SCIENTIFIC REPORTS
nature research

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Anesthetic agents affect urodynamic parameters and anesthetic depth at doses necessary to facilitate preclinical testing in felines

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Urodynamic studies, used to understand bladder function, diagnose bladder disease, and develop treatments for dysfunctions, are ideally performed with awake subjects. However, in small and medium-sized animal models, anesthesia is often required for these procedures and can be a research confounder. This study compared the effects of select survival agents (dexmedetomidine, alfaxalone, and propofol) on urodynamic (Δpressure, bladder capacity, bladder compliance, non-voiding contractions, bladder pressure slopes) and anesthetic (change in heart rate [ΔHR], average heart rate [HR], reflexes, induction/recovery times) parameters in repeated cystometrograms across five adult male cats. The urodynamic parameters under isoflurane and α-chloralose were also examined in terminal procedures for four cats. Δpressure was greatest with propofol, bladder capacity was highest with α-chloralose, non-voiding contractions were greatest with α-chloralose. Propofol and dexmedetomidine had the highest bladder pressure slopes during the initial and final portions of the cystometrograms respectively. Cats progressed to a deeper plane of anesthesia (lower HR, smaller ΔHR, decreased reflexes) under dexmedetomidine, compared to propofol and alfaxalone. Time to induction was shortest with propofol, and time to recovery was shortest with dexmedetomidine. These agent-specific differences in urodynamic and anesthetic parameters in cats will facilitate appropriate study-specific anesthetic choices.

Urodynamic studies such as cystometrograms (CMGs) are used to evaluate bladder function and study bladder pathophysiology. Generally it is recommended that the subject is awake and unanaesthetized or unsedated during these studies[1]. Anesthesia, which can confound urodynamic outcomes by generally increasing bladder capacity[2–4], decreasing peak pressure[2,5] and depressing the micturition response[3,4,6], is often required in animal models of urodynamic studies. This is especially true when performing urodynamic trials using minimally invasive transurethral catheterization (compared to surgical implantation of an indwelling suprapubic catheter), which often requires the animal to be stationary for placement and maintenance of the catheter. Even gold standard agents that are minimally inhibitory towards autonomic reflexes such as α-chloralose or urethane may alter bladder function[5,7,8].

It is important to understand how commonly used anesthetic agents affect the bladder, in order to distinguish between unintended anesthetic artifact and intentional experimental manipulation. However, comparative studies are limited. This is especially true in cats[9,10], which are a common animal model for spinal cord injury and associated bladder pathology[11] due to the similarity of their spinal cord in both length and anatomy to the human spinal cord[12–14].

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Aside from their impact on the urodynamic system, logistical concerns are also a consideration when choosing an anesthetic agent for a study. Current gold standard agents pose additional challenges due to concerns of prolonged recovery or carcinogenicity[15], making them only suitable for terminal studies. In contrast, survival agents allow for experimental testing across multiple time points, better control for intra-animal variability, and a reduction in animal use number. To our knowledge, no studies have characterized the effects of the following survival agents used in clinical veterinary medicine for cats on CMG parameters: (1) alfaxalone, (2) dexmedetomidine, and (3) propofol (without the use of another anesthetic).

Dexmedetomidine is commonly used as a sedative-anesthetic in veterinary medicine (and to previously facilitate CMG testing in our laboratory[16]), due to its ease of administration (intramuscular [IM], and rapid recovery through the reversal agent atipamezole). However, in a rat study, dexmedetomidine was inhibitory to the volume evoked micturition reflex and peak pressure compared to urethane and awake trials[17]. Propofol, an anesthetic agent used to facilitate CMG testing in clinical veterinary medicine[9,18], has not been compared directly to dexmedetomidine in cat trials, but is suspected to be less inhibitory than dexmedetomidine due to its mechanism of action (propofol has no analgesic properties, while dexmedetomidine does) and ability to carefully titrate anesthetic depth through intravenous [IV] infusion. Unfortunately, propofol can only be given IV, so it often requires another agent to secure IV access. Alfaxalone, recently approved in the United States for use in cats, has a similar mechanism of action and clinical application as propofol, but has not been used in cats in the context of urodynamic studies. Conveniently, alfaxalone is formulated for IM dosing and does not require an additional agent[19], so it provides logistical advantages over propofol.

In this study we evaluated the effects of anesthetic agents alfaxalone, dexmedetomidine, and propofol at the lowest doses practical to facilitate survival CMG testing prior to terminal procedures that used isoflurane and α-chloralose, and examined their effects on CMG and anesthetic parameters. We hypothesized that based on their properties, dexmedetomidine would be more inhibitory (increased bladder capacity, decreased non-voiding contractions, decreased peak pressure) compared to alfaxalone and propofol.

Results

Five cats were anesthetized at least 3 times with each agent (see Supplementary Table S1 online). At least 2 CMG trials were conducted per session. We only collected terminal (isoflurane and α-chloralose) trials from four of five cats due to one unexpected death.

Cystometrogram parameters. Bladder capacity differed significantly between agents (Fig. 1a, also see Supplementary Tables S2 and S3 online). Bladder capacity was greatest with α-chloralose (59.6 ± 9.2 mL), and significantly increased compared to alfaxalone (46.7 ± 8.6 mL, $p = 0.02$), dexmedetomidine (42.9 ± 8.6 mL, $p = 0.0008$), and propofol (44.2 ± 8.6 mL, $p = 0.003$). Bladder capacity under isoflurane (54.5 ± 9.1 mL) was significantly increased compared to dexmedetomidine ($p = 0.03$). Additionally, Δpressure also differed significantly between agents (Fig. 1b, also see Supplementary Tables S2 and S3 online). Trials under propofol had the greatest Δpressure (116.5 ± 9.6 cm H_2O), and were significantly increased compared to isoflurane (84.2 ± 13.1 cm H_2O, $p = 0.04$) (Fig. 1b). Compliance did not differ significantly between agents ($p > 0.05$ for all pairwise comparisons, see Supplementary Tables S2 and S3 online).

Non-voiding contraction rate was highest with α-chloralose (0.03 ± 0.005 NVCs/s) and significantly higher than all other agents ($p < 0.01$ for all comparisons; Fig. 1c, also see Supplementary Tables S2 and S3 online). Alfaxalone (0.01 ± 0.003 NVCs/s) had the second highest rate of NVCs, and was significantly greater than dexmedetomidine which had the lowest rate of NVCs (0.002 ± 0.003 NVCs/s, $p = 0.008$). The amplitude of non-voiding contractions was not different between agents ($p > 0.05$).

The trends of the CMG bladder pressure responses changed with agent, with different agents having variable periods of passive filling compared to active contraction over the trial. Notably, propofol CMG responses were characterized by an early and gradual rise (longer period of active contraction), compared to dexmedetomidine responses which were characterized by a long period of low pressure (passive contraction) and a rapid rise immediately before voiding (Fig. 2a, also see Supplementary Table S2 online). The bladder pressure trends for other agents fell between the propofol and dexmedetomidine extremes. This was further described through quantification of slopes at select time points (Fig. 2b). During slope 1 (infusion start to 100 s before void), propofol (0.06 ± 0.01 cm H_2O/s) was significantly increased compared to alfaxalone (0.03 ± 0.01 cm H_2O/s, $p < 0.0001$) and dexmedetomidine (0.02 ± 0.01 cm H_2O/s, $p < 0.0001$). During slope 2 (100 to 50 s before void), α-chloralose had the highest slope (0.5 ± 0.08 cm H_2O/s), significantly higher than dexmedetomidine (0.2 ± 0.05 cm H_2O/s, $p = 0.0004$) and alfaxalone (0.3 ± 0.05 cm H_2O/s, $p = 0.03$). During slope 3 (last 50 s before void) dexmedetomidine (1.5 ± 0.1 cm H_2O/s) was significantly greater than all other agents: alfaxalone (1.1 ± 0.1 cm H_2O/s $p = 0.009$), α-chloralose (0.6 ± 0.2 cm H_2O/s, $p < 0.0001$), isoflurane (0.6 ± 0.2 cm H_2O/s, $p < 0.0001$), and propofol (0.7 ± 0.1 cm H_2O/s, $p < 0.0001$). CMG parameters were influenced heavily by animal, moderately by session, or minimally by trial number of session (see Supplementary Fig. S1 and Supplementary Table S7 online).

