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Chronic social defeat, but not restraint stress, alters bladder function in mice

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HIGHLIGHTS

- Two chronic stressors on mouse behavior and bladder function were studied.
- The stressors generated different effects on locomotor behavior.
- Susceptibility to bladder dysfunction occurred only in response to social defeat.

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ABSTRACT

Background: Voiding disorders in humans, particularly in children are associated with increased incidence of behavioral issues as well as past history of childhood abuse. We hypothesized that creating stress in mice, utilizing either a chronic social defeat model (SD) or restraint stress in shallow water model (RSSW) would engender changes in bladder function, morphology, and behavior, thereby enabling us to study the resultant voiding dysfunction.

Methods: For SD stress (14 days), C57BL/6 male mice were exposed daily to a larger aggressive CD-1 male for 10 min, followed by sensory exposure in a barrier cage for 24 h. Control mice were similarly housed with no exposure. For RSSW (21 days), C57BL/6 mice were put in a perforated conical tube with feet immersed in water daily for 4 h, then returned to single housing cages. Control mice were also in single housing. After the stress period, voiding patterns were obtained on filter paper, followed by behavioral tests. At necropsy, blood was taken for corticosterone analysis, and bladder and body weights measured. Bladder cryosections were stained with hematoxylin and eosin (H&E) for morphological assessment. Sequential sections were immunostained with antibodies to Ki-67 as a proliferation marker, CD31 (endothelial cell marker), and uroplakin-II. ImageJ software was used to measure bladder wall thickness on blinded H&E photomicrographs as well as quantitate CD31 staining. Both Ki-67-positive and -negative nuclei were counted with Imaris software to obtain a proliferation index.

Results: Only SD mice had a single large void pattern. Bladder-to-body weight ratios increased in SD mice ($p \leq 0.02$) but not in RSSW mice. Plasma corticosterone levels were elevated in all stressed mice. SD mice exhibited lower levels of locomotor activity compared with controls; RSSW mice were hyperactive. In SD mice, bladder wall thickness was increased ($p \leq 0.003$) but no change was seen in Ki-67 proliferation index, consistent with hypertrophy. No difference with control mice was seen in vascularity as visualized by CD31 staining. Uniform uroplakin-II staining lined the urothelium of both SD and control mice.

Conclusions: Mice exposed to repeated SD (14 days) respond with altered voiding indicative of urine retention, and exhibit bladder wall changes consistent with hypertrophy while the urothelial barrier is maintained. These changes were not observed with repeated RSSW. SD, in contrast to RSSW, provides a model of psychological stress to further study the interplay of behavior and bladder dysfunction, enabling an improved understanding of voiding dysfunction, and the ability to create innovative and more effective management pathways for children who present with voiding dysfunction.

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1. Introduction

Disturbances of urinary bladder filling and voiding cycles are reflected in a number of diverse symptoms including urgency, incontinence, and changes in frequency of voiding, that are collectively termed LUTS (lower urinary tract symptoms). The prevalence of LUTS in adults, obtained from large-scale population studies, is high for both men and women [9,15] with symptoms such as urgency self-reported in 22.4% of men and 35.7% of women. In a cross-sectional study of school-age children LUTS were detected in 21.8%, with urgency reported in 13.7% [34]; a recent review estimated the prevalence of incontinence in children at 17% [11].

LUTS may arise from an underlying neurological abnormality (termed neurogenic LUTS) or from non-neurogenic abnormalities that may be due to anatomic malformation or be of unknown etiology. Behavioral and psychological disorders have been documented to coexist in children with LUTS more often than for children with normal voiding habits [19,24,30,36]. Findings from a number of studies agree that 20–25% of children with LUTS also have mental or behavioral health problems including anxiety, depression, attention deficit/hyperactivity disorder, or oppositional-defiant disorder. These findings have therapeutic implications for optimal treatment strategies for these children. Furthermore, it has been speculated that this close association may indicate an underlying role of psychosocial disorders in the etiology of LUTS in certain individuals [35]. While it is established that the central nervous system controls certain basic aspects of lower urinary tract function, the influence of psychosocial disorders and chronic stress on neural circuits controlling micturition has yet to be fully characterized and understood. This gap in knowledge is a significant barrier to improving clinical outcomes for those affected with LUTS.

For rodents, the subordination of one male by a more aggressive male (social defeat) provides a paradigm for socially-induced psychological stress and leads to alterations in behavior indicative of high anxiety and/or depression [13,16,17]. Similar study designs have also illustrated changes in immune function and visceral tissues, including the appearance of spontaneous colitis [28] and evidence of cardiac remodeling [8]. Splenomegaly occurs, accompanied by decreased sensitivity of immune cells to glucocorticoids, perhaps as an adaptive response to social defeat and the increased likelihood of sustaining a bite wound [3]. Of interest, it has been demonstrated that even age-matched mice of the same inbred strain and genetic background studied under identical conditions will vary in their responses to social defeat [2,17].

