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The amygdala central nucleus is required for acute stress-induced bladder hyperalgesia in a rat visceral pain model

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Abstract

Chronic stress has been implicated in the pathogenesis of chronic visceral pain conditions, such as interstitial cystitis (IC), and bouts of acute stress exacerbate clinical urological pain. Studies using animal models have shown that exposure to chronic footshock stress augments reflex responses to urinary bladder distension (UBD) in animal models, however acute effects in animal models are largely unknown, as are the central nervous system mechanisms of stress-related increases in nociception. The amygdala is a salient structure for integration of sensory and cognitive/emotional factors. The present study determined the role of the central nucleus of the amygdala (CeA) in stress-related bladder hypersensitivity. We examined the effects of CeA manipulations (lesions and chemical stimulation) on visceromotor responses (abdominal muscle contractions) to UBD in adult, female Sprague-Dawley rats. We report that acute footshock stress produces bladder hyperalgesia that can be prevented by bilateral CeA lesions, despite no effect of lesions on baseline somatic sensation, as indicated by flinch/jump thresholds to electrical shock. Further, acute glucocorticoid stimulation of the CeA recapitulated stress-induced hyperalgesia. Of note is that CeA lesions, but not chemical stimulation, significantly affected HPA axis activation, as indicated by measurements of circulating corticosterone. Our findings conclusively show that the CeA is necessary for the generation of bladder hyperalgesia in response to acute stress. The CeA may play multiple stress-related roles in nociceptive modulation, i.e., via direct facilitation of the HPA axis during acute stress, or via modulation of other systems that augment acute stress responsiveness.

Keywords

amygdala; visceral; hyperalgesia; stress; anxiety; glucocorticoid

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1. Introduction

Chronic bladder/pelvic pain associated with interstitial cystitis (IC) affects 3–8 million women in the United States (Berry et al., 2011). There is no single identified etiology of IC, although a prominent role for stress in its pathophysiology and presentation has been documented. Flares in bladder-related symptoms correlate with exposure to a laboratory stressor (Lutgendorf et al., 2000) and increased daily life stress (Rothrock et al., 2001). Those with a diagnosis of IC have a higher lifetime prevalence of panic disorder than healthy controls (Weissman et al., 2004), and patients with panic disorder are five times more likely than healthy controls to report IC symptoms (Talati et al., 2008).

We have previously shown that exposure to chronic footshock stress augments bladder nociceptive reflexes (Robbins and Ness, 2008), and both chronic and acute footshock alter spinal neuronal responses to noxious bladder stimulation and activate the stress-responsive hypothalamic-pituitary-adrenal (HPA) axis (Robbins et al., 2011). Peripheral and spinal nociceptive mechanisms are required for enhanced pain during periods of stress or following exposure to an aversive event; however, the transformation of individual emotional states or cognitive factors into modulation of peripheral afferent input requires cortical function. The amygdala is a limbic forebrain structure integral to both stress responsiveness and nociceptive processing (LeDoux, 2007). Clinical studies have reported that individuals with IC exhibit elevated autonomic nervous system activity (Lutgendorf et al., 2004), hypothalamic pituitary adrenal (HPA) axis dysregulation (Dimitrakov et al., 2001; Lutgendorf et al., 2002), and exaggerated startle responses to a visceral threat (Twiss et al., 2009), all of which may be influenced by amygdala activity.

The amygdala central nucleus (CeA) densely expresses glucocorticoid receptors (Reul and de Kloet, 1985), which bind the endogenous ligand cortisol (in humans) and its rodent analog, corticosterone. In rats, direct administration of corticosterone to the CeA via a sustained-release micropellet augments HPA axis responses to stress (Shepard et al., 2003), increases indices of anxiety (Shepard et al., 2000), and enhances nociceptive reflexes (Greenwood-Van Meerveld et al., 2001) and neuronal responses (Qin et al., 2003b) to colorectal distension.

In this study, experimental manipulations involving the CeA were performed to examine its contribution bladder pain produced by acute footshock stress. Our results indicate that the CeA is required for the expression of acute footshock-induced bladder hyperalgesia, and that acute CeA activation is sufficient to drive bladder hyperalgesia in the absence of footshock. Given newly evolving clinical therapeutic options involving non-invasive modulation of deep brain structures, these results have implications for the management of stress-related flares in bladder pain.

