

## TEMPORAL EFFECTS OF VAGINAL DISTENSION AND BILATERAL PUDENDAL NERVE TRANSECTION ON LEAK-POINT PRESSURE AND URETHRAL ANATOMY IN FEMALE MICE

### Hypothesis / aims of study

Vaginal distension (VD) and pudendal nerve injury (PNI) have been used for creation of stress urinary incontinence (SUI) in rats. We have recently reported successful induction of SUI in mice by VD. In this study, we aimed to examine the time course of recovery after VD and PNI in mice and to investigate the possible mechanism of SUI caused by these models.

### Study design, materials and methods

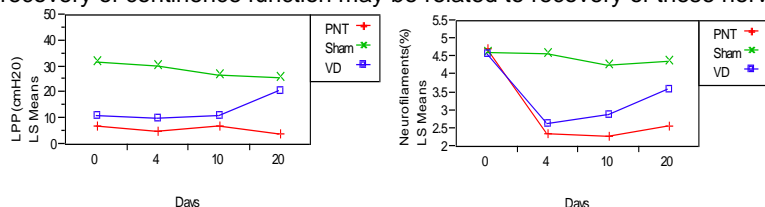
Seventy two virgin female C57BL/6 mice were randomly distributed into three groups. Vaginal distention (VD) group underwent VD for 1 hour, using a modified 6-Fr. Foley catheter with a balloon dilated to 0.3ml. Pudendal nerve transection (PNT) group received bilateral PNT, as described before. Sham VD group only received insertion of the uninflated 6-Fr Foley catheter for 1 hour. Each group was divided into four subgroups for performing leak-point pressure (LPP) at 0, 4, 10, and 20 days, after VD or PNI. All mice underwent suprapubic tube (SPT) implantation 2 days before performing LPP. After sacrifice, the urethras of the mice were harvested for histological examination and examination of NF-200 immunoreactive nerve terminals. The differences between groups were compared with two sided Student t-test at 0.05 significance level.

### Results

LPPs were significantly lower 0, 4 and 10 days after VD ( $10.88 \pm 1.52$ ,  $10.04 \pm 2.36$ , and  $10.93 \pm 2.11$  cm H<sub>2</sub>O,  $p < 0.05$ ) compared to sham VD ( $32.02 \pm 4.30$ ,  $30.20 \pm 5.90$ , and  $26.72 \pm 1.89$  cm H<sub>2</sub>O). LPPs 20 days after VD ( $20.47 \pm 2.98$  cm H<sub>2</sub>O) and sham VD ( $25.66 \pm 3.26$  cm H<sub>2</sub>O) showed no significant differences ( $p = 0.196$ ), indicating the recovery of the continence in mice occurs 20 days after VD. LPPs were significantly lower 0, 4, 10 and 20 days after PNI ( $6.85 \pm 2.63$ ,  $5.01 \pm 1.30$ ,  $6.90 \pm 2.43$ , and  $4.10 \pm 0.62$  cm H<sub>2</sub>O,  $p < 0.05$ ) than control (sham VD). Histological examination showed no significant difference in the thickness of urethral striated muscle between all subgroups. The density of immunoreactive neurofilaments in the urethra was significantly reduced 4 and 10 days after VD ( $2.61 \pm 0.05$ ,  $2.88 \pm 0.19\%$ ,  $p < 0.05$ ) and 4, 10, and 20 days after PNT ( $2.45 \pm 0.11$ ,  $2.27 \pm 0.28$ ,  $2.57 \pm 0.65\%$ ,  $p < 0.05$ ). The density of neurofilaments 20 days after VD ( $3.59 \pm 0.35\%$ ) was similar to 20 days of Sham VD ( $4.44 \pm 0.09\%$ ,  $p = 1.00$ ), indicating the source of recovery of continence function may be related to recovery of these nerve terminals.

### Interpretation of results

LPPs 20 days after VD and sham VD showed no significant differences, indicating the recovery of the continence in mice occurs 20 days after VD. The density of neurofilaments 20 days after VD was similar to 20 days of Sham VD indicating the source of recovery of continence function may be related to recovery of these nerve terminals.



### Concluding message

VD and PNI cause durable SUI in female mice, as measured by LPP. SUI after VD recovers by 20 days. Such recovery is temporally associated with recovery of the NF-200 immunoreactive nerve terminals in the urethra, indicating a key role for these elements in recovery of continence mechanisms after VD. Our created models could be used for mechanistic investigation of SUI by taking advantage of transgene and knock out technology in mice.

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<b>Is this a clinical trial?</b>	No
<b>What were the subjects in the study?</b>	ANIMAL
<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	Yes
<b>Name of ethics committee</b>	Cleveland Clinic's Institutional Animal Care and Use Committee