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Abstract: 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 animals on postoperative day 12-14. Measurements were repeated in the same animal on day 16 under urethane anesthesia. 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 versus bladder catheter and EUS EMG electrode groups. Urethane anesthesia evoked prominent changes in both urodynamic parameters and EUS EMG. Serum creatinine was within the normal limits in all animals. Bladder weight and bladder wall thickness were significantly increased in both the bladder catheter only and the bladder catheter and EUS EMG group compared to controls. CONCLUSIONS: Our novel urodynamic model allows repetitive measurements of both bladder and EUS function at different time points in the same animal under fully awake conditions and opens promising avenues to investigate LUTD in a translational approach.

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

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data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Abstract

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 animals on postoperative day 12-14. Measurements were repeated in the same animal on day 16 under urethane anesthesia. 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 versus bladder catheter and EUS EMG electrode groups. Urethane anesthesia evoked prominent changes in both urodynamic parameters and EUS EMG. Serum creatinine was within the normal limits in all animals. Bladder weight and bladder wall thickness were significantly increased in both the bladder catheter only and the bladder catheter and EUS EMG group compared to controls.

Conclusions: Our novel urodynamic model allows repetitive measurements of both bladder and EUS function at different time points in the same animal under fully awake conditions and opens promising avenues to investigate LUTD in a translational approach.
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 health care system (1). Particularly disastrous is detrusor sphincter dyssynergia 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 detrusor sphincter dyssynergia requires measurement of the function of both the detrusor and the external urethral sphincter (EUS). Critical to the development of new therapies to combat detrusor sphincter dyssynergia and other LUTD are rodent models that accurately measure both parameters. Unfortunately, current models either lack EUS assessments or utilize anesthesia 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 rodent 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

Animals (details in Supplement 1): Age-matched female Sprague Dawley rats (260-300 g, 5 mts, 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).

Experimental design (details in Supplement 1): Animals were divided randomly into 3 groups: 1) bladder catheter only group (n=4), 2) bladder catheter and EUS EMG group (n=6), and 3) control (i.e. naïve) group (n=4). Controls were used for creatinine assessment and histology only. To minimize implant-associated bladder dysfunction, urodynamics were not performed immediately but on postoperative day 12-14 on 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): Animals were anesthetized 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 subcutaneously 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 illustrated in Figure 1a and pictured in Figure 1b, awake animals 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 3 micturition cycles.

Post-mortem assessments (details in Supplement 1): At sacrifice, blood was obtained by heart puncture and creatinine assessed by standard Enzyme-Linked Immuno-Sorbent Assay (ELISA) techniques. Bladders were removed, weighed and the central third fixed, embedded and sectioned (5 µm). Sections were then stained with hematoxylin and eosin (H&E) or Masson’s trichrome stain using routine methodological techniques.

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 3 groups, one-way analysis of variance (ANOVA) was applied. 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; no losses (total n=10) were observed over the 3 weeks of the experiment. The animals were acclimated to the urodynamic measurement cabinet for 5 days, after which they stayed in the restraint position during the 1 hr 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 Figure 2a and includes a pressure tracing from the bladder, the determination of secreted urine (gr on scale) and the EUS EMG traces. An expanded graph of a single micturition is shown in Figure 2b. Micturition consists typically of four phases (2, 4) which are indicated on the figure. Phase α: initial increase of 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 micturition 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 demonstrate that there are no significant differences between the two groups.

