeCRF + 3.0 μg and 3.0 μg CRF treatment ($p < .05$). Follow up tests for changes in magnitude 20-30 min post-CRF yielded equivalent results.

2.2 Dose-dependent effects of ICV CRF on the latency of light evoked dLGN responses

Figure 5A shows the time course of dose-dependent effects of CRF ICV on the latency of dLGN neuronal responses to light flashes, displayed as percent control. dLGN neurons recorded from animals administered 0.1 μg CRF ICV showed no significant change in response latency over time ($F(3, 75) = 2.302, p > .05$) (Table 1B). As seen for response magnitude, there was a significant change in latency over time in dLGN neurons recorded from animals administered higher doses - 0.3 μg ($F(3, 81) = 38.483, p < .01$), 1.0 μg ($F(3, 72) = 38.142, p < .01$), 3.0 μg ($F(3, 72) = 35.144, p < .01$), 10.0 μg ($F(3, 75) = 23.604, p < .01$), and 3.0 μg CRF + DpHeCRF ($F(3, 75) = 14.746, p < .01$). Follow up tests confirmed that all of these decreases in response latency observed post-CRF were significantly different from control ($p < .05$) (Table 1B).

The latency of dLGN evoked responses post-CRF was significantly different between treatment groups (Treatment*Time, $F(1, 5) = 4.714, p < .01$). Follow up tests confirmed that the decrease in latency 10-20 min after administration of 0.3 μg ($-9 \pm 0.9\%, p < .01$), 1.0 μg ($-6 \pm 0.9\%, p < .01$), 3.0 μg ($-8 \pm 0.9\%, p < .01$), 10.0 μg ($-7 \pm 1.0\%, p < .05$), and DpHeCRF + 3.0 μg ($-5 \pm 0.9\%, p < .05$) was significantly different from 0.1 μg CRF ($-2 \pm 1.2\%$). Figure 5B summarizes these findings. There was no significant difference between 0.3, 1.0, 3.0, and 10.0 μg CRF ($p > .05$). The latency of evoked responses was significantly different between DpHeCRF + 3.0 μg and 3.0 μg CRF treatments ($p < .05$). Follow up tests for changes in latency 20-30 min post-CRF yielded equivalent results.

2.3 Dose-dependent effects of ICV CRF on dLGN neuron responses to previously sub-threshold light stimuli

Figure 6A shows a representative neuron displaying a „gating” effect post-CRF administration. The stimulus for the PTSHs shown was the lowest light intensity, which was initially sub-threshold for eliciting a response from dLGN neurons. Overall, CRF administration at progressively higher doses prompted a progressive increase in the number of cells exhibiting responses to the otherwise sub-threshold light stimulus. Figure 6B summarizes these results. Cells recorded across animals pretreated with CRF antagonist showed a marked decrease in the number of neurons recruited into the sensory response pool vs. those animals treated solely with 3.0 µg CRF ICV; 19% vs. 44%, respectively. The percentage of dLGN neurons whose responses were gated into the response pool was significantly different among doses, X^2 (5, $N = 130$) = 21.01, $p < 0.01$. The percent of cells gated in DpHeCRF pretreated animals was not significantly different from 3.0 µg CRF, X^2 (1, $N = 51$) = 2.57, $p = 0.0567$.

2.4 Effects of intra-LC infusion of CRF on on dLGN neuronal responsiveness to light stimuli

In a separate group of animals, dLGN neurons were recorded before and after direct infusion of CRF into the ipsilateral LC. Figure 7A shows a representative neuron whose responses to light stimuli were facilitated post-CRF (10 ng). Among dLGN neurons recorded from animals receiving 10 ng intra-LC CRF infusions, there was a significant change in response magnitude (F (3, 72) = 9.163, $p < .01$) and latency (F (3, 72) = 7.663, $p < .01$) over time. Follow up tests confirmed that these post-CRF increases in magnitude and decreases in latency were significant vs. control ($p < .05$). Twenty percent (5/25) of these cells exhibited gated visual responses after CRF administration. Similar albeit more moderate modulatory actions were observed following intra-LC infusions of 3 and 100 ng CRF. Schematic diagrams in Figure 7B and C illustrate cannula placements where intra-LC infusions of CRF were successful in producing modulatory effects vs. those where infusions were ineffective.

