Published in final edited form as:

Psychopharmacology (Berl). 2016 July ; 233(13): 2581–2592. doi:10.1007/s00213-016-4305-y.

Myocardial infarction sensitizes medial prefrontal cortex to inhibitory effect of locus coeruleus stimulation in rats

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Abstract

Rationale—Anxiety is a common comorbidity that develops after myocardial infarction and is now an established independent risk factor for cardiovascular mortality.

Objective—Here, we assessed anxiety and mapped neural activity of forebrain regions that regulate anxiety in a rat model of myocardial infarction in order to identify sites of dysregulation.

Methods—Anxiety responses to novel (open field) or aversive stimuli (discriminative auditory fear conditioning) were assessed in rats subjected to coronary artery ligation (CAL) or sham ligation. Forebrain metabolic activity was measured by cytochrome oxidase (CO) histochemistry. Changes in CO activity and the incidence of ventricular arrhythmias were also assessed during modulation of fear circuitry induced by electrical stimulation of the locus coeruleus.

Results—Coronary artery ligation had negligible effects on open-field behavior, but increased expression of learned fear and impaired fear cue discrimination. Cytochrome oxidase activity was increased in the medial prefrontal cortex and in the lateral amygdala after CAL. Locus coeruleus stimulation reduced CO activity in the infralimbic medial prefrontal cortex only in rats subjected to CAL. Stimulation of the LC also elicited new ventricular arrhythmias in rats subjected to CAL.

Conclusion—Coronary artery ligation sensitizes the infralimbic medial prefrontal cortex to the inhibitory effects of locus coeruleus stimulation. Suppression of infralimbic medial prefrontal cortical activity may impair the ability of rats subjected to CAL to discriminate between cues that signal aversive and neutral events which, in turn, may promote excessive sympathetic activation of the cardiovascular system in response to innocuous stimuli.

Keywords

Cytochrome oxidase; Anxiety; Amygdala; Blood pressure; Arrhythmia; Fear conditioning

Introduction

Anxiety is a common comorbidity of myocardial infarction (Frasure-Smith and Lesperance 2003; Friedmann et al. 2006; Gander and von Kanel 2006). The presence of anxiety after myocardial infarction (MI) is associated with increased cardiovascular morbidity (Frasure-Smith and Lesperance 2008; Huffman et al. 2008; Moser et al. 2007). Myocardial infarction and its associated sequelae may either lower the threshold for or increase the magnitude of emotional and sympathetic responses to perceived stress (Henze et al. 2008). Enhanced stress responsiveness may also contribute to induction of arrhythmias, vasospasm, or acceleration of ventricular remodeling (Frasure-Smith and Lesperance 2008; Vlastelica 2008; Ziegelstein 2007). However, the mechanisms that underlie increased anxiety after MI are not known.

A number of brain circuits involved in the regulation of emotion contribute to sympathetic activation and arrhythmia generation (Carpeggiani et al. 1992; Skinner and Reed 1981; Ter Horst 1999). Included among these are the amygdala and the medial prefrontal cortex (mPFC) which coordinate behavioral and autonomic responses to acute stress and learned aversive cues (Fryszak and Neafsey 1994; Grillon et al. 1996; Ramos et al. 2005). Excessive activation of the amygdala has been implicated in the development of anxiety disorders, as well as arrhythmia generation during expression of fear memory (Garakani et al. 2006; Nissen et al. 2010; Salome et al. 2007), while deficits in mPFC activity are associated with increased anxiety responses (Fredrikson and Faria 2013). Both the amygdala and mPFC receive noradrenergic input from the locus coeruleus (LC). Noradrenergic projections from the LC are activated when the salience of a cue is altered or during exposure to noxious stimuli (Chen and Sara 2007; Florin-Lechner et al. 1996; Proudfit and Clark 1991; Van Bockstaele et al. 1989). The subsequent release of norepinephrine (NE) activates both alpha- and beta-adrenergic receptors of cortical cells. Activation of alpha-adrenergic receptors negatively modulates responses to glutamatergic receptor activation of cortical cells, while beta-receptor activation appears to facilitate presynaptic glutamate release. Together, these processes are thought to increase the signal detection ratio of afferent input to cortical cells and thereby modulate the salience of environmental cues (Kobayashi et al. 2009). Interestingly, markers of chronic neuronal activation are increased in LC following coronary artery ligation (CAL) (Patel et al. 1993; Sole et al. 1982; Vahid-Ansari and Leenen 1998). Thus, the central noradrenergic system may become overactive after a coronary event and disrupt amygdalar and mPFC function necessary for appropriate processing of fear cues, and thereby contribute to post-MI anxiety. Here, we examined how amygdalar and mPFC metabolic activities are altered following CAL and LC stimulation, with the ultimate goal of identifying regions within the fear neurocircuitry that are susceptible to these stimuli. Metabolic activity was measured using cytochrome oxidase activity to enable identification of both increases and decreases in ongoing activity. To identify potential behavioral correlates of activity in fear circuitry, we also determined the effect of CAL on expression and discrimination of learned fear cues and on activity in a novel environment (open field) to assess innate fears.

