Social stress-induced bladder dysfunction: potential role of corticotropin-releasing factor

Abbreviated Title: Social Stress-induced bladder dysfunction

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Abstract

Psychological stress can impact on visceral function with pathological consequences, although the mechanisms underlying this are poorly understood. Here we demonstrate that social stress produces marked changes in bladder structure and function. Male rats were subjected to repeated (7 days) social defeat stress using the resident-intruder model. Measurement of the voiding pattern indicated that social stress produced urinary retention. Consistent with this, bladder size was increased in rats exposed to social stress. Moreover, this was negatively correlated to the latency to assume a subordinate posture, implying an association between passive behavior and bladder dysfunction. In vivo cystometry revealed distinct changes in urodynamic function in rats exposed to social stress, including increased bladder capacity, micturition volume, inter-micturition interval and the presence of non-micturition related contractions, resembling overactive bladder. In contrast to social stress, repeated restraint (7 days) did not affect voiding, bladder weight or urodynamics. The stress-related neuropeptide, corticotropin-releasing factor (CRF) is present in spinal projections of Barrington’s nucleus that regulate the micturition reflex and has an inhibitory influence in this pathway. Social stress, but not restraint, increased the number of CRF-immunoreactive neurons in Barrington’s nucleus. Additionally, social stress increased CRF mRNA in Barrington’s nucleus. Together, the results imply that social stress-induced CRF upregulation in Barrington’s nucleus neurons results in urinary retention and eventually, bladder dysfunction, perhaps as a visceral component of a behavioral coping response. This mechanism may underlie dysfunctional voiding in children and/or contribute to the development of stress-induced bladder disorders in adulthood.

Key words: Barrington’s nucleus, social defeat, micturition, cystometry, resident-intruder
Chronic or repeated stress has numerous adverse psychological and physiological consequences (33). The mechanisms by which stress produces adverse effects have been studied in rodents using a variety of stressors. However, many of these stressors lack ethological validity (i.e., footshock). One stressor that is more ethologically relevant and is currently being used to model stress-related pathology involves social defeat (44). This model, in which a rodent is forced into submission by a larger male conspecific, has been used by several groups to examine behavioral and endocrine consequences of social stress (3, 17, 24, 39, 44). A visceral effect of social stress that is often reported, but has not been systematically investigated, is urinary retention that can develop into bladder hypertrophy and even nephropathy due to reflux (10, 16, 29). The neural processes through which social stimuli can sufficiently impact bladder function to produce structural changes remain unknown.

Neural circuits linking the brain and bladder that are sensitive to stress likely mediate the impact of social stress on the bladder. One such circuit involves projections from Barrington’s nucleus in the pons to preganglionic parasympathetic neurons in the lumbosacral spinal cord (28). These projections mediate the efferent limb of the micturition reflex by regulating bladder contraction in response to distention (8). Barrington’s nucleus neurons contain the stress-related neuropeptide, corticotropin-releasing factor (CRF). In addition to its role in mediating endocrine and behavioral aspects of the stress response (1, 45), CRF is a neuromodulator in the micturition pathway from Barrington’s nucleus to the spinal cord where it has an inhibitory influence on micturition (35). Thus, this is a potential mechanism that can be recruited by social stress to produce urinary retention.

To better understand how social stress can produce bladder pathology, the present study characterized the effect of repeated social defeat stress on bladder structure and function by measuring bladder weight, voiding patterns and urodynamics using in vivo cystometry. Because CRF is prominent in circuits linking the brain and bladder, CRF expression in Barrington’s nucleus was also quantified. To determine whether the effects of social stress generalized to other stressors, the effects of repeated restraint stress on bladder function and CRF expression in Barrington’s nucleus were compared to those produced by social stress.
Materials and Methods

*Animals.* Male Long-Evans retired breeders (650-850 g) served as residents (Charles River) and all other subjects were male Sprague-Dawley rats (275-300 g). Rats were singly housed in a 12-hour light/dark, climate controlled room with food and water freely available. All studies were approved by the Children’s Hospital of Philadelphia IACUC and conformed to the guidelines set forth by the NIH Guide for the Use of Laboratory Animals.