Anesthetic parameters. Continuous HR readings from select survival sessions averaged by agent visually demonstrate that mean HR and mean ΔHR varied by agent (Fig. 3a). Two trials (both propofol) were excluded from the averaged continuous HR plot due to suspect artifacts.

Mean HR and mean ΔHR taken from all trials confirmed variation between agents (Fig. 3b, also see Supplementary Tables S4 and S5 online). Mean HR, compared across all five agents, was highest under alfaxalone (215.2 ± 5.7 bmp), and significantly greater than all other agents: α-chloralose (163.3 ± 9.5 bmp, $p < 0.0001$), dexmedetomidine (110.7 ± 5.8 bpm, $p < 0.0001$), isoflurane (130.7 ± 8.2 bmp, $p < 0.0001$), and propofol (176.7 ± 5.8 bmp, $p < 0.0001$). All other agents fell in between the two extremes. While there was some animal-specific

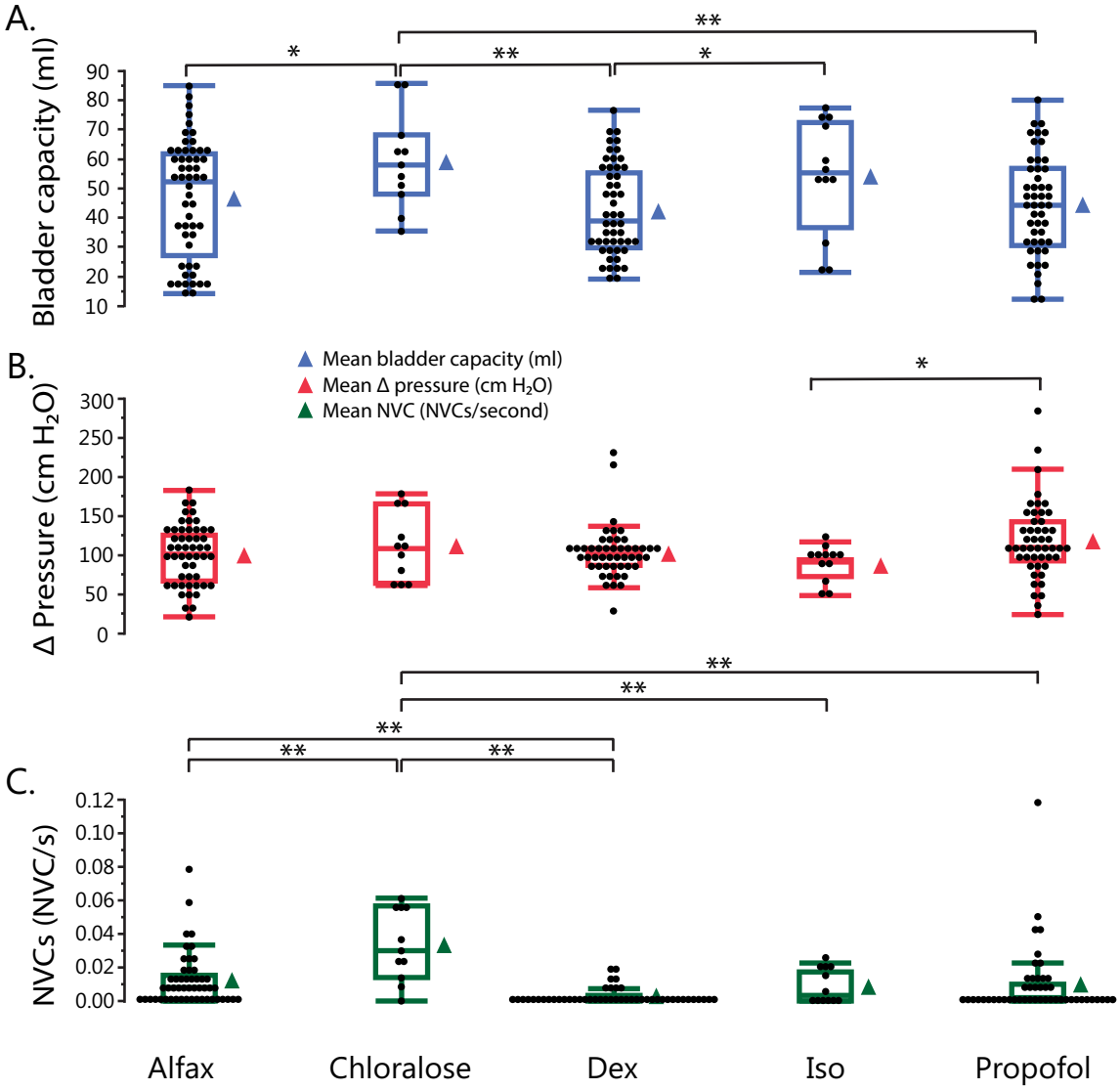

Figure 1. Boxplots of (**a**) bladder capacity, (**b**) Δpressure, and (**c**) NVC rate by anesthetic agent. Points represent individual samples. Adjacent triangle icons represent means of individual samples. Boxes represent 1st to 3rd quartile range (interquartile range [IQR]), with the line in the middle of each box representing the median. Tips of whiskers extending below and above box represent 1st quartile − 1.5*IQR and 3rd quartile + 1.5*IQR respectively. Significance: *$p < 0.05$, **$p < 0.01$.

variation, across animals the mean HR maintained these trends (see Supplementary Fig. S2 online). Mean change in heart rate (ΔHR), compared across only non-terminal agents, was lowest with dexmedetomidine (13.8 ± 6.8 bmp), and significantly different from propofol (37.5 ± 7.1 bmp, $p = 0.008$) and alfaxalone (45.4 ± 6.7 bmp, $p < 0.0001$). Mean ΔHR did not differ significantly between propofol and alfaxalone ($p = 0.8$).

Time to lateral recumbency and time to head up and walking differed significantly between the non-terminal agents (see Supplementary Tables S5 and S6 online). The time to lateral recumbency was shortest under propofol due to IV administration (2.4 ± 1.6 min), and significantly shorter than alfaxalone (9.5 ± 1.5 min, $p = 0.009$). The time to head up and walking were shortest under dexmedetomidine (1.7 ± 6.1, 2.5 ± 10.6 min) due to use of the reversal agent atipamezole compared to alfaxalone (37.2 ± 6.1 [$p < 0.0001$], 55.3 ± 10.6 [$p < 0.0009$] min) and propofol (43.2 ± 6.3 [$p < 0.0001$], 73.7 ± 10.6 [$p < 0.0001$] min) (Fig. 3c). Despite variation between individual cats (see Supplementary Fig. S3 online), reflexes showed greater anesthetic depth under dexmedetomidine compared to alfaxalone and propofol, although jaw tone was tighter under dexmedetomidine (Fig. 4). In general, average HR was influenced by both animal and session, and ΔHR was influenced only by animal (see Supplementary Fig. S1 and Supplementary Table S7 online).

Adverse consequences. Adverse consequences associated with VAP placement included surgical dehiscence (1/5) and port failure (1/5). In the cat with port failure, the last propofol trial was performed through isoflurane induction, in order to facilitate IV catheter placement. Dexmedetomidine caused vomiting immediately

