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Baseline cystometric parameters in conscious and anesthetized sheep: experimental data and systematic review

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OBJECTIVE
To characterize cystometry in conscious and anesthetized sheep, including bladder response to sacral root electrical stimulation, thereby providing a baseline set of values.

METHODS
Single-fill cystometries were repeated in adult mule ewes both conscious (n = 5) and under general anesthesia (18) using a commercial system. Parameters including bladder capacity, detrusor (bladder) pressure, urethral opening pressure, bladder compliance, number of nonvoiding detrusor contractions, and bladder pressure change in response to electrical stimulation of the sacral roots under general anesthesia are reported. Pubmed, Embase, and Web of Science databases were searched for studies relating to ovine cystometry, and a systematic review was conducted.

RESULTS
In awake sheep, mean ± SD bladder capacity was 79.6 ± 32.2 mL, urethral opening pressure was 26.0 ± 10.7 cm H_2O, and compliance was 3.5 ± 1.9 mL/cm H_2O. Peak detrusor pressures during micturition reached 57.7 ± 28.3 cm H_2O. In anesthetized animals, mean bladder capacity (endpoint, 50 cm H_2O) was 333 ± 191 mL, and mean bladder compliance was 7.7 ± 4.9 mL/cm H_2O. Values for these parameters from our systematic review are presented for comparison and reference. Electrical stimulation of the second and third sacral roots caused a greater increase in detrusor pressure than stimulation of the first and fourth sacral roots.

CONCLUSIONS
We present a comprehensive set of data for normal cystometry parameters in sheep, including the first report of detrusor response to sacral root stimulation in anesthetized sheep.

CLINICAL RELEVANCE
This report provides a valuable set of baseline values for a potential translational model of value to neurourologic research and may be a useful reference for clinicians.

Keywords: ovine cystometry, spinal cord injury, urinary incontinence, sacral nerve stimulation, large animal translational model

Spinal cord injury can cause disruption to the normal sensation of bladder filling and an inability to consciously trigger voiding in people and animals. In suprasacral injuries, plasticity in remaining intact spinal cord segments below the lesion can lead to an overactive bladder, with contraction of the detrusor against a closed urethral sphincter (detrusor dyssenergia) and intermittent uncontrolled voiding of small amounts of urine.[1] Additionally, there can be a lack of awareness of bladder fullness leading to urine retention and bladder distension. These have major impacts on the health and quality of life of affected individuals or animals (and their owners).

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Intermittent catheterization is commonly used to manage incontinence following spinal cord injury in people but is invasive and infection-prone; urinary dysfunction remains a significant cause of morbidity[2] and mortality[3] in people with chronic spinal cord injury. In dogs, bladder expression is most commonly used to manage incontinence after spinal cord injury. This is known to lead to incomplete bladder emptying,[4] and in 1 study,[5] 75% of chronically paraplegic dogs had at least 1 positive urine culture with 28% of dogs having recurrent infections (for a recent review, see also Granger et al[6]).

Sacral anterior root stimulation with an implant, notably using the Finetech-Brindley neuroprosthesis in people[7] and dogs,[8] provides an established means of replacing voluntary bladder voiding. However, posterior sacral roots frequently need to be cut during implantation in people to increase bladder compliance and urine storage, prevent reflex voiding, and aid voiding during electrical stimulation. This rhizotomy is irreversible and eliminates any remaining bladder fullness sensation and sexual function (reflex erections). Therefore, there is a clear need for improved neuroprostheses. More selective stimulation timed to suppress reflex bladder contractions, triggered by the detection of neural signals from the bladder, is a promising route demonstrated in rats.[9] One of the key barriers to the use of such a closed-loop control device in people and dogs is the development of a clinically applicable and noninvasive way of detecting bladder afferent signals from the sacral roots (ie, measuring fullness and/or pressure of the bladder via signals of stretch, tension, and/or nociception in afferent bladder fibers).[10] Such development requires an in vivo large animal model of comparable size to people, as this will better replicate the engineering challenges.

Nerve cuffs, which sit around the nerve and are therefore clinically safer than more invasive intraneural electrode devices, have particularly poor signal-to-noise ratios. The volume of a nerve can be assumed to be approximately cylindrical and therefore increases as a square of the diameter, ie, the volume increases markedly with small increases in diameter. This increased volume means that a nerve cuff sits further from individual axons and there is more tissue for electrical signals (ie, neural information) to penetrate and likely more unrelated signals due to a greater number of axons; hence, the signal-to-noise ratio is lower. This has major impacts on the design of nerve cuffs, amplification, and signal processing, and all these challenges must be addressed before translation to people.

Sheep are generally placid in temperament and tolerant of handling, requiring minimal facilities for housing and management in veterinary universities. Retrograde urethral catheterization of the female sheep urinary bladder can be performed with minimal restraint and without the need for sedation or anesthesia, therefore, avoiding the use of drugs (eg, opioids) known to affect normal voiding reflexes and bladder contractions.[11]

There have been isolated reports of urodynamics in conscious[12–21] and anesthetized[22–28] sheep, but no unified method or clear characterization of normal bladder physiological values exists for this species. We provide a more comprehensive summary of baseline cystometric values in conscious and anesthetized sheep than previously available in the literature. This report also describes a detailed method for cystometry in anesthetized and conscious sheep that can be replicated in future studies and therefore used as a standard. In anesthetized animals, we investigate urinary bladder pressure following direct electrical stimulation of the extradural sacral roots as would be required in a closed-loop neuroprosthesis. To provide a comprehensive reference for sheep cystometry, we have further compared our findings to existing studies reporting urodynamics in sheep via a brief systematic review.

