**ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)**
**Main Body**
**VERSION 4**

See Instructions for Completion of the Animal Component of Research Protocol (ACORP Instructions), for help in completing specific items.

## A. ACORP Status.

1. Full Name of Principal Investigator(s) ► [redacted] Ph.D., [redacted] Ph.D.

2. VA Station Name (City) and 3-Digit Station Number► Louis Stokes Cleveland DVAMC (541)

3. Protocol Title► Conscious ambulatory bladder monitoring to understand neural control of lower urinary tract function

4. Animal Species covered by this ACORP►Cat

5. Funding Source(s). Check each source that applies:

   ►( ) Department of Veterans Affairs.
   ►(**X**) US Public Health Service (e.g. NIH).
   ►( ) Private or Charitable Foundation -- Identify the Foundation:
   ►( ) University Intramural Funds – Identify the University and Funding Component:
   ►( ) Private Company – Identify the Company:
   ►( ) Other – Identify Other Source(s):

6. Related Documentation for IACUC reference.

   a. If this protocol applies to a project that has already been submitted to the R&D Committee for review, identify the project:

      **(1)** Title of project► Conscious ambulatory bladder monitoring to understand neural control of lower urinary tract function
      (2) If approved by the R&D Committee, give the date of approval►3-14-17

   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:

      (1) Identify the studies described in the previously approved ACORP that have already been completed
      ►We completed SA1 from our previous protocol, which was UroMOCA development. We came up with a guitar pick shaped form factor which allows us to easily insert the UroMOCA through a 1 cm incision into the bladder. We also were able to show wireless transmission of data and battery recharge of the device on the benchtop.

      We also completed section SA2a, which was implanting an active UroMOCA device in 1 anesthetized animal and gathering urodynamic data from the device along with reference pressures and volume. This was a terminal procedure.

We completed the 3 Sham Controls from section SA2b. This consisted of anesthetized cystotomies but without UroMOCA implantation. This allowed us to monitor the cats for any changes in behavior, including bladder function after the surgery.

We also completed 3 chronic experiments where a UroMOCA was implanted in the cat bladder. 1 of the cats had an inactive device and 2 had active devices. We monitored all cats and gathered data from the active devices for 4 weeks. In all experiments, the cats recovered quickly from the surgeries and the implant and we were able to gather valuable wireless data from the UroMOCA.

In our previous ACORP we stated we were going to use 6 animals for chronic implantation, but only used 3. We are requesting to use the 3 other animals in this renewal protocol.

(2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly
►We used 7 domestic short-haired male cats.
(3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other Items below.
►In this renewal protocol, we want to complete our validation of the UroMOCA and then use the validated device to map function of the lower urinary tract using neurostimulation.

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).

(1) Title of other protocol ► Conscious ambulatory bladder monitoring to understand neural control of lower urinary tract function
(2) IACUC approval number of other protocol ►17-012-CT-17-001-CC
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):

►(X) Research
►( ) Teaching or Training
►( ) Testing
►( ) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

## Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of people and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.

► Urodynamics is a study that assesses how the bladder and urethra are performing their job of storing and releasing urine. Urodynamic tests can help explain symptoms such as incontinence and frequent

urination which is a condition that affects more than 15 million Americans. There is an unmet need for a practical clinical device that can provide continuous bladder activity information during normal daily living routines without using catheters or wires. In response to this need, we have created the Urological Monitor of Conscious Activity (UroMOCA), which provides continuous, wireless, catheter–free, battery–powered bladder data for use in animal and ultimately human research of lower urinary tract function. We will continue to validate the UroMOCA and then use the UroMOCA as a tool to map bladder and pelvic floor activity during physiological bladder states and in response to neurostimulation. Studies of the neural mechanisms underlying control of the lower urinary tract are usually conducted in acute experiments under anesthesia and with bladder measurement methods that include a catheter and faster-than-physiological retrograde bladder filling, all of which can significantly affect the behavior of neural circuits and organ function. Chronic experiments in awake, behaving animals using measurement tools that minimize mechanical perturbations to the lower urinary tract would provide important new mapping data by more closely approximating normal physiological function and subject behaviors.–In contrast to current standard urodynamics methods, the UroMOCA will, for the first time, allow research subjects to sleep, ambulate, and conduct normal activities unencumbered by wires or catheters during experiments to figure out the underlying control of lower urinary tract function.
We have designed this study to minimize pain and distress and to provide for the psychological well-being of the cats. We feel that the benefits to spinal cord injured patients outweigh the pain and/or distress that may be caused in the animals in this study.

