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Lesions of the central amygdala and ventromedial medulla reduce bladder hypersensitivity produced by acute but not chronic foot shock

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Abstract

Both acute and chronic stress has been shown to exacerbate symptoms of chronic visceral pain conditions such as interstitial cystitis. Studies using animal models support these findings in that both acute and chronic exposure to foot shock induced stress (FS) augment nociceptive reflex responses to urinary bladder distension (UBD). Only a few studies have examined the neural substrates mediating these phenomena and it is not clear whether acute and chronic stress engage the same or different substrates to produce bladder hypersensitivity. The present studies examined the role of two important central nervous system structures – the amygdala (AMG) and the ventromedial medulla (VMM) – in mediating/modulating hypersensitivity evoked by acute versus chronic FS using responses to graded UBD in adult, female Sprague-Dawley rats. Bladder hypersensitivity produced by acute FS was significantly reduced by either bilateral central AMG or VMM lesions using measures generated by graded UBD, but these lesions had no significant effects using the same measures on bladder hyperalgesia produced by chronic FS. Our findings provide evidence that neural substrates underlying bladder hypersensitivity produced by chronic stress differ from those produced by acute stress. These findings suggest that while the AMG and VMM participate in pain processing during periods of limited exposure to stress, prolonged stress may recruit a new set of neural substrates not initially activated by acute exposure to stress.

Keywords

AMG; VMM, visceral; hyperalgesia; acute stress; chronic stress

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1. Introduction

The neuroanatomical substrates controlling normal bladder function are well-researched, but those underlying bladder pain are poorly understood (Birder et al, 2010; Lovick, 2016). Bladder pain is not only evoked by physical perturbations to the bladder, such as bacterial infections, but also can be both induced and exacerbated by stress (stress-induced hyperalgesia – SIH). In humans, 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). In rats, either acute or chronic footshock (FS)-evoked stress, or acute or chronic water avoidance-evoked stress augments bladder nociceptive reflexes and measures of bladder hypersensitivity (Lee et al., 2015; Robbins et al, 2007; Robbins and Ness, 2008; DeBerry et al., 2015; Smith et al., 2011). Both chronic and acute FS also alter spinal neuronal responses to noxious bladder stimulation (Robbins et al., 2011) and activate the stress-responsive hypothalamic-pituitary-adrenal (HPA) axis (DeBerry et al., 2015; Robbins and Ness, 2007; 2008).

Evidence has been found that the AMG mediates both stress responsiveness and nociceptive processing (c.f., LeDoux, 2007; Neugebauer, 2015 for reviews). Therefore, the AMG is a potential neuroanatomical site of interest that could mediate/modulate bladder pain and the influence of stress on bladder pain. The central nucleus of the AMG (CeA) densely expresses glucocorticoid receptors (Reul and de Kloet, 1985) also suggesting a possible site of action and role for peripherally-released glucocorticoids in response to exposure to stressors and modifications in the experience of bladder pain. 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. DeBerry et al. (2015) showed that the CeA is required for the expression of acute FS-induced bladder hyperalgesia and that acute CeA activation by corticosterone is sufficient to drive bladder hyperalgesia in the absence of FS.

The VMM is well established as a part of the primary neural network for either ascending or descending facilitatory and inhibitory nociceptive influences on somatic and visceral pain (Randich and Ness, 2010). There is some evidence that it also may play a role in bladder hypersensitivity since lesions of the nucleus raphe magnus in the central VMM attenuate bladder hypersensitivity produced by bladder inflammation and direct electrical stimulation of some sites localized in and around the VMM can increase the visceromotor responses (VMRs) to UBD and colorectal distension (Randich and Ness, 2010; Randich et al., 2008; Zhou and Gebhart, 2002; Imbe et al., 2002). Moreover, the VMM region projects to the CeA directly, and through relays in thalamic nuclei and the lateral parabrachial nuclei that may be independent of the direct projections (Roeder et al., 2016).