Methods

Animals

Eighteen adult mule ewes (cross Bluefaced Leicester and Swaledale) were used, obtained from an on-site farm at The Royal Veterinary College, UK. Animals were culled ewes all aged 4 to 5 years old, with a median weight of 63 kg (range, 39 to 107 kg). To be included in this study, animals had to be in good health with a normal clinical and neurological examination. All animals were included in the results. All procedures were performed at the Royal Veterinary College with local ethical approval (Animal Welfare and Ethical Review Body) and under UK Home Office regulations (project license P302A3B70). Sheep were group housed at pasture or indoors in large straw pens with ad libitum access to hay, water, and additional concentrate feed. All sheep had an anesthetic with cystometry, sacral dorsal laminectomy, nerve cuff implant, and electrical stimulation of the sacral roots as described in the following sections. Thirteen sheep were euthanized under this first anesthetic, and 5 were recovered for conscious cystometries and then euthanized under a second anesthetic. All animals were therefore euthanized at the end of experiments by an overdose of IV pentobarbital (150 mg/kg) while under anesthesia. The experimental unit was an individual sheep, and group numbers are listed below in relevant sections.

Cystometry

Cystometry in anesthetized sheep

Sheep (n = 18) were premedicated with a transdermal fentanyl patch the day before the experiment, induced with IV ketamine (7.5 mg/kg) and midazolam (0.5 mg/kg), and then intubated and maintained on sevoflurane in oxygen. Animals were ventilated, and physiological parameters were continuously monitored. Body temperature was maintained and animals received IV fluids (5% glucose in Hartmann solution). Sheep were positioned in sternal recumbency with hind legs flexed ("sphinx position" with hips flexed and hocks flat on the table).

After induction of anesthesia, single-fill cystometry was performed using a commercial fluid-charged system (Medica Pico Smart, which includes a peristaltic pump). A dual lumen 6-French catheter (Medica 2CYS-6LE) was placed via the urethra into the urinary bladder for intravesical pressure measurement and sterile saline filling and secured using sutures or tape around the tail. A rectal pressure catheter (Medica 1AR-5TC) was placed to measure abdominal pressure and therefore calculate detrusor pressure (ie, intravesical pressure − rectal pressure).[29] Rectal pressure is routinely used to estimate abdominal pressure and therefore elimate intravesical pressure changes not related to detrusor pressure.[29] Pressure was zeroed externally at a reference level of the urethral meatus.

A 12-French 10-mL balloon Foley catheter was used to block urethral outflow and prevent passive bladder emptying during filling. The Foley was placed by retrograde urethral catheterization into the urinary bladder (confirmed by obtaining urine through the catheter). The Foley balloon was then inflated and pulled caudally until resistance was felt with the balloon against the neck of the bladder and obstructing the internal urethral sphincter. The catheter was then clamped in place during filling of the urinary bladder. Without the Foley in place, urine drained from the bladder almost immediately after infusion was started in anesthetized animals; hence, leak point pressure (spontaneous) was not a useful measurement in our experiments and was not recorded. The bladder was emptied until negative pressure with syringe suction on the urinary catheter was achieved. The urinary bladder was palpated externally to change its position, and the suction was again applied; this was repeated until negative pressure was reliably obtained. Bladder filling was then performed with room temperature sterile saline at fill rates of 50 to 200 mL/min. Detrusor pressure and volume of saline infused were recorded. Saline infusion was stopped when saline leaked around the Foley catheter or detrusor pressure reached 50 cm H_2O. Change in volume divided by change in pressure from the empty bladder to this point was used to calculate compliance of the whole cystometry.

Initial "baseline" cystometries were taken within 1 hour of induction of anesthesia. Subsequent cystometries in each sheep were taken during the same anesthetic event after a nerve cuff was placed in apposition around 1 sacral root (S2 or S3 unilaterally) for the purpose of a separate experiment recording nerve signals. The number of cystometries was governed primarily by obtaining electroneurogram data for this separate experiment and limited by our ethical approval. All cystometries are included in the results. The total time the sheep had been under anesthesia at the start of each cystometry was noted, with time 0 as baseline cystometry.

Full details of the implanted nerve cuff are previously published.[30] Briefly, the nerve cuff was a "buckle" design of adjustable diameter with 10 equally spaced electrodes, manufactured by laser cutting silicon rubber and stainless-steel foil with a reinforced cast silicone block and two 5-core Cooper Cables exiting parallel to the nerve. Intravenous boluses of fentanyl (5 µg/kg) or ketamine (2 to 5 mg/kg) analgesia were given during a dorsal sacral laminectomy surgical approach as required for pain management.

Two distinct phases were noted during anesthetized cystometries: an initial slow increase in bladder pressure with constant rate infusion of saline into the urinary bladder (low gradient of curve) and then a transition to a much faster increase in bladder pressure with continued constant rate infusion (high gradient section of curve; **Figure 1**). The point of change in gradient ("breakpoint")[31] was estimated by smoothing the detrusor pressure curve, determining the equation of the tangent lines at 40% and 90% of bladder capacity in each cystometry, and calculating the pressure at which these lines intercepted using a custom script in MATLAB 2019 **(Supplementary Material S1)**. Compliances for the 2 phases were also calculated, termed initial and terminal compliance, respectively.[32] Initial compliance was calculated by change in volume divided by change in pressure from start of fill (empty bladder) to this breakpoint and terminal compliance by change in volume divided by change in pressure from breakpoint to endpoint (50 cm H_2O). Bladder pressure after accommodation (measured manually from the plateau of the bladder pressure curve immediately before emptying) was recorded for all baseline cystometries.

Cystometry in conscious sheep

A subset of animals (n = 5) were allowed to recover from anesthesia and had further conscious cystometries at a later date without chemical restraint or sedation. In these animals, transdermal fentanyl was continued for 3 days and intramuscular meloxicam (0.4 mg/kg) injection continued for up to 10 days postoperatively. These animals were euthanized as described above under a second anesthetic at the end of conscious experiments.