3. Discussion

Our results show that CRF ICV alters the magnitude and timing of stimulus evoked discharges within the sensory thalamus of the anesthetized rat. Specifically, CRF ICV increases the magnitude of individual dLGN neuronal responses to threshold level light stimuli according to a dose-response relationship that approximates an inverted-U, increases the speed of transmission of these signals through the thalamic visual circuitry, and causes a dose-dependent increase in the number of cells responding to previously sub-threshold light stimuli. These effects appear to depend on CRF-mediated activation of the LC-NE system, as animals pretreated unilaterally with intra-LC CRF antagonist showed blockade or attenuation of the latency and magnitude changes, as well as a reduction in the number of cells gated after CRF administration.

Together, these data suggest that stressor exposure and subsequent CRF release can modulate early stage sensory signal processing in the dLGN via output from the LC. Such effects depend upon the degree of activation of the CRF-LC pathway. Thus, stressor-

induced modulation of sensory signal processing in noradrenergic terminal fields does not occur as a discrete action, but instead likely operates along a continuum based upon the degree of CRF-mediated activation of the LC-NE pathway. The present findings also suggest a physiological basis for disruption of sensory signal processing that occurs during acute periods of intense stressor exposure or in conjunction with chronic stress disorders associated with dysregulation of the CRF system (Clark et al., 1986; Ermultlu et al., 2005; Grillon and Davis, 1997; Liu et al., 2011; Miyazato et al., 2000; Sutherland and Conti, 2011; Bakshi et al., 2012; Grillon et al., 1996).

3.1 Technical considerations

The facilitating effects observed after administration of 3.0 µg CRF ICV were reduced in animals pretreated with an ipsilateral intra-LC infusion of the CRF antagonist DpHeCRF. This suggests that stressor-related alterations in dLGN responsiveness to light stimuli are likely due to noradrenergic influences from the LC. The locations of the LC infusion cannulae were distributed throughout the nucleus and within the pericoerulear region (Fig. 2 B-D). Thus, we cannot attribute the observed effects to a specific portion of the LC or site within the pericoerulear region. The results from two animals were considered control data because the final location of the infusion cannula in each case was distal to the LC (see Fig. 2B-C). Of the 9 cells recorded from these animals; a decrease in response latency and increase in response magnitude (n = 7 cells), or gated response (n = 4 cells) was observed post-CRF. These actions were typical of those observed following 3.0 µg CRF ICV without infusion of DpHeCRF. While the majority of the innervation from LC to dLGN is ipsilateral, ~30-40% of the noradrenergic projection to the dLGN is derived from the contralateral LC (Kromer and Moore, 1980; Simpson et al., 1997). We believe the partial blockade of CRF influences on dLGN responses following unilateral CRF antagonist infusion was due to the bilateral nature of LC inputs to dLGN. Nevertheless, the large reduction in CRF-induced modulation of dLGN light evoked responses by CRF antagonist infusion provides evidence that the observed effects were the result of CRF-mediated activation of the LC-NE pathway.

To further examine the specificity of ICV CRF's noradrenergic action on dLGN evoked activity, a separate group of animals received direct infusions of CRF into the ipsilateral LC. dLGN neurons recorded from these animals showed significant increases in magnitude and decreases in latency post-CRF (see Results). These changes in magnitude and latency, as well as the increased number of cells responding to otherwise sub-threshold light stimuli after intra-LC CRF infusions (10 ng), were similar to the effects observed after administration of CRF ICV at moderate doses. No changes in dLGN neuronal responsiveness were observed in four animals where the cannula locations were distal to the LC (Fig. 7B-C). Together, these results provide further evidence that the changes in thalamic sensory neuron responsiveness observed after ICV CRF were specific to CRF-mediated activation of the LC-NE system.