*Social defeat (resident-intruder) paradigm and restraint.* The social defeat paradigm (4) was originally modified from the resident-intruder paradigm developed by Miczek (34). Intruder rats were placed into the cage of a novel, Long-Evans resident for 30 minutes on 7 consecutive days. The endpoint of defeat was met when the intruder assumed a supine position in response to the resident’s aggressive behaviors. At this point, a wire mesh enclosure was placed in the cage to prevent physical contact between the resident and intruder but allowing visual, auditory and olfactory contact to be maintained for the remainder of the 30-minute session. Controls were placed behind a wire partition in a novel cage for 30 minutes daily. Rats were returned to their home cage after each session. A separate cohort of rats was exposed to restraint (30 min) in a Plexiglas tube for 7 consecutive days. The control rats in the restraint study, unlike controls for defeated rats, were left in the home cage and not handled during the 7 day paradigm.

*Quantification of Voiding Pattern.* Voiding pattern measurements were conducted 24 hours before the first stress or control manipulation and 24 hours after the final manipulation. Subjects were placed individually in a cage with the floor lined with filter paper for 90 minutes. Urine spots on the filter paper were allowed to dry, illuminated with UV light and photographed. The number of urine spots was quantified using Image J by an individual blind to the treatment groups. In addition, magnification of the photographs allowed for easy identification of overlapping, but distinct spots.
**Quantification of Urodynamics.** Bladder catheters were surgically implanted from 5-24 hours after the last voiding pattern experiments. Urodynamic function was recorded using cystometry in the unanesthetized state 48 hours after surgery as previously described (22). Sterile saline was continuously infused (100 µl/min) through the bladder catheter while urodynamic endpoints were recorded on-line for 1 hour using cystometry equipment and software (Medical Associates, St. Albans, VT). At least 3 consecutive voiding events were used to calculate the urodynamic parameters of bladder pressure, bladder capacity (volume of infused saline in a micturition cycle), micturition volume, micturition threshold, resting pressure, peak micturition pressure, non-micturition related contractions, and inter-micturition interval. Non-micturition contractions were analyzed by Cystometry Analysis Software (SOF-522) from Catamount R&D (St. Albans, VT). The program considers only bladder contractions greater than 5 mm Hg and 3.5 s duration and not associated with urine expulsion as non-micturition related contractions. These were counted in a 10-minute interval. Residual volume was calculated as the difference between the infused volume and the voided volume averaged over 3 voiding events. Rats were sacrificed twenty-four hours after the cystometry session. Bladders from rats used in cystometry experiments were not included in bladder weight comparisons. Likewise brain tissue used for quantification of CRF mRNA or protein was not obtained from rats used in cystometry experiments.

**Quantification of CRF-immunoreactive neurons.** To determine whether social stress or restraint produced alterations in the expression of CRF protein, the number of CRF-immunoreactive neurons in Barrington’s nucleus was quantified. Rats were decapitated 24 hours after the final defeat, restraint or matched control manipulations and brains were removed and frozen. Coronal serial sections (14 µm-thick) were cut onto slides (Fisherbrand ProbeOn Plus; Fisher Scientific). Sections containing Barrington’s nucleus were processed to visualize CRF- and tyrosine hydroxylase (TH)-immunoreactivity, as previously described (47). Because TH-immunoreactivity reveals different levels of the nearby norepinephrine-containing nucleus, locus coeruleus, this was used as an anatomical landmark to match the rostro-caudal levels of sections used for quantification. Sections were post-fixed by incubating slides in 4% paraformaldehyde for 1 hour followed by rinses. Sections were incubated with a cocktail of rabbit
anti-CRF (C-70,1:2000, Wylie Vale, Salk Institute) and mouse anti-TH (1:5,000) for 48 hours at 4°C. The specificity of the anti-CRF antibody has been previously characterized by ourselves and others (5, 41, 49). These groups showed an absence of staining when sections were incubated with the antibody that has been preabsorbed with the antigenic peptide and when the primary antiserum was omitted. Figure 1 of Supplementary data verify the absence of CRF-immunoreactive staining in Barrington’s nucleus sections that have been incubated with the preabsorbed antibody or in the absence of the primary antibody. Using dot-blots, the concentration-dependence of the reaction was verified as well as lack of cross-reactivity with products of melanin concentrating hormone precursor (49). Finally, the antibody solution contains 1 mg/ml αMSH to assure no cross-reactivity with this peptide. Following rinses the sections were incubated with rhodamine-conjugated donkey anti-rabbit IgG antibody and fluorescein-conjugated donkey anti-mouse (1:200, Jackson Laboratories) for 90 min. Sections from stressed rats and their matched controls were processed at the same time using identical solutions. Immunoreactivity was visualized using a Leica fluorescent microscope and the exposure duration was maintained at a constant value for the comparison of all sections at the same time. Images were captured using a Hamamatsu ORCA-ER digital camera and Open Lab software (Improvision). CRF-immunoreactive cells within the core of Barrington’s nucleus were counted and averaged from 2 non-serial sections per rat by an individual blinded to the treatment condition. For CRF-expressing cell quantification, images were magnified and inverted in grayscale, such that cells showed up in black and background was white. This allowed for unambiguous distinction between staining within a cell and the background. The mean number of CRF neurons in Barrington’s nucleus for a particular group was determined by taking the mean of these averages for individual subjects within an experimental group.