Conscious cystometry was performed as described above, but with sheep standing and at a single fill rate of 50 mL/min. Conscious cystometry was performed at the highest rate considered safe from clinical experience (to minimize time of procedures for experimental animals). A Foley catheter to block the passive outflow of urine from the bladder was not required.

The standard cystometry data set in people[33] includes defined points such as first sensation of bladder filling, first desire to void, and strong desire to void, which are not possible to obtain in sheep. In conscious animals, we, therefore, recorded volume infused and detrusor pressure at the point of posturing to urinate and initiation of voiding flow (termed bladder capacity and urethral opening pressure, respectively) starting from an emptied bladder. Filling was stopped at posturing, and maximum detrusor pressure was recorded immediately before voiding (urine outflow) was recorded. Urine flow and volume were not measured.

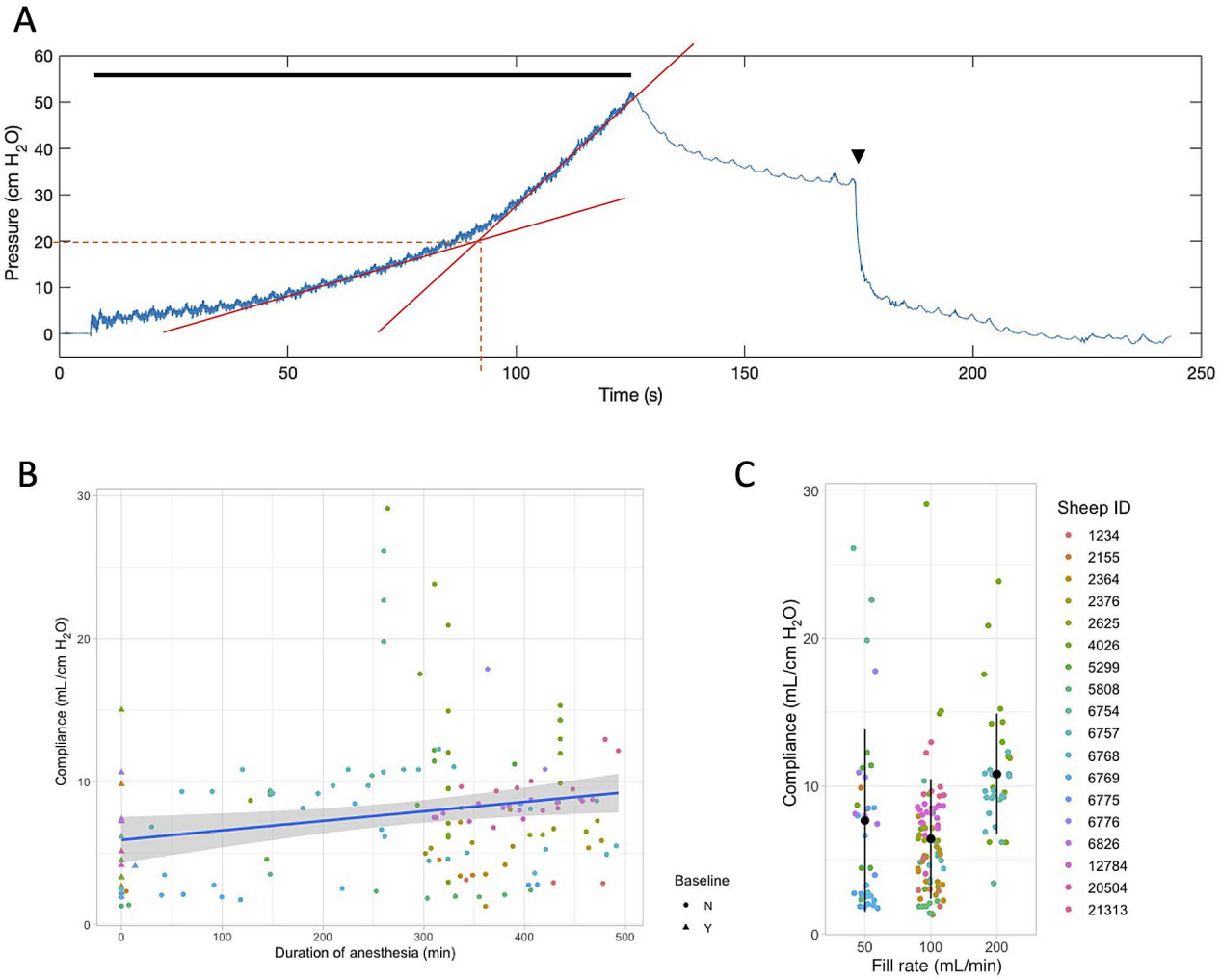

Figure 1—Cystometry under anesthesia. A representative trace of detrusor pressure recorded during 1 complete cystometry is shown (A). Black bar shows duration of bladder filling at 50 mL/min, and black arrowhead shows point where the Foley catheter was released and the bladder rapidly empties. Note the accommodation of the bladder and gradual decrease in pressure between end of filling and point of emptying associated with stress relaxation of the bladder wall. Red lines are the tangents drawn programmatically to identify gradient of the curve at 40% and 90% of final infused volume, and dotted red line illustrates the detrusor pressure at which the gradient of the curve is thereby defined to change. This highlights a change between 2 phases of filling, between initial and terminal compliance. Small oscillations at a rate of roughly 15 per minute throughout the trace are breathing artifacts. Higher frequency smaller magnitude noise during filling is from the infusion pump. A scatter plot (B) highlights that bladder compliance increases with increasing time under anesthesia (blue line shows line of linear regression, gray shading is 95% CI, and color identifies individual sheep). There was no significant difference in bladder compliance between fill rates (C; color identifies individual sheep, and individual points are offset on x-axis for visualization only, black points and vertical lines represent mean ± SD).