C. **Experimental Design.**

1. **Lay Summary**. Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.

   ► We will continue to validate the UroMOCA in conscious ambulating animals. We will then use the UroMOCA to conduct neurostimulation experiments in awake, behaving animals to generate new neural mapping data of the lower urinary tract and test hypotheses that are relevant to efforts for translating neurostimulation approaches for bladder function. Development of this innovative technology along with mapping bladder and pelvic floor states will be accomplished through the following (newly assigned) Specific Aims (SAs).
   SA1. Validate biocompatibility and function of the UroMOCA in chronic, awake animals. We will continue to test the UroMOCA in cats to validate wireless pressure and volume measurement and bladder contraction detection under anesthetized and conscious conditions in chronic experiments. We will also assess biocompatibility of the UroMOCA in the chronic feline experiments.
   SA2. Use the UroMOCA to map patterned sacral root stimulation on to bladder and pelvic floor functions in awake, behaving cats. We will map spontaneous bladder and pelvic floor activity to bladder state during normal physiological bladder states of filling and emptying; map low frequency sacral root stimulation to bladder and pelvic floor activity; and map kilohertz frequency sacral root stimulation to bladder and pelvic floor activity.

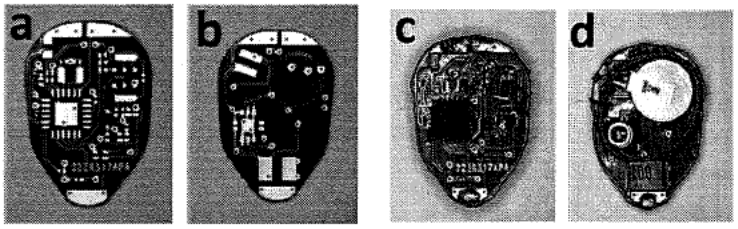
2. **Complete description of the proposed use of animals.** Use the following outline to detail the proposed use of animals.

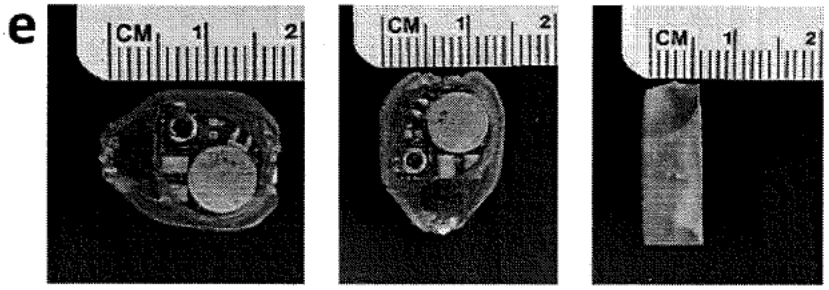
a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.

**UroMOCA**
PCB Board
(a) front
(b) back

**Assembled UroMOCA**
(c) front
(d) back

**Packaged**
UroMOCA (e)




► Purpose-bred adult (6 months and older) domestic cats of either sex will be used. There are two groups in this protocol (animal component):

SA1. Validate biocompatibility and function of the UroMOCA in chronic, awake animals

SA2. Map patterned sacral root stimulation on to bladder and pelvic floor functions in awake, behaving cats. After complete UroMOCA validation, we will conduct preliminary experiments testing sacral root stimulation in awake, behaving animals to (1) map spontaneous bladder and pelvic floor activity to bladder state during normal physiological bladder states of filling and emptying; (2) map low frequency sacral root stimulation to bladder and pelvic floor activity; and (3) map kilohertz frequency sacral root stimulation to bladder and pelvic floor activity.

Validate biocompatibility and function of the UroMOCA in chronic, awake animals (SA1):

Chronic UroMOCA only experiments will be conducted in up to three felines to validate the function and biocompatibility of UroMOCAs *in vivo* when animals are ambulatory.

For this experiment we request 3 animals to get meaningful data about the biocompatibility of the device implanted into the bladder, including measuring histological markers of tissue irritation or

damage, and conducting urinalyses to measure for the presence of heavy metals. We will confirm that device functions, including but not limited to pressure measurement, volume estimation, battery life, data transmission fidelity and distance, and recharge rate, meet design benchmarks as tested and confirmed by previous benchtop and in vitro experiments. We will validate that this functionality is maintained chronically (at least 30 days in vivo).