Changes in sensory evoked discharge following CRF administration, as observed in the present study, differ from effects observed following stressor exposure or intra-LC microinfusions of CRF. Direct infusions of CRF onto the LC have been shown to suppress, rather than enhance, thalamic sensory evoked responses in the ventral posteromedial (VPM)

thalamus in the waking animal (Devilbiss et al., 2012). In that study, two doses of CRF were tested. The opposite effects might be related to differences between the experimental preparations, anesthetized vs. awake. CRF is a more potent activator of LC neurons in the awake vs. anesthetized animal (Valentino and Foote, 1988). Accordingly, the doses administered by Devilbiss et al. (2012) may have elicited more intense activation the LC and greater downstream release of NE than in the present study. As such the LC-thalamic interactions observed may have been representative of the right limb of the inverted-U curve, suppressing stimulus-evoked neuronal responses rather than elevating them. We recently reported suppression of visually evoked neuronal responses in rat thalamus during hemodynamic stress and subsequent CRF-mediated activation of the LC (Zitnik et al., 2013). In contrast to more slowly acting ICV CRF, hemodynamic stress and direct infusion of CRF onto the LC have been shown to intensely activate LC neurons and cause rapid increases in terminal field concentrations of NE (Curtis et al., 1997; Page and Abercrombie, 1999; Palamarchouk et al., 2000; Zhang et al., 1998; Kawahara et al., 1999; Lavicky and Dunn, 1993; Swiergiel et al., 1998). We propose the mode of LC activation and time course of NE release in downstream targets, specifically the more gradual activation of LC and release of NE after ICV CRF administration likely account for the differences in the modulatory effects observed here and in our previous study using hemodynamic stress (Zitnik et al., 2013). Anesthesia affects the dynamics of sensory signal processing, a factor that could also account for differences between the results of the present study and prior reports (Devilbiss et al., 2012). Under isoflurane anesthesia (the anesthetic used in the current study), stimulus evoked responses and spontaneous activity in the thalamus are different from those in the awake animal (Detsch et al., 1999; Ries and Puil, 1999). Although we observed robust CRF-mediated modulation of stimulus evoked discharge in dLGN neurons, the limitations and advantages of the anesthetized preparation must be taken into consideration when comparing the results of the current study with changes in sensory processing that occur during normal physiological release of CRF in the waking animal. Anesthesia reduces neurotransmitter release, limits the size of sensory receptive fields and diminishes the responsiveness of neurons to synaptic inputs (Chapin et al., 1981; Nicolelis and Chapin, 1994). Although studies in the waking animal allow for investigations under the most physiologic of circumstances, the anesthetized preparation nevertheless affords an opportunity to assess drug actions under static and well-controlled experimental conditions.

Although local application of NE and activation of the LC-NE pathway are known to alter the responsiveness of dLGN neurons to synaptic inputs, other neurotransmitters have also been implicated in modulating neuronal activity in this region, including dopamine (Albrecht et al., 1996), serotonin (Kemp et al., 1982), and acetylcholine (Sillito et al., 1983). However, dopaminergic innervation in the rat dLGN is quite limited (Garcia-Cabezas et al., 2009). Serotonin- and acetylcholine-containing axons have been identified within the dLGN (Pasquier and Villar, 1982), but alterations in brain levels of serotonin and acetylcholine are not observed until 30 min after ICV CRF administration (Day et al., 1998; Desvignes et al., 2013; Price and Lucki, 2001); thus release of these transmitters cannot account for the CRF-mediated changes reported here which were observed in under 30 min and occasionally as early as 10 min post CRF infusion. CRF activation of serotonin and acetylcholine pathways

may however contribute to slower stressor-related changes in the responsiveness of thalamic neurons.