Methods and materials

Animals

Male Sprague–Dawley rats (275–300 g) obtained from Harlan (Indianapolis, IN) were provided with ad libitum access to food and water, while maintained on a 12-h light/dark cycle. All procedures were conducted in accord with NIH policies and were approved by the University's Institutional Animal Care and Use Committee.

Experiment 1

Rats were randomized to receive CAL or sham ligation surgery as described previously (Henze et al. 2008). Echocardiography (Acuson Sequoia C256, Siemens AG) was performed 1 and 7 weeks after surgery for determination of fractional shortening (FS) and left ventricular end-diastolic diameter (LVDD). The first echocardiogram was used to assess an initial level of ventricular dysfunction. The second was used to match rats with similar left ventricular function and diameter and assign pairs to receive mock or real LC stimulation and thereby ensure that each group had similar levels of left ventricular function. The second echo also permitted assessments of dysfunction over time. Behavior in the open field was determined 1 or 2 days prior to the second echocardiogram. Behavior was assessed for 12 min after placement of the rat in the center of the apparatus (100-cm square box with 33-cm high walls, delineated by 20 × 20-cm squares and four 4-cm holes located 17 cm from each corner). Behaviors were scored by an observer blinded to treatment group. Latency to leave the center square, number of wall or center squares entered, time spent in wall and center squares, number of rears, and nose-hole pokes were measured.

Locus coeruleus stimulation

One week after open field testing, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and implanted with indwelling femoral arterial and venous catheters for measurement of mean arterial pressure (MAP) and anesthetic delivery (80 mg/kg/h). Lead II electrocardiogram (ECG) electrodes were applied and the rat was placed in a stereotaxic frame. A stainless steel NE-100 concentric bipolar stimulating electrode (Rhodes Medical Instruments, Summerland, CA) was lowered into the right LC (0.8 mm caudal and 3 mm dorsal to the interaural line, and 1.3 mm from the midline, with the incisor bar positioned 5.8 mm below the interaural line) (Sved and Felsten 1987). Rats were matched for fractional shortening and assigned to receive either real or mock LC stimulation for 20 min beginning 5 min after electrode placement. Current pulses were generated by a Pulsar 6i stimulator (Frederick Haer & Co., Bowdoin, ME) in a pattern shown to increase NE release in the mPFC: 12-Hz trains of three (700 μ A/0.2 ms) pulses, with each train delivered at 1 Hz (Florin-Lechner et al. 1996).

Blood pressure was collected with a bridge amplifier (ADInstruments, Colorado Spring, CO) and Chart v5 data acquisition software. Electrocardiogram was measured using a bioamp amplifier (ADInstruments) and heart rate was determined using peak-to-peak detection of the ECG R wave. The ECG pattern recognition software ECG-Auto (EMKA Technologies, Falls Church, VA) was used to detect arrhythmias. Libraries of ECG waveforms were developed from baseline recordings for each rat following electrode placement. New

ventricular arrhythmias that developed during real or mock stimulation were identified as pre-ventricular contractions, bigeminy, or ventricular tachycardia according to the Lambeth Conventions (Walker et al. 1988). Four or more consecutive pre-ventricular contractions were defined as ventricular tachycardia.

Cytochrome C oxidase (CO) histochemistry

Rats were euthanized by decapitation under chloral hydrate anesthesia immediately after termination of the LC stimulation experiment as previously described (Tseng et al. 2006). Briefly, 16 µm thick coronal sections containing the prefrontal cortex and amygdala were collected and incubated at 37 °C in 0.1 M phosphate buffer containing 0.5 g/L 3,3'-diaminobenzidine, 0.33 g/L cytochrome c, 44 g/L sucrose, and 0.2 g/L catalase (Sigma-Aldrich) for 90 min. Sections were rinsed in phosphate buffer, dehydrated, and cover-slipped. The relative optical density (ROD) of regions of interest in the amygdala and prefrontal cortex (Paxinos and Watson 1997; Swanson 2004) was determined with ImageJ software by subtracting the mode of the optical density of the structure of interest from that of nearby white matter. Relative optical density was determined for each region by averaging values from three to four sequential sections. The resultant was normalized to maximum optical density.