The current set of neuroanatomical lesion studies examined whether the central AMG or VMM is required for the exacerbation of bladder pain produced by either acute or chronic FS stress in an animal model of bladder pain. Separate groups of rats were given either sham lesions, or lesions of either the bilateral central AMG or the VMM, followed by no

footshock (NFS), acute footshock (AFS) or chronic footshock (CFS) pretreatment procedures. They were subsequently examined for changes in VMRs to graded UBD quantified using dependent measures of either area under the curve (AUC) generated by all distending pressures or as responses to individual distending pressures.

2. Results

2.1 AFS and CFS Produce Bladder Hypersensitivity

We quantified abdominal EMG responses to graded bladder distension in rats with sham lesions and exposure to NFS, AFS, or CFS procedures. This part of the study was performed as a control experiment to confirm that AFS and CFS treatments significantly augment the VMR evoked by UBD. Mean AUC data and mean individual VMR stimulus response functions for sham-lesioned rats subsequently subjected to NFS, AFS or CFS procedures are presented in Figure 1. Typical examples of rectified EMG activity for three sham-lesioned rats which underwent NFS, AFS or CFS procedures are given on the right side of Figure 1.

In the AUC analyses, the mean VMRs of both AFS and CFS groups were significantly increased relative to sham-lesioned controls (p values < 0.01 and 0.05 , respectively). In the analysis of individual pressures, a repeated-measures ANOVA revealed significant effects of FS ($p < 0.01$), distending pressure ($p < 0.01$), and interaction between these variables ($p < 0.01$). Post-hoc comparisons of means indicated significantly greater abdominal EMG responses to bladder distension at 40, 50, and 60 mmHg relative to sham controls in both AFS and CFS conditions. These outcomes are consistent with our earlier reports related to stress and UBD where changes are observed at greater rather than lesser distending pressures (Robbins and Ness, 2008). These sham-lesioned rats served as the control rats for the subsequent data presented in Figure 3 demonstrating the effects of CNS lesions on stress-induced effects.

2.2 Effects of AMG and VMM lesions alone on VMRs

Abdominal EMG responses to graded UBD were quantified in groups of sham, bilateral AMG-lesioned, and VMM-lesioned rats in the absence of prior exposure to FS (the NFS group) to determine whether the lesions produced any changes in baseline responses to UBD. Notably, as observed in Figure 2A, a VMM lesion by itself, but not bilateral central AMG lesions alone, resulted in enhanced VMRs to UBD using either AUC ($p < 0.05$) or individual pressure data. In the VMM-lesioned condition, post-hoc comparisons of means obtained from the significant overall ANOVA indicated significant increases in VMRs at 40, 50, and 60 mmHg, relative to sham lesion controls.

2.3 AFS-induced bladder hypersensitivity is significantly attenuated by either bilateral central AMG or VMM lesions

Abdominal EMG responses to graded UBD were quantified in groups of sham-, bilateral central AMG-, and VMM-lesioned rats that underwent an AFS procedure. The middle panel of Figure 2 shows that both bilateral AMG and VMM lesions resulted in reduced VMRs to UBD in these rats using AUC data (p values < 0.05 relative to sham lesions). In the repeated-measures analyses, post-hoc comparisons of the bilateral AMG-lesioned treatment group

receiving AFS indicated the lesions prevented the development of hyperalgesia at 40, 50, and 60 mmHg (p values < 0.05), relative to sham lesion rats also receiving AFS.

2.4 CFS-induced bladder hypersensitivity is not affected by either bilateral central AMG or VMM lesions

Abdominal EMG responses to graded UBD were quantified in groups of sham-, bilateral central AMG-, and VMM-lesioned rats that underwent a CFS procedure. The far right panel of Figure 2 shows that neither AMG nor VMM lesions significantly altered the VMRs to UBD relative to sham controls using either AUC data or individual data.

2.5 Histology

An example of a lesion of the left central AMG is shown in Figure 3A and of the VMM in Figure 3B in order to show the typical size of the lesions. Composites of the location of lesions in the AMG and VMM are given in Figures 3C and 3D respectively. See *Section 4.6* for inclusion criteria regarding these lesions.