3.2 Putative Noradrenergic Mediation of CRF Effects

Previous studies have shown that electrical stimulation of the LC or local iontophoretic administration of NE increases the responsiveness of dLGN neurons (Kayama, 1985; Rogawski and Aghajanian, 1980, 1982) to afferent synaptic inputs via activation of the alpha-1 (α_1) adrenergic receptor (Rogawski and Aghajanian, 1980; Holdefer and Jacobs, 1994). However, high ejection currents of NE over a short period of time elicit a suppressant effect, which is mediated by the alpha-2 adrenergic receptor (α_2) (Rogawski and Aghajanian, 1980; Elmslie and Cohen, 1990). The facilitating effects observed in dLGN neurons after administration of ICV CRF at low to moderate doses mimicked the effects elicited by α_1 receptor activation or low level electrical stimulation of LC. We believe this CRF-mediated augmentation of evoked responses is due to low to moderate amounts of NE release and its activation of α_1 receptors, reflecting the linear relationship and interaction that exists between both forebrain NE release and LC output (Berridge and Abercrombie, 1999; Florin-Lechner et al., 1996), as well as ICV CRF dose and LC discharge (Curtis et al., 1997). We believe the temporal differences in NE release that result from strong electrical LC stimulation or iontophoretic application of NE at high ejection currents ICV CRF may account for the absence of suppressant effects observed in the present study.

The gating effect observed in the present study is consistent with previous demonstrations of NE- or LC-mediated gating of neuronal responses to previously sub-threshold synaptic stimuli (Devilbiss et al., 2006, Waterhouse et al., 1988, 1990). Our results show that unlike the observed changes in magnitude and latency of dLGN neuron responses to light stimuli, this effect did not approximate an inverted-U dose-response relationship, at least across the range of CRF doses tested. We postulate that the monotonically increasing relationship between ICV CRF dose and number of cells gated is receptor-specific. Although there is evidence that the ascending limb of the inverted-U relationship for noradrenergic modulation of excitatory neuronal responses is α_1 receptor mediated (Ramos and Arnsten, 2007), the receptor mechanisms underlying the gating effect have not been identified. As pointed out by Ramos and Arnsten (2007), adrenergic receptors have different binding affinities; low concentrations of NE activate α_1 receptors, while higher concentrations activate α_2 and beta-receptors. Thus, CRF-mediated increases in noradrenergic neurotransmission may engage a broad range of candidate adrenergic receptor mechanisms and actions as NE levels increase in LC terminal fields.

3.3 Behavioral and Functional Relevance

Several of the CRF doses tested elicited LC activation within a range observed during animal exposure to specific stressors (Curtis et al., 1997), e.g. predator odor (Curtis et al., 2012), bladder distention (Page et al., 1992), or colonic distention (Lechner et al., 1997). Overall, these results show that the modulatory effects of ICV CRF on sensory driven responses of downstream thalamic cells and circuits are dependent upon the degree of CRF-mediated activation of the LC. These dose-dependent effects suggest that low to moderate levels of stress may benefit performance of tasks requiring focused attention by facilitating

early stage sensory signal transmission, i.e. increased magnitude and reduced latency of sensory evoked dLGN neuronal responses. However, life threatening stressor exposure (e.g. predator odor) may trigger greater CRF-mediated activation of the LC-NE system and switch the dLGN circuitry to an operational mode that favors gating of responses to otherwise sub-threshold stimuli, a dynamic that would promote vigilance to a broader range of environmental cues and facilitate predator avoidance strategies.

