A novel urodynamic model for lower urinary tract assessment in awake rats

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Objectives
To develop a urodynamic model incorporating external urethral sphincter (EUS) electromyography (EMG) in awake rats.

Materials and Methods
Bladder catheters and EUS EMG electrodes were implanted in female Sprague Dawley rats. Assessments were performed in awake, lightly restrained rats on postoperative day 12–14. Measurements were repeated in the same rat on day 16 under urethane anaesthesia. Urodynamics and EUS EMG were performed simultaneously. In addition, serum creatinine and bladder histology was assessed.

Results
No significant differences in urodynamic parameters were found between bladder catheter only vs bladder catheter and EUS EMG electrode groups. Urethane anaesthesia evoked prominent changes in both urodynamic parameters and EUS EMG. Serum creatinine was within the normal limits in all rats. Bladder weight and bladder wall thickness were significantly increased in both the bladder catheter only and the bladder catheter and EUS EMG group compared with controls.

Conclusions
Our novel urodynamic model allows repetitive measurements of both bladder and EUS function at different time points in the same rat under fully awake conditions and opens promising avenues to investigate lower urinary tract dysfunction in a translational approach.

Keywords
urodynamics, rat, external urethral sphincter (EUS), electromyography (EMG), urethane
Experimental design (details in Supplement 1): Rats were divided randomly into three groups: (i) bladder catheter only group (four rats), (ii) bladder catheter and EUS electromyography (EMG) group (six), and (iii) control (i.e. naïve) group (four). Controls were used for creatinine assessment and histology only. To minimise implant-associated bladder dysfunction, urodynamics were not performed immediately but on postoperative day 12–14 in all groups with simultaneous EUS EMG measurement (where appropriate) [2]. On day 16 the same rats were administered 600 mg/kg urethane and urodynamics/EUS EMG assessed 30 min later.

Surgery (details in Supplements 1 and 2): Rats were anaesthetised with ketamine/xylazine and bladder catheters inserted into the bladder dome and secured with a purse string suture. Where indicated, EMG electrodes were affixed to the fat tissue beside the EUS and a ground wire sutured to the abdominal muscle. The bladder catheter and wires were tunneled s.c. to the back of the neck and the rat fitted with an infusion harness (QC Single, SAI Infusion Technologies, USA) and allowed 12–14 days to recover.

Urodynamic and EUS EMG measurements (details in Supplement 1): As shown in Fig. 1a and pictured in Fig. 1b, awake rats were positioned in a modified restrainer (modified from item # HLD-RM, Kent Scientific, Connecticut, USA) with a funnel situated under the urethra, as previously described [3]. The restrainer was then placed in a modified Small Animal Cystometry Lab Station (Catamount Research and Development Inc.; St. Albans, Vermont, USA) with a scale below the funnel. The bladder catheter was attached to a syringe pump with an in-line pressure transducer and the electrodes (where relevant) connected to an amplifier/converter. Saline was instilled (120 μL/min) and all parameters (pressure, scale, voltage) recorded simultaneously for at least three voiding cycles.
Post-mortem assessments (details in Supplement 1): At the end of the experiment, blood was obtained by heart puncture and creatinine assessed by standard ELISA techniques. Bladders were removed, weighed and the central third fixed, embedded and sectioned (5 μm). Sections were then stained with haematoxylin and eosin (H&E) or Masson’s trichrome stain using routine methods.

Statistical analysis (details in Supplement 1): Data are reported as mean ± standard deviation (SD). Comparing related and unrelated samples, the paired and unpaired t-test was used. To test for differences among the three groups, one-way ANOVA was used. The value of significance was considered at P < 0.05. Statistical analyses were performed using GraphPad Prism, version 6.01 (GraphPad Software, CA, USA).

Results
Urodynamic Investigation in Awake Rats
Rats tolerated the harness with the catheter port and the electrode plug very well; there were no losses (total 10 rats) over the 3 weeks of the experiment. The rats were acclimated to the urodynamic measurement cabinet for 5 days, after which they stayed in the restraint position during the 1-h measurement period without any signs of stress or discomfort.