3. Discussion

There has been considerable focus in recent years on the role of the AMG and VMM as neural substrates for the effects of stress and generation/exacerbation of pain – referred to as stress-induced hyperalgesia (SIH). As noted in the Introduction, SIH is most often reported in preclinical models using visceral systems and stands in contrast to somatic systems where stress often produces analgesia (stress-induced analgesia; SIA). Consistent with this, as previously noted, SIH is observed clinically in a variety of visceral pain-related disorders. Thus, SIH rather than SIA, is the rule rather than the exception when dealing with visceral disorders. Most of the visceral work on SIH has focused on irritable bowel syndrome and colon hypersensitivity (Zhou and Gebhart, 2002) but a few recent studies have examined substrates of stress-induced exacerbation of urinary bladder hypersensitivity (Black et al., 2009; Robbins et al., 2008, 2007; Smith, 2011, Pierce et al., 2016; Lee et al., 2015).

An organism's response to stress is thought to be generated by a network of integrated brain structures, in particular the hypothalamic paraventricular nucleus, the AMG, and the periaqueductal gray (Mayer, 2000). These integrated networks are activated in response to various stressors and their output initiates responses within the HPA axis, sympatho-adrenal axis, locus coeruleus/norepinephrine system, and endogenous pain modulatory systems (Valentino et al., 2011). Our studies focused on the central AMG because it receives nociceptive information via direct spinoamygdalar projections and via an indirect spinoparabrachial pathway (Bernard et al., 1994; Burstein and Potrebic, 1993; Gauriau and Bernard, 2002) and because of its known connectivity with physiological stress-response systems with relays to known pain modulatory regions. That is, the central AMG is in a notable position suitable for mediating the reciprocal relationship between pain and affective processes, and attaching emotional valence to sensory stimuli (Gauriau and Bernard, 2002; Price, 2002). We also examined the VMM for its capability to produce descending facilitatory modulation of spinal nociceptive transmission (Imbe et al., 2002; Randich and

Ness, 2010) and also as part of the network that communicates with areas such as the AMG and lateral parabrachial nucleus (Roeder et al., 2016).

In the present studies, AFS or CFS produced SIH in sham-lesioned animals, as has been reported previously in studies from our laboratory (Deberry et al, 2015; Robbins and Ness, 2008). Bilateral lesions of the central AMG or of the VMM eliminated SIH resulting from AFS. In contrast, neither of these lesions affected the SIH generated by CFS, despite having FS exposure that was identical to that experienced by the AFS group on the day of testing. Lesions of the VMM, but not AMG, increased the response to UBD in the absence of FS. We had no specific expectations related to data obtained in rats with AMG lesions. However, the data obtained in rats with VMM lesions would suggest that there is a tonic inhibitory influence of the VMM on the VMR evoked by UBD when there is no stress-induced alterations in central nervous system activity. This particular outcome is not surprising given that other studies have shown similar effects of VMM lesions (Randich and Ness, 2010). Studies not reporting this effect (e.g., Randich et. al., 2008) had methodological differences from the present study in that they used more punctate lesions of the nucleus raphe magnus alone, had differences in recovery times, and in some cases, had insertion of an electrode into the region without formation of an electrolytic lesion. However, since lesions of the VMM eliminated the SIH produced by AFS, it must be the case that AFS causes the predominant effect of the VMM to be a facilitatory influence on the response to UBD. This could be through direct activation of a descending facilitatory pathways associated with the VMM OR could be by an active inhibition of the tonic inhibitory effect associated with the VMM observed in the absence of AFS OR by a combination of the two phenomena. If a combination of facilitation and disinhibition were occurring due to VMM substrates, then our estimates of the effects of VMM lesions in attenuating the effects of AFS may underestimate the magnitude of the effect. Future experiments that record directly from neurons within the VMM following manipulations such as AFS might allow us to dissect out the subtleties of the mechanisms at work.

Our studies of the AMG as an important mediator of stress-related hypersensitivity are consistent with previous studies showing that AMG lesions reduce activation of the HPA axis following stress, while electrical stimulation of the CeA enhances it (Beaulieu et al., 1986, 1987, 1989; Feldman et al., 1982, 1994; Lilly et al., 2000; Van de Kar et al., 1991). Chronic or acute stimulation of the CeA with corticosterone or aldosterone enhances dorsal horn neuronal responses to colorectal distension (CRD) and UBD, and enhances nociceptive responses to CRD (Greenwood-Van Meerveld et al., 2001; Qin et al., 2003a, 2003b, 2003c; Myers and Greenwood-Van Meerveld, 2007; Myers et al., 2007).