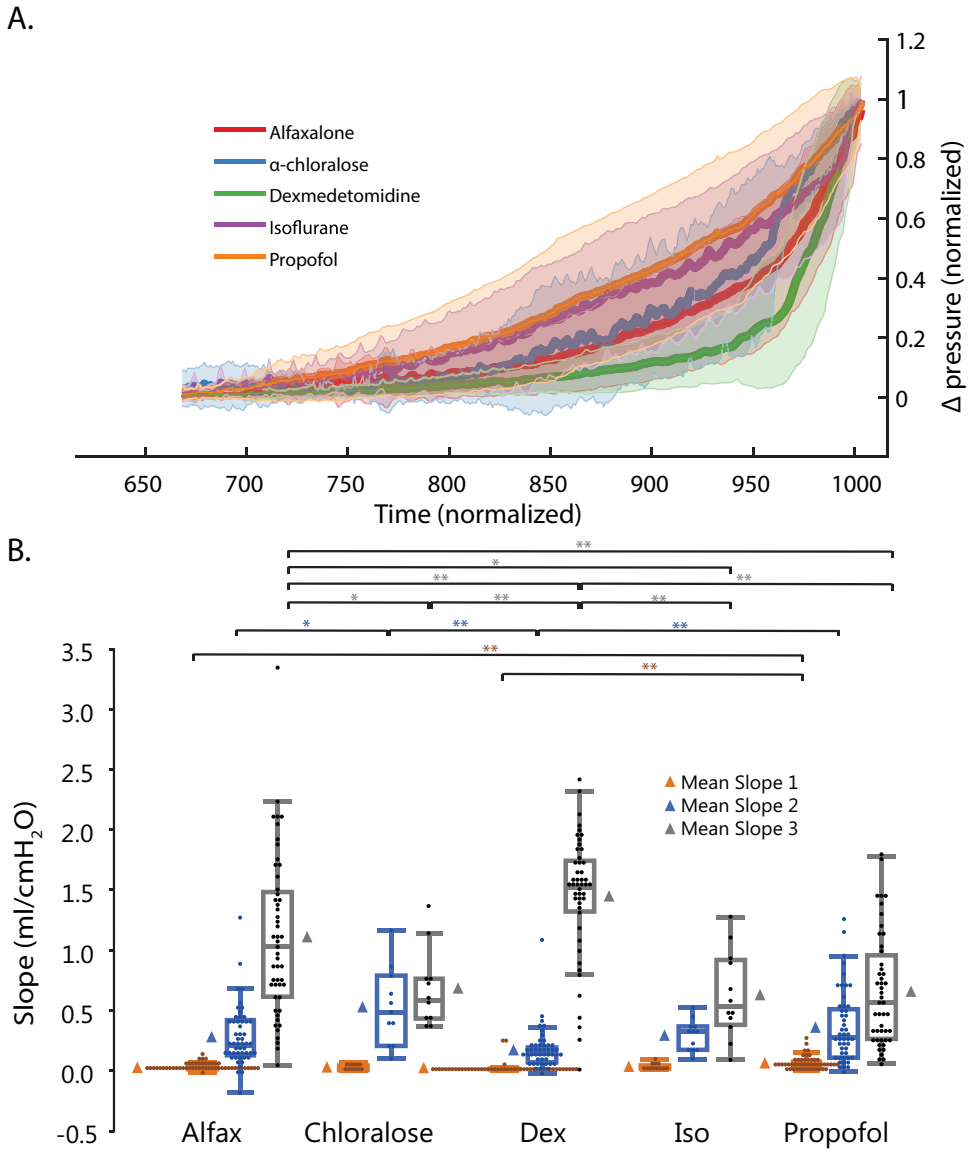

Figure 2. (**a**) Normalized CMG bladder pressure responses averaged by agent. Solid lines represent means, shading around each mean represents standard deviations. (**b**) Box plots of CMG slopes (slope 1) infusion start to 100 s before void, (slope 2) 100 to 50 s before void, and (slope 3) last 50 s before void. Data represented as in Fig. 1. Significance: *$p < 0.05$, **$p < 0.01$.

after injection in 7/15 (46.7%) of sessions. Cats did not vomit under other agents. Each cat vomited at least once under dexmedetomidine. One cat had an independent incidence of lethargy and vomiting unassociated with an anesthetic event, and was diagnosed with disseminated lymphoma at necropsy. One cat displayed significant bradycardia with 2nd degree atrioventricular (AV) block arrhythmia under dexmedetomidine in 2 separate anesthetized events. Echocardiogram and subsequent necropsy of this cat suggested evidence of left ventricular diastolic dysfunction and myofibril degeneration and atrophy. Finally, 1/13 (7.7%) alfaxalone trials resulted in unexpected death. During recovery, after the constant rate infusion (CRI) had been discontinued, one cat became apneic and was not able to be resuscitated.

Discussion

In this study we demonstrated differences in urodynamic parameters and anesthetic depth at minimal doses needed to perform CMG testing in male cats. To our knowledge, this was the first study to examine CMG parameters under alfaxalone, propofol in isolation without another agent, or dexmedetomidine in comparison to the other agents used here. Our study incorporates a novel method of CMG assessment (bladder pressure slopes) in conjunction with traditional CMG parameters and careful evaluation of anesthetic depth. Together, our results examine the interaction between urodynamic parameters and anesthetic depth, and provide recommendations for anesthetic choice based on study parameters of interest and logistic feasibility for CMG studies in male cats.

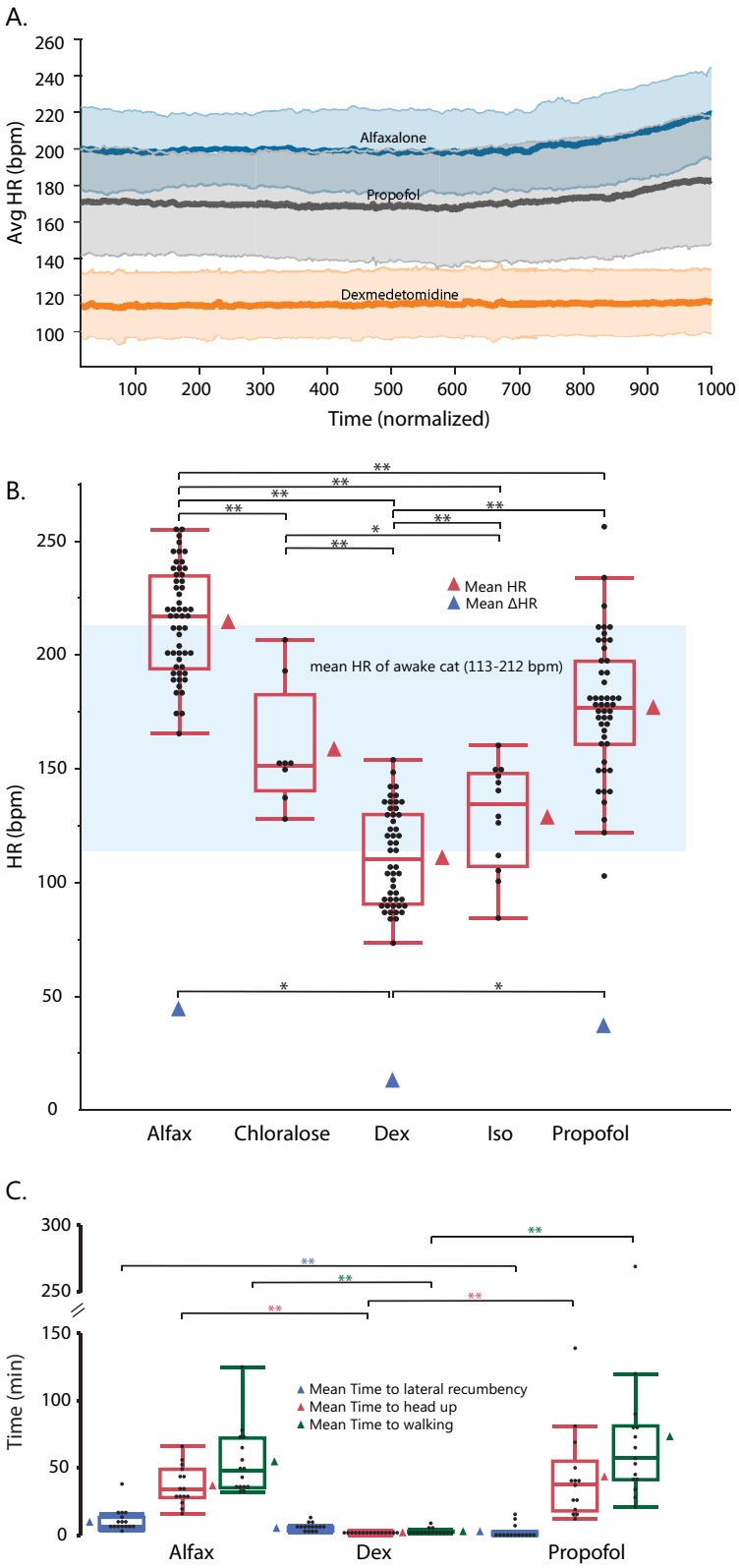

Figure 3. (**a**) Averaged continuous HR of last 3 cats during survival CMG trials by agent, normalized by time. Solid lines represent means, shading around each mean represents standard deviations. (**b**) Box plots of mean HR over all CMG trials by agent. ΔHR is shown for non-terminal agents only. Data represented as in Fig. 1. (**c**). Box plots for time to induction (approximated by time to lateral recumbency), time to recovery (approximated by time to head up), and time to walking for non-terminal agents. All panels: significance: *$p < 0.05$, **$p < 0.01$.