Compliance was calculated as bladder capacity divided by urethral opening pressure in conscious cystometries. Nonvoiding detrusor contractions were defined as a transient increase of more than 5 cm H_2O returning to preincrease levels not associated with movement, change in rectal pressure, or urine leakage[34] and were counted manually.

Electrical stimulation of the sacral roots

Under anesthesia, as described above (first anesthesia in the 5 animals recovered), a lumbosacral dorsal laminectomy was performed to expose the sacral roots S1-S4 bilaterally, identified based on anatomical landmarks (L7-S1 junction and differing course and size of L7 root). The bladder was filled to a pressure of 20 cm H_2O. Each visible extradural root was stimulated in turn once, unilaterally or bilaterally, at 30 Hz with consistently ramping voltage from 1 to 20 V using a voltage-controlled battery-powered surgical stimulator (Finetech Medical BSD260) and bipole hook electrodes (Finetech Medical). The order of stimulation was varied between sheep. For bilateral stimulation, both left and right roots were placed within this same bipole hook (containing 2 electrodes) at the same time. Detrusor pressure was measured continuously throughout electrical stimulation, and pressure was allowed to return to baseline between stimulation of each root.

Systematic search of the literature

A systematic review was conducted by searching Pubmed, Embase, and Web of Science with the search term "urodynamic* OR cystometr* OR (bladder AND pressure) OR (bladder AND volume) AND (sheep OR ovine OR ovis)". Searches were performed on February 5, 2021. Search libraries were combined in Endnote (X9.3.2; Clarivate), and duplicates were removed using an online deduplication tool.[35] Abstracts were screened using the CAMARADES Preclinical Systematic Review and Meta-analysis Facility with prespecified inclusion/exclusion criteria (criteria and data categories extracted are provided; Supplementary Material S1).

Statistical analysis

Descriptive statistics are reported as means ± SD unless otherwise stated. Normal linear regression models were used to analyze data with multiple dependent variables, and all variables were included as fixed effects. A threshold $P < .05$ was used for significance. Analysis was performed in Rstudio (The R Foundation).[36] Statistical analyses were not preplanned and are therefore considered exploratory and unpowered.

Results

Filling cystometry under anesthesia

The mean weight of the 18 sheep receiving cystometries under anesthesia was 62.7 ± 15.0 kg. Each animal had a median of 8 (range, 1 to 27) cystometries.

Detrusor pressure increased with continuous filling (Figure 1), initially slowly and then more rapidly in the last third of filling. This was reflected in a change between initial and terminal bladder compliance, from 12.7 ± 9.7 to 5.0 ± 3.2 mL/cm H_2O, respectively. The pressure at which this change in slope occurred was 23.3 ± 8.4 cm H_2O in the subset of cystometries where this could be calculated using the custom script (102 out of a total of 147 cystometries). In the remaining cystometries, an intercept could not be programmatically determined without changing parameters due most commonly to an irregular shape of the cystometry curve. Bladder volume at this breakpoint was 236 ± 118 mL.

Once filling stopped, a decrease in pressure was consistently seen as the bladder accommodated to the infused volume. The average pressure after accommodation was 32.1 ± 9.7 cm H_2O; this represents an average reduction to 62.4 ± 16.4% of peak bladder pressure. Pressure dropped almost instantaneously once the Foley catheter was released to drain the bladder. Detrusor contractions were not seen during filling under anesthesia.

Mean bladder capacity (endpoint, 50 cm H_2O) was 333 ± 191 mL (approximately 5.2 mL/kg), and mean bladder compliance for whole cytometry was 7.7 ± 4.9 mL/cm H_2O. There were significant differences in this whole cystometry compliance between individual sheep ($F[17,126] = 9.5$; $P < .0001$), and increasing duration of anesthesia significantly increased bladder compliance ($F[1,126] = 14.2$; $P < .001$; Figure 1).

The mean anesthesia duration was 426 ± 57 minutes. Baseline bladder capacity (at initial cystometry at time 0) was 337 ± 190 mL compared to 300 ± 200 mL in all later cystometries. These later cystometries occurred at a mean of 317 ± 121 minutes (range, 40 to 493 minutes into anesthesia). Baseline compliance was 5.7 ± 3.6 mL/cm H_2O compared to 8.0 ± 5.0 mL/cm H_2O across all later cystometries. The equation of the regression line for compliance as a function of anesthesia duration (Figure 1) is $y = 0.0067x + 5.9$, $R = 0.21$, and $R^2 = 0.54$.

There was no significant difference in compliance between the fill rates used in this study ($F[2,126] = 9.6$, $P < .001$, but $P > .05$ on post hoc Tukey honestly significant difference between rates – $P = .21$ for 50:100 mL/min, $P = .86$ for 50:200 mL/min, and $P = .53$ for 100:200 mL/min; Figure 1).

Conscious filling cystometry

The mean weight of the subset of 5 sheep also receiving conscious cytometries was 65.4 ± 14.8 kg. These sheep had a median of 9 cytometries (range, 5 to 19) on a median of 2 occasions (range, 1 to 4) up to 7 months (range, 13 to 221 days) after initial cystometries under anesthesia. Urethral catheterization and conscious filling cystometry were well tolerated with only mild restraint by one person.

Detrusor pressure progressively increased until voiding, with occasional nonvoiding contractions noted during filling **(Figure 2)**. Nonvoiding contractions were seen in 38% of cystometries (18 of 47) with a median number per cystometry of 0 (range, 0 to 3). Nonvoiding contractions were identified in 4 out of 5 sheep. There were no significant differences in the number of contractions between individual sheep or between the day of cystometry recording.

