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INNOVATIVE METHODOLOGY

Validation of an efficient and continuous urodynamic monitoring system for awake, unrestrained, chronic rodent studies

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Angoli D, Geramipour A, Danziger ZC. Validation of an efficient and continuous urodynamic monitoring system for awake, unrestrained, chronic rodent studies. *Am J Physiol Renal Physiol* 318: F86–F95, 2020. First published November 18, 2019; doi:10.1152/ajprenal.00349.2019.—The postvoid residual (PVR) is an important measure of bladder function, but obtaining PVR is burdensome because bladder volume must be measured at the time of voiding. The PVR measurement problem has led to experimental tricks in animal studies (infusing the bladder at supraphysiological rates and limiting animal observation windows) to keep the number of observed voids statistically robust while reducing the time an experimenter must be present. Our solution to the PVR measurement problem is a system called Automatic Monitoring for Efficient, Awake, Sensitive, Urine Residual Estimation (AMEASURE). AMEASURE combines metabolic cages and optimization algorithms to estimate continuously PVR for every voiding event 24 h/day for multiple weeks, without artificial bladder infusion, continuous experimenter supervision, anesthesia, or restraints. Using AMEASURE, we obtained voided volumes, PVRs, and other urodynamic parameters continuously for 21 days in 10 healthy female Sprague-Dawley rats. Importantly, this required only one manual measurement of animals' bladder volume every 12 h. We validated the accuracy of the system experimentally and in simulation. We detected marked differences in voiding frequency and efficiency between light and dark cycles and found that voiding frequency increased over time during the dark cycle (but not the light cycle), due to surgical recovery, cage acclimation, and socialization. This tool enhances the relevance of rodent models to the study of human lower urinary tract by expanding observation periods and obviating the need to infuse the bladder and facilitates the study of conditions for which behavioral, social, or circadian factors play essential roles.

bladder; cystometry; optimization; residual; ureter

INTRODUCTION

The postvoid residual (PVR) is a fundamental indicator of lower urinary tract (LUT) health (2). Studies using animal models maximize their potential for clinical relevance by measuring both PVR and voided volume (V_v) over as many voiding events as possible to generate statistically rigorous analyses comparing experimental conditions (28, 36, 42). PVR and V_v together yield many measures that are diagnostically salient and scientifically informative about LUT function, such as bladder voiding efficiency (VE), bladder capacity, and renal output (1, 9).

Getting accurate measurements of PVR during cystometry, however, is challenging. Transurethral catheters are a moderately invasive method of removing and measuring PVR; therefore, it is preferred in clinical urodynamics, but its reproducibility is limited (4, 18). In animal models, a typical solution for PVR measurement is to implant a suprapubic fluid-filled catheter into the dome of the bladder to access the bladder contents and remove PVR after each voiding event. The primary measurement problem with the suprapubic catheter approach is that an experimenter must be present for each void to withdraw and measure PVR, which is time prohibitive. The typical cystometric observation period for rodent studies is 1–3 h (10, 23, 36) and the natural intervoid interval for caged rats is ~45 min (35); therefore, only one to four voiding events would be observed, severely limiting data collection and statistical robustness. The most common workaround for this [in both awake (19) and anesthetized (32) preparations] is to infuse saline into the bladder through the suprapubic catheter to speed up the animal's voiding frequency. External infusion is undesirable because the bladder filling rate itself affects bladder capacity (31) and high-frequency voiding cycles may fatigue the bladder or introduce carryover effects (13, 22). Monitoring animals only during short observation windows is also undesirable because potentially relevant diurnal variations (19, 30, 35) cannot be monitored. The complications and drawbacks of measuring PVR lead some researchers to ignore PVR instead. Many chronic studies using metabolic cages for long-term monitoring do not implant a catheter and do not measure PVR (11, 35), which has the serious limitation of being unable to track fundamental urodynamic parameters.

Moreover, removing PVR for measurement after each void is nonphysiological when studying conditions like urinary retention, where the bladder does not begin the filling phase empty. To avoid frequently disturbing the bladder with PVR measurements, it is tempting to suppose that PVR for a given void equals the volume infused into the bladder minus the volume voided, which effectively assumes there is no renal input to the bladder; however, we know that computing PVR with this assumption accrues huge amounts of error (14). To avoid neglecting ureter inflow to the bladder the ureters can be diverted (7, 43), forcing ureter inflow to be zero and thereby make accurate the simple algebraic computation of PVR from voided and infused volume. Diverting the ureters is intrusive, however, and it is unclear if this alters voiding behavior or reflexes, and this technique is generally not suited for chronic monitoring of LUT health. As a noninvasive alternative, some studies have considered ultrasonic techniques to measure bladder contents (17, 24). However, this approach requires exten-

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sive preparation of the animal for measurement (resulting in only intermittent observations of bladder volumes), the bladder volume is not measured immediately after a void (so it cannot be considered PVR), and the measurement requires anesthesia. The problem of frequently, accurately, and nondisruptively obtaining PVR in animal studies, a critical measure LUT health, has been an active problem since the 1980s.

We propose a new solution to the PVR measurement problem in chronic, awake, unrestrained rodent studies. With the protocol presented here, we continuously tracked bladder pressure and accurately estimated PVR of every voiding event in a rat for 3 wk, without infusing saline into the bladder to artificially decrease intervoid intervals. We did this by extending our previously reported acute preparation PVR estimation technique (14) and combining it with modified metabolic cages. In this protocol, an experimenter measures bladder volume once each 12-h light-dark cycle and an optimization algorithm based on conservation of mass estimates the PVR of every voiding event during that cycle. Because a person does not need to be present for voiding events, we allow the bladder to fill naturally and do not limit observation to short windows, giving us a unique ability to estimate every PVR. We refer to the combined system as Automatic Monitoring for Efficient, Awake, Sensitive, Urine Residual Estimation (AMEASURE). In this work, we provide an introduction to AMEASURE, present a validation of its accuracy in experiments with healthy rats and computer simulations, and demonstrate how it can be used to track urodynamic parameters of every natural voiding event in a cohort of rats continuously for 3 wk.

METHODS AND MATERIALS

Surgical procedures. The protocol and animal use were reviewed and approved by the Institutional Animal Care and Use Committee of Florida International University. We used 10 female Sprague-Dawley rats (334.6 ± 10.6 g, 3–5 mo, Charles River). Rats were adapted to 12:12-h dark-light cycles for at least 1 wk before surgery. We anesthetized the animals with isoflurane (1.5–2% in oxygen), made a midline abdominal incision, and inserted into the apex of the bladder dome a 3-Fr silicon catheter (SAI Infusion) with a 4-mm fenestrated tip that extended into the intravesical space. We secured the catheter with a purse string suture around two beads attached to the catheter to prevent slip and create a watertight seal. We closed the musculature with suture and the skin with wound clips. We tunneled the catheter subcutaneously, externalized it dorsally, and connected it to a harness to which an external 21-gauge catheter was attached (Fig. 1) (42). Before insertion, we filled the catheter with taurolidine-citrate catheter solution (Access Technologies) to keep the line patent. We placed the animals in metabolic cages after the surgical procedure. All rats received buprenorphine (0.03 mg/kg) and cefazolin (33.3 mg/kg) in 3 mL saline solution twice daily for the first 5 days after surgery (34) in subcutaneous injections. For the rest of the study, we added the antimicrobial enrofloxacin to the drinking water (0.5 mg/mL). After 5–7 days, we removed the wound clips. Food and water were available ad libitum, and each animal was free to move inside the cage (the external portion of catheter was flexible and attached to a fluid commutator to minimize interference with the animals' activities).

