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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

Introduction

Lower urinary tract dysfunction (LUTD) is very common in neurological patients. It affects the lives of millions of people worldwide, has a major impact on quality of life and imposes a substantial economic burden for every healthcare system [1]. Particularly disastrous is detrusor–sphincter dyssynergia (DSD), where neuronal dyscoordination causes the detrusor to contract while preventing sphincter relaxation, resulting in dangerously high spikes in bladder pressure that may lead to kidney damage in the chronic state. Accurate diagnosis of DSD requires measurement of the function of both the detrusor and the external urethral sphincter (EUS). Critical to the development of new therapies to combat DSD and other LUTD are rodent models that accurately measure both parameters. Unfortunately, current models either lack EUS assessments or utilise anaesthesia that is likely to severely alter bladder function. Thus, we aimed to develop and establish an assessment protocol of lower urinary tract function in a rat model that incorporates the synchronous measurement of detrusor activity and EUS function in awake rats, in close analogy to the urodynamic assessment used clinically in humans.

Materials and Methods

Rats (details in Supplement 1): Age-matched female Sprague Dawley rats (260–300 g, aged 5 months, Harlan, Frederick, MD, USA) were used in all studies. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Medical University of South Carolina (USA).

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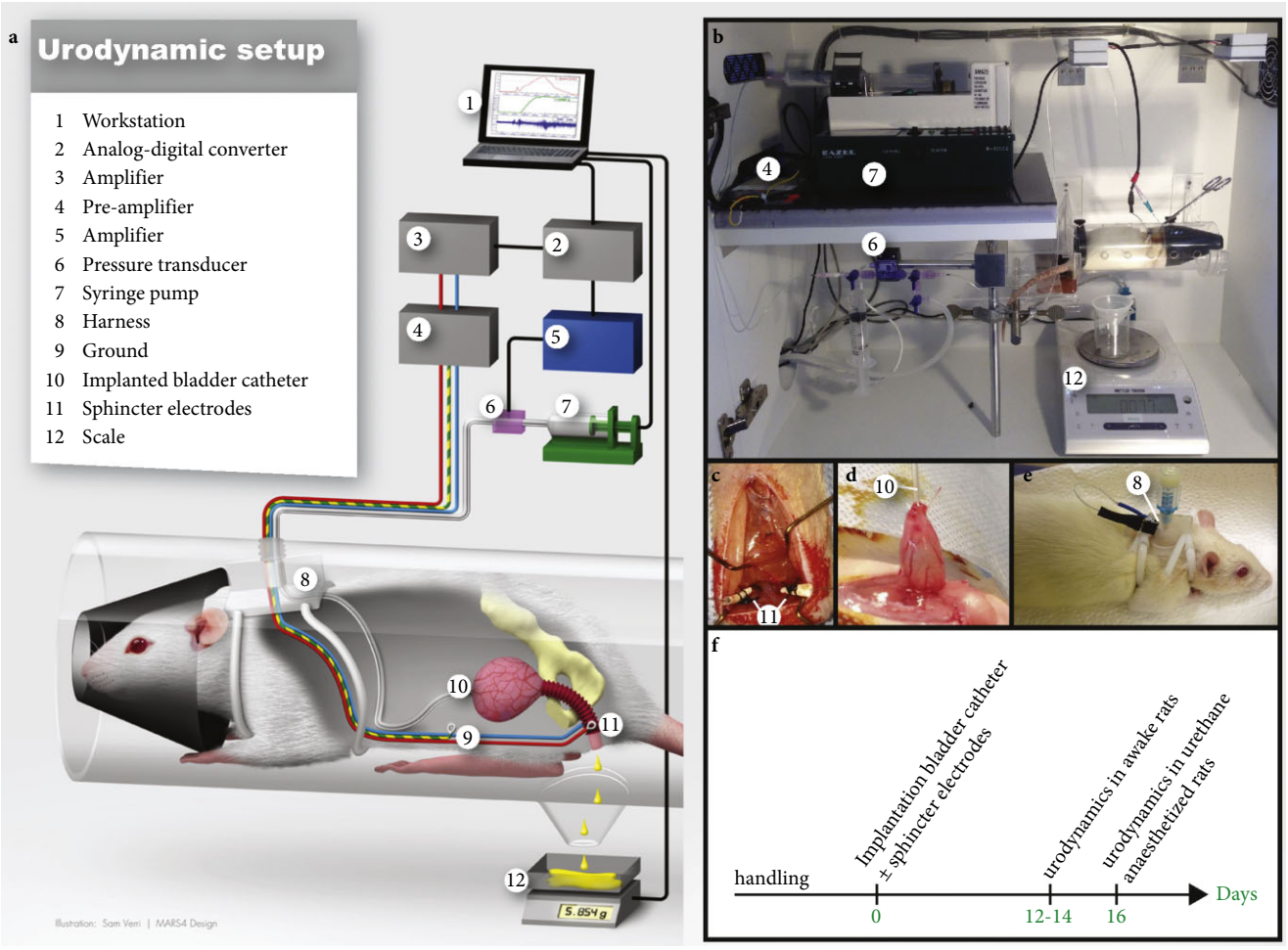
Fig. 1 (**a**) Scheme of the urodynamic setup. (**b**) Urodynamic laboratory station. (**c**) Intraoperative view of the urethra after bilateral implantation of the EUS EMG electrodes. (**d**) Intraoperative view of the bladder dome after implantation of the bladder catheter. (**e**) Rat with harness affixed. (**f**) Study timeline. Numbers in **b–e** relate to the legend in **a**.