Experiment 2

The effect of CAL on expression of learned fear and ability to discriminate between aversive and neutral conditioned cues was tested in separate groups of rats. Discrimination training was conducted in a Plexiglas chamber with a steel rod floor and dim light (32 lx). Rats were exposed to 22 trials each of 1 and 5 KHz, 90 decibel tones of 10-s duration presented in pseudorandom order (2 consecutive trials with the same tone) with a variable intertrial interval of 120 to 300 s. The first four trials of either CS type were presented without shock to habituate animals to the tones. In all subsequent trials, the last 500 ms of the CS+ coincided with a 0.5-mA shock. Rats were then randomly assigned to receive either the 1 or 5-KHz tone as the CS+. Assignment of CS+ tones was counterbalanced across groups. Freezing behavior, defined as the complete absence of movement, other than that associated with ventilation, was measured during the tone. One week later, rats were subjected to CAL or sham-ligation surgery. Echocardiography was conducted 1 and 6 weeks after surgery. Left ventricular function was determined at 6, rather than 7 weeks in order to reduce as much as possible any lasting effects of anesthesia on behavioral assessments. Measurement of two time points permitted assessment of dysfunction over time. Extinction training was performed 7 weeks after surgery. Extinction training consisted of 20 trials of each tone presented in pseudorandom order with intertrial intervals of 150 to 300 s. Extinction was conducted in a completely novel environment (25.4 × 50.8-cm Plexiglas chamber with 38-cm walls painted with black and white stripes and scented with dilute 5 % acetic acid). Freezing during exposure to the tone was measured as an index of fear.

Statistical analysis

Effects of CAL and Time on FS and LVDD were determined by two-way ANOVA with repeated measures. A two-way ANOVA was used to determine the effects of CAL and LC stimulation on baseline blood pressure and heart rate prior to stimulation. Effects of CAL on

postmortem measures of congestion (e.g., heart and lung weights) and open field measures were tested with Student's t test. Effects of CAL and LC stimulation on blood pressure was assessed by two-way ANOVA of area under the curve during the first 12 min of real or mock stimulation. Only the first 12 min was assessed in order to avoid effects of blood pressure drift due to electrode implantation (see "Results" section). Effects of CAL and LC stimulation on new arrhythmic beats was determined by two-way ANOVA on ranks due to lack of normal distribution of data. Effects of CAL on basal CO activity was determined by Student's t test comparison of data from unstimulated animals. Effects of CAL and LC stimulation on percent change in CO activity were determined by two-way ANOVA comparing data from stimulated rats normalized to the group mean of sham-ligated unstimulated rats. Interactions between CAL and LC stimulation were further investigated by normalizing data from stimulated rats to the group mean of its respective unstimulated group. This percent change was compared between sham and CAL groups by Student t test. For conditioned fear training, effect of CS type and trial on freezing behavior during fear acquisition was determined by two-way ANOVA with repeated measures. Initial fear expression during extinction was determined as freezing during the average of first two trial blocks for each CS type and compared by two-way ANOVA with repeated measures. To assess rate of extinction among animals with different initial freezing levels, freezing during each tone was normalized to the animal's own peak freezing expressed during the first CS+ trial. Cumulative freezing responses to CS+ and CS- cues over the first 15 trials was then determined, and effects of CAL and CS type was assessed by two-way ANOVA with repeated measures. Assessments were limited to the first 15 trials to avoid erroneously interpreting quiet rest as freezing once fear had been completely extinguished. Where appropriate, Tukey post hoc tests were used to make multiple mean comparisons. For all tests, P values of less than 0.05 were defined as significant.

Results

Overall, sham-ligated and CAL rats showed similar weight gain following surgery (Fig. 1a). No group differences in locomotor activity were found 7 weeks postsurgery in the open field test other than a slightly longer delay to leave the center square at the beginning of the trail among CAL rats (Table 1). Rats subjected to CAL showed evidence of left ventricular remodeling and dysfunction within 1 week of surgery as revealed by reduced FS and increased LVDD (Fig. 1b). Left ventricular remodeling and dysfunction became progressively worse by 7 weeks after surgery in CAL rats (Fig. 1b). Left ventricular remodeling and congestive heart failure were confirmed by increased heart and lung weight to body weight and tibia length ratios (Table 2).

Because CAL has been shown to cause chronic activation of LC neurons, we asked whether CAL altered basal or LC-dependent changes in cardiac sympathetic drive and metabolic activity in brain regions that regulate fear responding. Though heart rate was higher in the CAL group overall, baseline blood pressure did not differ between groups prior to stimulating electrode placement (Table 3). A gradual, but non-significant increase in blood pressure was observed following electrode insertion in unstimulated, sham-ligated animals. This effect became apparent by 12 min after start of the "mock" stimulation (Fig. 2a). When averaged, unstimulated CAL rats showed a non-significant decrease in blood pressure