2. Results

2.1 Acute footshock stress produces bladder hyperalgesia

We have previously shown that acute footshock exposure induces a significant increase in blood plasma corticosterone concentration (Robbins et al., 2011), indicative of HPA axis

activation. In the current study, we assessed fecal pellet output during acute footshock exposure as an additional measure of stress responsiveness (Tache et al., 2004). Compared to rats in the no footshock (NFS) condition, those exposed to footshock (FS) had increased fecal pellet output (0.00 ± 0.0 versus 6.14 ± 0.4 , respectively; $p < 0.001$; $N = 7/\text{group}$).

Abdominal EMG responses to graded bladder distension were quantified to determine whether acute footshock, like chronic footshock exposure, facilitates bladder nociceptive processing (Robbins et al., 2007). A repeated-measures ANOVA revealed significant effects of footshock ($p < 0.01$) and distension pressure ($p < 0.01$), and a significant interaction between these variables ($p < 0.01$). Post-hoc comparisons indicated significantly greater abdominal EMG responses to bladder distension at noxious pressures (40–80 mmHg) following footshock exposure (Figure 1).

2.2 Acute chemical stimulation of the CeA recapitulates footshock-induced hyperalgesia

Based on a series of aforementioned studies (Greenwood-Van Meerveld et al., 2001; Qin et al., 2003b; Shepard et al., 2000; Shepard et al., 2003), we first determined whether chronic application of corticosterone to the CeA via a sustained release micropellet increased bladder nociceptive responses to UBD. Compared to delivery of cholesterol, chronic corticosterone for seven days significantly augmented abdominal EMG responses to UBD at noxious distension pressures (Figure 2). It has previously been shown that the area of drug spread using this technique was 750 μm from the micropellet implantation site at five days post-implantation (Shepard et al., 2000).

Next, we tested acute administration of corticosterone, a model more relevant to acute footshock exposure, to determine if it produced effects similar to those of chronic delivery. Rats injected bilaterally with corticosterone (15 μg , but not 5 μg) onto the CeA 30 min prior to testing exhibited significantly more robust abdominal EMG responses to noxious distension pressures than those injected with cholesterol (Figure 3). We did not assess the area of drug diffusion from the injection site during the 30 min period between drug injection and testing; however a study by Gebhart et al. (Gebhart et al., 1983) suggests a minimal area of diffusion for an injected volume of up to 1 μl .

2.3 Bilateral CeA lesions prevent the development of footshock-induced hyperalgesia

To provide conclusive evidence that the CeA is required for expression of acute footshock-induced bladder hyperalgesia, we performed bilateral lesions of the CeA and tested bladder nociceptive responses. However, we first assessed flinch and jump thresholds to an ascending series of shock intensities in intact, lesioned, and sham surgery rats to determine whether our experimental manipulations impaired sensory transduction of the footshock ‘stress’ stimulus. There was no difference among these three groups for either flinch ($p = .412$) or jump ($p = .733$) thresholds.

For ease of presentation, data collected to address whether the CeA is required for acute footshock-induced bladder hyperalgesia will be described according to the manipulations preceding sensory testing, as follows: ‘Lesion’ indicates rats with bilateral lesions; ‘Control’ indicates rats that underwent sham surgery; ‘FS’ indicates rats that were exposed to acute footshock immediately prior to testing; ‘NFS’ indicates rats that were not exposed to

footshock. For each rat, the area of the CeA that was obliterated was assessed as a function of total CeA area in a blinded fashion by two independent observers. For lesions considered ‘hits,’ the obliterated area ranged from 68.3–100% of total CeA area (mean = $81.41 \pm 1.7\%$; Figure 4). As expected based upon our initial findings of footshock-induced bladder hyperalgesia, rats in the Control-FS condition had significantly greater responses than rats in the Control-NFS condition (Figure 5B). A comparison between Control-FS rats and “naïve” footshock-exposed rats showed no difference in abdominal EMG responses, indicating that there was no unexpected effect of sham surgery. Compared to Control-FS rats, those in the Lesion-FS group had significantly reduced responses that were no different from Control-NFS or Lesion-NFS conditions. To underscore the specificity of the effect of CeA lesions on EMG measures, quantitative data from individual rats with lesions that did not meet the criteria for CeA ‘hits’ are shown (Figure 5C).