A typical analysis from a postoperative day 12–14 rat with bladder catheter and EUS EMG is depicted in Fig. 2a and includes a pressure tracing from the bladder, the determination of secreted urine (g on the scale) and the EUS EMG traces. An expanded graph of a single void is shown in Fig. 2b. Voiding consists typically of four phases [2,4], which are indicated on the figure. Phases:

- **α**: initial increase of intravesical pressure with parallel increase of the EUS EMG activity due to the guarding reflex.
- **β**: intravesical pressure increased with high-frequency oscillations during pulsatile flow of urine. The EUS EMG shows the specific slow wave bursting.
- **γ**: rebound increase in intravesical pressure (end of pulsatile flow). The reappearance of the high-amplitude high-frequency bursting in the EUS EMG is indicative of a contraction and reappearance of the guarding reflex.
- **δ**: rapid intravesical pressure decline to the level before the voiding contraction.

**Fig. 2** (a) 1625-s window of a representative urodynamic tracing from a rat with bladder catheter and EUS EMG showing three voiding cycles. The first voiding cycle includes moving artefacts and serves for adaptation of the rat. The second and third voiding cycles are representative for an awake rat regardless of group. The top panel shows the bladder pressure tracing, the middle panel the secreted urine weight tracing and the bottom panel the EUS EMG tracing.

- **biP**: baseline pressure: lowest pressure between two voids;
- **TP**: threshold pressure: pressure shortly before the void is started;
- **Pmax**: maximum voiding pressure: highest pressure during the voiding cycle.

(b) 50-s window culled from (a). Top panel is the pressure tracing, middle panel the scale tracing and the bottom panel the EUS EMG tracing. The voiding consists of four phases (adapted from [3, 4]):

- **α**: initial increase of intravesical pressure with parallel increase of the EUS EMG activity due to the guarding reflex.
- **β**: intravesical pressure increased with high-frequency oscillations during pulsatile flow of urine. The EUS EMG shows the specific slow wave bursting.
- **γ**: rebound increase in intravesical pressure (end of pulsatile flow). The reappearance of the high-amplitude high-frequency bursting in the EUS EMG is indicative of a contraction and reappearance of the guarding reflex.
- **δ**: rapid intravesical pressure decline to the level before the voiding contraction.

(c) 4-s zoomed window from the EUS EMG from (b) before the voiding has started. Most prominent pattern is a low-amplitude high-frequency bursting.

(d) 4-second zoomed window from the EUS EMG from (b) during the voiding. Most prominent pattern is a high-amplitude low-frequency bursting with medium-amplitude high-frequency bursting between the slow-wave bursting.

(e) 4-s zoomed window from the EUS EMG from (b) after the voiding. Most prominent pattern is a high-amplitude high-frequency bursting.
intravesical pressure with parallel increase of the EUS EMG activity due to the guarding reflex. Phase β: intravesical pressure increase with high-frequency oscillations (pulsatile flow of urine). The EUS EMG shows the specific slow wave bursting. Phase γ: rebound increase in intravesical pressure (end of pulsatile flow). The reappearance of the high-amplitude high-frequency bursting in the EUS EMG is indicative of a contraction and reappearance of the guarding reflex. Phase δ: rapid intravesical pressure decline to the level before the voiding contraction.

Quantitation of the urodynamic parameters (bladder compliance, mean flow, voiding duration, maximum voiding pressure and voided volume) in the rats with bladder catheter only and rats with bladder catheter and EUS EMG electrodes were presented in Table 1 and show that there were no significant differences between the two groups.