following electrode insertion. However, this was due to a single outlier that demonstrated a large decline in blood pressure. This animal was otherwise remarkable only in that it had higher blood pressure at the start of insertion of the electrode compared to the group as a whole (89 vs. 70 mmHg). When this outlier was excluded, it was apparent that blood pressure did not change in the CAL group following electrode placement (Fig. 2a). During the course of LC stimulation, a marked elevation in blood pressure emerged in a subset of sham-ligated (6/8) and CAL (7/8) rats (Fig. 2b). The blood pressure response to stimulation in this subset of rats was determined as the area under the curve between time 0 and 12 min in order to avoid possible effects of blood pressure drift following insertion of the electrode (Fig. 2c). A main effect of LC stimulation on this value was due to the difference between stimulated and mock-stimulated sham-ligated groups. In CAL animals, stimulation produced a more gradual and less pronounced increase in blood pressure that did not differ from mock-stimulated CAL rats (Fig. 2c). There was no significant interaction between stimulation and surgery. Assessment of electrode placement within the rostral-caudal levels (Figs. 2d, e) revealed that rats that did not show any pressor response to LC stimulation had electrode tracts outside of the main cellular density of the LC. During stimulation, a subset of both sham-ligated and CAL rats showed an increased frequency of new ventricular arrhythmias that were not seen prior to stimulation. Arrhythmias typically developed after several minutes of LC stimulation (Fig. 3a). Two of nine sham-ligated rats and four of eight CAL rats demonstrated new incidences of bigeminy (Fig. 3b), while five of eight CAL rats and no sham-ligated rats developed isolated pre-ventricular contractions. Only one CAL rat developed ventricular tachycardia. Collectively, these results indicate that cardiac sympathetic drive was increased by LC stimulation in both groups. Thus, the lack of a significant pressor response to LC stimulation in CAL rats was likely due to the well characterized insensitivity to sympathetic-mediated vasoconstriction observed in heart failure models (Feng et al. 1999; Rudz et al. 2012).

To gain insight into whether key brain structures known to regulate fear responding were altered by CAL, changes in metabolic activity in the amygdala and mPFC were measured in sham-ligated and CAL rats by means of cytochrome oxidase (CO) histochemistry (Fig. 4). Relative to sham controls, rats that underwent CAL exhibited a significant elevation in baseline CO activity within the mPFC and orbitofrontal regions (Fig. 5a). Following LC stimulation, a slight non-significant increase in CO activity was observed in sham-ligated rats relative to their unstimulated group (Fig. 5b). In contrast, CAL rats showed reduced CO activity in the mPFC relative to their unstimulated group. The disparate responses in CAL and sham-ligated rats to stimulation (relative to their own unstimulated controls) was most notable in the infralimbic, prelimbic, and orbitofrontal regions of the frontal cortex (Fig. 5b inset). Assessment of amygdala showed that CO activity of the LA was increased in unstimulated CAL rats when data were normalized to the sham-ligated unstimulated group, but not when absolute values for ROD were compared (Fig. 6a, b). Overall, stimulation increased CO activity in the LA (Fig. 6b). However, the effect was only significant in sham-ligated rats.

To assess effects of CAL on fear expression and extinction, fear conditioning was implemented prior to surgery in order to avoid effects of surgery on fear acquisition. During fear conditioning, rats showed little freezing during tone presentation prior to shock

exposure (Fig. 7a). Rats began to freeze to CS+ and CS– tones only after presentation of the first reinforced tone (fifth CS+ trial) and continued to freeze during presentations of all subsequent tones. Freezing peaked during the tenth CS+ and CS– trials and trended downward in subsequent trials as animals began to move during the tones in order to adopt postures that avoided contact with the grid floor. Despite a small trend for increased freezing during CS+ trials for the first half of the training session, there was no effect of CS type on freezing duration.

It is common that freezing increases after the initial extinction trial despite the absence of the US, particularly when extinction is performed in a novel environment. This is likely due to the time required to recall the US CS association from long-term memory in the absence of contextual cues. To reduce the impact of this effect on our assessment of initial fear expression to the CS+, extinction training was initiated with the CS– trial, and fear expression was determined as the average freezing over the first two CS– or CS+ trials (Fig. 7b, c). Eight weeks after surgery, sham-ligated rats showed a greater freezing response to initial CS+ trials than to CS– trials indicating their ability to distinguish between the two cues (Fig. 7b, c). Rats subjected to CAL showed less freezing during the first CS– trial compared to the next CS+ trial, but then showed increased freezing in the three subsequent CS– trials. As a consequence, CAL rats showed similar freezing during presentation of the initial CS+ and CS– cues (Fig. 7c). CAL rats also showed greater fear expression than sham-ligated rats did during exposure to either the CS+ or the CS– cues. (Fig. 7c). In order to compare extinction between groups with different initial freezing responses, each animal's freezing response was normalized to its own maximal freezing duration observed during the first CS+ trial (Fig. 7d). A cumulative measure of normalized freezing over the first 15 trials for each CS type was used as an index of within-session extinction (Fig. 7e). When comparing this extinction index, we found that sham-ligated and CAL groups showed a similar rate of extinction of fear to the CS+. However, sham-ligated animals were able to suppress fear responding to the CS– more than CAL animals did.

Rats subjected to discriminative auditory fear conditioning that subsequently underwent CAL surgery showed deficits in left ventricular function (measured by FS), as well as left ventricular remodeling (measured by LVDD) both 1 and 6 weeks following surgery (Fig. 7f). Fractional shortening and LVDD measured 1 week after CAL surgery in rats subjected to prior fear conditioning did not differ from FS and LVDD measured in the rats from experiment 1 which were never exposed to fear conditioning (see Fig. 1b). However, FS of animals included in the CO study, but not those included in the fear conditioning study, declined significantly over the course of the experiment. The lack of change in FS over the course of the fear conditioning study may have resulted from the shorter time between echocardiographic studies (6 vs. 7 weeks). Alternatively, the slight, non-significant decrease in FS among animals subjected to conditioned fear may have resulted from the slightly (though not significant) lower FS. Nevertheless, left ventricular diameter increased similarly among CAL rats in both studies. Thus, it did not appear that prior fear conditioning contributed significantly to the extent of LV dysfunction.