Detrusor pressure during voiding showed 1 phase as previously reported,[37,38] although voiding volume and duration were not recorded (Figure 2). Average bladder capacity was 79.6 ± 32.2 mL, average urethral opening pressure was 26.0 ± 10.7 cm H_2O, and average compliance was 3.5 ± 1.9 mL/cm H_2O. Peak detrusor pressures during micturition reached 57.7 ± 28.3 cm H_2O.

Response to sacral root stimulation

The mean weight of the 17 sheep receiving sacral root stimulation (1 animal did not receive stimulation to the sacral roots due to experimental time constraints) was 64.8 ± 19.3 kg. An example graph showing bladder pressure in response to stimulation is provided **(Figure 3)**. Unilateral extradural stimulation of S1-S4, in turn, each increased detrusor pressure on average, but the increase was greater in S2 and S3 (10 ± 7 and 10 ± 8 cm H_2O, respectively) than S1 and S4 (3 ± 6 and 4 ± 3 cm H_2O, respectively). Differences reached significance ($F[3,104] = 21.9$; $P < .0001$) between S1-S2 ($P < .0001$ on post hoc Tukey honestly significant difference), S1-S3 ($P < .0001$), and S3-S4 ($P = .032$) and with $P = .073$ for S2-S4.

There was also significantly greater bladder response to bilateral stimulation compared to

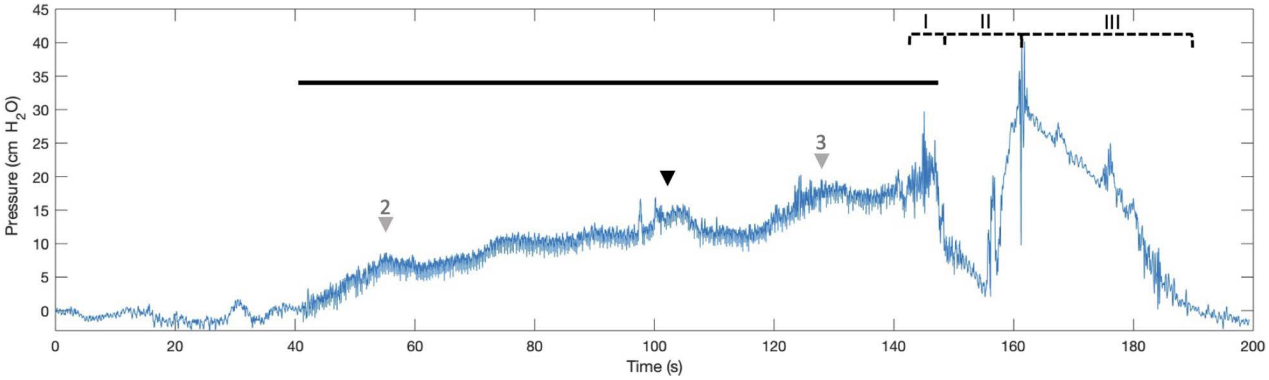

Figure 2—Conscious cystometry. A representative trace of detrusor pressure recorded during 1 conscious cystometry is shown. Solid black line marks duration of saline filling at 50 mL/min. Black arrowhead marks nonvoiding bladder contraction. Gray arrowheads mark nonvoiding variations in pressure that do not meet criteria for "nonvoiding contraction," and associated gray numbers are magnitude of change in pressure (cm H_2O). Dashed black line brackets labeled I, II, and III indicate respective phases of voiding. Please note these phases are indicative based on observation at the time of cystometry as voiding duration and volume were not measured.

unilateral stimulation (F[1,104] = 11.5; P < .001), differences between individual sheep in magnitude of response (F[16,104] = 8.7; P < .0001), but no difference between left and right unilateral stimulation (F[1,104] = 2.9; P = .093).

Stimulation of S1 caused marked hind limb musculature contraction; for stimulation of S2 to S4 tail, movement was primarily seen (as expected from their innervation).

Systematic review of filling cystometry in sheep

Search terms returned 166 studies in Pubmed, 138 studies in Embase, and 118 studies in Web of Science. After we removed duplicates, 257 studies remained. After the abstract screening, 52 studies were included for data extraction based on the following criteria. Inclusion criteria were as follows: studies in sheep of any age or breed; abstract reports of urodynamics or cystometry or mentions bladder volume, pressure, or compliance; and abstracts that provided sufficient information due to the small number of studies. Exclusion criteria were as follows: not primary research, no normal or control group, and preterm gestation animals. During extraction, a further 34 studies were excluded due to lack of reporting of a mean value for specified urodynamic parameters. Five studies included data on multiple experiments or groups of animals and 2 studies used the same control group, providing 21 included experiments (a PRISMA flow chart and all data from included studies are provided; Supplementary Material S1 and **Supplementary Table S1**).

Of these, 12 experiments reported urodynamic parameters with sheep conscious and 9 with sheep anesthetized. All studies in anesthetized sheep used halothane with or without nitrous oxide so anesthetic agent was not further considered. Sex of animals was reported in all except 3 studies, 8 experiments used females, and 8 used males (5 experiments not specified). Ages ranged from 1 month to 5 years, and median age was 12 months. Fill rate during cystometry was reported in 15 experiments, with 2 experiments reporting "incremental" increases (at 10 to 60 mL) and the remainder reporting continuous filling between 2 and 30 mL/min (30 mL/min being the most common, reported in 9 experiments). Nine experiments reported the temperature of infused saline, 8 reported position of cystometry (all conscious; 5 standing and 3 in lateral recumbency), and 3 reported weight of sheep.

All experiments reported bladder capacity, 6 experiments reported leak pressure (under anesthesia), 1 reported urethral opening pressure of 26.6 ± 14.4 cm H_2O (in conscious animals), 3 reported peak pressures during voiding (in conscious animals), and 8 reported bladder compliances (7 anesthetized and 1 conscious).