However, our findings indicate that the neural substrates underlying bladder hypersensitivity produced by chronic FS-related stress must differ from those produced by acute FS-related stress. In other words, while the AMG and VMM participate in pain processing during periods of limited exposure to FS stress, prolonged FS stress may recruit and be controlled by a new set of neural substrates. One possibility is that another structure(s), such as the parabrachial nucleus, set a new “tone” for neurons in typical pain pathways (Roder et al., 2016). Thus, chronic stress may cause a transformation of neural substrates mediating the effects of acute stress to a new set of substrates, perhaps due to changes resulting from the

individual's emotional state and cognitive factors relating to chronicity of stress. This may account for difficulties in treating chronic visceral disorders since data obtained by acute manipulations may not be relevant to those of chronic stress. One site that is likely changed by CFS is the spinal cord itself. Previous studies of spinal dorsal horn neurons excited by UBD (Robbins et al., 2011) observed that alterations in a neuronal subpopulation which are produced by AFS are abolished with acute spinal transection, but the qualitatively equivalent changes on the same spinal neuronal populations produced by CFS, which is observed in intact rats, is not abolished by spinal transection.

Our findings obtained with VMM lesions are surprising in the sense that this region is well established to serve as a final common substrate for descending modulation of nociceptive input. One might expect these lesions to affect SIH in the CFS-treated rats. However, as shown, it only affected hyperalgesia in those rats receiving AFS. One can argue about how “chronic” seven days of treatment may be, but in the present experiment, this was sufficient time to allow neuroplastic changes to occur which necessarily must have involved sites other than the VMM in association with footshock-related hyperalgesic phenomena.

Pain-related plasticity within the AMG has been demonstrated in rats in multiple models including acute-onset models of arthritis and colitis, in a model of neuropathic pain, and in a cyclophosphamide-induced cystitis pain model in mice (Han and Neugebauer, 2004; Ikeda et al., 2007; Neugebauer et al., 2003; Nishii et al., 2007). Neuroendocrine peptide expression is upregulated in the CeA following cyclophosphamide-induced cystitis of the urinary bladder, and chemical stimulation of the CeA via stereotaxic application of corticosteroids results in facilitation of spinal dorsal horn neuronal responses to bladder distension (Nishii et al., 2007; Qin et al., 2003c). At the present time, the complex pharmacology and intricate intraneuronal connections within AMG sites preclude any statements about possible pharmacological mediators, but they have been previously discussed by Neugebauer (2015).

We have demonstrated that the neural substrates mediating the effect of AFS and CFS on bladder SIH differ. However the influence of these brain regions on stress responsiveness may be stressor-specific () as distinct and contrasting patterns of neural activation have been demonstrated to occur that are dependent on the type of the stressor (Pacak et al., 1988; Sawchenko et al., 2000; Dayas et al., 2001; Herman and Cullinan, 1997). A study of c-fos mRNA expression within the AMG found that different patterns of expression emerge following either somatic or visceral stimulation (Nakagawa et al., 2003). Additional conditioned place aversion studies by the same group showed that discrete lesions of either the basolateral nucleus or CeA abolished the aversion following somatic stimulation while only CeA lesions reduced this measure following visceral stimulation (Tanimoto et al., 2003). Similar differences in patterns of ERK expression have been observed in the ventromedial medulla and locus coeruleus using forced swim and formalin injections (Imbe et al., 2004). Finally, recent optogenetic studies by Sadler et al. (2017) suggest even greater complexity in terms of right versus left central AMG influences on nociceptive responses which were not addressed by the present study.

In conclusion, the present study demonstrated a role for both the AMG and VMM in bladder hypersensitivity produced by AFS but not by CFS. Peripheral and spinal nociceptive

mechanisms clearly are required for enhanced pain during periods of stress or following exposure to aversive events. It is clear that changes in the individual emotional state or cognitive factors may transform responses to normal peripheral afferent inputs resulting in the presence of hypersensitivity. By understanding the mechanistic basis of the substrates of this hypersensitivity, it will be possible to derive therapeutic interventions that may reverse it. Future studies will be needed to identify these possibilities.