The UroMOCA will be inserted into 3 animals. The animals will be anesthetized and the bladder will be surgically exposed. For urodynamics, an intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter. In addition to the intraurethral catheter for bladder pressure monitoring, an inflatable balloon catheter will be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with normal saline at known volumes from 5 up to 50 mL. Standard urodynamics will be done prior to UroMOCA insertion to establish a baseline. Bupivacaine will be administered to the incision site to minimize discomfort after surgery. The UroMOCA will then be inserted suprapubically. After closing the bladder and the skin, a 3-D CT image will then be taken to demonstrate placement of the UroMOCA in the bladder and lack of bladder leakage. After imaging and confirmatory urodynamics, all catheters will be removed and the animal will be awoken. The cats will be observed after awakening and daily afterward for bladder spasms, which indicate bladder pain or irritation, and other changes in animal behavior denoting bladder health and function.

All animals will be monitored daily by study staff or ARF staff for changes in behaviors, especially relating to bladder function. Notes will be made in the cat's medical record of positional changes, voiding, bladder spasms, and other behaviors that occur during the daily observation (sham)/ recording time (device), to observe functionality of the UroMOCA. Investigators or study staff will conduct tests at least 3 times per week of the UroMOCA recharge and data transmission functions, collecting ambulatory bladder volume and pressure data. The cats will don a backpack harness that holds the data receiver circuit and coil with online storage, and the battery charger coil, as Dr. Bourbeau has done previously at the Cleveland VA. At least two weeks before other procedures are conducted, study staff will put the jacket or harness onto the animal during daily enrichment periods. The animal will wear the jacket for a brief period, about a half hour, and each day gradually work up to wearing the jack for longer periods, up to about 6 hours. Animals will be rewarded with veterinary-approved cat treats while the jacket is being put on and periodically while the animal is wearing it. Study staff will also engage the animals in playing with cat toys to encourage the animals to move around and acclimate to the jacket. For all animals in SA1, urodynamics and imaging tests will be conducted under anesthesia 14 days after initial surgery to observe the effects of the UroMOCA implantation or cystotomy (bladder incision) alone on bladder health and function. At 30 days after initial surgery, a terminal anesthetized urodynamics and imaging session will be done, device extracted under isoflurane, animals euthanized with pentobarbitol and bladder harvested for histological analysis.

<u>Map patterned sacral root stimulation on to bladder and pelvic floor functions in awake, behaving cats (SA2):</u>

Approaches using electrical stimulation of the sacral roots have been translated into clinical practice to evoke bladder emptying or improve urinary continence. Bladder inhibition for urinary continence can be achieved with low amplitude stimulation of a single sacral root, but the mechanism of action remains unclear. Bladder excitation can be achieved with higher amplitude stimulation across multiple sacral roots and this approach includes a posterior rhizotomy (severing the nerve roots in the spinal cord) to inhibit reflex sphincter activity, which also removes sensation and other reflex activity. *Inhibiting urethral sphincter activity without a posterior rhizotomy* remains an important clinical translation goal. Kilohertz frequency stimulation has been shown to inhibit conduction of action potentials in whole nerves. Kilohertz frequency nerve block (KFNB) of sacral roots could be used to inhibit unwanted urethral sphincter activity in humans because the neurons innervating the urethral sphincter run through the sacral roots. However, the feasibility and effectiveness of this approach remains unclear because neurons innervating other muscles and organs also run through these sacral roots (e.g. bladder, colon, anal sphincter, pelvic floor, lower limbs).

Results from previous studies testing sacral root stimulation to activate or block sacral roots have yielded important findings. Low frequency stimulation can directly activate motoneurons to produce functional responses and can modulate reflex pathways and produce coordinated activity of end organs. KFNB of the sacral roots can inhibit urethral sphincter activity that was evoked by proximal stimulation on the same root, which could improve voiding efficiency. However, these experiments have typically been conducted under anesthesia, which can significantly alter neural circuit functions and the effects of high frequency stimulation, and used urethral catheters, which affect lower urinary tract behavior. Therefore, new experiments conducted under normal physiological conditions without anesthesia should be undertaken to better map sacral root activity or stimulation to lower urinary tract functional state.