2.4 CeA lesions reduce HPA axis activation by footshock

Acute exposure to footshock has been shown to activate the HPA axis, the prototypic physiological stress response system (Robbins et al., 2011). To determine whether lesions of the CeA blocked HPA activation by footshock exposure, we assessed corticosterone release in lesioned and sham surgery rats with and without exposure to footshock. As expected based on our previous findings, footshock significantly increased plasma corticosterone concentration in rats that underwent sham surgery. CeA lesions effectively reduced corticosterone release in response to footshock, however, plasma concentration was not reduced to that of the Lesion-NFS counterparts (Figure 5A).

We also measured plasma corticosterone in rats that underwent acute application of corticosterone to the CeA to determine whether the mechanism of stimulation-induced hyperalgesia was similar to that of footshock. Corticosterone concentration 30 min post-injection was no different in these rats than in those administered cholesterol (15192.50 ± 1709.5 pg/mL versus 15900.00 ± 1389.54 pg/mL, respectively), providing further evidence in support of site-limited effects.

3. Discussion

In this study we report that acute exposure to a behavioral stressor, footshock, produces bladder hyperalgesia that requires the amygdala central nucleus for its expression. Further, we demonstrated that bladder hyperalgesia, as measured by assessment of abdominal EMG responses, was produced by manipulations with selective effects on the amygdala, and that stress-related hormones, through central administration, are able to produce bladder hyperalgesia like that produced by acute footshock. These observations support a role for the amygdala in the generation of clinical bladder hypersensitivity, particularly when generated by supraspinal mechanisms and/or in response to anxiety or psychological stress.

Our observations in this study underscore the clinical relevance of recent data showing increased amygdala gray matter density in individuals with chronic pelvic pain (CPP) (Bagarinao et al., 2014). In contrast to that study, decreased amygdala gray matter has been correlated with increased visceral sensitivity in healthy individuals (Elsenbruch et al., 2014). Taken together, these studies suggest a degree of complexity in the pathophysiology of

chronic visceral pain, including that of IC. Elucidation of the inter-related functions, precise connectivity, and underlying mechanisms of pain and stress systems at system, cellular, and molecular levels could advance the understanding of IC pathophysiology. An advanced understanding of IC pathophysiology, in turn, would provide a basis for the development of potentially useful novel pharmacological tools that may ultimately aid in improved quality-of-life for those suffering from IC.

While both acute stress and acute chemical stimulation of the CeA produced bladder hyperalgesia, only footshock stress produced an increase in circulating corticosterone suggesting the mechanisms driving the hyperalgesia produced by these two stimuli may be different. The lack of HPA axis facilitation by acute glucocorticoid application to the CeA has previously been reported (Akana et al., 2001). Rather, acute actions of glucocorticoid in the CeA may act to alter neural outflow to preganglionic sympathetic neurons (Veening et al., 1984) or to facilitate responses to heterotypic stressors experienced in conjunction with chronic stress (Bhatnagar and Dallman, 1998). The mechanisms whereby corticosterone injection into the CeA leads to hyperalgesia are unknown. The CeA projects to the periaqueductal gray (PAG), and directly and indirectly via the PAG to brainstem structures known to be involved in descending modulation of pain. It is therefore likely that the PAG is involved in the facilitation of visceral responses observed, although the contribution of direct brainstem projections cannot be ruled out. Inputs to the amygdala that might activate mechanisms related to hypersensitivity are many. There are profound nociceptive inputs to the amygdala from the ascending spinoparabrachial tract as well as extensive collateral branching with and to the spinothalamic tract. Neurons in the CeA change characteristics in responses to painful inputs (Crock et al., 2012; Neugebauer and Li, 2003) and these changes have been proposed as part of the central mechanisms of inflammation-induced hyperalgesia (Carrasquillo and Gereau, 2007). Since pains associated with IC are often mistaken as due to inflammation of the bladder as a consequence of infection, one must wonder whether convergent mechanisms are responsible for these abnormal sensations. Recent studies have implicated roles for novel pharmacologies associated with the amygdala in relation to pain (Carrasquillo and Gereau, 2007; Crock et al., 2012; Ji and Neugebauer, 2007), and so future studies will be able to examine whether these same pharmacologies may impact pains such as those experienced by individuals with IC.

