

## VALIDATION OF RAT OVERACTIVE BLADDER MODEL BY BLADDER WALL STRESS ESTIMATION

### Hypothesis / aims of study

In recent times, continuous efforts have been made to correlate afferent nerve activity in overactive bladder (OAB) models to a number of mechanical parameters [1], the most common being intravesical pressure. However relating afferent activity to pressure seems an oversimplification for the following reasons: - The pressure threshold for afferent nerve firing has been shown to be highly dependent on the filling rate [2] and, at comparable pressures, the afferent activity is higher before voiding than after it, which could be explained if bladder volume is considered a controlling factor [1]. In a model proposed by Le Feber et.al [1], it has been shown that afferent activity (in normal rats) correlates better with wall stress (and also with the product of intravesical pressure and volume, which is proportional to wall stress) than with intravesical pressure. In the present study we estimated bladder wall stress in the relaxation phase of voiding contractions of normal and Acetic Acid induced overactive rat bladders to validate this model for OAB.

### Study design, materials and methods

Male Wistar rats (mean weight  $395 \pm 37$ g) were anaesthetized with urethane (1 g/kg) and the bladder was exposed through an abdominal incision. Bladder filling (0.11 ml/min) and measurement of pressure ( $p_{ves}$ ) were performed by inserting a 23G needle at the top of the bladder, the other end of the needle was connected to a pressure transducer and an infusion pump. The bladder was filled repeatedly with saline (0.9% NaCl) or 0.5 % Acetic Acid (a proposed model for overactive bladder [3]) for 10 minutes or until a voiding contraction occurred, whichever happened first. The measured pressure was sampled at 25 Hz. Recorded signals were processed and analysed with a custom written MATLAB® program. After the measurements the rats were euthanized by injecting an overdose of KCL in the heart. A five second interval (T1-T2, fig 1) starting from the maximum pressure, immediately after cessation of the high frequency oscillation (T0-T1, caused by rapid contractions of the urethral sphincter during voiding of the male rat) was chosen for bladder wall stress estimation in normal (saline) and overactive bladder (AA) condition. The  $p_{ves}$  was normalized by dividing by  $p_{max}$  (fig. 1, the maximum pressure attained before voiding). Additionally urodynamic parameters like residual volume, voiding interval and number of spontaneous contractions were studied.

Data selection criteria: Only those measurements in which the pressure declined smoothly to baseline after T1 and the residual volume was more than 0.33 ml were chosen for bladder wall stress estimation.

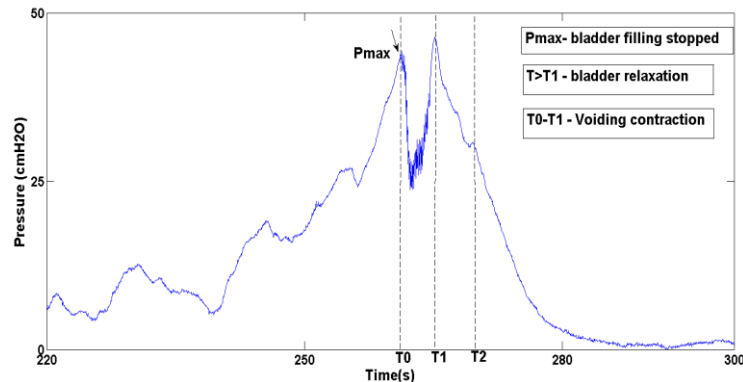


Fig.1 An example of bladder pressure during a voiding contraction (marked by high frequency oscillations of urethral sphincter, T0-T1) after Acetic Acid filling.

### Bladder wall stress estimation

Bladder wall stress has been shown to be proportional to  $p_{ves} \cdot V$  by Le Feber et. al (1), when bladder volume,  $V > 0.33$ cc.