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. Phase α: initial increase of

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]): Phase **α**: initial increase of intravesical pressure with parallel increase of the EUS EMG activity due to the guarding reflex. Phase **β**: intravesical pressure increased with high-frequency oscillations during 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. (**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.

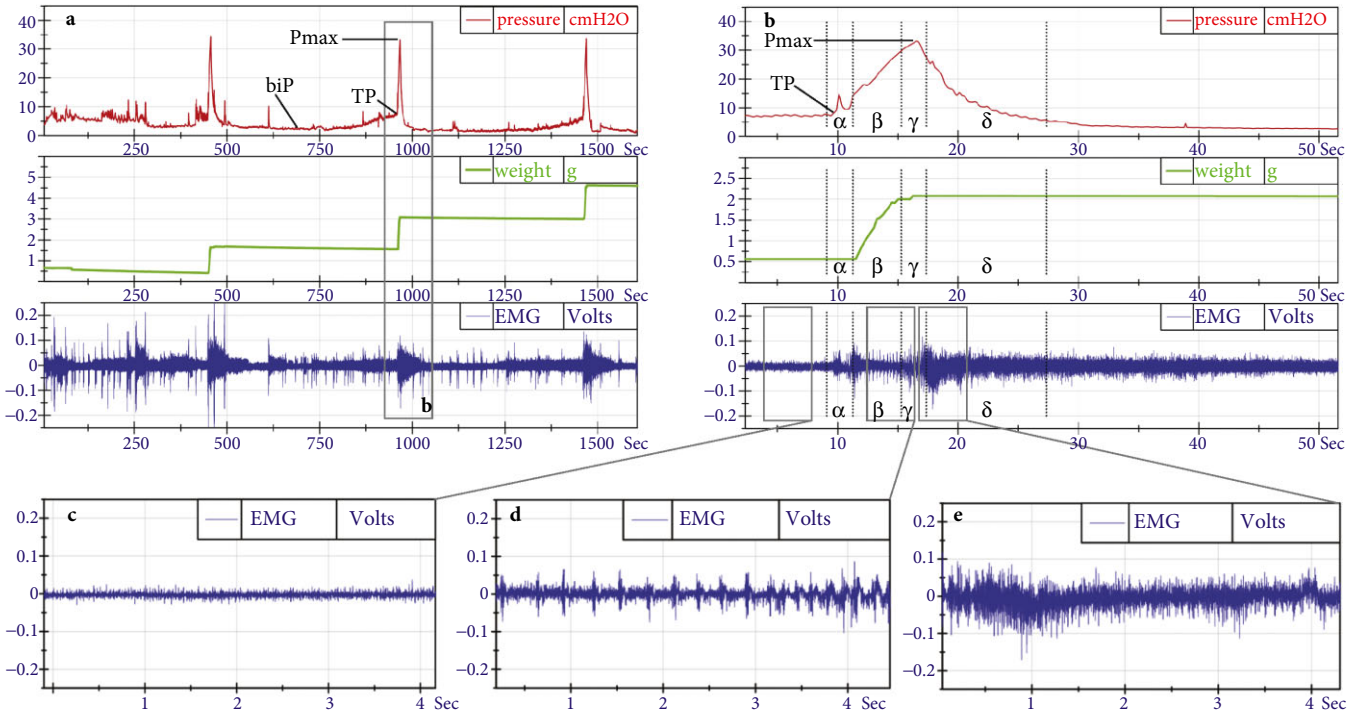


Table 1 Urodynamic variables in the bladder catheter only vs the bladder catheter and EUS EMG group.