We plan to conduct preliminary experiments testing sacral root stimulation in awake, behaving animals to (1) map spontaneous bladder and pelvic floor activity to bladder state during normal physiological bladder states of filling and emptying; (2) map low frequency sacral root stimulation to bladder and pelvic floor activity; and (3) map kilohertz frequency sacral root stimulation to bladder and pelvic floor activity. Bladder state (i.e. bladder filling or bladder emptying) will be an independent variable that may affect outcomes.

For the second aim experiments we request 6 animals.

Six animals are requested for this pilot study to determine if there is an effect on lower urinary tract function in response to electrical stimulation. Similar studies conducted in chronic felines to test lower urinary tract outcomes in response to electrical stimulation typically include 4-6 animals (Khurram et al. 2017, Boger et al. 2012). Our outcome measures include peak bladder pressure, and number of bladder contractions. This study will provide the data to power future studies.

Animal transport

The cats will be transported to the [redacted] in approved private vehicles, according to the LSCVAMC ARF SOP, the morning of the terminal or survival procedure and transported back to the VA (for survival procedure) when fully awake from anesthesia. The [redacted] have facilities necessary for CT imaging. The ability for the VA to provide group/floor housing of the cats makes up for the momentary distress that cats could experience during the

transportation.

Two employees from Dr. [redacted]s lab and at least one member of the ARF will be approved for transport in their private vehicles according to the VA SOP. This assures that there will be back up transportation in a situation where one of the employees isn't available to transport the animal.

For emergency overnight housing, the [redacted] has a small cat/dog recovery cage that can be utilized for an overnight stay and observation. The cage would be wheeled into a large, empty animal housing room where there is temperature, humidity and light control.

Photography

During surgery the animal will be partially draped. We will take photographs all through the surgery for documentation. We need to photograph key aspects of the anatomy and surgery to document our methods and to allow for retrospective identification of anatomical landmarks. Most photographs will only be seen by the study team. Some select photographs will be published in research journals. Photographs will be cropped to prevent animal identification. All collected materials will be stored on an encrypted laptop prior to upload to the [redacted] research drive. This network drive is password protected and only accessible to [redacted] employees.

We will also be using a FLIR camera for thermal imaging. This will allow us to see how warm the recharge coil gets, and if the animal's skin gets warm from the coil. The FLIR thermal imager we will use needs to be connected to a smart phone for it to work.

We will also be filming the room with a GoPro camera when the cat is wearing the jacket and coil. This way we can be out of the room, and then go back to the timestamp on the camera and correlate it with data -such as when the cat uses a litterbox.

All collected materials will be stored on an encrypted USB−key or laptop prior to upload to the [redacted] research drive. This network drive is password protected and only accessible to [redacted] employees.

b. Justify the group sizes and the total numbers of animals requested. A power analysis is strongly encouraged; see ACORP instructions.

► The total number of animals requested is 9.

| Experiment | Number of Animals |
|---|---|
| Validate biocompatibility and function of the UroMOCA in chronic, awake animals | 3 |
| Map patterned sacral root stimulation on to bladder and pelvic floor functions in awake, behaving cats | 6 |

Validate biocompatibility and function of the UroMOCA in chronic, awake animals (SA1): 3 animals

Chronic experiments will be conducted in up to 3 felines to validate the function and

biocompatibility of UroMOCAs in vivo when animals are ambulatory. Our previous protocol granted us permission to use 6 animals, but we were only able to test the device in 3 animals. These 3 animals will allow us to finish this aim.

Map patterned sacral root stimulation on to bladder and pelvic floor functions in awake, behaving cats (SA2): 6 animals

Up to 6 healthy adult cats of either sex will be tested. Six animals are needed to provide sufficient data to draw meaningful conclusions regarding lower urinary tract (LUT) function in response to electrical stimulation.

c. Describe each procedure to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

*Surgical Preparation, UroMOCA Insertion, Urodynamics and 3-D CT X-ray images will be performed at the* [redacted]

**SA1. Validate biocompatibility and function of the UroMOCA in chronic, awake animals**

SA1 Summary

1. Day 0 (CCF): Cystotomy with UroMOCA implantation; Urodynamics and Imaging
2. Day 1-13 (VA): Ambulatory testing of UroMOCA
3. Day 14 (CCF): Follow up urodynamics, imaging and charging of UroMOCA
4. Day 15-29 (VA): Ambulatory testing of UroMOCA
5. Day 30 (CCF): Urodynamics, imaging – terminal procedure

**Day 0: Surgery at** [redacted] **: UroMOCA only**

*For all drug dose information, see Biosafety Appendix 3, table 2*

Surgical Preparation: At least twelve hours prior to surgery, animals will begin fasting. Buprenorphine will be given pre-operatively and then at least 4 additional doses will be administrated (twice a day), and more if needed.