During an episode of social defeat the dominant mouse exhibits aggressive behavior, such as chasing, grappling and biting, while the subordinate displays avoidance behaviors including defensive upright posturing and immobility. Urinary voiding is affected by social rank in male mice so that the subordinate male withholds from voiding [12]. Previous work with rodent models of social defeat have shown that chronic social stress in the subordinate animal leads to changes in voiding patterns, and eventual physiological and molecular alterations in the bladder [6,37]. In this paper we sought to replicate the voiding dysfunction in mice caused by social defeat stress as well as examine the effects of another chronic psychogenic stress modality. For this we employed repeated daily periods of restraint during which the mice were also placed in shallow water.

2. Material and methods

2.1. Mice and housing

Mice were housed in a pathogen-free vivarium that uses the Modular Animal Caging System (Alternative Design, Siloam Spring, AR) with HEPA filtered air supplied via the Flex-Air System (Alternative Design, Siloam Spring, AR) at 30 air changes/h. Water was provided ad libitum by an automated system (SE Lab Group, Napa, CA). Each cage had ad libitum food, contained corncob bedding, and a nestlet to provide partial enrichment. Mice were maintained on a 14 h light:10 h dark (lights on at 600 h) schedule. Protocols were approved by the Institutional Animal Care and Use Committee. The vivarium is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

2.2. Social defeat model (SD)

Male C57BL/6N (B6) mice (46–63 days old) and CD-1 mice (4–6 months old, retired breeders) were obtained from Charles River Breeding Laboratories (Wilmington, MA). All mice were single housed in polysulfone plastic cages on stainless steel grid platforms over corncob bedding for 1 week prior to experimentation. Housing on a grid platform was required in order to minimize chewing and tearing of the filter papers used in obtaining urinary void patterns (see below). Daily social defeat encounters were carried out during the light period from 3–5 pm each day for 14 days, based on the protocol of Golden and colleagues [13] with the following modifications. Retired breeder CD-1 male mice were used as the resident (aggressor) mice. To test the aggressive behavior of the CD-1 mice, we used a separate cohort of B6 mice. Briefly, a naïve B6 mouse was added to the home cage of the CD-1 mouse and the time to attack was measured. Only CD-1 mice that attacked within 3 min were used in subsequent social defeat experiments. One week prior to the start of the experiment each CD-1 mouse was singly housed in a Thoren Weaning Cage ($31 \times 31 \times 14$ cm, Thoren Caging System, Inc., Hazleton, PA) with a stainless steel platform grid over corncob bedding and a nestlet. Each cage had a stainless steel double feeder with 2 water bottle holders, a top filter cover, and a perforated Plexiglas divider (crafted in house) that can be inserted in the middle to provide separate housing.

On day 1, a naïve B6 male ($n = 20$) was added to a Thoren cage containing a resident CD-1 mouse and a timer was started. The time to first attack was noted and the mice were kept in physical contact for a total of 10 min under constant observation. Then a perforated Plexiglas divider was inserted into the cage, separating the 2 mice physically but allowing sensory contact. Fresh nestlets were added to each compartment. If wounding occurred prior to 10 min, the mice were separated immediately. 24 h later the B6 mice were rotated to the home Thoren cage of a different CD-1 male, and the procedure repeated, for a total of 14 consecutive days. Platform grids were cleaned between each encounter. If no attack occurred within 3 min, the B6 mouse was removed and placed with another CD-1 mouse. Control B6 mice ($n = 16$) were singly housed on one side of the Plexiglas divider in grid-floored Thoren cages with no CD-1 mouse present. Each day, the grid platforms of the control cages were cleaned and the mice given new nestlets.

2.3. Restraint stress in shallow water model (RSSW)

Adult male C57BL/6J mice (~56 days of age) were obtained from Jackson Laboratories (Bar Harbor, ME) and singly housed in polysulfonate cages for 7 days prior to experimentation. Mice were randomly selected as controls (10 mice) or restraint condition (10 mice). Water-restraint stress was performed as described by Tomita and colleagues [31] with the following modifications. Mice were restrained in 50 ml conical tubes that had 10 holes to allow the mice to breathe. The tubes were then placed horizontally in a 12×8 cm plastic box and water (22 ± 1 °C) added to a depth of 0.9 cm to cover their ventral surface. Restraint occurred daily for 21 days for 4 h, beginning between the hours of 9 am and 1 pm each day. Control mice were left undisturbed in their individual home cages during this time.

2.4. Void pattern determination

Bladder voiding patterns for control and stressed mice were obtained on Whatman 3 MM chromatography paper (Fisher Scientific,