Variable	Bladder catheter group	Bladder catheter and EUS EMG group	*P*
Mean (SD):			
Bladder compliance, mL/cmH_2O	0.27 (0.07)	0.21 (0.09)	0.3
Mean flow, μL/s	259.0 (59.2)	251.3 (74.63)	0.9
Voiding duration, s	5.97 (0.21)	6.71 (1.53)	0.4
Maximum voiding pressure, cmH_2O	38.90 (13.44)	42.19 (11.65)	0.7
Voided volume, mL	1.58 (0.42)	1.63 (0.31)	0.9

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 are presented in Table 1 and show that there were no significant differences between the two groups.

Urodynamic Investigation: Awake vs Urethane Anaesthetised Rats

To assess the effect of urethane anaesthesia and to compare our findings in Fig. 2/Table 1 to previous studies, all 10 rats were administered urethane on postoperative day 16 and urodynamics (± EUS EMG where relevant) assessed 30 min later. Rats from both groups were included in the analysis. Of the 10 rats, two had to be excluded: one bladder catheter and EUS EMG electrodes-implanted rat died immediately after urethane administration and another (bladder catheter only) was excluded due to dripping overflow incontinence after urethane injection. As shown in Fig. 3, urodynamic parameters were significantly altered between awake and urethane anaesthetised rats. Anaesthesia provoked a decrease in maximum voiding pressure (Fig. 3h; $P = 0.008$), as well as an increase in compliance (Fig. 3g; $P = 0.04$) and voided volume (Fig. 3i; $P = 0.03$). The mean flow rate ($P = 0.6$) and voiding duration ($P = 0.15$) were similar between both groups (data not shown).

EUS EMG parameters were also altered after urethane administration. (Fig. 3, five rats). A high-frequency pre-micturition burst, similar to the post-micturition burst, was prominent in awake rats (Fig. 3a,c) but highly reduced (in two of the five) or not detectable (in three of the five) in urethane anaesthetised rats (Fig. 3b,d). In addition, baseline amplitude of fast frequency bursting before and during the voiding was reduced in the anaesthetised rats. During voiding of urethane anaesthetised rats, high-frequency bursting activity was almost absent in the intervals between slow-wave bursting (Fig. 3d).

Post-mortem Analysis

As shown in Fig. 4a, serum creatinine levels in the experimental, implanted rats were within the normal range (<88 μmol/L) with no significant differences to the controls. However, bladder weight and bladder wall thickness were increased more than two-fold in both the bladder catheter only and the bladder catheter and EUS EMG group compared with controls (Fig. 4b,c). These same groups displayed marked muscular hypertrophy and urothelial hyperplasia (Fig. 4d–f). Masson's trichrome staining for collagen was similar in all three groups (Fig. 4g–i) and there were no signs of bacterial infection.

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 anaesthetised rats, as less pressure is needed to overcome a lower infravesical resistance.