Quantification of CRF mRNA. Because social stress altered the number of CRF-immunoreactive neurons in Barrington’s nucleus, tissue from social stressed and control rats were also processed for CRF mRNA analysis. Sections adjacent to those used for immunohistochemistry were processed to quantify CRF mRNA as previously described (50). Sections were post-fixed by incubating in 10% formalin prior to hybridization with an antisense riboprobe to CRF mRNA (Dr. Audrey F. Seasholtz, University of
Michigan) and coated with Kodak NTB2 liquid autoradiographic emulsion (7-day exposure at 4°C). Following emulsion development, the tissue was lightly stained with cresyl violet. Brightfield microscopy was used in combination with Open Lab software to count cells within Barrington’s nucleus. For quantification, brightfield images were magnified to identify cresyl violet stained cells within the nucleus containing silver grains >2-4 times background. The average number of CRF-expressing cells from 2 sections representing the core of Barrington’s nucleus were quantified and averaged for each rat by an individual who was blind to the treatment condition.

**Statistical Analysis.** All data are presented as mean ±SEM. Unpaired Student’s t-tests were used to identify significant differences in absolute bladder weight or bladder to body weight ratios between defeated rats and their controls, as well as restrained rats and matched controls. In addition, all cystometry parameters were analyzed using unpaired Student’s t-tests to determine differences between defeated or restrained rats and their matched controls. Differences in CRF protein and mRNA expressing cell count between stressed animals and matched controls were also analyzed using unpaired Student’s t-tests. Voiding pattern experiments were quantified using a two-way analysis of variance (ANOVA) followed by Bonferoni post-hoc tests. Data were analyzed by evaluating treatment condition (stress vs. matched control) by day (day 0 vs. day 8) in defeated rats and matched controls as well as restrained rats and matched controls.
Results

Repeated social defeat, but not restraint, results in bladder hypertrophy. As an initial indication of stress-related bladder dysfunction, bladder weight was measured with respect to body weight and compared between rats exposed to the stressors and their matched controls. Rats subjected to social defeat had greater bladder:body weight ratios (0.37$\pm$0.01, n=67) than matched controls (0.32$\pm$0.01, n=46; p=0.0006). Moreover, the bladder:body weight ratio was negatively correlated with the latency to assume the defeat posture, suggesting a link between bladder dysfunction and submissive behavior (Fig. 1). Social stress did not decrease body weight although weight gain during the 7 days was attenuated for the intruders compared with controls (47g $\pm$3 vs. 35g $\pm$2; t(26)=3.8; p=0.0008). Importantly, because body weight alone was not correlated with defeat latency ($r^2=0.03$, p=0.15), the negative correlation between bladder:body weight ratio and defeat latency is unrelated to differences in body weight. In addition, the mean absolute bladder weight was also increased in social stressed rats above control values (121.1$\pm$2.9 mg vs. 112.2$\pm$2.5 mg, respectively; p=0.033). In contrast to social stress, repeated restraint had no effect on bladder:body weight ratio, which was 0.29$\pm$0.01 (n=19) in restraint rats and 0.30$\pm$0.01 (n=14) in matched controls (p=0.67), suggesting that the bladder hypertrophy produced by social stress does not generalize to all stressors.