Studies conducted under stress conditions provide behavioral support for many of the physiological alterations observed in the current study. For example, mild stress has been shown to reduce audiovisual reaction times (Saha et al., 1996). This may be related to the reduced latencies of neuronal responses to visual inputs as identified here. Stress has also been shown to increase distractibility, disrupting attention to task related cues (Clark et al., 1986; Ermultlu et al., 2005; Shackman et al., 2006; Pardon et al., 2000; an effect we would attribute to gating of otherwise subliminal sensory inputs. Gating may be a stressor-induced behavioral state in which dedicated attention to a singular task becomes difficult due to the broadly tuned detection of previously irrelevant or distracting stimuli. Such a mode of operation would promote a shift from focused to scanning or labile attention, thus allowing the organism to identify and respond to a broader array of low level sensory stimuli under circumstances where detection of sensory events is of greater importance to survival than *discrimination* of specific stimulus attributes (Valentino and Van Bockstaele, 2001; Aston-Jones et al., 1999).

3.4 Clinical Implications

Psychiatric disorders such as post-traumatic stress disorder (PTSD) (Aston-Jones et al., 1994; Adamec et al., 2010; Bangasser et al., 2010; Geraciotti et al., 2008), and anxiety (Bangasser, 2013; Bremner et al., 1996; Fossey et al., 1996) are related to both CRF and LC-system dysfunction. Patients with PTSD display various elements of altered sensory processing; both hyper-reactivity and hyper-vigilance to sensory inputs (Bakshi et al., 2012; Grillon et al., 1996; Ornitz and Pynoos, 1989; Orr et al., 2002). The results of the present study suggest these disruptions may be due in part to alterations in early stage sensory signal processing. Therefore possible targets for therapeutic intervention may need to consider changes in thalamic physiology that occur in conjunction with PTSD.

4. Experimental Procedure

4.1 Animals

The subjects, adult male Sprague-Dawley rats (Taconic Farms, Inc.; Hudson, NY) weighing 250-400 g, were housed two to a cage in a temperature and humidity controlled environment with *ad libitum* food and water. All procedures were conducted in accordance with the National Research Council *Guide for Care and Use of Laboratory Animals*. All protocols were approved by the Drexel University Institutional Animal Care and Use Committee.

4.2 Experimental protocol

Animals were anesthetized with isoflurane (induction at 4%, maintenance at 1-2%), then positioned in a stereotaxic apparatus. Burr holes were drilled over the dLGN (-4.8 AP, +4.1

ML) and lateral ventricle (-1.0 AP, +1.6 ML) (Paxinos and Watson, 1982). An ICV cannula (26 GA stainless steel) was then lowered into the lateral ventricle (-3.6 DV). A RadioShack® white, clear lens LED light (luminous intensity: 1100mcd, chromaticity coordinates: 660, viewing angle: 100 deg) was positioned centrally in front of the contralateral eye and the room lights were turned off. The light stimulus (highest level intensity) was delivered continuously at a rate of twice per sec for 20 ms duration. An 8-channel multi-wire (.0015" formvar insulated nichrome wire, A-M Systems®) recording electrode was lowered into the dLGN (-4.0 DV) (Fig. 1). The electrode's final location was determined by electrophysiological verification that the recording contained cells characteristic of LGN cell firing evoked by stimulus light flashes. Electrode signals were passed through a high input impedance amplifier (Plexon® MAP system; Dallas, TX) and monitored continuously online through Plexon® Sort Client. Once dLGN neurons were identified, control of the light stimulus was changed to a CED 1401 running a Spike 2® script in which 20 msec light flashes of three different light stimulus intensities were given in a pseudorandom order twice per sec. The highest stimulus intensity (~1,800 lux) was the same brightness used to identify and isolate waveforms from dLGN neurons. The intermediate stimulus was ~1,200 lux and the minimal level stimulus was ~250 lux. Across all animals studied the intermediate stimulus was sufficient to elicit responses from individual neurons whereas the minimal stimulus was routinely below threshold for evoking discharge from neurons that responded to the highest stimulus intensity. Spike train activity was then recorded and stored for subsequent analysis based upon peri-stimulus time histograms (PTSHs) and raster records of neuronal activity. Each recording session consisted of a 10 min period of pre-drug cell responses to light, followed by 60 sec administration of [0.1, 0.3, 1.0, 3.0, or 10.0 µg] CRF in 3.0 µL of artificial cerebral spinal fluid (aCSF), followed by a 30 min recording.