We noted robust increases in visceromotor responses at intensities of stimulation that are generally viewed as noxious, as they exceed normally observed intravesical pressures. The precise neurophysiological basis for this is unknown, although Qin et al. (Qin et al., 2003a) identified that amygdala corticosteroid administration produced complex changes in spinal neuronal responses to UBD. In that study, it is notable that some neuronal populations became more active and others less active. Similar differential effects on spinal neuronal responses to UBD have been documented following chronic footshock-induced stress (Robbins et al., 2011). Divergent effects on spinal neuron populations may result from differential sensitization of amygdala neuron populations, which can produce pro- versus anti-nociceptive effects (Ji and Neugebauer, 2008; Neugebauer and Li, 2003) or differential responses to noxious versus non-noxious stimulation (Myers and Greenwood-Van Meerveld, 2010).

It will now be the challenge of subsequent studies to elucidate subtleties related to the effects of the corticosterone-induced bladder hypersensitivity. As noted previously, extensive studies have been performed using colorectal distension-induced pain models. These have determined which receptors and neurotransmitters are of the greatest interest in those model systems, but it will be necessary to assess whether similar mechanisms are at work in urological pain systems. A logical next step would be to use experimental stress paradigms (i.e., footshock) in conjunction with other amygdala-related manipulations, such as lesions or administration of antagonist drugs, to precisely elucidate the role of the amygdala in bladder hyperalgesia. Furthermore, studies involving hypophysectomy or lesions of other brain regions (i.e., PAG) may provide a better understanding of the distinct neuroanatomical pathways underlying pain- and stress-related outcomes. Electrophysiological studies of spinal cord neuronal activity are likely necessary to determine the function of a spinal mechanism in CeA-related visceral nociceptive modulation. Finally, a hemispheric lateralization of nociceptive function has been demonstrated in the amygdala (Carrasquillo and Gereau, 2007) and whether/how this lateralization is involved in pain- and stress-related effects will be of interest.

The present study provides compelling support for a role of the amygdala and stress-related hormones in bladder hyperalgesia phenomena in rats. These same mechanisms may be present in humans with bladder hypersensitivity disorders such as IC/PBS. A precise delineation of the mechanisms associated with this phenomenon may allow an improved treatment of these disorders.

4. Experimental Procedures

4.1. Animals

Female, Sprague-Dawley rats (250–325 g, Harlan, Inc., Prattville, AL) were used in all experiments due to the 9:1 female to male bias in the prevalence of chronic, painful urological conditions. There was no attempt to control for estrous cycle, as the focus of this study was not on estrous-related changes in pain, and we have previously shown that hormone fluctuations due to the estrous cycle do not alter the vigor of distension-evoked visceromotor responses in rats without bladder inflammation (Ball et al., 2010). Rats were housed in standard cages with food and water available on an *ad libitum* basis with a 12:12-h light:dark cycle (lights off between 6:00 pm and 6:00 am). Rats were acclimated to the housing facility for one week between the time of arrival and any surgical or experimental procedures. Studies were approved by the UAB Institutional Animal Care and Utilization Committee.

4.2. Stereotaxic surgical procedure

Rats were anesthetized with inhaled isoflurane in oxygen (5% induction, 2.5–3% maintenance) and secured in a stereotaxic apparatus using non-puncture ear bars and a bite bar. Hair along the dorsal surface of the head was shaved and the skin was swabbed with a povidone-iodine solution. A midline incision was made in the skin along the top of the skull. The epidermis was retracted and the fascia cleared away. A flat-skull orientation was set using bregma and lambda as landmarks. A round, 2.0 mm burr attached to a Dremel device

was used to drill bilateral holes in the skull at the coordinates of 2.4 mm posterior to bregma and approximately 4.0 mm lateral to the midline (Paxinos and Watson, 1986).