**Urodynamic investigation: awake versus urethane anesthetized rats:** To assess the effect of urethane anesthesia and to compare our findings in Figure 2/Table 1 to previous studies, all animals (n=10) were administered urethane on...
post-operative day 16 and urodynamics (± EUS EMG where relevant) assessed 30 min later. Animals from both groups were included in the analysis. Of the 10 animals, 2 had to be excluded: one bladder catheter and EUS EMG electrodes implanted animal died immediately after urethane administration and another (bladder catheter only) was excluded due to dripping overflow incontinence following urethane injection. As shown in Figure 3, urodynamic parameters were significantly altered between awake and urethane anesthetized rats. Anesthesia provoked a decrease in maximum voiding pressure (Figure 3h; p=0.008) as well as an increase in compliance (Figure 3g; p=0.04) and voided volume (Figure 3i; p=0.03). 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 following urethane administration (Figure 3, n=5). A high frequency pre-micturition burst, similar to the post-micturition burst, was prominent in awake animals (Fig. 3a,c) but highly reduced (in 2 of 5) or not detectable (in 3 of 5) in urethane anesthetized rats (Fig. 3b,d). In addition, baseline amplitude of fast frequency bursting before and during the micturition was reduced in the anesthetized animals. During micturition of urethane anesthetized animals, high frequency bursting activity was almost absent in the intervals between slow wave bursting (Figure 3d).

Post-mortem analysis: As shown in Figure 4a, serum creatinine levels in the experimental, implanted animals 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 to controls (Figure 4b and 4c). These same groups displayed marked muscular hypertrophy and urothelial hyperplasia (Figure 4d-f). Masson’s trichrome staining for collagen was similar in all 3 groups (Figure 4g-i) and there were no signs of bacterial infection.

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Discussion

Our findings demonstrate 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 anesthesia 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 animal. Moreover, given the nondestructive nature of the measurements, this model allows for repetitive analysis at different time points in the same animal. Thus, our novel urodynamic rodent model opens promising avenues to investigate LUTD in a translational approach.

Anesthetic 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 anesthetics (8). In animals, however, all existing studies which included urodynamics and EUS EMG were carried out under anesthesia (9-11). Although urethane seems to be the best available anesthetic to maintain the micturition response (2, 12), it strongly impairs bladder function, leading to significant differences in urodynamic findings compared to the awake state (13). In the present study, we observed lower baseline amplitude of high frequency bursting before, during and after micturition 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 anaesthetized animals since less pressure is needed to overcome a lower infravesical resistance.
It is described in literature, basing on urethane anaesthetized 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 animals 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 anesthetics 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 normalizes 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 about 25% of the animals. 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 animals that did not display slow wave bursting were suffering from a LUTD. Moreover, there were significant differences in the electrode implantation techniques used in our study versus that of LaPallo et al. (15). In LaPallo’s study (15), 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 (Figure 1c and Supplement 2). These alternative approaches may contribute to the differences between the two studies.

Urethane is described by Hara et al. (16) as having no single predominant target channel but rather affecting multiple channels simultaneously, suggesting that neurotransmitter systems in the central nervous system might also be affected. Thus, careful use of urethane as an anesthetic for any neurophysiological measurements is highly warranted.
The pre-micturition high frequency burst detected in our awake animals was almost identical to the post-micturition burst. Interestingly, Kakizaki et al. (11) also observed similar high frequency bursting following 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 micturition begins. Under urethane anesthesia this pre-micturition burst disappeared in our study, similar to other reports in the literature (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. Since urodynamic assessment under urethane anesthesia necessitates sacrifice after investigation, large numbers of animals are needed per group to detect significant differences. Our novel urodynamic model allows for repetitive measurements at different time points in the same awake animal. 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 anesthetics affect bladder function, as shown by others (5-7) and the present study. Consequently, animal models that utilize anesthetics are problematic and the translational value of the findings is questionable. In line 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 jeopardize renal function so that an appropriate treatment is needed (18). Thus, the high spikes in pressure caused by detrusor overactivity and detrusor sphincter dyssynergia can cause significant kidney damage and accurate diagnosis in humans requires measurement of both detrusor and urethral sphincter function (1). Our 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 detrusor sphincter dyssynergia specifically and LUTD in general. The absence of urethane narcosis is critical for these future studies as anesthesia dampens pressure spikes. The risk that detrusor overactivity / detrusor sphincter dyssynergia are not recognized under urethane anesthesia is high and the effectiveness of a tested treatment may be underestimated.