Discussion

In the present study, we found that overall metabolic activity in the prefrontal cortex and lateral amygdala are elevated and that prefrontal cortex becomes more sensitive to inhibitory effects of LC input 8 weeks after CAL of male Sprague–Dawley rats. Expression of cue-induced fear was exaggerated in CAL rats. In addition, their ability to discriminate between distinct auditory cues that predicted neutral or aversive events was also impaired. In contrast, CAL rats showed minimal evidence of general anxiety in the open field. These findings support the view that altered neural activity in brain regions involved in fear association processing, particularly the mPFC, may underlie the anxiety that develops following MI.

Brain CO staining has been shown to reflect the overall metabolic activity of neuronal networks (Wong-Riley 1989). To our knowledge, this is the first study to show that CAL leads to metabolic alterations in cortical and subcortical regions of the forebrain, in particular in the prefrontal cortex and LA. Our evidence that metabolic activity was elevated in both the prefrontal cortex and LA suggests an increase in basal neural activity which in the region could conceivably be due to increases in activity of either GABA or glutamatergic cells or both. Chronic increases in prefrontal cortical activity such as those observed in our CAL model have also been observed in rat models of schizophrenia and early adolescent cocaine exposure. In both models, impaired cortical GABAergic function accompanies cognitive deficits (Cass et al. 2013; Tse et al. 2015). Recent work has also shown that acute blockade of prefrontal GABA receptors prior to testing of learned fear expression disrupts an animal's ability to discriminate between feared and neutral cues (Piantadosi and Floresco 2014). Thus, our findings of increased basal metabolic activity in the prefrontal cortex and deficits in discrimination may indicate derangement of ongoing inhibitory processes important in regulation of learned fear responses. Accordingly, CAL has been found to downregulate gene expression of *Npas4* and *Arc* in prefrontal cortex of mice (Frey et al. 2014). *Npas4* has been implicated in the maintenance of inhibitory synapses, while *Arc* is a cytoskeletal protein expressed during neuroplasticity (Lin et al. 2008; Lyford et al. 1995). Thus, inhibitory processes that regulate neuroplastic changes in medial prefrontal cortex during associative learning may be particularly vulnerable after CAL. Our findings that CAL rats expressed greater anxiety-like behavior during presentation of a feared auditory cue, but not during exposure to a novel environment, suggests CAL disrupts normal processing of learned auditory cues. This hypothesis is further supported by our evidence that metabolic activity was increased specifically in the LA. The LA is required for learning the predictive value of discrete auditory fear cues. In contrast, regions of the amygdala implicated in generalized anxiety (BLA) did not show a consistent effect of CAL.

Acute and chronic increases in noradrenergic neurotransmission in cardiac tissue develops during and following coronary artery ligation and myocardial infarction (Abrahamsson et al. 1982) (Parrish et al. 2008). Chronic noradrenergic activation of ventricular myocytes leads to well characterized disruption of noradrenergic signal transduction and myocyte dysfunction. An analogous surge in activity of LC noradrenergic cells also occurs following coronary artery ligation (Patel et al. 1993; Vahid-Ansari and Leenen 1998). To date, no one has examined the behavioral effects of CAL-induced LC overactivation in target brain regions. Here, we provide novel evidence that phasic electrical LC stimulation designed to

simulate a pattern of LC cell activity such as that elicited during exposure to a mild stressor, i.e., white noise (Abercrombie and Jacobs 1987) increased blood pressure in sham rats. In contrast, rats subjected to CAL showed a limited and inconsistent pressor effect during LC stimulation. Others have shown that pressor responses to short-term high frequency stimulation (90 Hz) of the LC is biphasic with two distinct pressor responses, only the latter of which was due to activation of catecholamine-containing cells of the LC (Drolet and Gauthier 1985). The LC-specific response is more long-lived and dependent upon intact forebrain projections and the adrenal medulla. In contrast, the non-specific pressor response dissipates immediately upon cessation of stimulation and is dependent upon sympathetic vasomotor axons. Here, our LC stimulation resulted in a relatively gradual and persistent rise in blood pressure despite the relatively low (1 Hz) frequency of burst stimulation. Thus, the pattern of blood pressure increase is more consistent with a specific stimulation of LC and a cumulative increase in circulating catecholamines. Our findings of delayed onset of arrhythmia with LC stimulation is also more consistent with a circulating source of catecholamines. Stimulation of β -adrenergic receptors in the heart normally contributes to increased blood pressure through elevated cardiac output. Deficits in β -adrenergic receptor transduction in the heart of rats subjected to CAL likely contributed to the limited pressor effect of LC stimulation. The upward drift in blood pressure expressed by sham-ligated animals during mock LC stimulation may have been due to local release of glutamate from cells damaged during cannula placement with subsequent activation of surrounding intact LC neurons. We would expect that such effects in CAL rats would lead to an attenuated rise in pressure given predicted deficits in their ability to generate sympathetic-mediated increases in cardiac output.