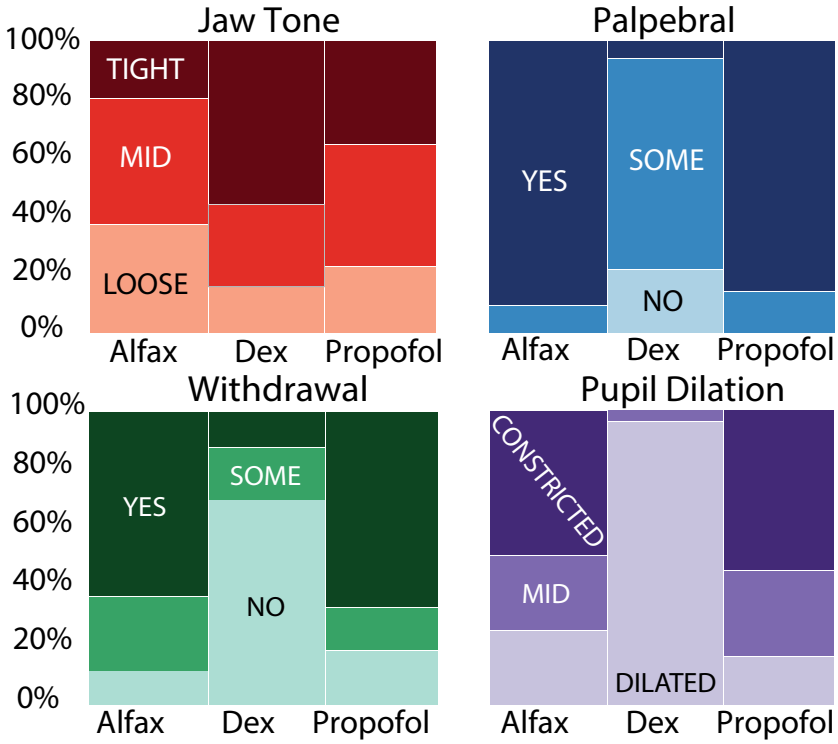

Figure 4. Reflex distribution by survival agents across all trials. Darker colors indicate a lighter plane of anesthesia.

Due to commonalities between species in response to anesthetic agents, select information from this study may be able to be guide preliminary anesthetic choice to other species, including humans. However, feasibility and effect on urodynamic and anesthetic parameters should be confirmed for the species in question.

Bladder capacity increases due to inhibition of central nervous system reflexes under anesthesia[3]. In this study, the bladder increases seen under terminal agents (isoflurane and α-chloralose) (Fig. 1a) were strongly correlated with prolonged anesthesia (isoflurane [2–6 h before CMG trials, 8–10 h total], followed by α–chloralose [12–22 h before CMG trials]) and repeated bladder fills for other experimental objectives. Although we did not see significant differences between survival agents, dexmedetomidine has been reported to cause polyuria through blockage of arginine-vasopressin release[20,21].

Maximum detrusor pressure [Pdet(max)] can also be used to assess bladder function[18]. We reported Δpressure to account for any positioning differences between trials that may have affected baseline pressures. Consistent with previous studies examining Pdet(max)[2,9], we found that Δpressure was higher with propofol, and lower with isoflurane (Fig. 1b). While this may be attributed to the lighter plane of anesthesia under propofol (general anesthetics depresses Pdet(max) in a dose-dependent manner[2,9]), a human study showed that even under comparable anesthetic depth, propofol showed less suppression of cortical somatosensory evoked potentials compared to isoflurane[22]. Alternatively, the increase in Δpressure may be related to loss of function and initiation of the voiding reflex under propofol, as evidenced by bladder slopes drastically different from the awake state. While the results of propofol are difficult to interpret, isoflurane should be avoided in studies where Pdet(max) or Δpressure is a key parameter of interest. Our Δpressure values trended slightly higher than previously reported values both in cats under α-chloralose[5,16,23], isoflurane[9], propofol[9], and dexmedetomidine[16]. Individual animal variation or experimental logistics could explain these trends. The placement of a transurethral catheter is likely a primary factor in this difference, as it provided a partial blockage of the bladder outlet. Transurethral catheters have been documented to increase voiding pressure in rats compared to suprapubic catheters[24]. Our catheterization approach was consistent across all agents, so relative pressure trends are generalizable.

Non-voiding contractions are phasic pressure increases seen during bladder filling[25] that may be linked to sensations relating to bladder volume[26]. They are more likely and more frequently occur with urinary bladder pathologies[25,27,28]. Consistent with our understanding that α-chloralose maintains spinal reflexes relative to other anesthetic agents[5], the greatest number of NVCs were observed with α-chloralose (Fig. 1c). Alfaxalone trials had the next greatest number of NVCs, and the most out of the non-terminal agents studied. Notably, dexmedetomidine almost completely eliminated NVCs compared to other agents. The α_2 mediated analgesic properties of dexmedetomidine may block the sensation of bladder filling at the spinal or supra-spinal level[19,29]. Clinically, dexmedetomidine has been used for analgesia and prevention of post-operative catheter-related bladder discomfort[30]. However, awake CMG trials have significantly less NVCs than anesthetized trials[16], so absence of NVCs is not necessarily pathological. When NVCs are desired (ex: overactive bladder models), α-chloralose (non-survival) and alfaxalone (survival) can be considered, and dexmedetomidine should be avoided.

In this study, CMG bladder pressure slopes differed between agents (Fig. 2), interpreted as previously described variable periods of passive filling and active contraction[31]. Pressure slopes under dexmedetomidine (characterized by a flat filling period, followed by a sharp rise to the void event) are most similar to awake or decerebrate animal cystometrograms[5,10,32,33]. In contrast, pressure slopes under propofol had the most obvious change from the awake slope, characterized by an early rapid rise and less obvious void event. Although there are no standardized criteria for defining passive filling and active contraction, we found propofol consistently reached a higher normalized pressure compared to dexmedetomidine across normalized time, with the most obvious separation noted between 900 and 950 normalized time, where the mean normalized pressure ± standard deviation range of dexmedetomidine is distinctly separate from the corresponding range for propofol (Fig. 2a). These findings suggest dexmedetomidine was associated with more awake-like bladder responses compared to GABA-ergic agonists.

Anesthetic depth was increased under alfaxalone and propofol compared to dexmedetomidine as demonstrated by reflexes, HR, and ΔHR. The rise in bladder pressure was closely associated with a rise in heart rate for propofol and alfaxalone (around 200 normalized time units in both measures for both agents). This phenomenon is likely driven by sympathetic activity in response to urinary bladder distension, as previously described in cats by the viscerovascular reflex[34], and was notably not observed in the dexmedetomidine trials.

The exact significance of bladder pressure slopes and their association with anesthetic depth is unclear. In a previous rat study comparing varying doses across a single anesthetic agent (tiletamine-zolazepam), lighter anesthesia was associated with more awake-like bladder curves[32]. In this experiment, comparing across anesthetic agents, we found the opposite, likely due to differences in mechanism of action between agents. We recommend further investigation of bladder pressure response trends in future studies in association to anesthetic depth, pharmacokinetics, preservation of spinal reflexes, and potential bladder pathology.

In addition to affecting CMG and urodynamic parameters, each agent offers unique logistical benefits and constraints. Dexmedetomidine (α_2 agonist), conveniently administered through IM boluses (CRI only recommended for analgesia, not for sedation or anesthesia[19]) and rapidly reversed through atipamezole, provides a convenient method of anesthetizing cats. Due to the nature of IM bolus administration, depth of anesthesia and therefore bladder function can vary as plasma levels of dexmedetomidine increase or decrease. Common adverse effects of dexmedetomidine include bradycardia, arrhythmias (AV block), and vomiting[19], all of which were noticed in our experiments.