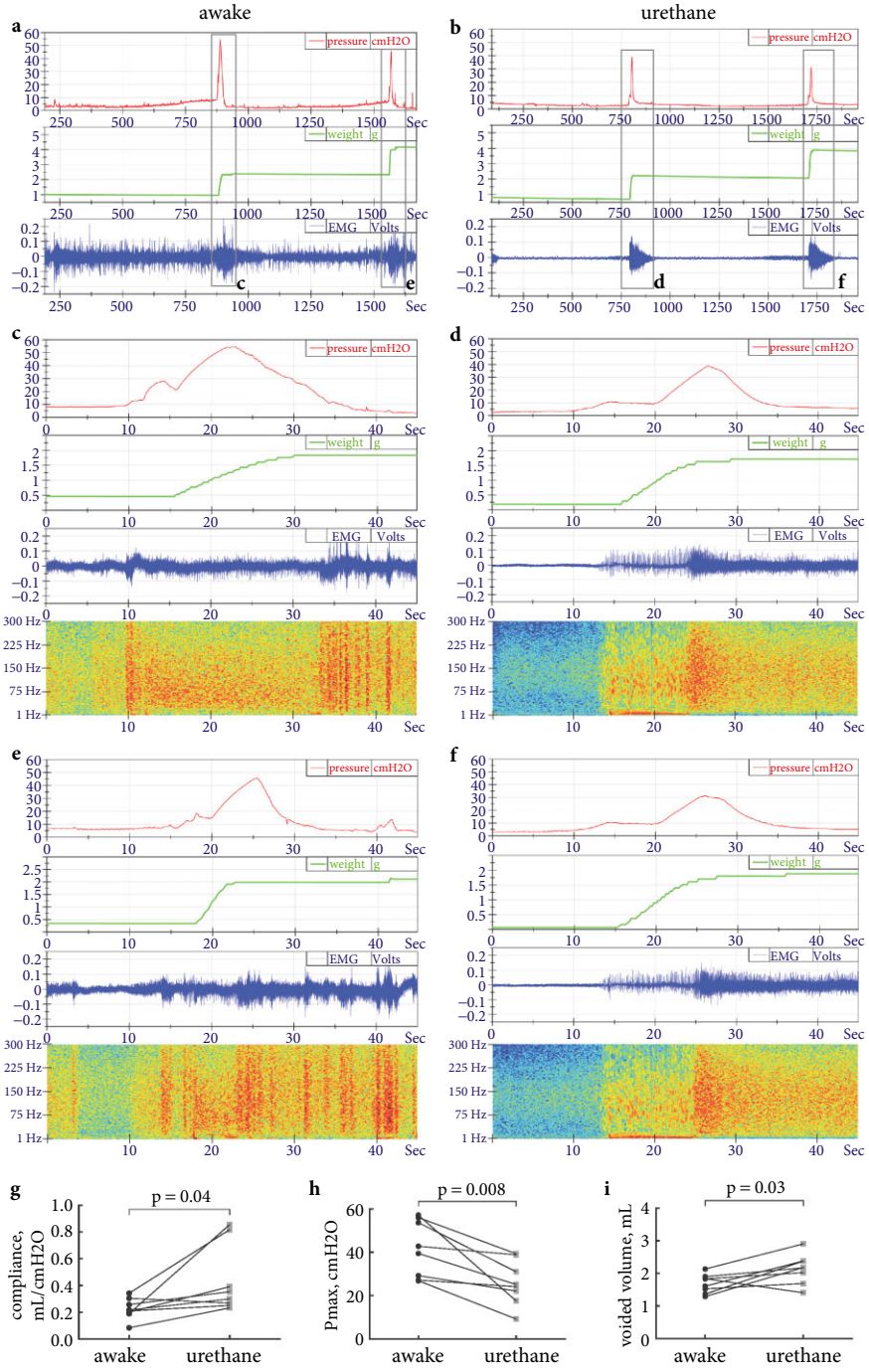
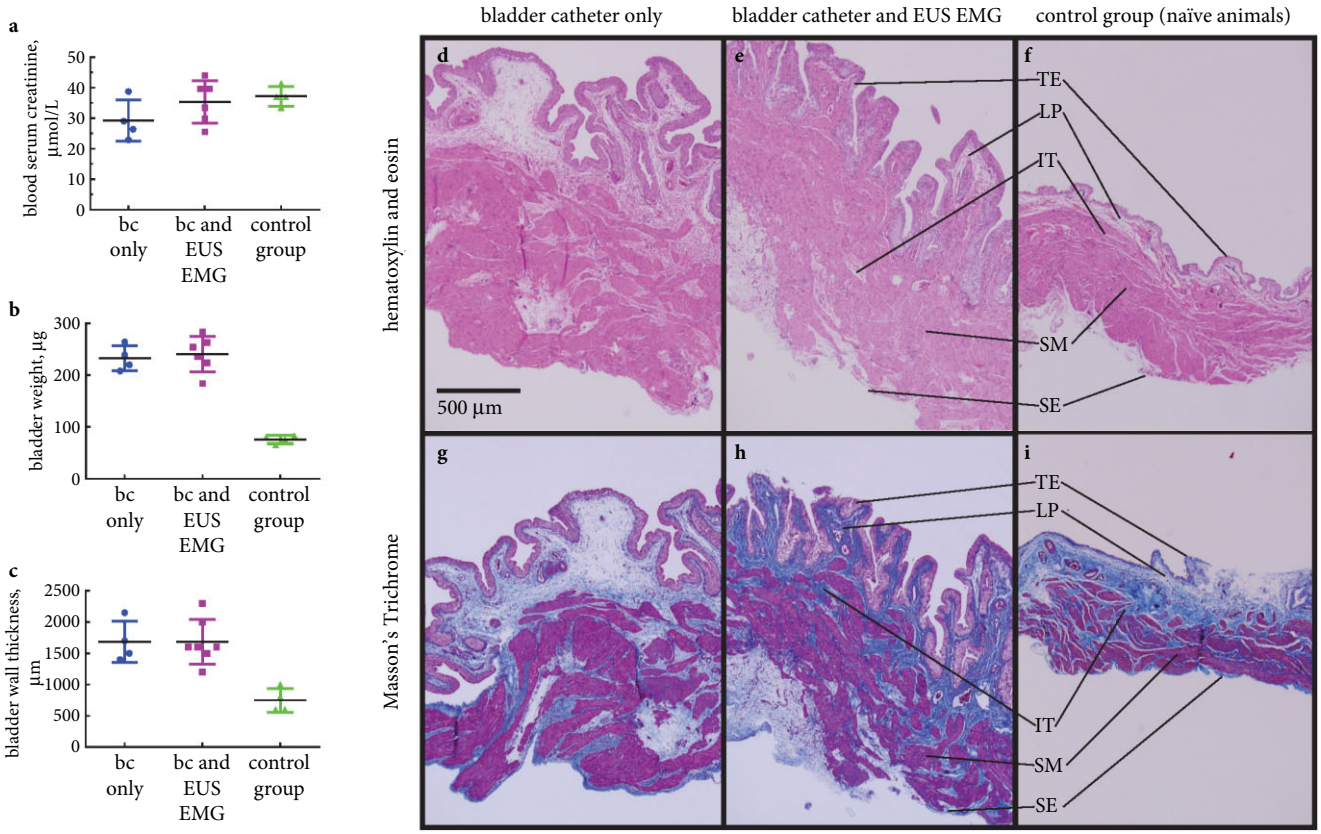