Robenacoxib will also be administered the morning of surgery for multimodal analgesia and at least 2 days post-op.

The morning of the surgery, the cats will be transported to the [redacted] in private vehicles, according to the LSCVAMC ARF SOP.

10–15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam and Torbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15 minutes the animal remains bradycardic, a dose of glycopyrrolate will be given. Cefazolin and Baytril will be administered for pre-operative antibiotic prophylaxis.

A dose of Famotidine will be given during anesthesia to decrease nausea/inappetence.

The front limbs will be shaven for placement of IV catheter. An intravenous cephalic vein catheter will be placed for IV access during pre-operative preparation.

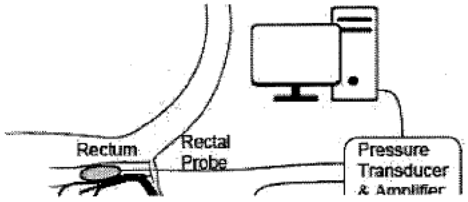
General anesthesia will be induced by isoflurane. Constant rate infusion (CRI) Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

The hind legs and the pelvic region, including the base of the tail and the lower abdomen, will be shaven. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, end-tidal CO2, respiratory rate, non-invasive blood pressure, and temperature. Using these vital signs in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels. Intraoperative monitoring and will be recorded at regular intervals (e.g., every 15 minutes). This record will become a part of the animal's medical record. Body temperature (38.5 degree Celsius) will be maintained using a hot-water circulated heating pad and drapes. Once physiological parameters have been stabilized a sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures. Aseptic procedures by trained personnel will be utilized during the surgery. All tools will be steam sterilized and the UroMOCA will be gas (ethylene oxide) sterilized prior to insertion.

Aseptic techniques will be used during surgery. The surgeon and assistant will scrub hands, wear clean scrubs, shoe covers, mask, bonnet, sterile gown and sterile gloves. Observers/anesthetist will wear clean scrubs, shoe covers, bonnet and mask. All participants will avoid touching non-sterile surfaces. Any instrument that touches outside the surgical field will be replaced and the hands of the surgeon and/or assistant will be re-gloved.

Urodynamics-Baseline

The first urodynamics measurement will be done prior to UroMOCA insertion to establish baseline bladder function for that cat. The animal will be transitioned to CRI propofol anesthesia and off isoflurane. An intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter and a urine sample will be collected and submitted for analysis, including culture and sensitivity. In addition to the intraurethral catheter for bladder pressure monitoring, an inflatable balloon catheter may be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with saline at known volumes from 5 to 50 ml. A 3-D CT X-ray image will be taken to demonstrate the shape of the bladder, by infusion of contrast solution into the bladder.



Standard urodynamics method. A urethral catheter is inserted into the bladder for pressure monitoring and for fluid infusion using a volume syringe. A rectal balloon catheter is inserted into the rectum to measure abdominal pressure. A similar system is used clinically in humans. *Image courtesy of* [redacted]

Cystotomy and UroMOCA implantation

The animal will be transitioned back onto isoflurane and 1mg/kg bupivacaine will be administered at the incision site before and 1mg/kg bupivacaine after incision, for a total of 2mg/kg. With the animal in supine position, a midline suprapubic incision will be made to expose the bladder. The dome of the bladder will be incised and a UroMOCA will be placed into the bladder through this small, approximately 1 cm incision. The bladder incision will then be sutured closed with 4−0 synthetic, monofilament, absorbable suture; tapered needle swaged to suture and closed using Lembert suture pattern (continuous stich). Bladder will be filled with saline to check for leaks. The abdominal wall will also be sutured closed with the 4-0 absorbable suture, using a simple continuous pattern with gentle tissue handling. A simple interrupted pattern is recommended for abdominal wall closure in animals as they may lick at their incisions and a suture break will cause failure of the entire incision. The skin will then be closed using a continuous subcuticular pattern and then a loosely placed simple interrupted or simple cruciate pattern with 4-0 prolene,(non-absorbable, monofilament suture with a cutting needle). A 3−D CT X−ray image will be taken to demonstrate placement of the UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder, and lack of leakage through the bladder wall by infusion of contrast solution into the bladder. The skin sutures will be removed 10-14 days after the operation.