Propofol and alfaxalone ($GABA_A$ agonistics) are used clinically to both induce (IV) and maintain (CRI or repeat IV bolus) anesthesia[15]. Maintenance of anesthesia using a CRI allows for steady-state drug plasma levels and a more stable plane of anesthesia. While propofol is limited to IV administration, alfaxalone can also be given IM for single-agent anesthesia. Common side effects of both propofol and alfaxalone are apnea, although one study showed alfaxalone had less respiratory depression compared to propofol[35]. Unexpectedly, we experienced a death during the recovery phase (most common time of anesthetic-related death[36]) of one alfaxalone trial. To our knowledge, this was an isolated incident and not representative of the general safety of alfaxalone.

In this study, we were able to decease concentrations of GABA-ergic agonists below recommended labelled dosing in part due to the stable plane of anesthesia associated with CRI administration. This could be one explanation for the lighter plane of anesthesia (more preserved reflexes, higher average heart rate, and greater change in heart rate over trial) observed in GABA-ergic agonistics compared to dexmedetomidine (Figs. 3 and 4). However, another concurrent explanation for increased heart rate under the GABA-ergic agonists is hypotension with reflex tachycardia[37]. This is in contrast with dexmedetomidine, which causes vasoconstriction, leading to hypertension and reflex bradycardia[19]. Consistent with previous studies in dogs showing alfaxalone causes less cardiovascular depression than propofol[37], we observed the mean heart rate under alfaxalone (at or slightly above the upper limit of awake reference range[38]) was significantly higher than under propofol (Fig. 3).

Isoflurane (exact mechanism of action unknown[19,39], but suspected to affect GABA receptors[40]) is the primary inhalant anesthetic used in veterinary medicine, especially in prolonged invasive procedures[19]. Although in this study it was only used during terminal procedures, in clinical veterinary medicine isoflurane is commonly used during recovery procedures. Its inhaled route of administration allows dosing to be easily titratable to adjust the depth of anesthesia. However, inhaled agents are logistically more challenging, either requiring technical expertise to intubate the animal, or risking personnel exposure. Since recovery from isoflurane is rapid, and isoflurane has been previously used to facilitate propofol administration for urodynamic procedures[41], the effects of isoflurane on subsequent α-chloralose and propofol trials in this study are assumed to be minimal.

The non-survival hypnotic α-chloralose (mechanism unknown) provides light long-lasting anesthesia with minimal analgesia, and minimal effect on normal spinal and sympathetic reflex function[5,15]. Unfortunately, α-chloralose is not recommended for survival studies due to involuntary excitement and prolonged recovery[15] although several studies have successfully recovered animals following α-chloralose anesthesia[42,43]. In this study, isoflurane and α-chloralose were only used in terminal surgeries, and thus had fewer test sessions than for the other agents. Given this relative disparity, results from isoflurane and α-chloralose should be interpreted with caution.

The independent effects of animal, session, and trial number of session are confounders to this study (see Supplementary Fig. S1 and Supplementary Table S7 online). Animal-specific changes in compliance and slope are linked to animal-specific changes in bladder capacity and Δpressure from which compliance and slope are calculated. The differences in slope and average HR over sessions can be attributed to the specific agents tested during that session, rather than an independent session-specific effect. The variation in NVCs, Δpressure, and slope may be attributable to changes in plane of anesthesia during the experiment. Although differences in urodynamic and anesthetic parameters were found between the examined variables, the mixed model analysis, as well as similar number of trials and sessions per agent and per animal control for this variation.

Limitations of this study include the following: (1) lack of direct comparison to awake CMG trials, (2) use of transurethral catheterization, which may cause obstructive changes to voiding response[24], (3) inability for full randomization due to logistical constraints (drug availability, ability to secure IV access, etc.), although effort was made to spread out agents, so that each agent was tested once within the first 3 trials, once within the next 3 trials, and once within the final 3 trials, (4) inability to extrapolate data to female cats, (5) limited data for terminal agents (isoflurane and α-chloralose) due to nature of the experiments and the unexpected death of one animal, (6) possible influence of previously administered agents on future trials (especially analgesics which may decrease the sensation of bladder filling), both between and within sessions, especially during the terminal studies, (7) confounding effects of prolonged anesthesia, repeat CMG trials on subsequent CMG trials, and an invasive laminectomy for terminal studies, (8) lack of blinding in data analysis and experiments, especially for qualitative measures such as anesthetic reflexes, (9) lack of monitoring data other than heart rate due to measurement artifact and animal movement during trials, (10) inability to control for different doses and routes of administration of a single drug due to logistical considerations and sample size, and (11) confounding effects of animal-specific and anesthetic-specific factors, though attempts were made to control this variability using a mixed model statistical analysis.

Despite these limitations, this study compares and contrasts urodynamic and anesthetic parameters between survival agents, including a novel agent alfaxalone (previously unstudied in cats in the context of urodynamic parameters), and gold standard terminal agents. The differences identified between agents in this study can help (1) interpret the results of existing urodynamic studies by differentiating unintended anesthetic artifact from intentional experimental manipulation, and (2) guide selection of anesthetic agents based on logistic or experimental considerations. Future studies to extend this work and address the limitations of this study include additional routes and doses of single agents, concurrent CMG in awake cats for direct comparison to anesthetized cats, observing bladder contractions outside of the active fill period, and filling of ex-vivo or post-mortem bladders to provide a truly passive bladder control.

Methods

Animals. Five adult, intact male domestic shorthair cats (7–18 months, 4–6 kg, Marshall BioResources, Inc., North Rose, NY) were included in this study. These animals were available from another study, and the cohort size is similar to other relevant studies[2,9,44]. Animals were group housed in a temperature (72 ± 2 °F) and humidity (30–70%) controlled room on a 12:12 h light/dark cycle with ad libitum access to water and fed a complete diet for adult cats (Purina One Urinary Tract Health Formula and Purina ProPlan EN, Nestle Purina, Neenah, WI). Cats were free of feline herpesvirus, calicivirus, panleukopenia, coronavirus, feline immunodeficiency virus, chlamydia, and toxoplasmosis. All experimental procedures were performed at an AAALAC accredited institution, according to the standards established by the Guide for the Care and Use of Laboratory Animals, and were approved by the University of Michigan Institutional Animal Care and Use Committee.

Vascular access port. Each cat was implanted with a VAP (ClearPort Mid VAPs 5Fr with a rounded tip, 24″ catheter; Access Technologies, Skokie, IL) to guarantee immediate IV access. IV access was essential for avoiding a second agent in propofol experiments, and useful in providing a constant rate infusion (CRI) in the alfaxalone and propofol experiments to maintain anesthesia. Cats were induced with dexmedetomidine (0.02 mg/kg, Dexmedseed, Dechra, Overland Park, KS), ketamine (6.6 mg/kg, Zetamine, Vet One, Boise, ID), and butorphanol (0.66 mg/kg, Torbugesic, Zoetis, Kalamazoo, MI), and maintained on isoflurane (0–2.5%, Fluriso, Vet One, Boise, ID). Incisions were made on the left ventral neck and dorsal neck or back. A catheter was inserted and secured in the left jugular vein, and tunneled to a subcutaneous port secured to the muscle between the shoulder blades[45,46]. The port was accessed with a non-coring Huber needle (Access Technologies, Skokie, IL), and flushed regularly (weekly, or after each use) with a locking solution (TCS Lock Solution, Access Technologies, Skokie, IL) or heparinized saline. Post-operative medications (buprenorphine [0.01–0.02 mg/kg, Par Pharmaceutical, Chestnut Ridge, NY], robenacoxib [6 mg/kg, Onsior, Elanco, Greenfield, IN] and famotidine [0.5 mg/kg, Westward, Eatontown, NJ]) were given for 24 h post-operatively. The cats were monitored daily until suture removal 7–14 days later.