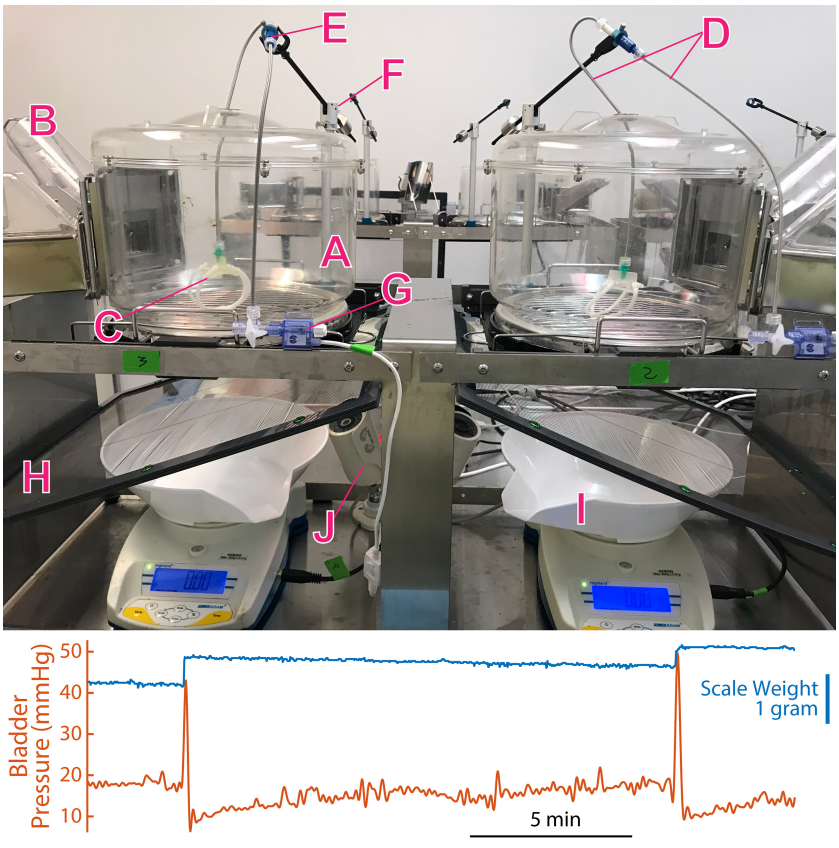

Fig. 1. Metabolic monitoring setup. *Top*: animals were housed in the Techniplast metabolic chambers (*A*) and graduated cylinders were used to measure water intake (*B*). The bladder catheter was externalized dorsally and passed through a harness for stabilization (*C*), which interfaced with an external fluid-filled catheter (*D*) that transmitted pressure via a fluid commutator (*E*), supported by a rotating counterbalanced arm (*F*), to a pressure transducer (*G*). The coarsely grated cage floor allowed objects to pass through, and the extremely fine wire mesh below the cage floor (*H*) blocked solid waste from landing on the weigh boat (*I*) resting on the digital scale that monitored voided urine. Pressure and voided volume data were displayed in real time on a PC terminal running custom MATLAB software (not shown). Animals were monitored by video to help maintain their health and to gather behavioral data (*J*). Bioamplifiers, the analog-to-digital converter, and serial input hardware are not visible. *Bottom*: example trace of a simultaneous voided volume and bladder pressure time series. The orange trace shows bladder pressure, as measured by the indwelling catheter and external pressure transducer; the blue trace shows volume voided by the animal, as measured by the scales under each cage.

Data collection was performed in two cohorts of four and six animals separated by 1 mo. Each animal was followed for 3 wk and then euthanized. In both cohorts, approximately two new animals were added to the monitoring chambers per week, and no more than four animals were observed simultaneously. We observed no weight loss on average throughout the study (gain of 2.5 ± 20 g, $P = 0.703$), which may indicate some increased stress levels compared with animals in traditional cages since typical weight gain during this portion of their life cycle is ~50 g (8).

Data collection. The physical components of AMEASURE consisted of the top housing portion of Techniplast metabolic cages. We replaced the bottom portions with a steel fine wire mesh (Med Associates) placed at a 20−23° angle to the horizontal that allowed over 99.5% of urine to pass through immediately and blocked feces pellets and dropped food, which rolled down the slope to avoid interference with falling urine. Digital scales (HCB 302, Adam Equipment) below the filter weighed falling urine. (Anecdotally, we found that the commercially available metabolic cage urine collection funnels introduced significant delay between voiding and the urine landing on the scale and that a substantial amount of urine, up to 30% of the total volume, would adhere to the funnel walls and not be recorded, which motivated our use of the mesh filter instead.) The external catheter extended from the harness to a fluid commutator on a counterbalanced rotating lever arm (SAI Infusion) that allowed animals to move freely, created a continuous fluid column with a pressure transducer that transmitted data to bioamplifiers (WPI), and allowed removal of PVR. We embedded the external catheters in metal springs to prevent rats from damaging the lines. We used a DAQ (USB-6218, National Instruments) for analog-to-digital conversion and custom MATLAB software to integrate data streams, save, and observe urine weight (1 Hz) and bladder pressure (40 Hz) in real time. Animals were videorecorded to monitor health. We measured bladder volume (meaning simply the contents of the bladder, not bladder capacity) at the transitions between each 12:12-h light-dark cycle and measured 12-h water consumption. We identified voiding events from the scale weight time series by computer algorithm, the results of which were audited by two experimenters to verify accuracy. The setup is shown in Fig. 1.

To validate AMEASURE experimentally, an experimenter watched a subset of animals for a 7- to 12-h period and removed the PVR after every natural voiding event (see Fig. 4). Unlike typical cystometry, after we removed and measured PVR, we reinserted it back into the bladder. This procedure allowed us to validate experimentally AMEASURE's estimates by comparing every measured PVR to every estimated PVR.

Data analysis. In Fig. 3, the boxplot edges mark the 25th and 75th percentiles of the data, the centerline shows the median value, and whiskers extend to the most extreme nonoutlier (±1.5 times the central 50% of data). Figure 3 data were aggregated by computing the cycle average of a given urodynamic variable (e.g., V_v) for each animal in each cycle. These (10 animal × 2 cycles/day × 21 days/animal = 420 cycles) 420 cycle averages were then averaged over animals to display changes across days or averaged over days to display interanimal variation. Averaging within each cycle first balanced descriptive and inference statistics by preventing any one animal from dominating the group averages (e.g., if one animal had many more voids in a given cycle than others, the PVRs of that animal would bias the population means if we did not first average over cycles). Of the 420 total 12-h cycles, 6 cycles were excluded from analysis as outliers because their mean cycle value was outside the average value of all cycles ± 3 SD. VE was defined as the ratio of V_v to total bladder contents (where V_r is PVR) as follows:

$$\mathrm{VE} = \frac{V_v}{V_v + V_r} \tag{1}$$

Mathematical estimation of PVRs. The PVR of the kth voiding event $[V_r^{(k)}]$ in a series of voids is not computable algebraically because it is a function of the unmeasured ureter inflow rates into the bladder leading up to the void, $\dot{V}_u^{(k)}$ [neglecting $\dot{V}_u^{(k)}$ results in unacceptable levels of error (14)]. In our previous work, we showed that a volume balance over the urethra, ureters, and bladder catheter for the kth voiding event can be expressed as follows:

$$V_r^{(k)} = \dot{V}_u^{(k)} t^{(k)} + V_i^{(k)} - V_v^{(k)} + V_r^{(k-1)} \tag{2}$$

That is, the PVR of the kth void $[V_r^{(k)}]$ is equal to the volume entering the bladder through the ureters $[\dot{V}_u^{(k)} t^{(k)}]$ plus any volume infused through the catheter $[V_i^{(k)}]$ minus V_v $[V_v^{(k)}]$ plus any volume left over in the bladder from the previous void $[V_r^{(k-1)}]$ (14). To solve the linear recurrence equation (*Eq. 2*), we previously assumed that the rate of ureter inflow was constant, i.e., $\dot{V}_u^{(k)} = \dot{V}_u$. This assumption achieved accurate results in acute studies where intervoid intervals were short and $V_r^{(k)} \ll V_i^{(k)}$. This approach is insufficient for chronic studies, however, where variations in $\dot{V}_u^{(k)}$ accrue over long durations and violate the constant flow assumption and where $V_i^{(k)}$ may be zero.

