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THE EFFECT OF ACUTE AND CHRONIC STRESS AND ANXIETY-LIKE BEHAVIOUR ON BLADDER MOTOR AND SENSORY FUNCTION

Hypothesis / aims of study

Stress appears to play a role in the exacerbation and possibly the development of functional urinary tract disorders including painful bladder syndrome (PBS) and overactive bladder (OAB). Acute stress increases bladder pain and urgency in many of these individuals. To better understand the mechanisms underlying this relationship, we aimed to characterize changes in micturition frequency, interval, and anxiety-related behaviour in rats exposed to 10 days of chronic psychological stress.

Study design, materials and methods

24 adult female Wistar rats (200-300g, Charles River, Wilmington, MA) were subjected to water avoidance (WA) stress or sham 1 hour a day for 10 consecutive days. This model represents a potent psychological stressor with elevations of ACTH and corticosterone [1]. Estrous cycles were not controlled for.

WA stress protocol. Rats were placed on a glass platform in the center of a plastic container filled with 25°C water up to 1 cm below the top of the glass platform. In the sham group, the plastic container was empty.

Colonic Assessment. The number of fecal pellets excreted was counted as a measure of stress induced colonic motility [2].

Voiding assessment. Immediately after day 1 of WA stress or sham (acute stress) and on day 10 (chronic stress) rats were placed in a metabolic cage (Tecniplast USA, Exton, PA) for a 2 hour voiding assessment.

Urine markers. Urine norepinephrine levels were determined by ELISA (Rocky Mountain Diagnostics, Colorado Springs, CO) on days 1 and 10 and compared to baseline values.

Light-dark box transition test. To quantify anxiety-like behaviour of the rats we used a light-dark box (LDB) transition test. The apparatus consisted of a 76 x 30 x 30 cm glass aquarium divided into two sections. The lighted section was brightly illuminated (360 lux) using a 60 W white light bulb. The dark chamber was painted black and covered with a light-proof lid. Animals were placed on the light side and allowed to move freely between the two chambers for 10 minutes and recorded by video camera.

Histological examination. Four animals from each group were sacrificed on day 10 and the bladders removed, fixed, sectioned and stained with hematoxylin and eosin. Bladder tissue was evaluated for structural changes in three non-contiguous locations.

Repeat voiding assessments. The remaining 8 animals in each group underwent repeat voiding assessment every three days for one month.

Statistical analysis. Student's t-test was used to determine statistical significance.

Results

Rats exposed to WA stress but not sham developed persistent significant increases in anxiety-like behaviour, micturition frequency and decreases in latency to first void, voiding interval and volume of first void when compared to baseline (Table 1). Stressed rats also showed enhanced fecal pellet excretion and increased bladder angiogenesis. No detectable difference in urinary norepinephrine levels was appreciated. Alteration in micturition parameters following the termination of the stress protocol persisted for at least one month.

Table 1. Micturition parameters following water avoidance stress or sham, n=24.

	Acute WA Stress	**Acute WA Sham**	**p-value**	**Chronic WA Stress**	**Chronic WA Sham**	**p-value**	**Baseline**
Total # Voids/2 Hours	5.8	3.4	**0.05**	7.2	3.8	**0.02**	**2.9***
Latency to 1st Void (min)	9.6	35.4	**<0.01**	8.0	29.2	**<0.01**	**42.0***
# Minutes btw Voids (min)	20.4	31.8	**0.05**	18.3	34.4	0.11	**42.1***
Volume of 1st Void (ml)	0.5	1.0	**0.02**	0.5	0.8	**0.03**	**0.92***
Total Volume Voided (ml)	4.6	2.4	0.08	5.2	3.2	0.21	2.4
Volume per Void (ml)	0.7	0.9	0.53	0.6	0.8	0.19	**1.01***

***** Indicates statistically significant difference between baseline and both acute and chronic stress.

Interpretation of results

Chronic psychological stress in rats results in a robust and long lasting alteration of micturition parameters. The response appears to be related to hypothalamic-pituitary axis activation secondary to stress resulting in end organ functional manifestations. Similar effects on the gastrointestinal system together with these findings argue for initiation of this response centrally.

Concluding message

We present a novel model of urinary frequency in the rat subjected to WA stress. Previous data suggests this model of stress simulates irritable bowel syndrome with respect to fecal pellet output and mucosal changes and confirms elevation in stress hormone levels. Together, our data suggest this model may represent a valid tool for studying PBS and OAB.

References

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