4.3 Intra-LC microinfusions

A 31 GA stainless steel cannula was used to directly administer drugs into the LC ipsilateral to the dLGN recording site (AP -10.0, ML +1.2 mm, DV -5.1 mm). For animals receiving D-PheCRF₁₂₋₄₁, 10 ng (in 100 nL of aCSF) was infused 6 min prior to administration of 3.0 µg CRF ICV. Intra-LC administration of this drug has been shown previously to prevent tonic LC activation produced by CRF ICV (Curtis et al., 1997). At the conclusion of these experiments, cannula locations were marked by a 100 nL infusion of 2% pontamine sky blue dye (Fig. 2A). Animals were eliminated from the study if leakage into the 4th ventricle occurred, as evidenced by pontamine sky blue dye in the ventricular system post-mortem (n = 2 animals). Successful cases were those where the cannula was proximal to LC and no leakage into the ventricular system was observed (n = 5 animals). A separate group of animals received intra-LC infusions of 3-100 ng CRF (in 100 nL aCSF), a dose range that increases LC output similar to low to intermediate doses of ICV CRF (Curtis et al., 1997).

4.4 Histology

Animals were perfused with 0.9% saline followed by a 10% formalin solution. After euthanasia, the brains were removed, stored for 48 hrs in phosphate buffer solution containing 20% sucrose, sectioned, and stained with neutral red in order to verify electrode and cannula locations. Only data from animals where drug infusions and unit recording were

confirmed in the lateral ventricle, LC and dLGN, respectively, were included in this analysis.

4.5 Extracellular recording and analysis

Recording methods and offline sorting of single unit waveforms, using Plexon® Offline Sorter were identical to Zitnik et al. 2013. Computer-generated cumulative rasters and PSTHs were constructed from the data recorded from each cell in order to characterize and quantify unit responses to light stimuli before and after ICV CRF infusions. The spontaneous firing rate, control magnitude and latency of light evoked responses for each cell were determined during control recordings prior to ICV CRF. The level of spontaneous (baseline) activity was subtracted from the stimulus evoked discharge. Onset of the stimulus evoked response was identified as the time lag of the first of two consecutive bins in the PSTH (bin size = 1 ms) with firing frequencies one standard deviation above baseline firing rate. Offset of the response was defined as the lag of the first bin following onset in which firing rate fell below one standard deviation above the initial baseline firing rate for two consecutive bins in the PSTH. The primary stimulus evoked response was then identified as the height of the tallest bin between this onset and offset. Changes in the magnitude and timing of light evoked discharges were quantified by comparing discharge rates and times for primary response peak histograms generated for each neuron. The highest level light stimulus was used to calculate the peak amplitude (impulses/sec) of the primary stimulus-evoked discharge and the mean latency of the peak response (ms). 'Gating' was defined as cells that demonstrated no response to the lowest intensity stimulus during control, but a prominent peak after CRF administration.

All statistical tests were conducted using SPSS (version 19). Drug-mediated changes in magnitude and latency of stimulus evoked responses over time within each treatment group were measured using one-way repeated measures analysis of variance (ANOVA). Bonferroni-corrected follow up paired t-tests were made to compare individual time segments (0-10, 10-20, 20-30 min post-CRF) to control values. Additionally, two-way mixed factor ANOVAs (dose as between subjects factor and time post-infusion as within subjects factor) was used to compare changes in dLGN response latency and magnitude post-CRF between treatment groups. Post hoc tests were conducted using Tukey's HSD to identify significant individual pairwise comparisons between treatment groups at 10-20 and 20-30 mins post-CRF. A 5×2 chi-squared test for independence was performed to determine if there was a dose-dependent difference in the number of dLGN neurons gated after CRF administration. A 2×2 chi-squared test for independence was performed to determine if the number of dLGN neurons gated after 3.0 µg CRF vs. DpheCRF + 3.0 µg treatment was different.