Anesthesia. Each cat was anesthetized at least three times ("sessions") with each of three agents: (1) dexmedetomidine (0.02–0.04 mg/kg IM bolus; reversed with matched volume of atipamezole [Antisedan, Orion Corporation, Espoo, Finland] after session), (2) alfaxalone (5 mg/kg IM bolus + 0.08 mg/kg/min IV CRI, Alfaxan Multidose, Jurox Inc. Kansas City, MO), and (3) propofol (2 mg/kg IV bolus + 0.15 – 0.20 mg/kg/min IV CRI, Hospira, Inc., Lake Forest, IL). Effort was made to randomize sessions (each agent was tested once within the first 3 sessions, once within the next 3 sessions, and once within the final 3 sessions), but full randomization and consistency among animals could not be achieved due to logistical constraints (agent availability, repeat sessions, etc.). Each session was on a different day, at least 2 days after the previous anesthetized session. Sessions ranged from 2–5 h in length, with CRIs running continuously during alfaxalone and propofol sessions, and intermittent IM bolus injections given to maintain anesthesia during dexmedetomidine sessions. Occasionally, 1 mL IV boluses of alfaxalone and propofol were given in addition to the CRI to keep the cats at an appropriate plane of anesthesia.

During terminal procedures in conjunction with an unrelated research study, cats were anesthetized with the same dexmedetomidine/ketamine/butorphanol combination as for the VAP surgery, and transitioned to isoflurane. Surgical procedures for the other study were performed under isoflurane (2–4 h) before the isoflurane CMG trials of this study (1–2.5 h). The cats remained on isoflurane for an addition period of time (3–5.5 h) before being transitioned to α-chloralose over a duration of 45 min (70 mg/kg induction, 20 mg/kg maintenance

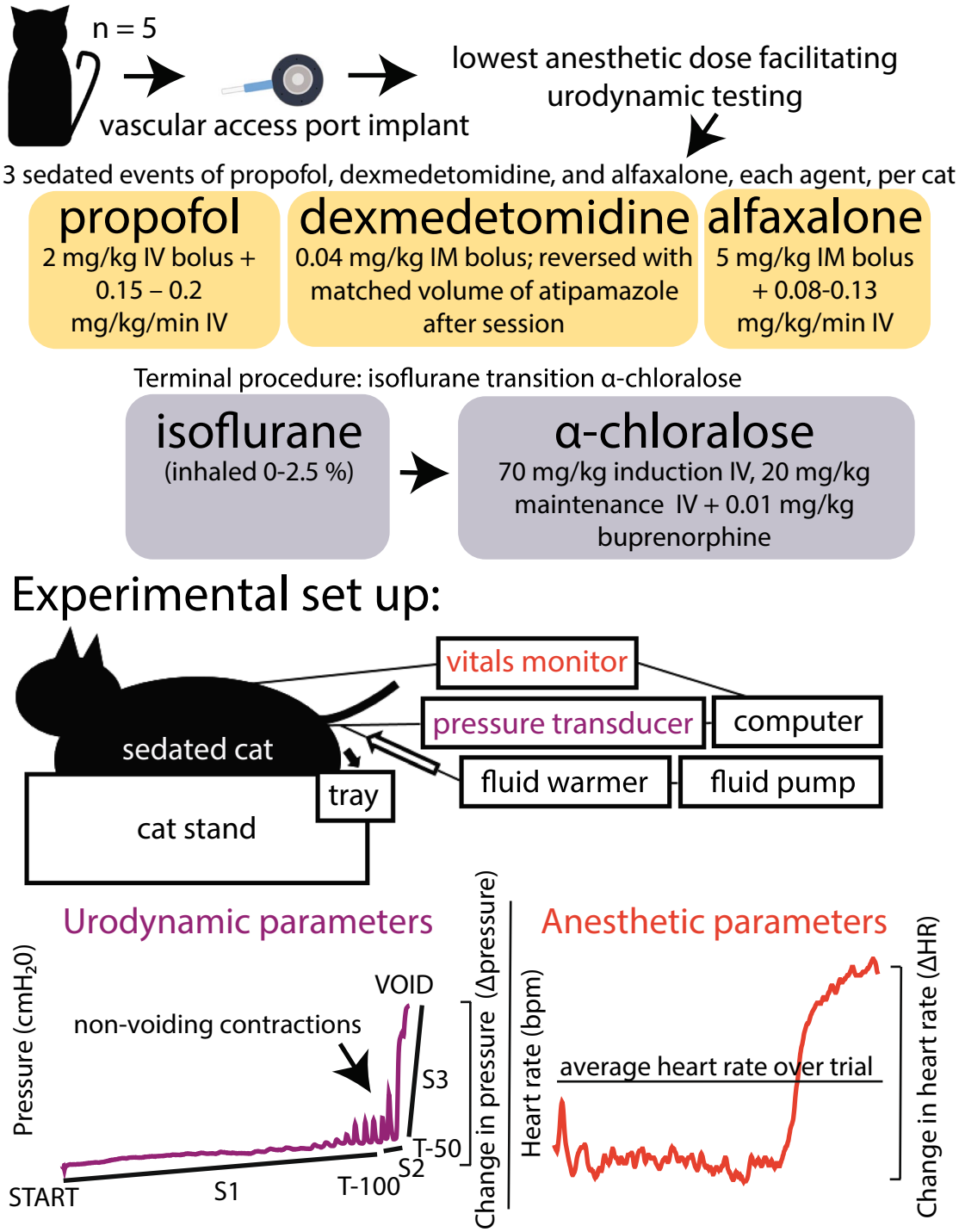

Figure 5. Experimental overview. Cats were implanted with VAPs and anesthetized at the lowest doses necessary to facilitate urodynamic testing. At least 3 sessions of propofol, dexmedetomidine, and alfaxalone each were conducted, in addition to a terminal experiment under isoflurane and α-chloralose in conjunction with another study. During anesthetized sessions at least 2 CMG trials were performed in each session to assess urodynamic parameters. Heart rate and reflexes were used to assess anesthetic depth, and time to induction/recovery was noted.

every 4–6 h or as needed [MilliporeSigma, Burlington, MA]) supplemented with 0.01 mg/kg buprenorphine[47]. The total time under isoflurane anesthesia ranged from 8 to 10 h. Experimental testing including CMGs for the unrelated research study were carried out under α-chloralose (12–22 h) before α-chloralose CMG trials for this study were conducted.

Cystometrograms. At least two CMG trials were conducted in each session. Warmed saline (41 °C) was pumped at 2 mL/min (AS50 infusion pump, Baxter, Deerfield, IL; or PHD 2000, Harvard Apparatus, Holliston, MA) through a fluid warmer (Hotline Fluid Warmer, Smiths Medical, Minneapolis, MN) and one lumen of a dual-lumen urethra catheter (Umbili-Cath™ 3.5 Fr Polyurethane UVC Catheter, Utah Medical, Midvale, UT) into the urinary bladder. For non-terminal sessions, sterile preparation of the perineal region and catheter was performed to mitigate against infection. The other lumen of the catheter was connected to a pressure transducer (V6402 pressure transducer, Smiths Medical, Minneapolis, MN) and Labchart (ADInstruments, Colorado Springs, CO) for recording. At bladder capacity, a void event occurred and urine would leak around the catheter into a collecting tray (Fig. 5). The bladder was emptied and a break of at least 10 min was given between trials to allow the bladder to relax. Bladder capacity volume was calculated using two methods: (1) the total volume infused and (2) the sum of urine collected from the bladder and collecting tray. The greater of these two values was used in subsequent analysis. The variables analyzed for each CMG were bladder capacity, change in pressure from baseline to maximum (Δpressure), compliance (capacity/Δpressure), number and amplitude of non-voiding contractions (NVCs), and slopes of the bladder pressure ("pressure slopes") over three intervals as defined below.

Anesthetic parameters. The time to lateral recumbency after initial agent injection and times to head up and walking (ten consecutive steps without falling) were recorded for each anesthetic session with non-terminal agents. During each session, vitals (heart rate [electrocardiogram or pulse oximetry], O_2 hemoglobin saturation (pulse oximetry; tongue, lip, or ear), non-invasive blood pressure [forelimb] and temperature [rectal]) were monitored once every 15 min using a vitals monitor (Surgivet, Smiths Medical, Minneapolis, MN). Respiratory rate was taken manually. The average heart rate (mean HR) for a trial appeared to be a consistent indicator of anesthetic plane and was later included in statistical analysis. Other vitals either did not fluctuate across sessions or were subject to artifact and were not analyzed across sessions. During non-terminal procedures for three of the five cats, a data logger was used for continuous vitals recording once per second and the calculation of change in heart rate over trial (ΔHR). Reflexes (palpebral, withdrawal, jaw tone, pupil dilation) were checked before each CMG trial. Palpebral and withdrawal reflexes were categorized as "yes (strong withdrawal with paw pinch)", "some (weak withdrawal with paw pinch)" or "no (no withdrawal, unresponsive to paw pinch)". Jaw tone was categorized as "tight (significant pressure required to open jaw)," "mid (intermediate pressure required to open jaw)", or "loose (lack of muscle tone, jaw opened easily)". Pupil dilation was categorized as "constricted," "mid (intermediate)", or "dilated". In general, as anesthetic depth increases, palpebral and withdrawal reflexes decrease, jaw tone loosens, and pupils become more dilated[48].