### Results

From 29 voiding contraction measurements in 8 rats, 19 measurements (Table 1) were selected for the bladder wall stress estimation. The other 10 measurements were excluded from the analysis based on the criteria described in the preceding section. The mean value of normalized  $p_{ves}$  in the interval (T1-T2, fig1) was ~38% higher in saline as compared to AA measurements, (ANOVA,  $p < 0.005$ ). The mean value of estimated stress (normalised  $p_{ves}$  times  $V$ ) was ~45% higher in saline as compared to AA measurements (ANOVA,  $p < 0.005$ ). The stress and pressure did not vary significantly between animals (ANOVA,  $p > 0.05$ ). The urodynamic measurements suggested bladder overactivity on acetic acid fillings, as evidenced by a decrease in voiding interval and an increase in number of spontaneous contractions (observed visually during data acquisition) in a given period of time (fig 2). The residual volume was 7% higher in AA as compared to saline measurements, however the difference was not significant ( $p > 0.05$ ). A summary of results is provided in table 2.

	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8
Saline (n=7)	-	1	1	2	2	-	1	-
AA (n=12)	1	-	4	2	1	3	-	1

Table 1. The selected Saline and Acetic acid (AA) measurements

	Stress	Pressure(normalized)	Residual volume (ml)
Saline	$0.49 \pm 0.1$	$0.75 \pm 0.1$	$0.65 \pm 0.11$
Acetic Acid	$0.26 \pm 0.1$	$0.47 \pm 0.2$	$0.6 \pm 0.19$

Table2. A summary of results (mean  $\pm$  standard deviation

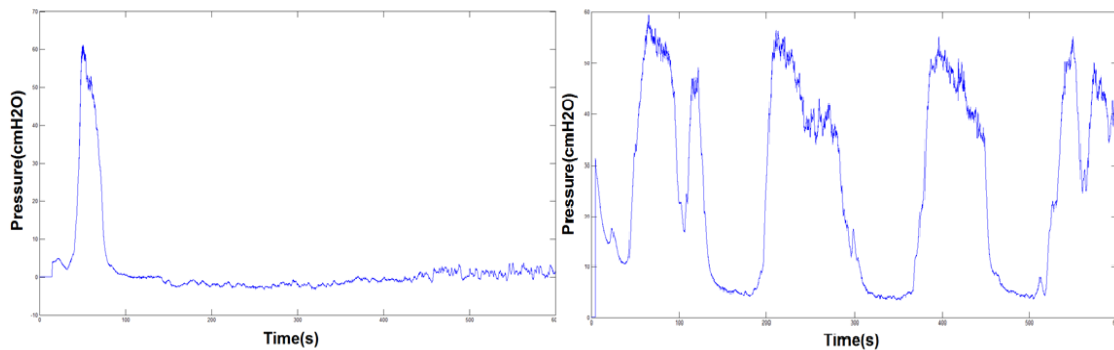


Fig 2 Example of 10 min. of pressure recording showing spontaneous contractions in the same rat with a single filling of a) saline, and b) Acetic Acid.

#### Interpretation of results

Using the Acetic Acid model of OAB, we observed overactivity in rat bladders in terms of a decrease in inter-voiding interval and an increase in the number of spontaneous contractions (findings consistent with the literature). As a next step we estimated bladder wall stress in normal (saline) and OAB (AA) condition. In our analysis bladder wall stress during the relaxation phase was significantly lower in AA than in saline. Assuming that stress is related to afferent nerve activity as shown before, it can be deduced that afferent nerve activity in the relaxation phase after voiding is lower in the overactive rat bladder model than in the normal rat bladder. In addition to the lower  $p_{ves}$ , this might also be related to a change in bladder capacity as; - afferent fibers become active at lower volumes due to their increased sensitivity i.e. their threshold for firing becomes lower.

#### Concluding message

Bladder wall stress estimation along with afferent activity recording in a rat overactive bladder model could provide a better understanding of the underlying mechanism behind the bladder overactivity. As a first step we have validated an Acetic Acid instillation OAB model by bladder wall stress estimation. A further validation of the used model and the obtained results by including afferent nerve activity measurements is needed.

#### References

1. le Feber J, van Asselt E, van Mastrigt R. Afferent bladder nerve activity in the rat: A mechanism for starting and stopping voiding contractions. *Urol Res.*32:395–405, 2004
2. Moss, N., W. Harrington, and M. Tucker. Pressure, volume, and chemosensitivity in afferent innervation of urinary bladder in rats. *Am. J Physiol.* 272: R695-R703, 1997.
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#### Disclosures

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