Table 1 Urodynamic variables in the bladder catheter only vs the bladder catheter and EUS EMG group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bladder catheter group</th>
<th>Bladder catheter and EUS EMG group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder compliance, mL/cmH2O</td>
<td>0.27 (0.07)</td>
<td>0.21 (0.09)</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean flow, μL/s</td>
<td>259.0 (59.2)</td>
<td>251.3 (74.63)</td>
<td>0.9</td>
</tr>
<tr>
<td>Voiding duration, s</td>
<td>5.97 (0.21)</td>
<td>6.71 (1.53)</td>
<td>0.4</td>
</tr>
<tr>
<td>Maximum voiding pressure, cmH2O</td>
<td>38.90 (13.44)</td>
<td>42.19 (11.65)</td>
<td>0.7</td>
</tr>
<tr>
<td>Voided volume, mL</td>
<td>1.58 (0.42)</td>
<td>1.63 (0.31)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Discussion

Our present findings show that chronic, combined bladder catheter and EUS EMG electrodes in the same animal do not impair bladder function in the awake rat. On the other hand, urethane anaesthesia significantly alters both detrusor and EUS activities. To the best of our knowledge, this is the first presentation of a rodent urodynamic model for repetitive lower urinary tract assessment that includes EUS EMG analysis in an awake rat. Moreover, given the nondestructive nature of the measurements, this model allows for repetitive analysis at different time points in the same rat. Thus, our novel urodynamic rodent model opens promising avenues to investigate LUTD in a translational approach.

Anaesthetic drugs are well known to impair lower urinary tract function [5–7]. Thus, to represent the situation in everyday life as close as possible, human urodynamics (which includes EUS EMG) is performed in an awake state without anaesthetics [8]. However, in animals, all existing studies that included urodynamics and EUS EMG were carried out under anaesthesia [9–11]. Although urethane seems to be the best available anaesthetic to maintain the micturition response [2,12], it strongly impairs bladder function, leading to significant differences in urodynamic findings compared with the awake state [13]. In the present study, we observed lower baseline amplitude of high-frequency bursting before, during and after voiding in the urethane-treated rat, showing the lower basal EUS activity. Decreased EUS activity results in lower bladder outlet resistance, which might explain the lower maximum voiding pressure in the anaesthesised rats, as less pressure is needed to overcome a lower intravesical resistance.
It is described in literature, based on urethane anaesthetised measurements, that the slow-wave bursting, the most prominent pattern during voiding, facilitates a sufficient urination [10]. Leung et al. [14] generally supported this opinion in a series of experiments using restrained, awake rats tested shortly after the implantation of the bladder catheter and EUS EMG electrodes. However, their model is hampered by the fact that measurements were performed immediately after surgery where postoperative pain and the anaesthetics used for the implantation surgery are likely to have affected bladder function. Additional, as mentioned by Andersson et al. [2], the implantation causes acutely smaller voiding volumes that corresponds with a frequency symptomatic that normalises after some days. In contrast, LaPallo et al. [15] assessed EUS EMG activity over time in unrestrained awake rats and did not detect EUS slow-wave bursting activity during voiding in ≈25% of the rats. Correlation of those studies with the present one is difficult since LaPallo et al. [15] did not
assess bladder function with simultaneous intravesical pressure measurement. It is possible that the 25% of rats that did not display slow-wave bursting had LUTD. Moreover, there were significant differences in the electrode implantation techniques used in our present study vs that of LaPallo et al. [15]. In the LaPallo et al. [15] study, the EUS EMG electrodes were affixed intra-abdominally to the pelvic bone, whereas in the present study we have used an extra-abdominal pelvic approach and affixed the electrodes to the fat tissue beside the EUS (Fig. 1c and Supplement 2). These alternative approaches may contribute to the differences between the two studies.

Urethane is described by Hara and Harris [16] as having no single predominant target channel but rather affecting multiple channels simultaneously, suggesting that neurotransmitter systems in the CNS might also be affected. Thus, careful use of urethane as an anaesthetic for any neurophysiological measurements is highly warranted.

The pre-micturition high-frequency burst detected in our awake rats was almost identical to the post-micturition burst. Interestingly, Kakizaki et al. [11] also observed similar high-frequency bursting after induced reflex bladder contractions. One possible explanation for this phenomenon is that the pre-micturition burst might be due to an EUS contraction induced by the guarding reflex just before voiding begins. Under urethane anaesthesia this pre-micturition burst disappeared in our present study, similar to other published reports [10,17]. This result highlights the significant influence urethane exerts on lower urinary tract function.