Data acquisition and analysis. Bladder pressure data was processed using MATLAB (R2019a, MathWorks, Natick, MA) and Excel (Microsoft, Redmond, WA). Bladder pressure was smoothed using a 1 s moving average. NVCs were identified in MATLAB using the findpeaks function, defining a peak as at least 3 cm H_2O, at least 0.5 s away from another peak, and then manually confirmed. To account for the range of trial lengths, NVC counts were divided by the total length of the trial to calculate NVCs per second. To quantify overall bladder pressure trends seen between agents, pressure slopes were calculated for three distinct time periods: (1) infusion start to 100 s before void, (2) 100 to 50 s before void, and (3) last 50 s before void. To visualize the changes seen during slope calculations over different pressure and time ranges across cats and test sessions, the bladder pressure was normalized from 0 (start) to peak/void (1,000) on the x (time) axis. After noting that pressures did not significantly fluctuate from resting baseline pressure until about 675 normalized time units, the 675–1,000 normalized time unit range was targeted, and the pressure was normalized from 0 to 1 within that window. Continuous heart rate readings during non-terminal trials for the last 3 cats were also processed similarly, but over the entire length of the trial. Statistics were analyzed using JMP Pro 14.2.0 (SAS Institution Inc. Cary, NC), using a mixed model approach, setting anesthetic agent as a fixed variable and animal as a random variable. For analysis of bladder capacity, the cat weight was set as an additional fixed variable. Nominal categorical data was converted to ordinal categorical data in order to fit the mixed model, for reflex data. Yes/some/no, tight/mid/loose, and constricted/mid/dilated were converted to 1/0.5/0 respectively. Pairwise comparisons were made using Tukey's HSD to examine the effect of anesthetic agents on the bladder. Pairwise comparisons using a Steel–Dwass test (non-parametric version of Tukey's HSD) was used to determine the effect of confounding factors (session, trial number of session, and animal) on urodynamic and anesthetic parameters, using only survival agents, the first 9 sessions of each animal, and first 3 trials of each session. Significance was defined as $p < 0.05$. Where relevant, data is reported as mean ± standard deviation.

Data availability

The datasets generated and analyzed during the current study and the MATLAB code used for data analysis are available in the Open Science Framework repository, (https://osf.io/8zjkp/ [https://doi.org/10.17605/OSF.IO/8ZJKP]).

Received: 3 January 2020; Accepted: 22 June 2020
Published online: 09 July 2020

References

1. Blaivas J, C. M., Weiss, J., & Verhaaren, M. *Atlas of Urodynamics*. 2nd edn. Vol. 25 (Blackwell, 2007).
2. Ghoniem, G. M., Shoukry, M. S. & Monga, M. Effects of anesthesia on urodynamic studies in the primate model. *J. Urol.* **156**, 233–236 (1996).