We extended our previous work by relaxing the constant ureter inflow rate assumption and recasting *Eq. 2* as a constrained, regularized optimization problem:

$$\arg\min_{\dot{V}_u} \left(\frac{1}{E\left(\dot{V}_u t\right)^2} \left\{ \sum_{k=1}^{N} \left[\dot{V}_u^{(k)} t^{(k)} + V_i^{(k)} - V_v^{(k)} \right] + V_r^{(0)} - V_r^{(N)} \right\}^2 + \frac{\sigma\left(\dot{V}_u\right)}{E\left(\dot{V}_u\right)} \right)$$

The sum of all ureter inflow $[\dot{V}_u^{(k)} t^{(k)}]$ and externally infused volume $[V_i^{(k)}$, zero in this study] to the bladder minus each associated V_v $[V_v^{(k)}]$ for the entire series of N voids must equal the final PVR of the series $[V_r^{(N)}]$ by conservation of mass (practically equivalent to conservation of volume under physiological temperatures and pressures). The first term in *Eq. 3* represents the squared difference (error) between those two quantities. Since $V_r^{(N)}$ is the one bladder measurement we make at the end of each 12-h cycle, it is known. Therefore, we can minimize *Eq. 3* by finding the ureter inflow rates, $\dot{V}_u^{(k)}$, that minimize the squared error. Note that $V_v^{(k)}$ and $t^{(k)}$ are measured by the digital scales automatically (they are known), and $V_r^{(0)}$ is the initial bladder fill condition (which is zero in these

experiments because we remove all bladder volume at the end of the previous series for measurement).

We regularized the optimization by assuming that the variability in ureter inflow rate to the bladder is low within a batch of N voids, which is expressed as the second term in *Eq. 3*, $\sigma\left(\dot{V}_u\right)$. This assumption allows the optimization to select one set of $\dot{V}_u^{(k)}$ from among the many possible that minimize the squared error of the first term equally well. We normalized both terms by their expected values to maintain dimensional consistency and weight, the tradeoff between error and permissible $\dot{V}_u^{(k)}$ variability. Here, $\sigma(\cdot)$ is the standard deviation and $E(\cdot)$ is the expected value.

Finally, the optimization was subjected to the following $2N$ constraints for all voids from 1 to N, $\forall k \in \{1,..., N\}$, which express that all PVRs must be non-negative and all ureter inflow rates must be positive, meaning that we assumed there was no retrograde flow through the ureters, such that all volume is either voided or stays in the bladder.

$$\sum_{k=1}^{N} \dot{V}_u^{(k)} t^{(k)} \geq \sum_{k=1}^{N} V_v^{(k)} - V_r^{(0)} \text{ and } \dot{V}_u^{(k)} > 0$$

In summary, we generated our estimate of each $\dot{V}_u^{(k)}$ such that the squared difference between the one measured bladder volume per series, $V_r^{(N)}$, and our prediction of $V_r^{(N)}$ was as small as possible without violating non-negative volume constraints. Our estimations occurred under a second regularizing assumption that the variation of $\dot{V}_u^{(k)}$ between voids within a single batch of voids is small but not necessarily constant (an assumption we validated; Fig. 4).

Here, we discuss some experimental details related to the PVR estimation algorithm. We executed the PVR estimation algorithm (*Eq. 3*) once per batch of N voids, where a batch is all voids between the previous and current bladder volume measurement. As mentioned above, we assumed that the intervoid ureter inflow rate variability is low within a single batch of voids. We did not assume that ureter inflow rate has low variation between batches, and we had no assumptions about the behavior of any parameter across timescales longer than a single batch. Since ureter inflow between active (dark) and dormant (light) cycles is slightly different, we minimized intrabatch variations by separating dark cycle voids and light cycle voids into separate batches by performing bladder volume measurements at the transition between cycles (every 12 h). This partitioned the ureter inflow variance into different batches, which minimized violation of the low intrabatch variance assumption. Therefore, if an intervention will alter ureter inflow rate, the batch size (meaning the interval between bladder volume measurements) should be reduced to a short enough period that within the batch ureter inflow rate changes only slightly. Finally, we note that the bladder volume measurement needed to complete a batch of voids does not need to occur immediately after a voiding event; it can be taken at any time between voids and treated as a void with a VE of zero (see Fig. 4*A*). The PVR estimations remain accurate using this procedure, and the zero VE event was discarded from urodynamic analysis.

Simulation of serial cystometrograms. We performed simulations of serial cystometry (see Fig. 4*B*) by drawing each PVR, ureter inflow rate, and intervoid duration from normal distributions and then algebraically solving for the associated V_v that satisfied conservation of volume (*Eq. 2*). We added variability to the true values to represent measurement noise and compared our optimization algorithm's estimation of all VEs (using only the last measured PVR) and the simulated measurements of VEs. We used an average ureter flow rate of 0.37 mL/h (the average of our measured ureter flow rates from validation experiments; Fig. 4), an intervoid duration of 0.75 ± 0.16 h (the average for the 10 validation serial cystometrograms), and PVR of $15 \pm 3\%$ of bladder volume at the time of the void (Fig. 3). We used normally distributed measurement noise of ± 0.05 mL for PVR and ± 0.005 mL for V_v, obtained from our prior work (14).

RESULTS

Continuous, long-term urodynamics with AMEASURE. For the first time, we tracked the V_v and PVR of every voiding event in awake behaving rats continuously for 3 wk without infusing saline into the bladder. Figure 2 shows measured voided and estimated residual volumes from a typical animal. Qualitatively, PVRs were markedly larger during the dark cycle than the light cycle. This difference may be driven by rats (nocturnal) being more active during the dark cycles. Light cycle PVRs appeared to stabilize at low values ~5 days after implantation, consistent with the previously reported time required for recovery from suprapubic catheter implantation (42), whereas dark cycle PVRs never approached low values. These data illustrate how AMEASURE can monitor LUT activity continuously in chronic, awake behaving rodent models.

Longitudinal voiding comparison between light and dark cycles. We observed differences in urodynamic parameters between the light and dark periods. Far fewer voiding events occurred on average across animals during the light cycle (14.5 ± 1.6) than the dark cycle (26.6 ± 2.8, $P < 0.001$). The average volume per void was higher in the light cycle (0.32 ± 0.05 mL) than the dark cycle (0.21 ± 0.06 mL, $P < 0.001$). The average total V_v per cycle was slightly lower in the light cycle (4.52 ± 0.83 mL) than the dark cycle (5.12 ± 1.10 mL, $P = 0.003$). The average intervoid interval was larger in the light cycle (51.1 ± 4.3 min) than the dark cycle (29.5 ± 4.4 min, $P < 0.001$). Animals drank less water (available ad libitum) on average during the light cycle (9.3 ± 1.0 mL) than the dark cycle (15.9 ± 2.5 mL, $P < 0.001$). We show these measurements in boxplots as distributions over animals in Fig. 3, *A–E*). We reported the measures above as means ± SD and computed P values using a matched-pairs t test between each animal's average light and dark cycle values.