Upon completion of behavioral testing, all rats were perfused with saline followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde followed by 25% sucrose, and sectioned at 40 μ m by cryostat. Light microscopy was used to determine the site of pellet implantation or injection.

4.2.1. Chronic CeA chemical stimulation procedure—A 25-gauge stainless steel microinjector loaded with a 30 μ g micropellet of either corticosterone or cholesterol was lowered 6.8 mm ventral to the brain surface toward the dorsal margin of the CeA. The micropellet was expelled and the microinjector removed. Skin was closed and the rats allowed to recover. Abdominal EMG measures were collected seven days post-surgery. Rats were administered acetaminophen via drinking water (0.16 mg/ml) daily for three days prior to and two days following surgery, and carprofen (5 mg/kg, s.c.) and buprenorphine (0.1 mg/kg, s.c.) upon completion of surgery and twice on the day following surgery.

4.2.2. Acute CeA chemical stimulation procedure—A 25-gauge stainless steel microinjector loaded with a solution consisting of sterile saline with heat-dissolved corticosterone (5 or 15 μ g/0.5 μ l) or cholesterol (15 μ g/0.5 μ l) was lowered 6.8 mm ventral to the brain surface to the dorsal margin of the CeA. The solution was injected gradually in one hemisphere, left in place for one minute following drug injection, and the microinjector was then removed and re-positioned for injection in the opposite hemisphere using the same procedure. Rats remained anesthetized for 30 min following injections until time at which VMR testing began or blood was taken for plasma corticosterone measurement as described in Section 4.7.

4.3. CeA electrolytic lesion procedure

Rats were surgically prepared using the same protocol described above. An electrode (00 insect pin insulated to within 1.0 mm of the tip) was lowered 7.0 mm ventral to the brain surface and lesions were produced by passage of anodal direct current (0.8 μ A, 9 s). Sham surgery was identical except no electric current was passed. Gel foam was placed in the trephine holes and the skin incision was closed. Animals were returned to their home cage to recover. Behavioral experiments (Section 4.8) or blood draw for measurement of plasma corticosterone concentration (Section 4.7) were performed seven days later. The same post-surgical protocol for administration of acetaminophen, carprofen (5 mg/kg, s.c.), and buprenorphine (0.1 mg/kg, s.c.) as described above was followed. Fixed brain tissue was stained with cresyl violet and light microscopy was used to determine lesion sites. Micrographs were matched to templates (Paxinos and Watson, 1986) and the obliterated areas were assessed as a function of total CeA area. Data from cases without bilateral CeA lesions were not included in statistical analyses.

4.4. Flinch and jump thresholds

Flinch and jump thresholds were used to determine whether CeA lesions impaired sensory transduction of the footshock stimulus. Following recovery from lesion or sham surgery,

each rat underwent a total of six (2× daily; 15 min) accommodation sessions to an operant conditioning chamber enclosed in a sound-attenuating cubicle. During the seventh session, an ascending series of electric shocks (100 ms) was delivered via the grid floor of the chamber. The initial shock intensity was 0.00 mA and subsequent shocks increased in increments of 0.05 mA (30 s inter-shock interval). The presentation of an incrementally stronger shock was dependent upon the rat maintaining all four paws in contact with the grid floor during the previous shock. A flinch response was defined as the lowest shock intensity that elicited withdrawal of a single forepaw from the grid floor; a jump response was defined as the lower of two successive shock intensities that elicited simultaneous withdrawal of both hindpaws from the grid floor. Three trials were conducted for each rat, and the thresholds from each trial were averaged to obtain a single flinch and single jump threshold for statistical analysis. An additional group of “naive” rats that received no surgery were handled in the same fashion and tested to assess the presence of nonspecific surgical effects.

4.5. Footshock stressor

Chronic or acute exposure to footshock, a classic stressor, can facilitate physiological responses in a rodent model of urinary bladder pain (Robbins et al., 2007; Robbins and Ness, 2008; Robbins et al., 2011). In the current study, rats underwent a total of six (2× daily; 15 min) accommodation sessions to an operant conditioning chamber. During the seventh session, rats in the ‘stress’ condition received footshock (1 mA) on a variable interval schedule (30 shocks in 15 min) while in the chamber; rats in the control condition were treated identically but did not receive any footshock.