Unique ECG waveforms developed during and persisted after the 20-min stimulation period in almost all stimulated MI rats, while only two sham-ligated rats developed bigeminy during LC stimulation. In the absence of stimulation, sham-ligated rats did not develop any ventricular arrhythmias, providing further support that activation of the LC promotes the generation of ventricular arrhythmias. Together, these results suggest that stimuli, including mild psychological stressors, which activate the LC can induce cardiac arrhythmias in the susceptible heart.

Increased sympathoadrenal activation is thought to be responsible for arrhythmia generation after MI, as cardiac sympathetic nerve stimulation results in arrhythmias in rats subjected to MI, and β AR blockade prevents this effect (Du et al. 1999). LC activation increases sympathetic output, though the mechanism is unclear. Although the LC does project to the rostral ventrolateral medulla (RVLM), a region that provides tonic excitation of sympathetic preganglionic neurons of the spinal cord, this projection is thought to be primarily inhibitory by virtue of the action of NE on α 2ARs (Van Bockstaele et al. 1989; Hayar and Guyenet 1999). However, the LC also projects directly to the spinal cord though not directly to sympathetic preganglionic cells (Proudfit and Clark 1991). Alternatively, LC stimulation may increase sympathetic drive through its projections to cardiorespiratory forebrain regions, such as the amygdala. The central nucleus of the amygdala regulates autonomic responses to stressful stimuli via direct projections to the RVLM and nucleus tractus solitarius (nTS) (Cassell and Gray 1989; Zardetto-Smith and Gray 1990; Hopkins and Holstege 1978). The mPFC has also been shown to contribute to sympathoexcitation in response to stressors, as

lesion of the ventral mPFC results in the attenuation of the sympathetically mediated tachycardiac response to a tone previously paired with footshock (Skinner and Reed 1981; Fryszak and Neafsey 1994), through its projections to cardio regulatory regions of the brainstem (nTS, RVLM, dorsal vagal motor nucleus) and spinal cord (Neafsey et al. 1986; Hurley et al. 1991). Furthermore, transsynaptic labeling studies using pseudorabies virus have demonstrated pathways from the PFC, including infralimbic and prelimbic cortices, to the left myocardium (Ter Horst 1999), providing further support for potential mPFC control of cardiac function.

A major novel finding of this study was the consistently lower metabolic activity observed in the prefrontal cortex of CAL rats following LC stimulation. Recent work has shown that acute 20-s bursts of LC stimulation increases activity of both inhibitory interneurons and glutamatergic neurons of the frontoparietal cortex in anesthetized rats (Toussay et al. 2013). In contrast, we observed a non-significant increase in metabolic activity in sham-ligated animals. This discrepancy may be due to the lower frequency of our stimulation. Nonetheless, the same stimulus parameter reduced metabolic activity in CAL rats. In this study, we did not identify the origin of altered metabolic response in CAL rats. Interestingly though, chronic overactivation of adrenergic input to cardiomyocytes produces deficits in β -adrenergic signaling, but not α -adrenergic signaling. After CAL, β -adrenergic receptors expressed by cardiomyocytes shift their coupling from predominantly Gas to Gai (Kompa et al. 1999). Thus, the same stimulus can activate divergent transduction pathways in CAL and sham-ligated animals. Such switching of β -adrenergic receptor coupling is also seen in hippocampal cells following chronic cold stress (Schutsky et al. 2011). It is tempting to speculate that responses to noradrenergic input may change in response to chronic overactivation of the LC in part by altering adrenergic receptor density or second messenger coupling either in the prefrontal cortex or in regions affected by LC stimulation that project to and regulate prefrontal cortical activity. If chronic overactivation of LC does mediate such effects, then regions that receive a greater density of noradrenergic might be more vulnerable.

Of interest in this regard are our findings that metabolic inhibition with LC stimulation was most robust in the infralimbic prefrontal cortex. This region is more densely innervated by noradrenergic terminals than by neighboring cortical areas (Kritzer 2003). Infralimbic prefrontal cortex is involved in extinction of contextual and cued fear (Quirk et al. 2006; Thompson et al. 2010). Here, we showed that the rate of extinction of the CS+ was similar between CAL and sham-operated rats when freezing over trials was normalized to maximal freezing. Both groups of rats showed freezing responses to CS- cues. However, sham-operated rats were able to extinguish fear to the CS- more rapidly whereas CAL rats showed no difference in the rate of extinction or CS+ and CS- cues. It remains to be determined whether LC-induced adrenergic input to mPFC interferes with an animal's ability to discriminate between distinct cues after CAL. Greater sensitivity to inactivation of mPFC processes during LC stimulation may interfere with discrimination between important and innocuous events by reducing the difference in vigilance elicited by either cue (Aston-Jones et al. 1994).