An important advantage of AMEASURE is that we can also obtain estimates of urodynamic parameters that are not available in traditional continuous chronic protocols. Cycle differences in our estimated urodynamic parameters were consistent with the measured parameters. Average PVR per void was far smaller across animals in the light cycle (0.12 ± 0.04 mL) than the dark cycle (0.21 ± 0.05 mL, $P < 0.001$). The average VE per void was larger in the light cycle (0.72 ± 0.05) than the dark cycle (0.54 ± 0.05, $P < 0.001$). The average renal output per void was slightly lower in the light cycle (0.44 ± 0.08 mL/h) than the dark cycle (0.48 ± 0.08 mL/h, $P = 0.042$). We show these boxplots in Fig. 3, *F–H*.

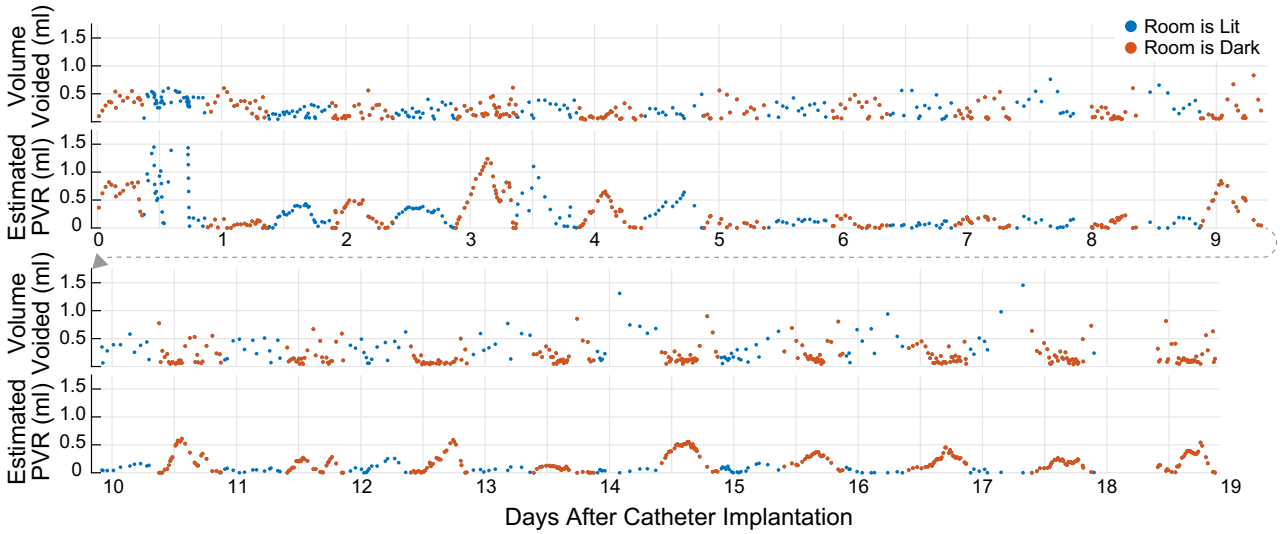

Fig. 2. Example data from a typical animal using Automatic Monitoring for Efficient, Awake, Sensitive, Urine Residual Estimation showing the ability to obtain voided volume (*top* rows) and estimated postvoid residual volume (PVR; *bottom* rows) of every voiding event for 19 consecutive days without externally infusing the bladder.

Animals' urodynamic parameters changed throughout the study. Figure 3, *left*, shows across-animal averages for each cycle since catheter implantation. Qualitatively, there appeared to be three phases. The first phase is the period of surgical recovery lasting ~5 days after implantation (42). The second phase is a slow change in voiding parameters, especially in the dark cycle, which stabilized at ~15 days postimplant. The third phase is a more stable period from 16 to 21 days.

As a check on our algorithm's estimation fidelity, we computed the absolute difference between the measured total volume voided in every cycle and the measured intervoid interval multiplied by the estimated ureter inflow rate for that cycle, $\left|\sum V_v^{(k)} - \sum \dot{V}_u^{(k)} t^{(k)}\right|$, which by design should be as close to zero as possible given the first term in the optimization function (*Eq. 3*). That is, we computed the discrepancy between total volume measured and what our ureter inflow estimates predicted the total volume should be. Over all animals and cycles, the average difference between the measured V_v and computed V_v was 0.065 mL in the light cycle and 0.067 mL in the dark cycle. This represents a discrepancy of 0.013% and 0.014% of the total V_v in the light and dark cycles, indicating that the minimization of *Eq. 3* (subject to *Eq. 4*) yields ureter flow estimates that are consistent with measured volumes.

Validation of PVR estimates. To establish the validity of AMEASURE, we compared our estimates with actual PVR measurements. To achieve this, we observed a subset of animals continuously for 7–12 h and measured PVRs after every natural void and then reinserted the PVR into the bladder after each measurement. We compared the results of our estimation procedure, giving it access to only the last measured bladder volume in the series, to the measurements made throughout the observation period. This was done six separate times among four animals. The estimation showed good agreement with measurements of VE at 9.4 ± 8.3% over the 50 observed voids, spanning a wide range of VEs (Fig. 4*A*). We performed validations using VE since it normalizes to the size of each animal's bladder. Note that we obtained 0 VE for the last measurement in each series for procedural reasons; once the observation period was complete, we removed the bladder volume to obtain a final measurement even though the animal had not actually voided, thereby obtaining a zero efficiency. This illustrates a helpful feature of AMEASURE, namely, we can measure the bladder volume at any time (we do not have to wait for the animal to void) and still accurately estimate all PVRs in the series. We then removed the final 0 VE event post hoc for cystometric analysis.

Although the experimental validation was supportive of the estimation method, it only allowed us to compare our estimates with PVR measurements (which themselves are subject to noise), not to ground truth; therefore, we performed computer simulations of series of voids to analyze a situation where we know all cystometric values exactly. Even under experimental conditions conducive to measuring PVR via suction through suprapubic catheter, i.e., when the animal is anaesthetized and the bladder is exposed for visual inspection, we found a PVR measurement error of ±0.05 mL (14). Anecdotally, this measurement error may be due to experimental factors such as portions of bladder volume that failed to be collected, high suction drawing fluid out of the catheter itself rather than only the bladder, precision limits on measurement tools (e.g., scales and syringes), and similar technical details. For example, occasionally the bladder wall position relative to the indwelling catheter prevented us from withdrawing PVR; in these cases, inserting a small amount of saline (~50 μL) through the catheter repositioned the bladder walls, which then allowed us to withdraw the PVR (we subtracted off the added volume from the total PVR measurement).