4.6 Drugs

Ovine CRF was supplied by AnaSpec, Inc. (San Jose, CA). D-PheCRF₁₂₋₄₁ was supplied by Tocris Bioscience® with permission of The SALK Institute (San Diego, CA).

Acknowledgments

This work was supported by the National Institute on Drug Abuse (NIDA DA017960) to BDW.

Abbreviations

LC	locus coeruleus
dLGN	dorsal lateral geniculate nucleus
CRF	corticotropin releasing factor
NE	norepinephrine
ICV	intracerebroventricular

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Highlights

- We examined how different doses of CRF ICV alter LC-target sensory neurons.
- dLGN single unit responses to light stimuli were recorded in the anesthetized rat.
- Dose-dependent changes in magnitude and latency of response were observed.
- CRF-induced changes were blocked in animals pretreated with CRF-antagonist.
- Changes in dLGN activity are based on the degree of CRF-LC-NE activation.

Figure 1.

Brightfield photomicrograph illustrates the location of a bundle of recording electrodes within the dLGN. The section is counterstained with neutral red. dLGN, dorsal lateral geniculate nucleus; vLGN, ventral lateral geniculate nucleus; VPM, ventral posteromedial thalamus. * denotes location of microwire bundle protruding from the 26 GA guide cannula.

Figure 2.

A. Brightfield photomicrograph illustrates the location of a microinfusion cannula adjacent to the LC. The section is counterstained with neutral red. Final location of the cannula tip is outlined in black. 4V, fourth ventricle; Cb, cerebellum; Me5, mesencephalic trigeminal nucleus. B-D: Plots of DpHeCRF microinfusion sites. Shown are representative rat brain atlas sections through the brainstem at the level of the LC. "X" represents the location of the individual cannula placements (filled X = confirmed location within or proximal to LC, unfilled X = confirmed location distal to LC). B. -9.8 bregma, C. -10 bregma, D. -10.3 bregma.

Figure 3.

Peri-stimulus time histograms (PSTHs) illustrating the facilitating effects of 1.0 μ g CRF ICV on the responsiveness of a single dLGN neuron. Time zero represents onset of a 20 ms stimulus (highest intensity light stimulus). On average, the light was presented once every 1.5 sec. The y-axis represents the magnitude of evoked responses, based on frequency of dLGN firing (impulses/sec). The vertical dotted line represents the mean peak response latency during control. Note the increase in evoked dLGN discharges and decrease in response latency occurring after administration of ICV CRF.

Figure 4.

Effects of ICV CRF on the magnitude of dLGN neuron responses to light stimuli. A. Time course of effects. All data are expressed as percentage of mean control (pre-CRF) discharge rate. Each point represents the mean of all cells recorded in animals administered CRF at each doses/treatment group. Paired comparisons with control values: $*p < .05$. B. Comparisons between treatment groups. Each bar represents the change in magnitude 10-20 min post-CRF administration, expressed as percent change from control. Intra-LC administration of the CRF antagonist, DpHeCRF, prior to 3.0 μ g CRF prevented the enhancement of evoked responses normally observed following administration at this dose. Paired comparisons with 0.1 μ g CRF: # $p < .05$, ## $p < .01$. Paired comparisons with DpHeCRF + 3.0 μ g CRF: † $p < .05$. Vertical lines indicate ± 1 SEM.

Figure 5.