4. Experimental Procedures

4.1 Animals

Naïve female Sprague-Dawley (SD) rats (11-12 weeks of age) were obtained from Harlan (Prattville, AL) and maintained in separate cages. All rats were housed with food and water available on an *ad libitum* basis. A 12:12-h light:dark cycle was maintained, where lights were off between 6:00 pm and 6:00 am. 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 UBD-evoked VMRs in rats without bladder inflammation when the current methodology is employed (Ball et al., 2010). It is not known whether central nervous system lesions might unmask estrous cycle effects. To limit non-specific stress, rats were allowed to accommodate to their new location for one week after being received by the laboratory. A timeline for the procedures described below is summarized in Figure 4. These studies were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC).

4.2 Stereotaxic surgical procedures for bilateral AMG and VMM lesions

Rats were administered carprofen (5 mg/kg s.c.) one day prior to surgery, on the day of surgery, and one day after surgery. Ampicillin (50 mg/kg s.c.) and buprenorphine (0.05 mg/kg s.c.) were administered the day of surgery. All rats were anesthetized with inhaled isoflurane (2-5%) in oxygen. Hair along the dorsal surface of the head was clipped and the skin surrounding the surgical area was swabbed with a 70% alcohol and povidone-iodine solution. The rat was then secured in a stereotaxic apparatus.

For bilateral central AMG lesions, a midline skin incision was made along the top of the skull, beginning just behind the eyes and extending approximately 2 cm caudally. For VMM lesions, a midline skin incision was made along rear of the skull and extending approximately 2 cm caudally. The epidermis was retracted and the fascia cleared away, and a flat-skull orientation was set using bregma and lambda as landmarks. A 2.0 mm burr attached to a Dremel device was used to drill holes in the skull. The lesion electrode was constructed of a 00 insect pin insulated to within 1.0 mm of the tip with epoxy. For AMG lesions, bilateral lesions were 2.4 mm posterior to bregma, 4.0 mm lateral to the midline, and 6.8 mm ventral to the brain surface (Paxinos and Watson, 1986). The tip was targeted at the CeA but lesions usually encompassed more of the AMG but normally did not include the basolateral nuclei of the AMG. For VMM lesions, a unilateral midline lesion was made 2.5 mm posterior to lambda and 8.6 mm ventral to the brain. All lesions were produced by passage of anodal 1.0 mA direct current (10 sec for AMG and 8 sec for VMM). Two rats were

excluded from further study as they demonstrated obvious neurological motor deficit after recovering from anesthesia.

The sham lesion control surgery procedure involved drilling burr holes in the brain but no electrode was passed into the brain. Two holes were drilled for comparison with the bilateral AMG-lesioned condition and one hole for comparison with the VMM-lesioned condition. The data from the two different sham lesion groups were combined for all analyses, as the data from these groups did not statistically differ from each other (see below in *Section 4.7*). The skin incision was closed and animals returned to their home cage to recover from the procedure for seven days.

4.3 AFS, CFS, and NFS Procedures

AFS and CFS were administered in an operant conditioning chamber enclosed in a sound-attenuating chamber similar to our previous studies (Robbins and Ness, 2008). In all experiments, FS and accommodation (NFS) procedures did not start until a minimum of seven days after recovering from surgery.

In the AFS procedure, each rat was placed in an operant conditioning chamber for a 15 min accommodation session twice daily for three days prior to UBD testing. These accommodation sessions served as a control procedure in order to reduce stress-related variability due to animal handling and novel environment exposure, and were matched to CFS rats in the number of exposures to the chambers. On the day of UBD testing, AFS rats were placed in the operant conditioning chamber and received intermittent FS (15 min, 1.0 mA, 1 sec duration, total of 30 shocks) administered via a parallel rod floor on a variable interval (VI) schedule. This procedure has been used previously in our laboratories and demonstrated to produce robust stress-related effects (Robbins et al., 2008; DeBerry et al., 2015). Rats in the corresponding NFS control condition were treated identically except they did not receive any FS while in the operant conditioning chambers.