Using the simulations, we compared the accuracy of determining VE by measuring every PVR to AMEASURE, which used only one bladder volume measurement. Figure 4*B* shows multiple different simulated conditions, with increasing intervoid ureter inflow rate variation on the *x*-axis and increasing durations of the serial cystometrograms (curves). For the experimental conditions described above in *Longitudinal voiding comparison between light and dark cycles* (marked by the "x"), simulations predicted our method to be ~9% worse than actu-

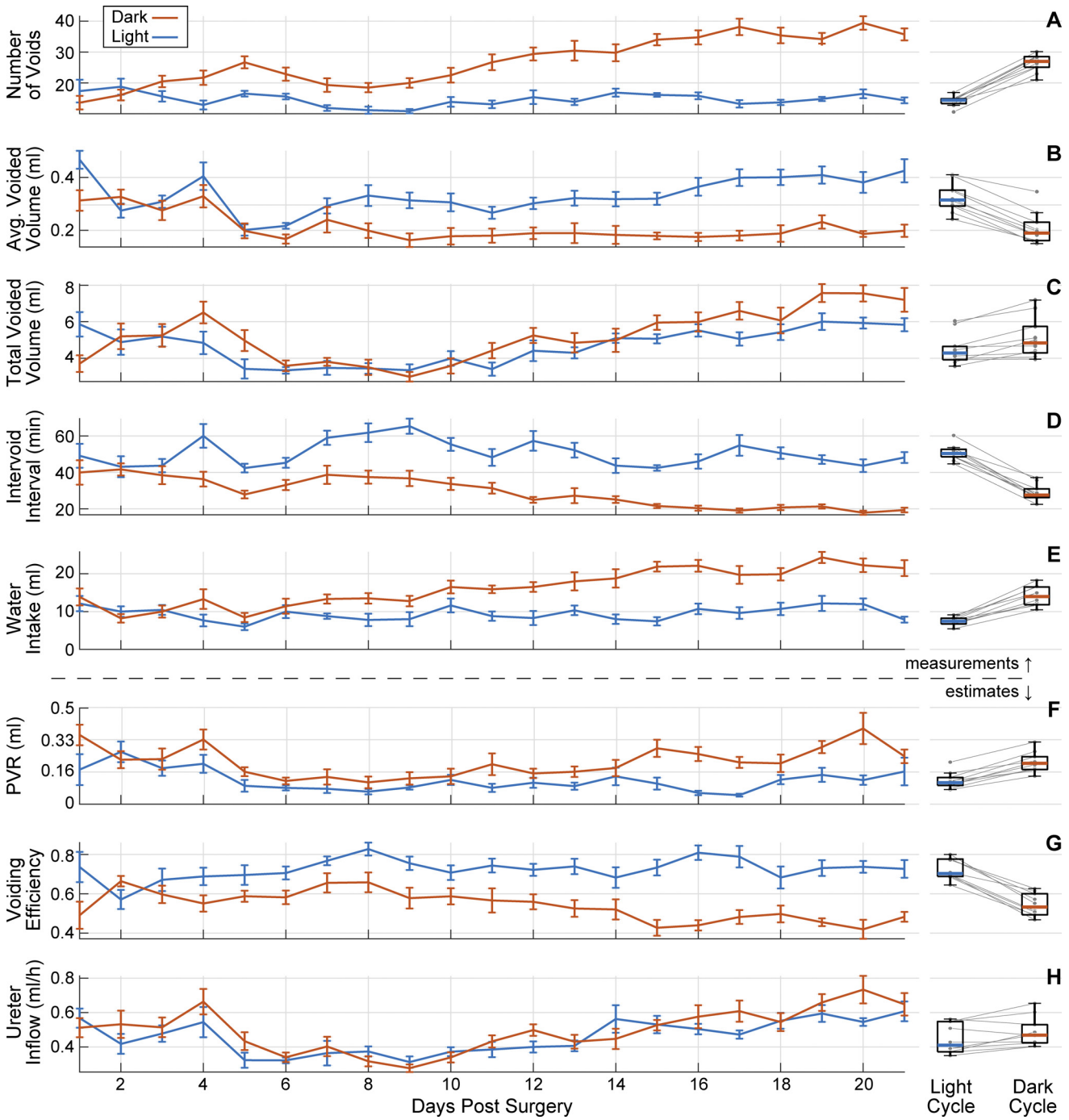

Fig. 3. Summary data for chronic continuous urodynamic parameters of 10 rats over 3 wk. *Left*: longitudinal plots showing the average over all animals' 12-h cycle averages for each day postsurgery (±SE), illustrating differences in behavior over time and between light and dark cycles. *Right*: boxplots displaying the average across all days of each animal's cycle averages, illustrating cycle differences. *A*: number of voids; *B*: average voided volume; *C*: total voided volume; *D*: intervoid interval.

ally measuring the PVR after each void, which is in close agreement with the validation experiments. (We used 12-h serial cystometrograms and measured the median ureter inflow rate SD of the 6 validation experiments, red dots, to be 0.13 mL/h.) Prediction accuracy degraded with increasing ureter inflow rate variation because we assumed that variation was low in our optimization method (*Eq. 3*, second term) and degraded with increasing series duration because error slowly accrues with each void in the conservation of volume balance. The ureter inflow variation we measured in the experimental validation is an overestimate of the true variation because the measurement process itself introduces noise; therefore, the "x" represents an upper bound on the expected error of AMEASURE relative to measuring all PVRs directly for actual experiment conditions. When intervoid ureter flow rate variation is lower than the precision

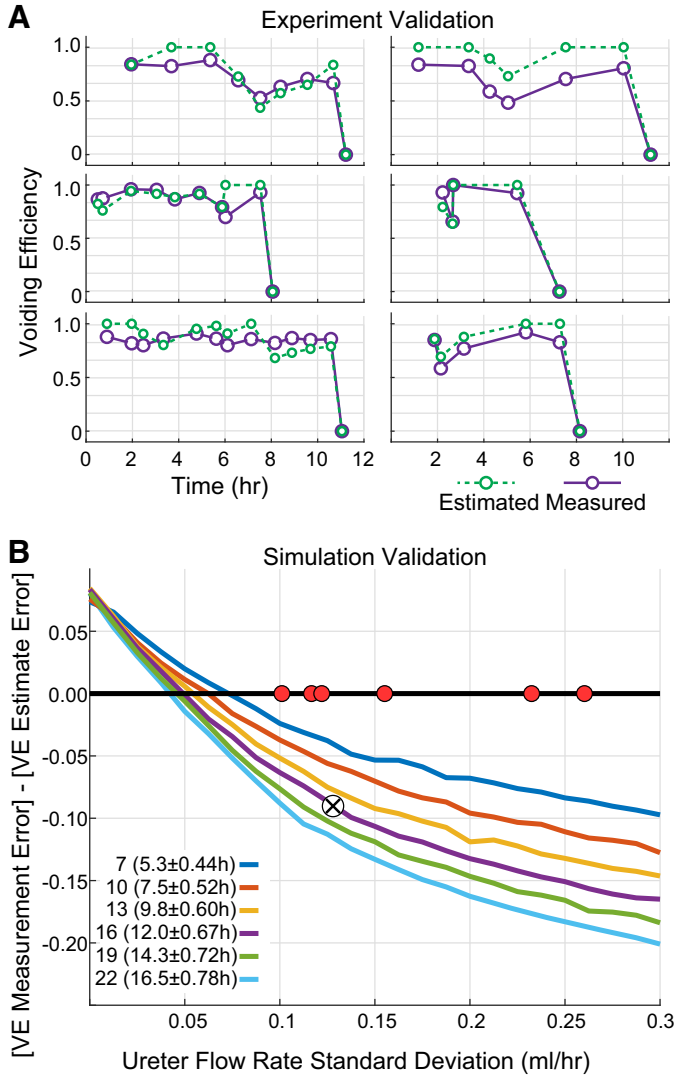

Fig. 4. Validations of Automatic Monitoring for Efficient, Awake, Sensitive, Urine Residual Estimation (AMEASURE). *A*: comparison of the AMEASURE estimate of voiding efficiency (VE) to direct measurement of VE in six light cycle serial cystometrograms, lasting 9.5 ± 1.9 h with an average of 8.3 voids per series. Estimates were in good agreement with experimental measurements, indicating that the technique is accurate despite using only one volume measurement to estimate VE for all events in the series. *B*: simulation of the difference in error between measuring VE after each void and the AMEASURE estimates. Each curve (representing different numbers of voids per series) is composed of the average of 2,000 simulations at each ureter inflow rate SD (horizontal axis). Ureter inflow rate SD was evaluated in increments of 0.0125 mL/h. Red circles denote the ureter inflow rate variation measured from each the six validation experiments shown in *A*. The black horizontal line marks where the AMEASURE estimate is equally good at determining VE as measuring VE after every void. The "x" denotes the approximate parameters in the main experimental study (Figs. 2 and 3).

associated with PVR measurements (14), AMEASURE is more accurate than measuring PVR directly after every void.