Repeated social defeat, but not restraint alters bladder function. Quantification of the voiding pattern after 7 days of social stress was consistent with urinary retention in social stressed rats compared with controls. Prior to any manipulation (day 0), both groups urinated ubiquitously throughout the cage as indicated by a comparable number of voiding spots (Fig. 2B). However, after 7 days of social defeat or control manipulation there was a significant stress effect (F(1,46)=4.4; p=0.04) whereby defeated rats had fewer urination spots than control animals on day 8 (Fig. 2A,B). Furthermore, control animals continued to void throughout the cage, similar to day 0, whereas defeated subjects urinated in the corners of the cage.
(Fig. 2A). On the other hand, repeated restraint stress had no effect on the number of voiding spots compared to controls (F(1,24)=1.03;p=0.32) (Fig. 2C).

Cystometry records revealed marked changes in urodynamic function produced by social stress that in some ways resembled those produced by partial bladder outlet obstruction, a prevalent bladder disorder (38). Representative cystometrograms illustrate numerous non-micturition related contractions indicative of overactive detrusor in the rat exposed to social stress that were not present in controls (Fig. 3A). Additionally, the urodynamic profile of the social stressed rat was characterized by fewer micturition events occurring during an equivalent period of time, and larger bladder capacity and micturition volume compared with the control rat (Fig. 3A). Figure 3A shows that the same number of micturition events (4) occur over a much longer time for the social stressed rat (1200 s) compared to the control rat (420 s). Quantification of the mean urodynamic parameters revealed a significant increase in the intermicturition interval in social stressed subjects compared to matched controls, consistent with urinary retention (Fig. 3B). As a result, both bladder capacity and micturition volume were greater in social stressed rats (Fig. 3B). Additionally, the number of spontaneous non-micturition contractions was also greater in defeated subjects (Fig. 3A,B). One third of the rats exposed to social stress (5 of 15) displayed dribbling incontinence that was absent in unstressed controls. Finally, there was a trend for greater residual urine (202±86 vs. 44±25 l, p=0.07, unpaired t-test) in social stressed rats. Micturition threshold, resting pressure, and peak micturition pressure were comparable between groups (Fig. 3B). Notably, there was no difference in any of these urodynamic parameters in rats exposed to repeated restraint stress compared to their matched controls (Fig. 3C).

Social defeat stress increases CRF-immunoreactive- and CRF mRNA-expressing neurons in Barrington’s nucleus. Given evidence for an inhibitory influence of CRF in the pontine micturition circuit (35), the effect of social stress on CRF expression in Barrington’s nucleus was examined. Figure 4A,B shows representative fluorescent photomicrographs of CRF immunolabeling in the core of Barrington’s nucleus. Quantification revealed that social stress increased the number of CRF-immunoreactive neurons in Barrington’s nucleus (Fig. 4C1). In contrast, restraint stress had no effect on CRF-immunoreactivity in
Barrington’s nucleus (Fig. 4C). Notably, the number of CRF-immunoreactive neurons in Barrington’s nucleus in controls matched for social stress (38.9 ± 3.9, n=9), controls matched for restraint stress (34.9 ± 5.4, n=7) and restraint stress (34.6 ± 6.6, n=8) rats were nearly identical. This experiment was repeated in another cohort of social stressed rats and matched controls at another time and a similar finding that the mean (±SEM) number of CRF-immunoreactive neurons in Barrington’s nucleus was greater in social stressed rats (34.0±2.4; n=17) than controls (23.2±3.6; n=13) was repeated (p=0.02, unpaired Student’s t-test).

To determine whether effects of social stress to increase the number of CRF-immunoreactive neurons in Barrington’s nucleus was the result of increased gene transcription, CRF mRNA was quantified using in situ hybridization. Figure 5A,B shows representative dark field photomicrographs of CRF mRNA expression in Barrington’s nucleus of control and social stressed rats. Quantification revealed that social stress increased the number of CRF mRNA-expressing cells within Barrington’s nucleus (Fig 5C).