Under anesthesia, all studies used a suprapubic or percutaneous catheter, and all but 1 study measured bladder capacity as the total volume infused at the leak point. Average leak point pressure from studies where this was specified (n = 6; values are provided; Supplementary Table S1) was 27.0 ± 3.3 cm H_2O. The remaining study[26] measured capacity at a similar endpoint of 30 cm H_2O. Where defined, compliance was calculated as volume divided by pressure at the endpoint, but 2 studies[22,28] did not report this calculation.

One study[18] in conscious sheep used a suprapubic catheter; all others used urethral catheters. All studies measure bladder capacity at voiding; defined as assuming a voiding posture or sustained pressure above 30 cm H_2O in 8 studies, as increased detrusor pressure for 20 s in 3 studies,[15,19,21] and was not further defined in 1 study.[18]

Bladder capacity reported in anesthetized sheep was 207 ± 65 mL, while in conscious sheep it was 119 ± 51 mL **(Figure 4)**. Leak pressure was 26.5 ± 3.0 cm H_2O (n = 6 experiments), and peak pressure during voiding was 51.2 ± 10.7 cm H_2O (3 experiments). Compliance was reported as 11.7 ± 5.3 mL/cm H_2O in anesthetized animals (n = 7 experiments) and 36 mL/cm H_2O in 1 conscious experiment, although it is unclear how compliance was calculated in this report.

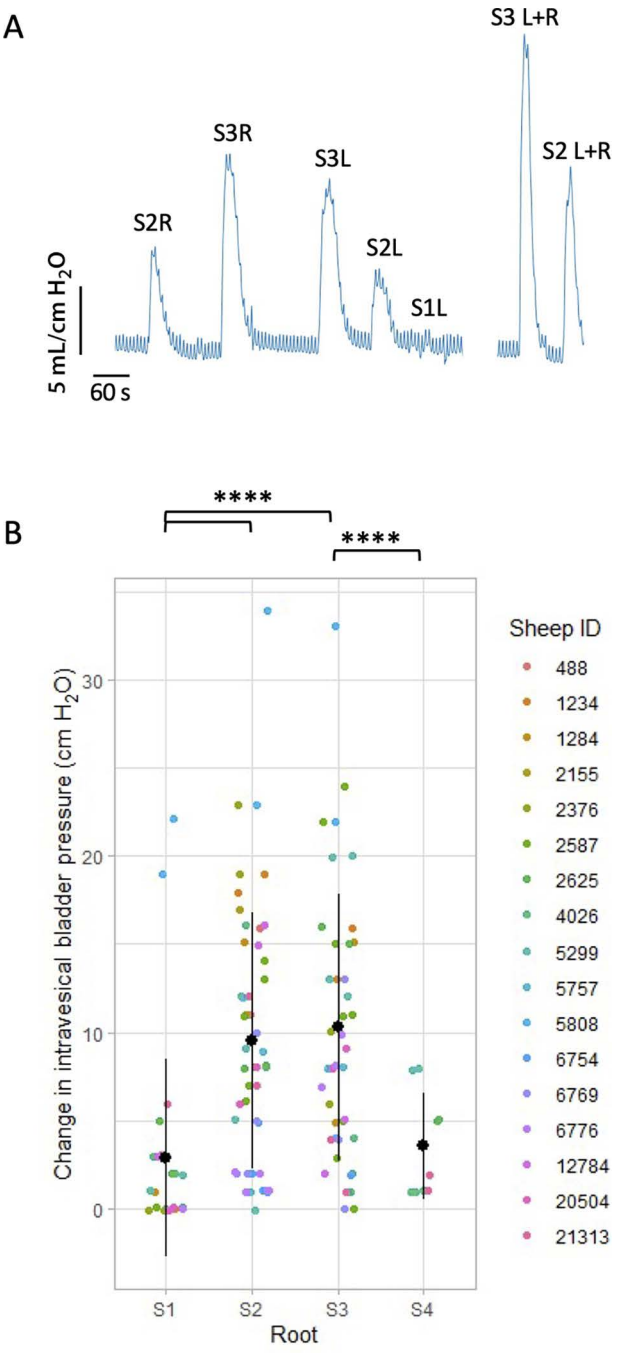

Figure 3—Bladder pressure response to electrical stimulation of extradural sacral roots (S1, S2, and S3). Detrusor pressure response to stimulation is shown for an individual example sheep (A; ID no. 2587). Peaks are labeled with root(s) stimulated (left [L], right [R], and bilateral stimulation (L + R) are shown; note that minimal bladder pressure increase was seen in response to stimulation of S1L). On average (B), unilateral electrical stimulation of the S2 and S3 extradural sacral roots in turn caused greater increases in detrusor pressure from baseline than S1 or S4 (****P < .0001 on post hoc Tukey honestly significant difference). Large black dots represent mean, and black lines represent SD). Significant variation was seen in magnitude of response between individual sheep (identified by color).