DISCUSSION

We have developed and validated AMEASURE, a combined metabolic cage system and optimization technique capable of obtaining, for the first time, V_v and PVR of every void made by a rat for weeks at a time (Figs. 2 and 3). We achieved this without the need for continuous human monitoring, without artificially infusing the bladder with saline, and without disruptions to the LUT caused by frequent removal of bladder volume. Compared with the gold standard method of measuring PVR directly after every void, AMEASURE is more flexible and comprehensive and is, at most, 9.5% less accurate (upper bound, Fig. 4). With this system, we found that LUT function evolves over the first 2 wk of observation in chronic studies with an implanted suprapubic catheter and that these changes are significantly different between light and dark cycles (Fig. 3).

We present a comparison between current practices for studying the LUT using rodent models in Table 1. In chronic studies of awake animals, AMEASURE is the only approach that can obtain PVR, bladder pressure data, and collect data in both light and dark cycles. AMEASURE preserves physiologically natural conditions at least as well as other currently used methods, although no method using a metabolic cage or anesthesia perfectly matches the rodents' natural state. In contrast to typical chronic studies, which use intermittent monitoring in metabolic cages that require artificial infusion rates to facilitate cystometric analysis (19, 23, 36, 42), AMEASURE requires very few volume measurements, which obviates the need for saline infusion and requires minimal experimenter oversight. Since volume is removed infrequently, AMEASURE will not interfere with the study of conditions such as urinary retention or underactive bladder (UAB) where the pathology is such that bladder is rarely empty.

The AMEASURE design is flexible, fundamentally requiring only the metabolic cages and scales to record time-stamped V_v. To reduce cost of the setup, fewer and cheaper metabolic cages can be used, provided they efficiently and accurately separate and gather liquid waste. For example, although we have presented AMEASURE with an indwelling catheter, this is not a strictly necessary component of the system. If bladder pressure is not required the animal does not need to be catheterized (and transducers are not required) and manual expression of the bladder into a collection vessel (or ultrasound) can be used to intermittently measure bladder volume for the estimation algorithm (see asterisks in Table 1). Likewise, water intake, real-time display of voiding, and video surveillance of behavior are not necessary to estimate PVRs, even though we include them here. The system can also be expanded to capture additional information. For example, if a drug under investigation may interfere with the natural phasic activation of the rat external urethral sphincter, a chronic sphincter electromyographic monitoring device (26) can be included in this system to determine how the treatment's effect on sphincter control affects voiding parameters (25, 38).

We found a large difference in voiding parameters between light and dark cycles that became more pronounced throughout the study (Fig. 3), starting at approximately *day 5*, highlighting the relevance of diurnal variation in LUT behavior. We attribute the increase in number of voids during the dark cycle throughout the experiment (slope: 1.23 voids/day, $R^2 = 0.85$, $P < 0.001$), in part, to increasing socialization among rats in adjacent cages because during the light cycle there was no corresponding change in the number of voids over time (slope: −0.09 voids/day, $R^2 = 0.07$, $P = 0.26$). The cages were set at 5- to 7-in. intervals and had open bottoms, which allowed animals to be aware of each other through vocalizations and scent marking through urination, which is a common method

Table 1. *Comparison of currently used methods of tracking urodynamic parameters in rodent models*

	Acute Anesthetized Preparations With a Catheter	Intermittent Long-Term Monitoring With an Indwelling Catheter	Continuous Long-Term Monitoring Without an Indwelling Catheter	AMEASURE System
		Processes monitored		
PVRs and voiding efficiency data	✓	✓	–	✓
Bladder pressure data	✓	✓	–	✓*
Voiding throughout the circadian cycle	–	–	✓	✓
Longitudinal studies supported	–	✓	✓	✓
		Physiological relevance		
Does not require frequent PVR collection	–	–	NA	✓
Requires minimal supervision	–	–	✓	✓
Bladder filled at a natural renal rate	–	–	✓	✓
Voiding without anesthesia	–	✓	✓	✓
Animals are unrestrained	NA	–/✓	✓	✓
Does not require invasive procedures	–	–	✓	–*

For acute anesthetized preparations with a catheter, the acute preparations obtain postvoid vesiduals (PVRs) but require anesthesia. While acute preparations are tightly controlled and statistically well powered, they are the least physiologically relevant. Restraining the animals does not apply since they are anesthetized. For intermittent long-term monitoring with an indwelling catheter, chronic studies where catheterized animals are placed in metabolic cages for short observation periods each day require the infusion of saline into the bladder to generate enough voids to achieve sufficient statistical power, and PVR measurements must be taken after each void. Some methods restrain animals, to measure electromyography, for example (36). For continuous long-term monitoring without an indwelling catheter, some chronic studies leave animals in metabolic cages continuously to measure voided volume but do not implant a catheter to measure PVR (15). This preserves many key physiological features but comes at the cost of obtaining PVRs and bladder pressure. For the Automatic Monitoring for Efficient, Awake, Sensitive, Urine Residual Estimation (AMEASURE) system, AMEASURE is able to collect all urodynamic measures and maximize physiological relevance, with the exception of using an invasive implanted catheter. *Fields that reverse if manual expression is used to measure bladder volume instead of an indwelling catheter; without an indwelling catheter, AMEASURE becomes equivalent to continuous long-term monitoring without an indwelling catheter with the important exception that estimating PVR is still possible. NA, not applicable.

of communication in rodents (5, 6). If rats in the dark cycle were voiding, in part, for the purposes of communication their goal may not have been complete evacuation of urine with each void. If true, this may explain why PVR was on average much larger (and correspondingly VE was lower) in the dark cycle; namely, when rats were active, they more often used voiding as a method of communication, and when rats were dormant, voids were used more often to simply empty the bladder.