Discussion

The present study demonstrated that the social stress of the resident-intruder model produced urinary retention and bladder hypertrophy, consistent with other social stress models (10, 16, 19, 29). This is the first study to systematically characterize the urodynamic changes induced by social stress and to provide evidence for a putative neural mechanism. Cystometry revealed overactive detrusor and pathology similar to that produced by partial bladder outlet obstruction (42). However, unlike partial bladder obstruction, micturition pressure was not elevated, suggesting that the stress-related urinary retention resulted from a decreased drive to the bladder rather than increased outflow resistance. Because CRF has an inhibitory influence in Barrington’s nucleus spinal projections that regulate the micturition reflex (35), CRF upregulation in Barrington’s nucleus by social stress is a putative mechanism for urinary retention. This was further supported by the findings that a non-social stressor, restraint stress, neither
upregulated CRF protein in Barrington’s nucleus, nor produced bladder dysfunction. Differences between social and restraint stress on bladder urodynamics could not be attributed to differences in the magnitude of stress habituation as this is comparable between the two stressors (4, 20). CRF upregulation in Barrington’s nucleus and the associated urinary retention may be a visceral component of an adaptive passive coping strategy that is specific to social stress. However, if maintained, it can result in pathological alterations in bladder structure and function. This process may be involved in dysfunctional voiding in children that is characterized by voiding postponement or may contribute to bladder disorders in adulthood.

Relation to previous studies. Previous studies that have focused on social stress or subordination in rodents report evidence of urinary retention (10, 29). Social subordination in rodents was also reported to produce bladder hypertrophy, a response to long term urine retention (16, 29). Even a mild social stress model that did not involve aggressive encounters but was based on competition for a preferred food, resulted in greater bladder weight in subordinate rats (19). A recent report in mice has further demonstrated that increases in bladder weight induced by social stress is associated with changes in signaling molecules that have been linked to hypertrophy produced by partial bladder obstruction (54). Nonetheless, a systematic characterization of social stress-induced bladder dysfunction has been lacking and the question of how a social stimulus could produce bladder pathology has not been previously addressed.

Bladder dysfunction produced by social stress. The urodynamic profile associated with repeated social stress resembled overactive detrusor and shared characteristics with the pathology seen with partial bladder obstruction. These characteristics include increased bladder capacity, micturition volume, non-micturition related contractions and dribbling incontinence (30). An important distinction between the urodynamic profile associated with social stress and that associated with partial obstruction is the absence of increased micturition pressure in the social stress model. This indicates that in contrast to partial obstruction, which results in increased resistance at the bladder neck, the effects produced by social stress most likely result from decreased central drive to the bladder during the micturition reflex, i.e., decreased
parasympathetic tone. Nonetheless, in the absence of direct measurements of urethral tone, an additional effect on the urethral outlet cannot be completely ruled out. The finding that micturition threshold was unaffected suggests that afferent input from the bladder to the brain is intact. Alterations in sympathetic tone do not explain the urodynamic profile produced by social stress because sympathetic activation increases micturition threshold, an effect not observed with social stress (9). Interestingly, with stress models characterized by increased sympathetic tone, such as the spontaneously hypertensive rat (36) or models using cold stress (6), micturition volume, bladder capacity and intermicturition interval are all decreased and these effects are sensitive to alpha adrenergic antagonists. This profile is in complete contrast with the effects reported here with social stress. Finally, non-voiding contractions have been suggested to result from increased spinal regulation of the micturition reflex, an effect indicative of decreased central regulation (43, 52). Thus, the urodynamic profile associated with social stress best fits one that would be predicted by decreased central excitatory drive to the bladder during the micturition reflex.

The abnormal urodynamic pattern produced by social stress was observed 3-4 days after the last exposure to stress, suggestive of an enduring effect. Previous studies in mice demonstrated that three exposures to social defeat resulted in inhibition of voiding (as determined by voiding pattern) that persists for five weeks after the last stress (29). Whether bladder hypertrophy and abnormal urodynamics following social stress in the rat resident-intruder model endures for weeks after the stress or whether it is reversible is a direction of future studies.

Another compelling question is whether this effect occurs in females. The resident-intruder model used in the present study is not a consistently effective stressor in females. Rather, social instability or isolation may better model social stress and potential urodynamic dysfunction in females (15).