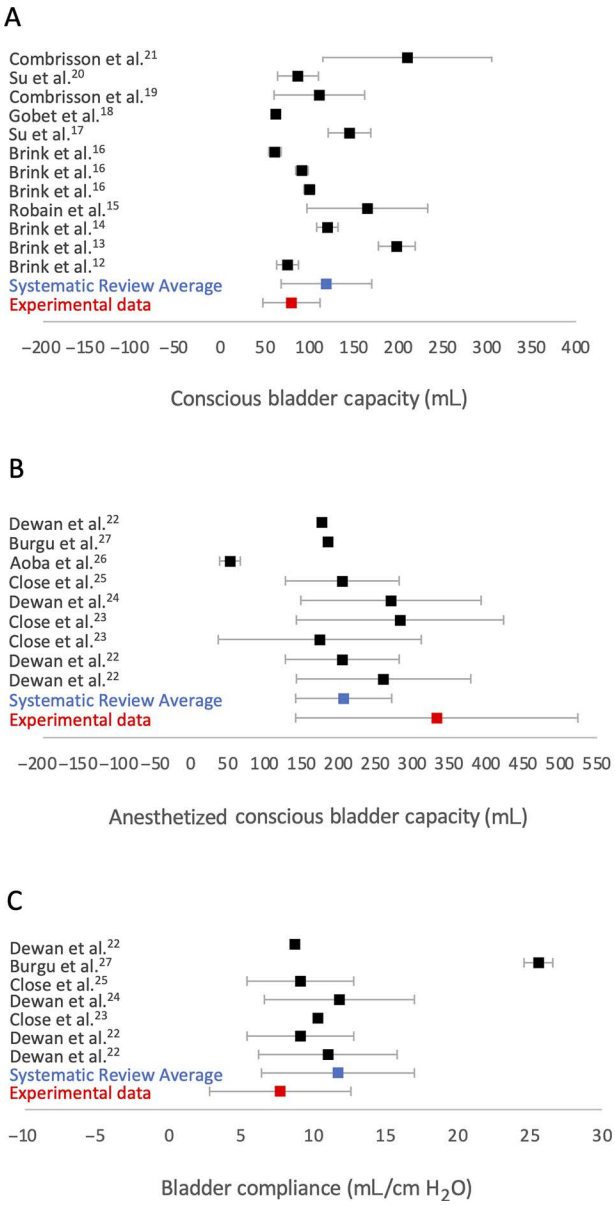

Figure 4—Summary values from systematic review of ovine cystometry. The forest plots show means ± SD for each study in the systematic review that reported data on bladder capacity in conscious (A) or anesthetized (B) sheep and bladder compliance in anesthetized sheep (C). Overall means ± SD for included studies are shown in blue, and experimental data obtained in this report are shown in red. Other parameters are not included due to minimal data being available in the systematic review. Duplicated study references indicate multiple experiments per study.

Discussion

We describe a reproducible method to use urodynamics in anesthetized and conscious sheep and report values for bladder capacity, urethral opening pressure, peak detrusor pressure during micturition, and bladder compliance. Although data about some of these individual parameters can be extracted from

the previous literature, this study is the first to present a complete set of values. As shown (Figure 4), these values are broadly within the range of that previously reported in the literature and identified in our review.

The systematic review highlighted there is considerable variation in the methods and reporting of urodynamics in sheep, with missing information from methods in many studies. This makes comparison between studies challenging and may account for the variation in values seen. For example, 5 of 21 experiments do not specify the sex of the animals used, fill rate is only reported in 15 of 21 experiments, position of animals during cystometry is reported only in 8 of 21 experiments, and position is not reported for any experiment under anesthesia. Nonetheless, there is reasonable consistency in variables that are reported in multiple studies (such as bladder capacity and leak point pressure under anesthesia). Leak point pressure under anesthesia was not a useful measure in our experiments as urine leaked almost immediately after starting bladder filling, perhaps due to urethral catheterization in our study (all other studies used less clinically applicable suprapubic catheters under anesthesia) and/or positioning in sternal recumbency in our study (position was not reported in anesthetized sheep in other studies) or the fact that female sheep were used in our study (leak point pressure is only reported for male sheep in the literature). Hydrodistension of the bladder using pressure on the urethra to prevent leaking is reported in people[39]; with the urethral catheter blocked and a Foley catheter obstructing the urethral outflow, we reached detrusor pressures similar to the peak pressures seen during voiding in conscious sheep (51.2 ± 10.7 cm H_2O). This is of particular importance to our intended purpose as a model for bladder afferent recording device development.

Although our experimental study presents the greatest number of animals for anesthetized cystometries and reports the most complete set of cystometric data all conducted with the same methods, some information remains limited. We have only performed cystometries in one lab, with one urodynamic setup, with animals in one position, in one breed of sheep, and only in female sheep. We therefore cannot comment on whether these results will be reliable across different labs or with different instrumentation. Factors such as sex of animals and position could have an impact on cystometric parameters. To our knowledge, there is no comparative information in the literature on these factors in sheep, but in people, cystometry is normally performed in a vertical position (seated or standing, depending on usual preference of patient) to replicate the normal situation.[40] However, sitting is known to be more provocative for abnormal detrusor activity than the supine position in people.[29] Arguably, keeping sheep in a sphinx position while anesthetized and standing for conscious cystometries as we have done in this study most closely approximates the "normal" physiological position for this species. Sex differences in cystometric parameters are reported in people, with males having a higher bladder capacity[41]; this is also reported in other species including rhesus macaques.[42]

The 2 phases of bladder filling we identify under anesthesia, and characterize with initial and terminal compliance, are not previously reported in sheep but have been reported in mice,[43] cats,[44] and people.[45] The change in filling profile in these species occurs at similar pressures (breakpoint, ~27 cm H_2O) and relates to the volume at which the detrusor is unable to accommodate further and filling becomes "supraphysiological." It is interesting to note that in our experiments this pressure, with a mean of 23.3 cm H_2O, roughly equates to the bladder pressure at voiding in conscious animals. These 2 phases are important to consider in future experiments aiming to determine bladder fullness from neural signals. The initial phase has been considered a clinically safe phase in which the bladder should be emptied to prevent upper urinary deterioration in a subset of patients.[32,46] If the detection of bladder fullness is to guide emptying, this phase (or the end of it) must be determined for the device to be useful. Further, it may be that afferents responding to different modalities (stretch, tension, or nociception) are firing during the different phases of filling[10] or are firing at different rates. In particular, afferent bladder activity has been shown to change from predominantly myelinated A∂ (from stretch receptors) to unmyelinated C fibers (carrying nociceptive information) between these phases.[43] Discrimination of these fiber types from neural signals by implanted neuroprostheses may aid in determination of bladder fullness. However, while anesthetized supraphysiological filling may provide useful proof of concept for these devices, it is likely not representative of conscious filling and voiding. Work in conscious animals will also be necessary.