We found that despite animals drinking much more water in the dark cycle than light cycle (Fig. 3*H*), there was a comparatively modest increase in urine production (Fig. 3*E*). We attribute the high initial ureter inflow rates in *days 1–5* to analgesic and antibiotic injections in saline boluses. Thereafter (*days 6–21*), ureter inflow increased slightly over days in both cycles (light: 0.019 $mL \cdot h^{-1} \cdot day^{-1}$, $R^2 = 0.83$, $P < 0.001$; dark: 0.026 $mL \cdot h^{-1} \cdot day^{-1}$, $R^2 = 0.84$, $P < 0.001$). During this same period, water intake increased in the dark cycle but not in the light (light: 0.10 mL/day, $R^2 = 0.10$, $P = 0.23$; dark: 0.76 mL/day, $R^2 = 0.86$, $P < 0.001$). Furthermore, there is a very large amount of water loss in both cycles, meaning that urine output is far less than water intake. Although the data gathered in this study do not clarify why this water loss occurs, the result is consistent with observations from the literature. Water intake is highly correlated with urine production (with a slope always <1 in linear models, indicating water loss) during observation of animals in metabolic cages that have been fasted; however, in animals that have been provided food ad libitum before (or during) observation there is no correlation between water intake and urine output (27, 30). The water loss between intake and urine production is also far larger in nonfasted animals (27), likely because water is diverted for food digestion and processing. Rats also eat more and generate more feces during the dark cycle, exhibiting a diurnal pattern that tracks voiding behavior closely (16). Since rats in this study ate and drank ad libitum, we expect a relatively low correlation between water intake and urine production (we found an average R^2 value across the 10 animals in *days 6–21* of 0.19 ± 0.12). Furthermore, we suspect that rats ate more frequently during the dark cycle (based on Ref. 16), which created far greater water loss in the dark cycle compared with the light cycle and which may explain the water loss patterns we observed (Fig. 3).

The purpose of this work is to develop a tool that facilitates translatability of basic urodynamic findings by maximizing the relevance of rodent models to human LUT physiology and context without sacrificing the acquisition of key urodynamic parameters. It is likely that one productive application of AMEAUSRE will be the study of UAB, the etiology of which remains unclear despite its large impact and calls for more research (3, 29, 41), because UAB is associated with high PVR (40) and a high prevalence of nocturia (39). AMEASURE can quantify changes in PVR caused by experimental interventions without needing to remove bladder volume after each void. This more closely models UAB because patients with UAB rarely have empty bladders. AMEASURE can also track diurnal changes in the LUT, both in frequency and PVR between active and dormant cycles. A potential experimental design to investigate new therapeutic compounds for UAB, such as PGE_2 (20, 21, 37), is to select a UAB model [e.g., aged animals (12, 33) or acute neurogenic injury (37)] and compare sets of treated and untreated rats analyzed with the AMEASURE system. The indwelling catheter can be implanted and rats left untreated for 5 days or until their bladder has completely recovered and no further pain medication is required. Thereafter, administration of drug to the treatment group can begin, and changes to the PVR, frequency, and diurnal voiding behavior can be used to assess the therapeutic effects of the compound on VE, nocturia, and voiding pressure. If the drug cannot be added directly to the water supply, it can be given by injection or fluid supplement, and in this case a corresponding vehicle-treated group would be required to control for the

additional animal handling and fluid intake. The duration of the study will depend on the timescale of action of the compound, but we have shown here that weeks-long monitoring is possible. If the effect of the drug on renal output is not known in advance, the first treated animals should be monitored for the time course of any potential changes in ureter inflow rate, and if the rate changes quickly, batch sizes shorter than 12 h should be used during the period of rapid change to maintain PVR estimation accuracy. If the drug has a rapid effect on renal output and must be delivered by injection, we recommend taking a bladder volume measurement (i.e., creating a new batch of voids) just before administration of the drug to curtail the effect of ureter inflow variance. The AMEASURE platform is especially well suited to study LUT dysfunction that involves long-term changes or washout effects, relevant diurnal variations, poor VE, free behavior, or sensitive bladders that may be affected by supraphysiological bladder filling rates.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.A., A.G., and Z.D. conceived and designed research; D.A., A.G., and Z.D. performed experiments; D.A., A.G., and Z.D. analyzed data; D.A., A.G., and Z.D. interpreted results of experiments; D.A., A.G., and Z.D. prepared figures; D.A., A.G., and Z.D. drafted manuscript; D.A., A.G., and Z.D. edited and revised manuscript; D.A., A.G., and Z.D. approved final version of manuscript.

REFERENCES

1. **Abrams P.** Bladder outlet obstruction index, bladder contractility index and bladder voiding efficiency: three simple indices to define bladder voiding function. *BJU Int* 84: 14–15, 1999. doi:10.1046/j.1464-410x.1999.00121.x.
2. **Abrams P.** *Urodynamics*. London: Springer-Verlag, 2006.
3. **Aggarwal H, Zimmern PE.** Underactive bladder. *Curr Urol Rep* 17: 17, 2016. doi:10.1007/s11934-016-0582-6.
4. **Bellucci CH, Wöllner J, Gregorini F, Birnböck D, Kozomara M, Mehnert U, Kessler TM.** Neurogenic lower urinary tract dysfunction−do we need same session repeat urodynamic investigations? *J Urol* 187: 1318–1323, 2012. doi:10.1016/j.juro.2011.11.107.
5. **Beynon RJ, Hurst JL.** Urinary proteins and the modulation of chemical scents in mice and rats. *Peptides* 25: 1553–1563, 2004. doi:10.1016/j.peptides.2003.12.025.
6. **Birke LIA.** Scent-marking and the oestrous cycle of the female rat. *Anim Behav* 26: 1165−1166, 1978. doi:10.1016/0003-3472(78)90106-9.
7. **Boggs JW, Wenzel BJ, Gustafson KJ, Grill WM.** Bladder emptying by intermittent electrical stimulation of the pudendal nerve. *J Neural Eng* 3: 43–51, 2006. doi:10.1088/1741-2560/3/1/005.
8. **Brower M, Grace M, Kotz CM, Koya V.** Comparative analysis of growth characteristics of Sprague Dawley rats obtained from different sources. *Lab Anim Res* 31: 166–173, 2015. doi:10.5625/lar.2015.31.4.166.
9. **Chancellor MB.** Urodynamic evaluation after spinal cord injury. *Phys Med Rehab Clinics* 4: 273–298, 1993.
10. **Chen SC, Grill WM, Fan WJ, Kou YR, Lin YS, Lai CH, Peng CW.** Bilateral pudendal afferent stimulation improves bladder emptying in rats with urinary retention. *BJU Int* 109: 1051–1058, 2012. doi:10.1111/j.1464-410X.2011.10526.x.
11. **Chun AL, Wallace LJ, Gerald MC, Levin RM, Wein AJ.** Effect of age on in vivo urinary bladder function in the rat. *J Urol* 139: 625–627, 1988. doi:10.1016/S0022-5347(17)42546-8.
12. **Coelho A, Charrua A, Oliveira R, Cavaleiro H, Cruz CD, Cruz F.** Underactive bladder in aging rats is associated with a reduced number of serotonin-expressing cells in the urethra and is improved by serotonin application to the urethra. *Low Urin Tract Symptoms* 11: 248−254, 2019. doi:10.1111/luts.12273.
13. **Colhoun AF, Klausner AP, Nagle AS, Carroll AW, Barbee RW, Ratz PH, Speich JE.** A pilot study to measure dynamic elasticity of the bladder during urodynamics. *Neurourol Urodyn* 36: 1086–1090, 2017. doi:10.1002/nau.23043.
14. **Danziger ZC, Grill WM.** Estimating postvoid residual volume without measuring residual bladder volume during serial cystometrograms. *Am J Physiol Renal Physiol* 311: F459–F468, 2016. doi:10.1152/ajprenal.00516.2015.
15. **Dogishi K, Okamoto K, Majima T, Konishi-Shiotsu S, Homan T, Kodera M, Oyama S, Oyama T, Shirakawa H, Yoshimura N, Nakagawa T, Kaneko S.** A rat long-lasting cystitis model induced by intravesical injection of hydrogen peroxide. *Physiol Rep* 5: e13127, 2017. doi:10.14814/phy2.13127.
16. **Eriksson E, Royo F, Lyberg K, Carlsson HE, Hau J.** Effect of metabolic cage housing on immunoglobulin A and corticosterone excretion in faeces and urine of young male rats. *Exp Physiol* 89: 427–433, 2004. doi:10.1113/expphysiol.2004.027656.
17. **Griffiths CJ, Murray A, Ramsden PD.** Accuracy and repeatability of bladder volume measurement using ultrasonic imaging. *J Urol* 136: 808–812, 1986. doi:10.1016/S0022-5347(17)45086-5.
18. **Gupta A, Defreitas G, Lemack GE.** The reproducibility of urodynamic findings in healthy female volunteers: results of repeated studies in the same setting and after short-term follow-up. *Neurourol Urodyn* 23: 311–316, 2004. doi:10.1002/nau.20039.
19. **Herrera GM, Meredith AL.** Diurnal variation in urodynamics of rat. *PLoS One* 5: e12298, 2010. doi:10.1371/journal.pone.0012298.
20. **Hindley RG, Brierly RD, Thomas PJ.** Prostaglandin E2 and bethanechol in combination for treating detrusor underactivity. *BJU Int* 93: 89–92, 2004. doi:10.1111/j.1464-410X.2004.04563.x.
21. **Hokanson JA, Langdale CL, Sridhar A, Grill WM.** OAB without an overactive bladder in the acute prostaglandin E_2 rat model. *Am J Physiol Renal Physiol* 313: F1169–F1177, 2017. doi:10.1152/ajprenal.00270.2017.
22. **Hokanson JA, Langdale CL, Sridhar A, Grill WM.** Stimulation of the sensory pudendal nerve increases bladder capacity in the rat. *Am J Physiol Renal Physiol* 314: F543–F550, 2018. doi:10.1152/ajprenal.00373.2017.
23. **Hsieh TH, Lin YT, Chen SC, Peng CW.** Chronic pudendal neuromodulation using an implantable microstimulator improves voiding function in diabetic rats. *J Neural Eng* 13: 046001, 2016. doi:10.1088/1741-2560/13/4/046001.
24. **Keirstead HS, Fedulov V, Cloutier F, Steward O, Duel BP.** A noninvasive ultrasonographic method to evaluate bladder function recovery in spinal cord injured rats. *Exp Neurol* 194: 120–127, 2005. doi:10.1016/j.expneurol.2005.01.027.
25. **LaPallo BK, Wolpaw JR, Chen XY, Carp JS.** Contribution of the external urethral sphincter to urinary void size in unanesthetized unrestrained rats. *Neurourol Urodyn* 35: 696−702, 2016. doi:10.1002/nau.22789.
26. **LaPallo BK, Wolpaw JR, Chen XY, Carp JS.** Long-term recording of external urethral sphincter EMG activity in unanesthetized, unrestrained rats. *Am J Physiol Renal Physiol* 307: F485–F497, 2014. doi:10.1152/ajprenal.00059.2014.
27. **Lee KM, Reed LL, Bove DL, Dill JA.** Effects of water dilution, housing, and food on rat urine collected from the metabolism cage. *Lab Anim Sci* 48: 520–525, 1998.
28. **Leung PY, Johnson CS, Wrathall JR.** Comparison of the effects of complete and incomplete spinal cord injury on lower urinary tract function as evaluated in unanesthetized rats. *Exp Neurol* 208: 80–91, 2007. doi:10.1016/j.expneurol.2007.07.013.
29. **Li X, Liao L.** Updates of underactive bladder: a review of the recent literature. *Int Urol Nephrol* 48: 919–930, 2016. doi:10.1007/s11255-016-1251-x.
30. **Longhurst PA, Eika B, Leggett RE, Levin RM.** Comparison of urinary bladder function in 6 and 24 month male and female rats. *J Urol* 148: 1615–1620, 1992. doi:10.1016/S0022-5347(17)36981-1.