4.6. Measurement of fecal pellet output

Fecal pellet output is a reliable measure of autonomic system activation and modulation of colonic motility (Tache et al., 2004). At the termination of each footshock/control session, fecal pellets were collected from the operant conditioning chamber and counted.

4.7. Enzyme-linked immunosorbent assay (ELISA)

Plasma corticosterone concentration was determined using ELISA (Cayman Chemical Company, Ann Arbor, MI) as per kit instructions. Rats were anesthetized with isoflurane in oxygen and blood was collected via cardiac puncture into heparinized vials and centrifuged (14,000 ×g, 15 min). Plasma was extracted and plasma samples (50 µl) were aliquotted onto a 96-well plate in duplicate. The plate was covered and developed in the dark on an orbital shaker for 60–90 min. Optical densities (OD) at 412 nm were obtained using a plate reader (BioRad Laboratories, Hercules, CA). A standard curve was plotted and the concentration of corticosterone in each sample was calculated from the curve based on its OD value.

4.8. Urinary bladder distension (UBD) and response measures

Rats were anesthetized with isoflurane in oxygen (5% induction, 2% maintenance) via a tight-fitting mask. A 22-gauge polytetrafluoroethylene angiocatheter (Johnson and Johnson, Arlington TX) was placed through the urethra into the bladder and held in place by a tight suture around the distal urethral orifice. Silver wire electrodes were inserted into the external oblique musculature immediately superior to the inguinal ligament for recording of

abdominal electromyography (EMG) activity. Following surgery, isoflurane was reduced (typically 1–1.25%) until flexion withdrawal reflexes were present in the hind limbs in response to noxious stimulation in the absence of escape behavior or evidence of tonic sympathetic nervous system activation (e.g., piloerection). UBD consisted of pressure-controlled air distension of the urinary bladder via the transurethral catheter using a previously described distension control device (Anderson et al., 1987). An in-line, pneumatically-linked, low volume pressure transducer was used to monitor intravesical pressure. Abdominal EMG activity was recorded using standard differential amplification and rectification and saved on a computer (Spike 2 software, Cambridge Electronic Design, UK). Approximately 15 min after initial anesthesia induction, three 60 mmHg distensions (3 min inter-trial interval) were administered to establish stable responses (Castroman and Ness, 2001) and were followed by graded, constant-pressure air distensions of the urinary bladder (20 sec duration; 1 min inter-trial interval) at ascending pressures in intervals of 10 mmHg. EMG responses were quantified as: (rectified EMG activity during UBD - rectified baseline EMG prior to UBD) / (rectified baseline EMG).

4.9. Statistical analyses

Quantitative comparisons of group mean abdominal EMG responses were performed using repeated-measures ANOVA on noxious distension pressures (40–60 mmHg) (Ness et al., 1991; Randich et al., 2006). Post-hoc effects were assessed by simple contrast analyses of group data at each distension pressure within the same range. Flinch and jump thresholds and plasma corticosterone concentration were compared by ANOVA and independent samples *t*-tests. Fecal pellet output was compared by independent samples *t*-tests. Statistical significance for all analyses was set at $p = 0.05$. Holm's correction (Holm, 1979), a step-wise modification of the Bonferroni procedure, was used to maintain family-wise α for each set of individual group comparisons of EMG responses, flinch and jump thresholds, and plasma corticosterone concentration.

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Highlights

- Acute exposure to footshock stress augments reflex responses to bladder distension.
- Chemical stimulation of CeA recapitulates footshock-induced bladder hyperalgesia.
- Electrolytic lesions of the CeA block footshock-induced bladder hyperalgesia.
- Acute footshock, but not chemical stimulation of the CeA, activates the HPA axis.