In the CFS procedure, rats were placed in the same operant conditioning chamber as the AFS procedure and received similar intermittent FS once per day but for 7 days. Rats in the corresponding NFS control condition remained in the chambers for 15 min once per day for 7 days, but did not receive FS. Thus, the total number of exposures to the chambers was kept constant between NFS, AFS, and CFS procedures.

4.4 UBD and VMR Procedures

Animals were anesthetized with isoflurane (5% induction, 2-3% maintenance) in oxygen via a tight-fitting mask and a 22-gauge polytetrafluoroethylene angiocatheter (Johnson and Johnson, Arlington TX) was placed into the bladder via the urethra and held in place by a tight suture around the distal urethral orifice. Platinum or silver wire electrodes were inserted into the external oblique musculature immediately superior to the inguinal ligament for recording of abdominal EMG activity. Following surgery, anesthesia was reduced until flexion reflexes were present in the hind limbs but spontaneous escape behaviors were absent (1-1.25% isoflurane). All rats then received a series of UBDs.

The primary response measure was the VMR evoked by distension of the urinary bladder via the intravesical catheter. An in-line, pneumatically-linked, low volume pressure transducer was used to monitor intravesical pressure. Pressure within the bladder during stimuli was controlled using a pressure control device similar to previous reports (Ness et al., 2001). In the various treatment groups, 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; 3 min inter-trial interval) of ascending pressures at intervals of 10 mmHg up to 60 mmHg. Contraction of the abdominal musculature, recorded as EMG activity, was measured via the electrodes using standard differential amplification and rectification and saved on a computer (Spike 2 software, Cambridge Electronic Design, UK). EMG responses were quantified as a “signal-to-noise ratio” as done previously (Robbins et al., 2008) using the formula: (rectified EMG activity during UBD - rectified EMG measured immediately prior to UBD) / (rectified baseline EMG measured in the absence of UBD at a single time point for each experiment)) and thereby expressed as a normalized VMR value.

4.5 Evaluation of Effect of Lesions and Footshock on VMRs

Three groups of rats were studied to demonstrate the effects of AFS and CFS in sham-lesioned animals. All groups received a sham-lesion procedure and each group then received the AFS, CFS or NFS procedure. On the day of testing, rats received the UBD and VMR procedures described above.

Two additional groups of rats were studied to demonstrate the effects of bilateral central AMG or VMM lesions in the absence of FS. These were compared against sham-lesioned rats that underwent the NFS procedure (part of group described above). These groups received the NFS procedure prior to testing and received the UBD and VMR procedures described above.

Two additional groups of rats were formed to evaluate the effects of the bilateral central AMG and VMM lesion procedures in animals receiving AFS. These were compared against sham-lesioned rats that underwent the AFS procedure (part of group described above). These groups received the AFS procedure. On the day of testing, rats received the UBD and VMR procedures described above.

Two additional groups of rats were formed to evaluate the effects of the bilateral central AMG and VMM lesion procedures in animals receiving CFS. These were compared against sham-lesioned rats that underwent the CFS procedure (part of group described above). These groups received the CFS procedure. On the day of testing, rats received the UBD and VMR procedures described above.

4.6 Histology

Upon completion of the experiments, brain tissue was processed and stained with cresyl violet. Light microscopy was used to verify lesion sites. AMG sections were matched to templates ranging from -1.92 to -3.00 mm posterior to bregma using successive sections as shown in Paxinos and Watson (1986). For a lesion to be considered successful, it was

required that 90% of the central AMG was destroyed or replaced by gliosis in at least two of five sections examined on each side. Similarly, VMM sections were matched to additional templates ranging from -9.68 to -10.52 mm posterior to lambda. In that case, an animal was included in analyses only if the lesion eliminated the entire nucleus raphe magnus and damaged the surrounding gigantocellular pars alpha in three of the sections.

4.7 Statistical Analyses

Statistics are presented as the mean $\pm$ S.E.M in the graphs. Two response measures were used. In AUC measures, analysis consisted of unpaired t-test comparison of each lesion/treatment group with its appropriate control group. In analyses of individual pressures, a repeated measures ANOVA was used. Post-hoc contrasts were performed when appropriate and Holm's procedure (1979) maintained family-wise α at 0.05.