31. **Maggi CA, Santicioli P, Giuliani S, Furio M, Meli A.** The capsaicin-sensitive innervation of the rat urinary bladder: further studies on mechanisms regulating micturition threshold. *J Urol* 136: 696–700, 1986. doi:10.1016/S0022-5347(17)45030-0.
32. **Maggi CA, Santicioli P, Meli A.** The nonstop transvesical cystometrogram in urethane-anesthetized rats: a simple procedure for quantitative studies on the various phases of urinary bladder voiding cycle. *J Pharmacol Methods* 15: 157–167, 1986. doi:10.1016/0160-5402(86)90064-1.
33. **Pagala MK, Tetsoti L, Nagpal D, Wise GJ.** Aging effects on contractility of longitudinal and circular detrusor and trigone of rat bladder. *J Urol* 166: 721–727, 2001. doi:10.1016/S0022-5347(05)66050-8.
34. **Robinson MA, Herron AJ, Goodwin BS, Grill RJ.** Suprapubic bladder catheterization of male spinal-cord-injured Sprague-Dawley rats. *J Am Assoc Lab Anim Sci* 51: 76–82, 2012.
35. **Schmidt F, Yoshimura Y, Ni RX, Kneesel S, Constantinou CE.** Influence of gender on the diurnal variation of urine production and micturition characteristics of the rat. *Neurourol Urodyn* 20: 287–295, 2001. doi:10.1002/nau.1006.
36. **Schneider MP, Hughes FM Jr, Engmann AK, Purves JT, Kasper H, Tedaldi M, Spruill LS, Gullo M, Schwab ME, Kessler TM.** A novel urodynamic model for lower urinary tract assessment in awake rats. *BJU Int* 115, *Suppl* 6: 8–15, 2015. doi:10.1111/bju.13039.
37. **Sekido N, Kida J, Mashimo H, Wakamatsu D, Okada H, Matsuya H.** Promising effects of a novel EP2 and EP3 receptor dual agonist, ONO-8055, on neurogenic underactive bladder in a rat lumbar canal stenosis model. *J Urol* 196: 609–616, 2016. doi:10.1016/j.juro.2016.02.064.
38. **Streng T, Santti R, Andersson KE, Talo A.** The role of the rhabdosphincter in female rat voiding. *BJU Int* 94: 138–142, 2004. doi:10.1111/j.1464-4096.2004.04875.x.
39. **Uren AD, Cotterill N, Harding C, Hillary C, Chapple C, Klaver M, Bongaerts D, Hakimi Z, Abrams P.** Qualitative exploration of the patient experience of underactive bladder. *Eur Urol* 72: 402–407, 2017. doi:10.1016/j.eururo.2017.03.045.
40. **Uren AD, Drake MJ.** Definition and symptoms of underactive bladder. *Investig Clin Urol* 58, *Suppl* 2: S61–S67, 2017. doi:10.4111/icu.2017.58.S2.S61.
41. **Valente S, DuBeau C, Chancellor D, Okonski J, Vereecke A, Doo F, Lajiness M, Diokno A, Chancellor M.** Epidemiology and demographics of the underactive bladder: a cross-sectional survey. *Int Urol Nephrol* 46, *Suppl* 1: S7–S10, 2014. doi:10.1007/s11255-014-0811-1.
42. **Yaksh TL, Durant PAC, Brent CR.** Micturition in rats: a chronic model for study of bladder function and effect of anesthetics. *Am J Physiol Regul Integr Comp Physiol* 251: R1177–R1185, 1986. doi:10.1152/ajpregu.1986.251.6.R1177.
43. **Yang Z, Dolber PC, Fraser MO.** Differential vulnerabilities of urethral afferents in diabetes and discovery of a novel urethra-to-urethra reflex. *Am J Physiol Renal Physiol* 298: F118–F124, 2010. doi:10.1152/ajprenal.00281.2009.