**CRF in Barrington’s nucleus as an underlying mechanism for stress-related bladder dysfunction.**

Excitatory input to the bladder during micturition arises from the sacral parasympathetic preganglionic neurons that give rise to the pelvic nerves (8). These neurons are regulated by Barrington’s nucleus neurons in the pons that terminate in the parasympathetic preganglionic column and use excitatory amino
acid neurotransmission to drive the parasympathetic preganglionic neurons (31, 32, 53). CRF, a stress-related neuropeptide, is prominent in Barrington’s nucleus spinal projections and is therefore a potential mediator of stress effects on micturition (51). CRF has an inhibitory influence in this pontine-spinal pathway. For example, bladder contractions elicited by chemical stimulation of Barrington’s nucleus are increased by intrathecal microinjection of a CRF antagonist and intrathecal microinjection of CRF decreases bladder contractions elicited by Barrington’s nucleus activation (35). Given evidence for the inhibitory function of CRF within this pathway, CRF upregulation in Barrington’s nucleus neurons produced by social stress can account for the associated urinary retention and its enduring consequences on bladder structure and function.

It should be noted that CRF can impact on urination at sites other than the pontine micturition circuit because in addition to this pathway, it is present in bladder urothelium and at sensory levels of the spinal cord (25, 26). Cystometry studies of the effects of CRF agonists and antagonists on bladder urodynamics have reported equivocal results. A study comparing effects in female Wistar-Kyoto rats (WKY) with Spontaneously Hypertensive rats (SHR) demonstrated that systemic or intrathecal administration of CRF increased urinary frequency and decreased micturition threshold and volume in WKY rats only (23). Another cystometry study using male Sprague Dawley rats showed that intrathecal CRF decreased micturition frequency and increased bladder capacity and micturition volume and these effects were dependent on the state of arousal, occurring only in the awake state (22). These discrepant results may be attributed to differences in the sex and strain of experimental subjects as well as the state of arousal during which CRF effects are measured. These studies underscore the importance of these determinants on the effects of CRF-related agents when administered to the whole animal. Additionally, in these studies CRF could be exerting its effect at multiple sites. The comparison of bladder contraction elicited by direct stimulation of Barrington’s nucleus neurons before and after intrathecal administration of CRF antagonists was the only study to directly examine the role of CRF in this specific pathway and revealed an inhibitory influence of CRF that may serve to balance the excitatory drive to preganglionic parasympathetic neurons (35).
**Clinical relevance.** The present findings provide a neurobiological mechanism for the clinical association between social stress and bladder dysfunction. Dysfunctional voiding remains the most common presentation to pediatric urologists (13). Voiding postponement is one form of dysfunctional voiding that can be sufficiently severe as to result in reflux (18). The lack of anatomical pathology and a positive response to psychological treatments suggest a potential psychogenic basis for the disorder and trauma or abuse during critical times in development have been implicated (2, 12). Consistent with this, children with voiding postponement have a less balanced family environment and more psychiatric co-morbidity compared to children with other types of voiding dysfunction (27). The impact of childhood social stress on bladder function can extend into adulthood and childhood sexual or physical abuse are associated with urinary disorders characterized by retention in adults (7, 37). Adult traumatic social events (e.g., loss of a loved one, broken marriage) have also been reported to produce urinary retention (14). Our results suggest that targeting CRF may be a rational approach for the development of drugs to treat these disorders.

**Perspectives and Significance.** Here we show that a social stimulus can profoundly affect the structure and function of viscera. The correlation between subordinate behavior and the visceral response implies that urinary retention in subordinate animals is a visceral component of a passive behavioral repertoire that is engaged in response to social threats. Although transient urinary retention during social stress is acutely adaptive, repeated exposure to the stimulus maintains this visceral response and results in pathological changes in bladder structure and function. One brain region that is poised to coordinate these behavioral and visceral responses is the periaqueductal gray, a region involved in determining active vs. passive coping style (21) that is also a major afferent to Barrington’s nucleus (46). Recent findings that social stress in cichlids cause CRF-mediated bile retention and gall bladder hypertrophy suggest that this passive behavioral-visceral repertoire and its underlying substrates are phylogenetically conserved (11). The conservation of this response in humans can contribute to voiding dysfunction and bladder disorders in susceptible individuals. Future studies designed to identify the neural mechanisms by which social
stimuli impact on the viscera will advance our understanding of how social experiences shape physical health.
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References