A direct comparison between bladder capacity in anesthetized and conscious animals was not considered reasonable due to the use of voiding as an endpoint in conscious animals and a prespecified pressure in anesthetized animals. However, we do see a considerably higher bladder capacity under anesthesia at both 50 cm H_2O and at breakpoint, even though the pressure at breakpoint is similar to the pressure at voiding in conscious animals. The effect of anesthetic agents on urodynamic parameters has not been studied in sheep but is reported in other species.[34,47] Most general anesthetics induce smooth muscle relaxation that may affect bladder function[48,49] and increase bladder capacity.[11] Detrusor activity is also known to be affected by ketamine, opioids, and volatile agents such as isoflurane or sevoflurane, reducing the number of nonvoiding contractions seen during bladder filling.[11,34,47,50] We note this effect in sheep primarily anesthetized with sevoflurane; occasional nonvoiding detrusor contractions were seen in conscious cystometries but none were seen under anesthesia. However, this is an important consideration for experiments investigating therapeutics for reflex incontinence, which could not be assessed in the anesthetized model we present here and would need to be tested during conscious cystometries in sheep, or after further investigation of the differential effects of anesthetic agents on urodynamics in sheep.

It is possible that the duration of anesthesia may exacerbate anesthetic agent effects as described above (for example, reducing smooth muscle tone), and we indeed saw a statistically significant increase in bladder compliance with increasing duration of anesthesia. However, we perceive the clinical effect to be minimal as there is only a small absolute change in compliance and bladder capacity between baseline and later cystometries, the gradient of the line of best fit is very low (0.0067), and correlation coefficient and R^2 are low indicating that the absolute effect of duration of anesthesia on bladder compliance is likely low.

Fill rate was not found to have an effect on bladder compliance under anesthesia in our study. Theoretically, the filling rate might be expected to affect compliance due to the viscoelastic structural properties of the bladder wall.[31] However, experimentally in rats, increased fill rate seems to have little effect on bladder volume or pressure at voiding (parameters used to calculate compliance).[51] Clinically, in people, a higher fill rate predominantly increases detrusor overactivity leading to more nonvoiding detrusor contractions.[40] This is a possible reason why we see nonvoiding detrusor contractions in conscious cystometries in our study of normal sheep, as we used the upper end of what is considered the "medium" filling rate (25 to 50 mL/min) appropriate for most studies in people.[29] However, the low number of detrusor contractions we see in conscious animals is perhaps expected, given that nonvoiding contractions are predominantly seen with an overactive detrusor, often as a result of spinal cord injury,[29] and would therefore not be expected at a high level in normal sheep.

We present data on bladder pressure under anesthesia in response to electrical stimulation of the sacral roots. Electrical stimulation of the sacral roots is used clinically to trigger voiding and would be needed as part of a closed-loop-control device to prevent reflex incontinence, in this instance to apply a conduction block to prevent detrusor contraction. Sacral neuromodulation is also used as a therapy for overactive bladder, but because we do not see spontaneous detrusor contractions under anesthesia, this model would not be suitable for testing this therapy; conscious sheep could potentially be used but we have not explored this.

Only 2 reports[12,20] of sacral root stimulation in sheep were found. These studies do not assess bladder pressure response to stimulation and to the authors' knowledge there are no prior reports of sacral root stimulation in anesthetized sheep so the data presented here provide the only baseline values. The bladder pressure rises we see in response to stimulation in sheep are lower than those in people, pigs,[52] and dogs where increases up to 100 cm H_2O have been reported.[8] The bladder pressure rises we see are also not as high as those seen in conscious animals during voiding, and we therefore do not know if stimulation at the sacral roots would be sufficient to trigger voiding in sheep. Ultimately, this is an open question that would have to be answered experimentally by stimulating the sacral roots in conscious sheep, something beyond the scope of this study.

We note the greatest response is seen when stimulating S2 and S3, with individual variation most likely due to normal variation in anatomy.[53,54] In people, it is reported that the S3 root tends to have the predominant effect on detrusor contraction, with contribution from S2 and S4.[55–57] In rats, the S1 root appears to be most important.[58] In 2 reports[8,59] in dogs, it is the S2 root that elicits the greatest bladder pressure rise most commonly, although in foxhounds the S3 root has greater effect[60] suggesting there may even be breed-specific variation. Characterizing these differences facilitates translation from experimental models to people, our findings in sheep that S2 and S3 stimulation causes the greatest bladder pressure increase are similar to findings in people.

Sheep could provide a useful experimental model for translation of neurourological devices. Adult sheep are of comparable weight to people with comparable size of spinal canal; in our experience, the canal at the sacrum in sheep is approximately 10 mm in diameter, and it has been reported to be approximately 20 mm in humans at the same level.[61] Sheep and humans have similar mammalian neurophysiology, with fasciculated peripheral nerves of similar dimensions[62] and myelinated afferent nerve conduction velocity of around 41 m/s[63] in sheep compared to human bladder afferents of 38 to 41 ms.[10] Facsicularization of peripheral nerves, in particular, seems to vary between species with the dog having very limited vascularization compared to larger mammals such as sheep, pigs, and sheep.[64] Further, the bladder capacity of sheep under anesthesia in our experiments is comparable to the bladder capacity reported in people (300 to 400 mL).[65] The logistical and engineering challenges for sensory recording at the sacral roots (for example, size of implant and signal-to-noise ratio of neural signals) are therefore similar in these species to humans making this model particularly relevant for this purpose.

We have demonstrated a method for anesthetized and conscious urodynamic recording in sheep and provided comprehensive baseline values for future research. These values may also provide a useful clinical reference.

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Supplementary Materials

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