Locus coeruleus neurons provide dense innervation of the basolateral nucleus and to a lesser extent, the lateral subnucleus of the BLA complex of the amygdala (Fallon et al. 1978). Direct iontophoretic application of NE in the basolateral and lateral amygdala reduces spontaneous activity in the majority of amygdalar projection neurons but produces relatively large excitatory responses in a minority of neurons in anesthetized rats (Buffalari and Grace 2007). The heterogeneous response has been attributed to the opposing effects of α 2- and β -adrenergic receptors, the latter of which mediate the excitatory effect of NE in these neurons. Interestingly, excitatory effects of NE in basolateral amygdala (lateral amygdala was not tested) are enhanced following chronic stress, possibly by an upregulation of β -adrenergic receptor expression or coupling (Buffalari and Grace 2009). Thus, increased activity in the lateral amygdala of unstimulated CAL rats may reflect the effects of chronic physiological stress on spontaneous amygdala activity. In any case, it is appreciated that responses to conditioned auditory cues arise from the lateral amygdala and are passed on for processing to the BLA complex which contributes to behavioral expression of fear as well as sympathetic activation (Davis and Whalen 2001; Goosens and Maren 2001). As such, heightened conditioned responses elicited by CAL may arise from changes in excitability of cells in lateral amygdala.

Together, our findings show that CAL causes changes in the basal activities of the prefrontal cortex and the lateral amygdala. Our additional evidence that medial PFC is more susceptible to inhibition during LC stimulation after CAL coupled with findings that CAL exaggerated fear expression and attenuates discrimination of conditioned fear cues suggest that altered adrenergic neurotransmission in the PFC during fear may be a primary mediator of anxiety and increased cardiovascular responsiveness after MI.

Acknowledgments

This study was supported by 09PRE2260787 from Mid-West Affiliate of the American Heart Association (JG), by funds from Loyola University Chicago (KES), and R01-MH-086507 (K-YT). A subset of the data herein was published in abstract form: FASEB J March 17, 2011 25:646.3

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Fig. 1.

(a) Time line of experiment superimposed on weekly body weights of rats subjected to coronary artery ligation (CAL) or sham ligation (Sham). Body weight data are group means $\pm$ SEM. (b) Dot plot of fractional shortening (FS) and left ventricular end-diastolic diameter (LVDD) in individual CAL and Sham rats 1 and 7 weeks after surgery. Thicker line joining the two sides of the plots indicate group averages at each time point. ** $p < 0.01$ between groups (vertical line) and within groups over time (thicker line over weeks)

Fig. 2.

Change in blood pressure starting 6 min after placement of LC electrode in CAL and Sham rats receiving mock stimulation (a) or real stimulation (b). Outliers that showed either a larger depressor or no response to stimulation are shown by individual lines. Data from all other animals are depicted as group average $\pm$ SEM. Change in blood pressure over time was determined as area under the curve between time 0 and 12 min of mock or real stimulation for each group (c). Two-way ANOVA revealed a main effect of stimulation ($p < 0.05$), but only sham animals showed a significant effect in comparison to sham mock-stimulated controls. Values are group means $\pm$ SEM. * $p < 0.05$ between groups. An example of track and electrode localization are shown in a coronal section stained with neutral red (arrows indicate track) (d). Inset shows magnified view of LC contralateral (left) and ipsilateral (right) to electrode placement. Number in lower right indicates distance caudal to Bregma

(mm). A schematic shows placement of tip of stimulating electrodes in Sham (back row) and CAL (front row) in coronal hemisections through the LC (e). Numbers across bottom indicate distance caudal to Bregma. Symbols represent location of electrode tip and maximal pressor response elicited by stimulation; white square <0–5, black square 5–10 mmHg, plus sign <10 mmHg. Electrode placement rostral to the most rostral sections shown were omitted due to space limitations. Omitted are sham-ligated rat, ~–9.47 (no response); CAL rat, ~–9.72 (>10 mmHg)

Fig. 3.

Example of ECG waveforms collected during LC stimulation in a rat subjected to CAL (a). Three sequential ECG waveforms are shown in each row. Sequential wave forms are shown before start of stimulation (Pre-stim), during stimulation and after stimulation (Post-stim). Note that arrhythmias unique from the prestimulus baseline rhythms develop midway through stimulation and did not revert immediately upon cessation of LC stimulation. bVertical plots indicating percent bigeminy, preventricular contraction (PVC), and ventricular tachycardic (VT) rhythms detected during real or mock LC stimulation in rats subjected to CAL and sham ligation. Symbols represent percent of total waveforms (beats) detected during real or mock stimulation that were of the specified waveform subtype and distinct from any waveform detected during the prestimulus time period for each individual animal. Horizontal bar indicates group mean, and vertical bar is SEM; **P < 0.01

Fig. 4.