Figure Legends

Figure 1. Social stress produced bladder hypertrophy that is negatively correlated to latency to assume a subordinate posture. The abscissa indicates the latency to defeat and the ordinate indicates the bladder:body weight ratio. The horizontal line indicates the mean bladder weight in control rats. Each point represents an individual subject exposed to social stress. There was a significant negative correlation between these endpoints, suggesting that bladder hypertrophy is associated with passive behavior (r= -0.32, p=0.015).

Figure 2. Social stress, but not restraint, resulted in urinary retention. (A) Representative voiding patterns from a control and social stressed rat 24 hours after the seventh and final manipulation. Each image is a montage of two halves of the voiding pattern paper that was cut in half to accommodate the size of the UV light box. (B) Bars indicate the mean number of urine spots for controls (n=9) or social stressed (n=15) rats on day 0 or 24 h after the final manipulation (day 8). (C) Bars indicate the mean number of urine spots for controls (n=6) or restraint stressed (n=8) rats on day 0 or 24 h after the final manipulation (day 8). *p<0.05, Bonferroni post-hoc versus matched controls.

Figure 3. Social stress, but not restraint, altered the urodynamic profile. (A) Representative cystometrograms from a control and defeated rat. From top to bottom the traces show bladder pressure, volume of saline infused (i.e., bladder capacity), and micturition volume. Figure 3A shows that the same number of micturition events occur over a much longer time for the social stressed rat (1200 s) compared to the control rat (420 s). Therefore, the time scale on the abscissae differs between the two examples to illustrate 4 voiding events for each animal. (B) Bars are the mean values of controls (open bars, n=9) and social stressed (solid bars, n=15) rats for inter-micturition interval, bladder capacity (BC), micturition volume (MV), micturition threshold (MT), micturition pressure (MP), resting pressure and non-micturition contractions. (C) Bars are the mean values of controls (open bars, n=6) and restraint stressed
(solid bars, n=8) rats for the same urodynamic parameters as shown in B. *p<0.05, **p<0.01, ***p<0.001 unpaired Student’s t-test.

Figure 4. Social stress, but not restraint, increased the number of CRF-immunoreactive neurons in Barrington’s nucleus. (A) Fluorescent photomicrographs of CRF-immunoreactive neurons in Barrington’s nucleus of representative control rats from the defeat (A1) and restraint (A2) study. (B) Fluorescent photomicrographs of CRF-immunoreactive neurons of a social stressed (B1) and restrained (B2) rat 24 hours after the 7th manipulation. Photomicrographs were grouped and contrast and brightness was equally adjusted using Adobe Photoshop. The arrows point to the cluster of CRF-immunoreactive neurons in Barrington’s nucleus. The arrowheads point to a CRF-immunoreactive terminal field dorsolateral to Barrington’s nucleus that derives from cell bodies in the central nucleus of the amygdala (40, 48). Top is dorsal in all photomicrographs. In A1 and B1 medial is to the right. In A2 and B2 medial is to the left. The calibration bar represents 100 μm. (C1) Bars represent the mean number of CRF-immunoreactive cells in Barrington’s nucleus for controls (n=9) and social stressed (n=12) rats. (C2) Bars represent the mean number of CRF-immunoreactive cells in Barrington’s nucleus for controls (n=7) and restraint stressed (n=8) rats. **p<0.01, Student’s unpaired t-test.

Figure 5. Social stress upregulated CRF mRNA expression in Barrington’s nucleus. (A and B) Dark field photomicrographs showing hybridization signal for CRF mRNA in Barrington’s nucleus of a representative control (A) and social stressed (B) rat 24 hours after the 7th manipulation. Photomicrographs were grouped and contrast and brightness was equally adjusted using Adobe Photoshop. Calibration bar represents 100 μm. (C) Bars represent the mean number of CRF mRNA expressing cells in Barrington’s nucleus in control (n=8) and social stressed (n=11) rats. **p<0.01, Student’s unpaired t-test.