The main limitation of our study is the small number of animals 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 animals. 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. There was no increase in collagen content, however, 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 spincter 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 animal model. So far, EUS EMG signals were only analyzed semi-quantitative, 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 animal under fully awake conditions, 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

Dr. Schwab reports grants from Swiss National Science Foundation, grants from Christopher and Dana Reeve Foundation, grants from European Research Council, advanced grant, during the conduct of the study; grants from Swiss National Science Foundation, grants from Christopher and Dana Reeve Foundation, grants from European Research Council, advanced grant, outside the submitted work; . All other authors have nothing to disclose.
References:


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**Table 1**: Urodynamic parameters in the bladder catheter only versus the bladder catheter and external urethral sphincter (EUS) electromyography (EMG) group.

**Figure 1**: a) Scheme of the urodynamic setup. b) Urodynamic lab station. c) Intraoperative view of the urethra after bilateral implantation of the external urethral sphincter electromyography 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.

**Figure 2**: a) 1625 second window of a representative urodynamic tracing from a rat with bladder catheter and external urethral sphincter (EUS) electromyography (EMG) showing three micturition cycles. The first micturition cycle includes moving artifacts and serves for adaptation of the animal. The second and third micturition 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. *bP*: baseline pressure; lowest pressure between two micturitions; *tP*: threshold pressure: pressure shortly before the micturition is started; *Pmax*: maximum voiding pressure: highest pressure during the micturition cycle.

b) 50 second window culled from a. Top panel is the pressure tracing, middle panel the scale tracing and the bottom panel the EUS EMG tracing. The micturition 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 micturition contraction.

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c) 4 second zoomed window from the EUS EMG from b) before the micturition 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 micturition. Most prominent pattern is a high amplitude low frequency bursting with medium amplitude high frequency bursting between the slow wave bursting. e) 4 second zoomed window from the EUS EMG from b) after the micturition. Most prominent pattern is a high amplitude high frequency bursting.

Sec: second.

Figure 3: a) 1500 second window of a representative urodynamic tracing with two micturition 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 external urethral sphincter (EUS) electromyography (EMG) tracing b) 1875 second window of a representative urodynamic tracing with two micturition cycles (d and f) of the same but urethane anesthetized rat. Top panel is the pressure tracing, middle panel the scale tracing and the bottom panel the EUS EMG tracing. c/e) 45 second 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 micturition a band of 4-12 Hz burst simultaneous with a second band of 30-300 Hz bursting is most prominent. During the micturition the 30-300 Hz bursting is less prominent (in 5 out of 5 animals). At the end of micturition the 4-12 Hz slow bursting disappears and the 30-300 Hz bursting gets very prominent for 5-10 seconds. d/f) 45 second 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 micturition there is only very little bursting in any frequency. During the micturition the 4-12 Hz slow wave bursting is very prominent (in 5 out of 5 animals). At the end of micturition the 4-12 Hz slow bursting disappears and the 30-300 Hz bursting gets very prominent for 5-10 seconds.

g) Bladder compliance of the individual animals in the awake compared to the urethane anesthetized state (n=8, p=0.04). h) Maximum voiding pressure (Pmax) of the individual animals in the awake compared to the urethane anesthetized state (n=8, p=0.008). i) Voided volume of the individual animals in the awake compared to the urethane anesthetized state (n=8, p=0.03).
Figure 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) animals (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 demonstrating muscular hypertrophy, urothelial hyperplasia and increased edema between the mucosal layer and the detrusor in the experimental groups as compared to the controls.

g/h/i) Histological sections of bladders dissected from the same groups depicted in a and stained with Masson’s trichrome demonstrating a proportional increase in collagen without increased fibrosis in the experimental groups as compared to the controls.

TE: Transitional epithelium; LP: Lamina propria; IT: Interstitial connective tissue; SM: Smooth muscle bundles; SE: Serosa.
Table 1. Urodynamic parameters in the bladder catheter only versus the bladder catheter and external urethral spincter (EUS) electromyography (EMG) group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bladder catheter group</th>
<th>Bladder catheter and EUS EMG group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<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/sec]</td>
<td>259.0 ± 59.2</td>
<td>251.3 ± 74.63</td>
<td>0.9</td>
</tr>
<tr>
<td>Voiding duration [sec]</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>