Fig. 3 (**a**) 1500-s window of **a** representative urodynamic tracing with two voiding cycles (**c** and **e**) in an awake rat. Top panel is the pressure tracing, middle panel the scale tracing showing the secreted urine and the bottom panel the EUS EMG tracing (**b**) 1875-s window of a representative urodynamic tracing with two voiding cycles (**d** and **f**) of the same but urethane anaesthetised rat. Top panel is the pressure tracing, middle panel the scale tracing and the bottom panel the EUS EMG tracing. (**c/e**) 45-s zoomed window from **a** showing urodynamic tracings with time matched frequency spectrograms (bottom panel) of the EUS EMG tracing. Red stands for high amplitude of the specific frequency at this time point, deep blue for low amplitude. Shortly before voiding a band of 4–12 Hz burst simultaneous with a second band of 30–300 Hz bursting is most prominent. During the voiding the 30–300 Hz bursting is less prominent (in five out of five rats). At the end of voiding the 4–12 Hz slow bursting disappears and the 30–300 Hz bursting gets very prominent for 5–10 s. (**d/f**) 45-s zoomed window from **b** showing urodynamic tracings with time matched frequency spectrograms (bottom panel) of the EUS EMG tracing. Red stands for high amplitude of the specific frequency at this time point, deep blue for low amplitude. Before voiding there is only very little bursting in any frequency. During the voiding the 4–12 Hz slow-wave bursting is very prominent (in five out of five rats). At the end of voiding the 4–12 Hz slow bursting disappears and the 30–300 Hz bursting gets very prominent for 5–10 s. (**g**) Bladder compliance of the individual rats in the awake vs the urethane anaesthetised state (eight rats, $P = 0.04$). (**h**) Maximum voiding pressure (Pmax) of the individual animals in the awake compared to the urethane anaesthetised state (eight rats, $P = 0.008$). (**i**) Voided volume of the individual rats in the awake vs the urethane anaesthetised state (eight rats, $P = 0.03$).

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

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.



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

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 cmH_2O 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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All other authors have nothing to disclose.

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Abbreviations: DSD, detrusor–sphincter dyssynergia; EMG, electromyography; EUS, external urethral sphincter; H&E, haematoxylin and eosin; LUTD, lower urinary tract dysfunction; VCUG, videocystourethrography.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Supplement 1 Methods.
Supplement 2 Video of catheter and electrodes implantation.