Figure 1. Acute footshock stress produces bladder hyperalgesia

Abdominal EMG responses to graded UBD (10–80 mmHg) were quantified following acute exposure (1 mA, 1 sec, 30 shocks in 15 min) to footshock (FS) or no footshock (NFS) after six accommodation sessions. Data are presented as group mean normalized responses; $N=7$ /group. A repeated-measures ANOVA revealed significant effects of footshock ($p<0.01$) and distension pressure ($p<0.01$), and a significant interaction between these variables ($p<0.01$). * indicates $p<0.05$ acute footshock > no footshock at noxious distension pressures. *B*) Raw EMG responses to bladder distension (60 mmHg; 20 sec) are shown for a rat exposed to footshock (FS; *upper trace*) and a rat in the no footshock condition (NFS; *lower trace*).

Figure 2. Chronic delivery of corticosterone to the CeA produces bladder hyperalgesia
A) Abdominal EMG responses to graded UBD (10–80 mmHg) were quantified one week after implantation of a micropellet containing either corticosterone (CORT, 30 μ g) or cholesterol (CHOL, 30 μ g). Data are presented as group mean normalized responses; N=7/group. A repeated-measures ANOVA revealed significant effects of drug treatment ($p<0.05$) and distension pressure ($p>0.05$). * indicates $p<0.05$ chronic CORT > chronic CHOL at noxious distension pressures. *B)* Raw EMG responses to bladder distension (60 mmHg; 20 sec) are shown from a rat with corticosterone (CORT; *upper trace*) or cholesterol (CHOL; *lower trace*) stereotactically applied to the CeA one week prior to testing. *C)* Histological location of micropellet implants at –2.40 mm posterior to bregma are shown for rats that received CORT (*triangles*) or CHOL (*circles*).

Figure 3. Acute application of corticosterone to the CeA produces bladder hyperalgesia

A) Abdominal EMG responses to graded UBD (10–80 mmHg) were quantified 30 min following bilateral microinjection of a solution containing corticosterone (CORT, 15 μ g) or cholesterol (CHOL, 15 μ g). Data are presented as group mean normalized responses; N=6/group. A repeated-measures ANOVA revealed significant effects of drug treatment ($p<0.05$) and distension pressure ($p>0.01$). * indicates $p<0.05$ acute CORT > acute CHOL at noxious distension pressures. **B)** Raw EMG responses to bladder distension (60 mmHg; 20 sec) are shown from a rat with CORT (*upper trace*) or CHOL (*lower trace*) stereotactically applied to the CeA 30 min prior to testing. **C)** Histological location of microinjection sites at -2.40 mm posterior to bregma are shown for rats that received CORT (*triangles*) or CHOL (*circles*).

Figure 4. Histological verification of CeA lesions

Fixed brain tissue was stained with cresyl violet and light microscopy was used to determine lesion sites. Micrographs were matched to templates (Paxinos and Watson, 1986) and the obliterated areas were assessed as a function of total CeA area. Lesions were targeted to -2.40 mm posterior to bregma, and templates are shown for sites ranging from -2.04 to -2.64 mm posterior to bregma. For lesions considered 'hits,' the obliterated area ranged from 68.3–100% of total CeA area (mean = $81.41 \pm 1.7\%$). For each level shown, the lighter shaded area represents the maximum lesion areas and the darker shaded area represents the minimum lesion areas. CeA=central nucleus, MeA=medial nucleus, LA=lateral nucleus, BA=basal nucleus.

Figure 5. Electrolytic lesions of the CeA prevent the development of acute footshock-induced hyperalgesia

A) An increase in plasma corticosterone concentration normally produced by acute exposure to footshock is blocked by bilateral CeA lesions. * indicates $p < 0.05$ lesion footshock < sham surgery footshock; # indicates $p < 0.05$ sham surgery footshock > sham surgery no footshock. B) Abdominal EMG responses to graded UBD (10–80 mmHg) were quantified one week after bilateral electrolytic lesions of the CeA were performed. * indicates $p < 0.05$ sham footshock > sham no footshock; sham no footshock and lesion footshock groups do not differ. *Inset*: representative photograph of CeA lesion in cresyl violet-stained tissue. Scale bar = 2 mm. C) Abdominal EMG responses from individual rats in which lesions did not hit the CeA are shown to demonstrate specificity of CeA lesion effects shown in B. D) Raw EMG responses to bladder distension (60 mmHg; 20 sec) are shown from rats exposed to footshock that underwent sham surgery (*upper trace*) or with bilateral CeA lesions (*lower trace*).