3. Elsamra, S. E. & Ellsworth, P. Effects of analgesic and anesthetic medications on lower urinary tract function. *Urol. Nurs.* **32**, 60–67 (2012).
4. Darrah, D. M., Griebling, T. L. & Silverstein, J. H. Postoperative urinary retention. *Anesthesiol. Clin.* **27**, 465–484. https://doi.org/10.1016/j.anclin.2009.07.010 (2009).
5. Rudy, D. C., Downie, J. W. & McAndrew, J. D. Alpha-chloralose alters autonomic reflex function of the lower urinary tract. *Am. J. Physiol.* **261**, R1560-1567. https://doi.org/10.1152/ajpregu.1991.261.6.R1560 (1991).
6. Ozkurkcugil, C. & Ozkan, L. Effects of anesthetics on cystometric parameters in female rats. *Int. Urol. Nephrol.* **42**, 909–913. https://doi.org/10.1007/s11255-010-9745-4 (2010).
7. Schneider, M. P. *et al.* A novel urodynamic model for lower urinary tract assessment in awake rats. *BJU Int.* **115**(Suppl 6), 8–15. https://doi.org/10.1111/bju.13039 (2015).
8. Crook, J. & Lovick, T. Urodynamic function during sleep-like brain states in urethane anesthetized rats. *Neuroscience* **313**, 73–82. https://doi.org/10.1016/j.neuroscience.2015.11.027 (2016).
9. Cohen, T. A., Westropp, J. L., Kass, P. H. & Pypendop, B. H. Evaluation of urodynamic procedures in female cats anesthetized with low and high doses of isoflurane and propofol. *Am. J. Vet. Res.* **70**, 290–296. https://doi.org/10.2460/ajvr.70.2.290 (2009).
10. Li, W. J., Kim, J. M. & Oh, S. J. Effects of level of consciousness on urodynamic procedure in female cats. *J. Korean Med. Sci.* **26**, 803–806. https://doi.org/10.3346/jkms.2011.26.6.803 (2011).
11. Fry, C. H. *et al.* Animal models and their use in understanding lower urinary tract dysfunction. *Neurourol. Urodyn.* **29**, 603–608. https://doi.org/10.1002/nau.20903 (2010).
12. Hultborn, H. & Nielsen, J. B. Spinal control of locomotion—from cat to man. *Acta Physiol.* **189**, 111–121. https://doi.org/10.1111/j.1748-1716.2006.01651.x (2007).
13. Burkholder, T., Ledesma-Feliciano, C., VandeWoude, S. & Baker, H. J. In *Laboratory Animal Medicine* (Elsevier, Amsterdam, 2015).
14. Perese, D. M. & Fracasso, J. E. Anatomical considerations in surgery of the spinal cord: A study of vessels and measurements of the cord. *J. Neurosurg.* **16**, 314–325. https://doi.org/10.3171/jns.1959.16.3.0314 (1959).
15. Flecknell, P. *Laboratory Animal Anaesthesia*. 4th edn, 109–140 (Elsevier, Amsterdam, 2016).
16. Khurram, A. *et al.* Chronic monitoring of lower urinary tract activity via a sacral dorsal root ganglia interface. *J. Neural Eng.* **14**, 036027. https://doi.org/10.1088/1741-2552/aa6801 (2017).
17. Harada, T. & Constantinou, C. E. The effect of alpha 2 agonists and antagonists on the lower urinary tract of the rat. *J. Urol.* **149**, 159–164. https://doi.org/10.1016/s0022-5347(17)36030-5 (1993).
18. Goldstein, R. E. & Westropp, J. L. Urodynamic testing in the diagnosis of small animal micturition disorders. *Clin. Tech. Small Anim. Pract.* **20**, 65–72. https://doi.org/10.1053/j.ctsap.2004.12.009 (2005).
19. Plumb, D. C. *Plumb's Veterinary Drug Handbook* 9th edn. (Wiley, New York, 2018).
20. Ji, F. & Liu, H. Intraoperative hypernatremia and polyuric syndrome induced by dexmedetomidine. *J. Anesth.* **27**, 599–603. https://doi.org/10.1007/s00540-013-1562-3 (2013).
21. Granger, S. & Ninan, D. Intraoperative dexmedetomidine-induced polyuric syndrome. *Cureus* **9**, e1218. https://doi.org/10.7759/cureus.1218 (2017).
22. Liu, E. H. *et al.* Effects of isoflurane and propofol on cortical somatosensory evoked potentials during comparable depth of anaesthesia as guided by bispectral index. *Br. J. Anaesth.* **94**, 193–197. https://doi.org/10.1093/bja/aei003 (2005).
23. Bruns, T. M., Bhadra, N. & Gustafson, K. J. Bursting stimulation of proximal urethral afferents improves bladder pressures and voiding. *J. Neural Eng.* **6**, 066006. https://doi.org/10.1088/1741-2560/6/6/066006 (2009).
24. Smith, P. P., Hurtado, E., Smith, C. P., Boone, T. B. & Somogyi, G. T. Comparison of cystometric methods in female rats. *Neurourol. Urodyn.* **27**, 324–329. https://doi.org/10.1002/nau.20512 (2008).
25. Drake, M. J. *et al.* The potential role of unregulated autonomous bladder micromotions in urinary storage and voiding dysfunction; overactive bladder and detrusor underactivity. *BJU Int.* **119**, 22–29. https://doi.org/10.1111/bju.13598 (2017).
26. Streng, T., Hedlund, P., Talo, A., Andersson, K. E. & Gillespie, J. I. Phasic non-micturition contractions in the bladder of the anaesthetized and awake rat. *BJU Int.* **97**, 1094–1101. https://doi.org/10.1111/j.1464-410X.2006.06137.x (2006).
27. Sekido, N. *et al.* A novel animal model of underactive bladder: Analysis of lower urinary tract function in a rat lumbar canal stenosis model. *Neurourol. Urodyn.* **31**, 1190–1196. https://doi.org/10.1002/nau.21255 (2012).
28. Fowler, C. J., Griffiths, D. & de Groat, W. C. The neural control of micturition. *Nat. Rev. Neurosci.* **9**, 453–466. https://doi.org/10.1038/nrn2401 (2008).
29. Michel, M. C. & Vrydag, W. Alpha1-, alpha2- and beta-adrenoceptors in the urinary bladder, urethra and prostate. *Br. J. Pharmacol.* **147**(Suppl 2), S88-119. https://doi.org/10.1038/sj.bjp.0706619 (2006).
30. Singh, T. K., Sahu, S., Agarwal, A., Gupta, D. & Mishra, P. Dexmedetomidine for prevention of early postoperative catheter-related bladder discomfort in voluntary kidney donors: Prospective, randomized, double-blind, placebo-controlled trial. *J. Anaesthesiol. Clin. Pharmacol.* **34**, 211–215. https://doi.org/10.4103/joacp.JOACP_287_16 (2018).
31. Choudhary, M., van Asselt, E., van Mastrigt, R. & Clavica, F. Neurophysiological modeling of bladder afferent activity in the rat overactive bladder model. *J. Physiol. Sci.* **65**, 329–338. https://doi.org/10.1007/s12576-015-0370-y (2015).
32. Matsuura, S. & Downie, J. W. Effect of anesthetics on reflex micturition in the chronic cannula-implanted rat. *Neurourol. Urodyn.* **19**, 87–99 (2000).
33. Ouyang, Z. *et al.* In *2019 9th International IEEE/EMBS Conference on Neural Engineering (NER)* 1058–1061 (IEEE, San Francisco, 2019).
34. Medda, B. K., Koley, J. & Koley, B. Sympathoadrenal activity in the visceral (viscerovascular) reflexes to distension of the urinary bladder. *Jpn. J. Physiol.* **46**, 3–92. https://doi.org/10.2170/jjphysiol.46.83 (1996).
35. Campagna, I., Schwarz, A., Keller, S., Bettschart-Wolfensberger, R. & Mosing, M. Comparison of the effects of propofol or alfaxalone for anaesthesia induction and maintenance on respiration in cats. *Vet. Anaesth. Analg.* **42**, 484–492. https://doi.org/10.1111/vaa.12231 (2015).
36. Brodbelt, D. Feline anesthetic deaths in veterinary practice. *Top Companion Anim. Med.* **25**, 189–194. https://doi.org/10.1053/j.tcam.2010.09.007 (2010).
37. Okushima, S., Vettorato, E. & Corletto, F. Chronotropic effect of propofol or alfaxalone following fentanyl administration in healthy dogs. *Vet. Anaesth. Analg.* **42**, 88–92. https://doi.org/10.1111/vaa.12166 (2015).
38. Abbott, J. A. Heart rate and heart rate variability of healthy cats in home and hospital environments. *J. Feline Med. Surg.* **7**, 195–202. https://doi.org/10.1016/j.jfms.2004.12.003 (2005).
39. Fish, R. E., Brown, M. J., Danneman, P. J. & Karas, A. Z. *Anesthesia and Analgesia in Laboratory Animals* 2nd edn. (Elsevier, Amsterdam, 2008).
40. Moody, E. J., Suzdak, P. D., Paul, S. M. & Skolnick, P. Modulation of the benzodiazepine/gamma-aminobutyric acid receptor chloride channel complex by inhalation anesthetics. *J. Neurochem.* **51**, 1386–1393. https://doi.org/10.1111/j.1471-4159.1988.tb01102.x (1988).
41. Wu, C. H., Buffington, C. A., Fraser, M. O. & Westropp, J. L. Urodynamic evaluation of female cats with idiopathic cystitis. *Am. J. Vet. Res.* **72**, 578–582. https://doi.org/10.2460/ajvr.72.4.578 (2011).
42. Grad, R., Witten, M. L., Quan, S. F., McKelvie, D. H. & Lemen, R. J. Intravenous chloralose is a safe anesthetic for longitudinal use in beagle puppies. *Lab Anim. Sci.* **38**, 422–425 (1988).

43. Luckl, J., Keating, J. & Greenberg, J. H. Alpha-chloralose is a suitable anesthetic for chronic focal cerebral ischemia studies in the rat: A comparative study. *Brain Res.* **1191**, 157–167. https://doi.org/10.1016/j.brainres.2007.11.037 (2008).
44. Dybowski, B. *et al.* Optimum anesthesia for reliable urethral pressure profilometry in female dogs and goats. *Int. J. Urol.* **23**, 701–705. https://doi.org/10.1111/iju.13114 (2016).
45. Guerios, S., Matos, D., Hm Souza, C. & Bacon, N. Surgical placement and management of jugular vascular access ports in dogs and cats: Description of technique. *Revista Colombiana de Ciencias Pecuarias* **28**, 265–271. https://doi.org/10.17533/udea.rccp.v28n3a7 (2015).
46. Farrow, H. A., Rand, J. S., Burgess, D. M., Coradini, M. & Vankan, D. M. Jugular vascular access port implantation for frequent, long-term blood sampling in cats: Methodology, assessment, and comparison with jugular catheters. *Res. Vet. Sci.* **95**, 681–686. https://doi.org/10.1016/j.rvsc.2013.03.021 (2013).
47. Ouyang, Z., Sperry, Z. J., Barrera, N. D. & Bruns, T. M. Real-time bladder pressure estimation for closed-loop control in a detrusor overactivity model. *IEEE Trans. Neural Syst. Rehabil. Eng.* **27**, 1209–1216. https://doi.org/10.1109/tnsre.2019.2912374 (2019).
48. Guedel, A. E. Stages of anesthesia and a re-classification of the signs of anesthesia. *Anesth. Analg.* **6**, 157–162 (1927).

Acknowledgements

We thank Zachariah Sperry, Ahmad Jiman, Elizabeth Bottorff, Hannah Parrish, and other members of the Peripheral Neural Engineering and Urodynamics (pNEURO) Lab for their assistance with surgeries, data collection, and analysis. We also thank Anna Skorupski, Samantha Eckley, Robert Sigler, and other members of the University of Michigan Unit for Laboratory Animal Medicine (ULAM) for their assistance with animal diagnostics and care. We thank the University of Michigan Consulting for Statistics, Computing and Analytics Research (CSCAR) for their advice on statistical analysis. This study was funded in part by the University of Michigan Cohen Comparative Medicine Research Award. No other funding sources contributed to this study.

Author contributions

J.J.X., T.M.B., P.A.L., and T.M. conceived and designed research. J..J.X., Z.O., and E.K. developed logistics for performing experiment. J.J.X., Z.Y., and E.K. performed experiments. J.J.X. and Z.Y. analyzed data. J.J.X., Z.Y., Z.O., and T.M.B. interpreted results of experiments. J.J.X. and Z.Y. prepared figures. J.J.X., Z.Y., and T.M.B. drafted manuscript. J.J.X., Z.Y., Z.O., P.A.L., T.M., and T.M.B. edited and revised manuscript. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-68395-3.

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