It is our view that the AUC measure provides a better dependent measure of the observed effects since it includes all the data and avoids problems associated with the use of a large number of post-hoc contrasts requiring corrections. As noted above, in all experiments, the sham-lesioned data from the two-hole and one-hole surgical groups were combined because an ANOVA revealed no significant difference between the vigor of VMRs associated with the two types of sham lesions.

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Highlights

Acute and Chronic Footshock Paradigms Produce Bladder Hypersensitivity

Lesions of the Bilateral Central Amygdala or Ventromedial Medulla Attenuate Hypersensitivity

Produced by Acute Footshock

Lesions of the Bilateral Central Amygdala or Ventromedial Medulla Do Not Attenuate Hypersensitivity Produced by Chronic Footshock

Sham lesion groups

Figure 1. AFS and CFS produces bladder hyperalgesia

Visceromotor responses (VMRs) to graded urinary bladder distension (UBD; 10-60 mmHg) were quantified as abdominal EMG responses following exposure to no footshock (NFS; $n=12$), acute footshock (AFS; $n=16$), or chronic footshock (CFS; $n=9$) procedures in rats which had received sham lesion surgeries. Data in the main panel are presented as group means $\pm$ S.E.M. In the Area-Under-the-Curve (AUC) analyses shown in the inset, both AFS and CFS significantly increased the VMRs to UBD relative to NFS sham-lesioned controls. Similarly, a repeated-measures ANOVA revealed significant effects of footshock ($p < 0.01$), UBD pressure, ($p < 0.01$), and a significant interaction between these variables ($p < 0.01$). Post-hoc contrasts of means revealed significant increase at 40-60 mmHg in both AFS and CFS groups:

* indicates $p < 0.05$ and ** indicates $p < 0.01$. In the right panel are three typical examples of VMRs following AFS (top), CFS (middle), and NFS (bottom) procedures. These data represent rectified EMG measures of the VMR to UBD (60 mmHg, 20 s – period of distension indicated by bar).

Figure 2. Effects of NFS, AFS and CFS in bilateral AMG, VMM, and Sham-lesioned animals
Visceromotor responses (VMRs) to graded urinary bladder distension (UBD; 10-60 mmHg) were quantified as abdominal electromyographic responses following exposure to No footshock (NFS; panel A), acute footshock (AFS; panel B), and chronic footshock (CFS; panel C) procedures in sham-, bilateral amygdala (AMG-), and ventromedial medulla (VMM-) lesioned rats. Insets indicate Area-Under-the-Curve (AUC) data and larger graphs represent VMRs for individual pressures. Data are presented as group means $\pm$ S.E.M. A. In NFS rats, only the VMM-lesioned rats (n=7) showed a significant increase in the VMR to UBD; (AMG-lesioned rats n=8). B. In the rats which experienced AFS, both the AMG- (n=8) and VMM-lesioned (n=8) groups showed a significant decrease in the VMR to UBD. Following a significant repeated-measures analysis, post-hoc comparisons of the bilateral AMG-lesioned rats indicated significant increases at 40, 50, and 60 mmHg (p values < 0.05), relative to sham lesion controls. C. In the rats which experienced CFS, the bilateral AMG- (n=9) and VMM-lesioned (n=9) groups had no significant difference in VMRs to UBD in comparison with sham-lesioned rats. * indicates $p < 0.05$ and ** indicates $p < 0.01$.

Figure 3. Histological reconstruction of central AMG and VMM lesions

Fixed brain tissue was stained with cresyl violet and light microscopy was used to determine lesion sites. A typical example of a central AMG lesion is given in panel A and of a VMM lesion in panel B. Composites of multiple sites of lesion centers are given on representative coronal sections for AMG lesions in panel C and for VMM lesions in panel D. Numbers in C and D correspond to the distance posterior to bregma using Paxinos and Watson (1986) atlas criteria. See Section 4.6 for description of lesion inclusion criteria.

Figure 4. Experimental timeline

Schematic diagram indicating timeline for major components of the experimental protocol. See text for full explanation.