Typical CO label in a PFC and b amygdala of the rat brain. Inset in B shows location of subnuclei of the amygdala assessed in the study. c Examples of CO label after increasing incubation time in prefrontal cortex and more rostral cortical areas, along with average relative optical density measures from the prefrontal and basolateral nucleus of the amygdala (BLA) from three unstimulated animals. Values are mean $\pm$ SEM. Data show linear response in both regions up to 90 min. Infralimbic medial prefrontal cortex (IL), prelimbic medial prefrontal cortex (PL), cingulate cortex (Cg), motor cortex (M), somatosensory cortex (SS), insular cortex (I), orbitofrontal cortex (OF), basolateral nucleus of the amygdala (BLA), central nucleus of the amygdala (CeA), lateral nucleus of the amygdala (LA)

Fig. 5.

(a) Relative optical density (ROD) representing CO activity in PFC of unstimulated sham-ligated (Unstim Sham) and CAL rats (Unstim CAL) that were implanted with a stimulating electrode, but not subjected to stimulation (Unstim CAL, A). (b) Relative optical density of all PFC regions in all groups normalized to sham-ligated unstimulated rats. Significant interactions between surgery and LC stimulation were found in prelimbic, infralimbic, and orbitofrontal cortices. Inset shows change in ROD of stimulated rats normalized to their respective unstimulated group mean. Group n is shown in parentheses. Values shown in (a) and (b) are group means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$. Prelimbic medial mPFC (PL), infralimbic medial mPFC, orbitofrontal cortex (OF), cingulate cortex (CG), motor cortex (MC), somatosensory cortex (SS), and insular cortex (IC)

Fig. 6.

(a) Relative optical density (ROD) of CO label in the basolateral nucleus (BLA), central nucleus (CeA), and lateral nucleus (LA) of the amygdala in unstimulated Sham and CAL rats. (b) Relative optical density of all groups normalized to the mean of the unstimulated sham-ligated group. Values are group means $\pm$ SEM; * $p < 0.05$

Fig. 7.

(a) Freezing during CS+ and CS- tones presented during fear conditioning. First four trials of each CS type were presented without shock. All subsequent CS+ tone presentations were terminated by shock. (b) Freezing behavior during CS+ and CS- tones without shock 8 weeks after CAL or sham surgery. Lines are curves fitted to the groups extinction data using a three-parameter exponential decay equation. (c) Fear expression 8 weeks after surgery averaged over the first two CS+ and CS- trials for Sham and CAL groups, * $p < 0.05$ vs. Sham CS+, ++ $p < 0.01$ vs. sham CS-. (d) Freezing over extinction trials normalized to each animal's own maximum freezing expressed during first CS+ presentation. (e) Normalized freezing during tone presentation summed over the first 15 trials for each stimulus type, ** $p < 0.05$ vs. sham CS+, + $p < 0.01$ vs. CAL CS-. (f) Fractional shortening (FS) and left ventricular diastolic diameter (LVDD) in CAL and sham-ligated rats 1 and 6 weeks after CAL or sham surgery among rats that underwent prior fear conditioning. ** $p < 0.01$ between groups (vertical line) and within groups over time (thick gray line across weeks)

Table 1

Open field assessment

Activity	Sham (17)	CAL (14)
Time to leave center	5.8 ± 0.09	12.8 ± 3.4 *
Time in squares		
Center	47.1 ± 12.6	48.0 ± 12.5
Wall	673.4 ± 12.3	627.0 ± 12.5
Squares entered		
Center	9.6 ± 2.3	6.8 ± 1.5
Wall	138.9 ± 17.2	154.8 ± 11.7
Rears	8.8 ± 1.6	10.2 ± 2.3
Nose Pokes	4.6 ± 1.0	3.4 ± 1.0

Values are group mean ± SEM,

*
 $p < 0.05$

Table 2

Body weight and indices of congestion

	Sham (17)	CAL (14)
Body weight (BW, kg)	400.1 ± 6.5	395.9 ± 7.3
Lung/BW (g/kg)	3.50 ± 0.13	5.41 ± 0.65 *
Heart/BW (g/kg)	3.29 ± 0.06	3.98 ± 0.17 **
Lung/tibia length (g/cm)	0.35 ± 0.01	0.53 ± 0.07 **
Heart/tibia length (g/cm)	0.33 ± 0.01	0.39 ± 0.02 **

Values are group means ± SEM,

*
 $p < 0.05$;

**
 $p < 0.01$

Table 3

Baseline blood pressure and heart rate prior to LC stimulation

Group	Mean arterial pressure	Heart rate
Unstim Sham (8)	64.3 ± 4.8	319 ± 9
Stim Sham (9)	66.8 ± 5.7	331 ± 13
Unstim CAL (6)	73.9 ± 6.3	416 ± 35 *
Stim CAL (8)	64.7 ± 6.6	385 ± 23

Values are mean ± SEM, main effect of CAL on heart rate, $p < 0.01$;

* $p < 0.05$ vs. Unstim Sham group; *Unstim* unstimulated, *Stim* stimulated