Effects of ICV CRF on latency of dLGN neuron responses to light stimulus. A. Time course of effects. All data are expressed as percentage of mean control (pre-CRF) response latency. Each point represents the mean of all cells recorded in animals administered CRF at each doses/treatment group. Paired comparisons with control values: $*p < .05$. B. Comparisons between treatment groups. Each bar represents the change in latency 20-30 min post-CRF ICV, expressed as percent change from control. Intra-LC pretreatment with the CRF antagonist, DpHeCRF, prior to 3.0 μ g CRF ICV attenuated the decrease in latency normally observed following administration at this dose. Paired comparisons with 0.1 μ g CRF: # $p < .05$, ## $p < .01$. Paired comparisons with DpHeCRF + 3.0 μ g CRF: ‡ $p < .05$. Vertical lines indicate ± 1 SEM.

Figure 6.

Effects of ICV CRF on dLGN neuron response to a previously sub-threshold light stimulus. A. PSTHs illustrate the “gating” effect of CRF (10ug, ICV) on responsiveness of a single dLGN neuron to light stimulus. Time zero represents onset of a 20 ms stimulus (light flash). On average, the light was presented once every 1.5 sec. The y-axis represents the magnitude of evoked responses, based on frequency of dLGN firing (impulses/sec). Note that control recordings show that the cell was initially un-responsive to the light stimulus, but demonstrated a robust light evoked discharge after administration of ICV CRF. B. Percentage of dLGN cells exhibiting gating as a function of dose; i.e. the number of cells exhibiting gating divided by the total number of cells recorded in each treatment group. Note the large decrease in the percentage of cells exhibiting gating of visual signals in those animals receiving intra-LC infusions of the CRF antagonist, DpHeCRF, prior to administration of 3.0 µg CRF ICV vs. those animals solely administered 3.0 µg CRF ICV.

Figure 7.

A. PSTHs illustrating the facilitating effects of local CRF administration (10 ng) on the responsiveness of a single dLGN neuron to light stimulus. Note the progressive increase in response magnitude and decrease in response latency post-CRF. These changes mimic those observed after ICV administration of moderate CRF doses. B-C: Plots of CRF microinfusion sites. Shown are representative rat brain atlas sections through the brainstem at the level of the LC. "X" represents individual cannula placements for 10 ng CRF (in 100 nL aCSF) infusions (filled X = confirmed location within or proximal to LC, unfilled X = confirmed location distal to LC). Open circle and open triangles represent confirmed individual cannula placements where 3 and 100 ng CRF (in 100 nL aCSF) infusions were ineffective in producing changes in dLGN neuron responses to light stimuli, respectively. B. -9.8 bregma, C. -10 bregma.

Table 1

Summary of results for response magnitude (A) and latency (B) over time for all treatment groups tested.

A		Magnitude		B		Latency			
ICV CRF dose (µg)	Control	0-10 min Post-CRF	10-20 min Post-CRF	20-30 min Post-CRF	ICV CRF dose (µg)	Control	0-10 min Post-CRF	10-20 min Post-CRF	20-30 min Post-CRF
0.1	127 ± 8	131 ± 7	129 ± 5	127 ± 4	0.1	39 ± 0.8	39 ± 1.0	38 ± 1.0	38 ± 1.2
0.3	118 ± 6	134 ± 7*	136 ± 7*	137 ± 7*	0.3	40 ± 0.8	37 ± 0.8*	37 ± 0.7*	36 ± 0.8*
1.0	137 ± 9	164 ± 9*	171 ± 9*	153 ± 7*	1.0	40 ± 0.7	38 ± 0.7*	37 ± 0.9*	37 ± 0.8*
3.0	110 ± 6	123 ± 6*	125 ± 6*	126 ± 6*	3.0	44 ± 0.7	42 ± 0.6*	41 ± 0.6*	40 ± 0.5*
10.0	115 ± 9	117 ± 10	123 ± 10	124 ± 9	10.0	41 ± 0.8	39 ± 0.8*	38 ± 0.7*	37 ± 0.8*
DpheCRF+3.0	134 ± 6	97 ± 8	103 ± 10	99 ± 8	DpheCRF+3.0	42 ± 0.9	40 ± 0.8*	39 ± 0.9*	39 ± 0.9*

* Paired comparisons with control values: $p < .05$.