One major issue in urodynamics in rats is the high inter-animal variability. As urodynamic assessment under urethane anaesthesia necessitates killing after investigation, many rats are needed per group to detect significant differences. Our novel urodynamic model allows for repetitive measurements at different time points in the same awake rat. Testing an animal before and after treatment allows that animal to serve as its own control and allows assessment relative to that animal’s individual baseline. This eliminates the problems associated with inter-animal variability and

Fig. 4 (a) Blood serum creatinine levels in rats with bladder catheter only (bc only), combined bladder catheter and EUS EMG electrodes (bc and EUS EMG), or in control (naïve) rats (control group). (b) Bladder weights of the same groups depicted in a. (c) Bladder wall thickness of the same groups depicted in a. (d/e/f) Histological sections of bladders obtained from the same groups depicted in a and stained with H&E showing muscular hypertrophy, urothelial hyperplasia and increased oedema between the mucosal layer and the detrusor in the experimental groups as compared with the controls. (g/h/i) Histological sections of bladders dissected from the same groups depicted in a and stained with Masson’s trichrome showing a proportional increase in collagen without increased fibrosis in the experimental groups as compared with the controls. TE, transitional epithelium; LP, lamina propria; IT, interstitial connective tissue; SM, smooth muscle bundles; SE, serosa.
dramatically reduces the number of animals needed to detect significant changes, ultimately reducing experimental time, costs, and resources without compromising statistical quality.

The evidence is clear that anaesthetics affect bladder function, as shown by others [5–7] and the present study. Consequently, animal models that use anaesthetics are problematic and the translational value of the findings is questionable. Consistent with the International Continence Society Guidelines on Urodynamic Equipment Performance in humans [8], it is suggested that all urodynamic assessments in animal models be performed in an awake state to avoid major bias by narcotics.

A high-pressure system puts at risk the upper urinary tract. In humans, intravesical pressures that spike to >40 cmH2O during the storage phase are generally agreed to jeopardise renal function, so that an appropriate treatment is needed [18]. Thus, the high spikes in pressure caused by detrusor overactivity and DSD can cause significant kidney damage and accurate diagnosis in humans requires measurement of both detrusor and urethral sphincter function [1]. Our present model allows for simultaneous detrusor and EUS assessment in awake rats for the first time and thus promises to be a very useful tool for future translational research on detrusor overactivity and DSD specifically, and LUTD in general. The absence of urethane narcosis is critical for these future studies as anaesthesia dampens pressure spikes. The risk that detrusor overactivity/DSD are not recognised under urethane anaesthesia is high and the effectiveness of a tested treatment may be underestimated.

The main limitation of the present study is the small number of rats investigated. However, our findings are well in line with the literature and our model combines for the first time bladder and EUS assessment in awake rats. Another limitation is that histology showed urothelial hyperplasia and detrusor hypertrophy in both the bladder catheter only, as well as the combined bladder catheter and EUS EMG electrode implanted rats. However, there was no increase in collagen content, suggesting that bladder catheter implantation did not cause bladder fibrosis. The implantation-induced tissue alterations need to be considered when bladder-specific processes are assessed. In humans, combined pelvic floor EMG and videocystourethrography (VCUG) during urodynamic investigation are the most acceptable and widely agreed methods for diagnosis of DSD [19], especially considering that both detrusor internal and external sphincter dyssynergia can be investigated. VCUG is not yet available in rats but we are working on some additional improvements and in the optimal case a video-urodynamic assessment could be established. Thus, detrusor internal sphincter dyssynergia (bladder neck dyssynergia) is currently not evaluated in our rat model. So far, EUS EMG signals were only analysed semi-quantitatively, this is according to urodynamic investigations in humans.

However, software for quantitative assessments is under development.

In conclusion, our novel urodynamic model allows repetitive measurements of both bladder and EUS function at different time points in the same rat under fully awake conditions and opens promising avenues to investigate LUTD in a translational approach. In future studies, we will use this model to investigate major neurological diseases causing LUTD such as spinal cord injury [20], multiple sclerosis [21] and stroke [22], where we expect it to provide better understanding of the underlying mechanisms involved. In addition, our model can be used to assess new causal therapeutic options for these diseases.

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Conflicts of Interest

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Lower urinary tract assessment in awake rats

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