Proper anesthesia will be maintained by Monitoring the vital signs ECG, heart rate, respiratory rate, expired CO2, SPO2, noninvasive blood pressure or arterial blood pressure, and temperature in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels.

Urodynamics-UroMOCA

Animal will be transitioned to CRI propofol anesthesia, and off isoflurane. In addition to volume and pressure data from standard urodynamics, volume and pressure data from the UroMOCA will also be recorded as a function of infused bladder volume. Multiple urodynamics measurements will be done simultaneous with UroMOCA recordings to validate the UroMOCA and the automated analysis software.

Recovery

After UroMOCA insertion, confirmatory urodynamics and 3-D CT imaging, all catheters will be removed and the animal will be awoken. When the cats have become ambulatory, they will be transported back to the VA where the post-operative care will be provided until the next procedure. An Elizabethan collar will be placed to prevent licking of the incision. The Elizabethan collar may be removed intermittently while the cat is under direct observation. The cat will be observed after awakening and frequently afterward for bladder spasms, which indicate bladder pain or irritation, and other changes in animal behavior denoting bladder health and function.

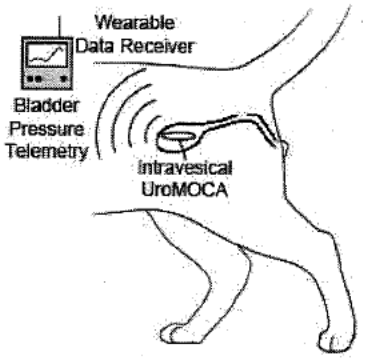
Buprenorphine will be administered twice daily every (8-12 hours) as needed starting the first post-op day, to reduce post-operative pain, with additional doses if deemed necessary according to daily observations by veterinary/investigative staff. If additional pain management is needed after the surgery, a NSAID, Robenacoxib will be administrated, once a day (every 24 hours) for up to 3 days total. Acepromazine may be given as needed for excessive urinary bladder spasms. Doses will be given if deemed necessary according to daily observations by veterinary/investigator staff. The antibiotic Convenia (cefovecin sodium) will be given once after initial surgery, and additional with veterinary approval.

The cat will be group housed in the cat room but will be isolated in a cage (cage rested) in the room for two weeks after surgery. The cat will be brought out under observation for Interim/Ambulatory Testing during that time. After that time the cat will no longer need the cage and will be able to socialize with the other cats. Stiches and the Elizabethan collar will be removed at 10-14 days post-op.

Ambulatory Testing – UroMOCA; Day 1-13 at VAMC

Animals will be monitored at least once per day for changes in behaviors, especially relating to bladder function by study staff and by staff at the Animal Research Facility (ARF). The cat will be crate rested for the first two weeks so the frequency of using the litterbox, and estimated amount and frequency of input/output can be recorded by the ARF staff and lab staff. Notes will be made in the cat's medical record of positional changes, voiding, bladder spasms, and other behaviors that occur during the observation. Investigators or study staff will conduct tests 3-5 times/week of the UroMOCA recharge and data transmission functions, collecting ambulatory bladder volume and pressure data. Data from the UroMOCA will be collected with an external antenna coil. This receiving antenna coil is either attached to a long cable and then to a data receiver circuit attached to a computer or attached directly to a little box that has the receiver circuit in it. If the receiving antenna coil is attached to the long cable, we will walk around holding the receiver antenna near the cat to get data. If the receiving antenna coil is directly attached to the small box, it will be powered by battery and have no cables attached. When used wirelessly, the antenna/box combo will be placed in a belt or backpack harness. These data collection and recharge sessions are expected to last approximately 2-6 hours during animal enrichment in the VA animal research facility. During this time out of the cage, we will attempt to prevent the cat from jumping, due to its recent cystotomy.

A recharge coil may be built into a bedding unit for the cats for recharge while the cat is sleeping. All electronic components will be shielded from the cats.




The UroMOCA is similar to a Holter monitor for the bladder. This sensor enables wireless, catheter-free bladder volume and pressure measurements. *Image courtesy of* [redacted]

**Backpack harness/jacket**

The cats will don a backpack harness that holds the data receiver circuit and coil with online data storage, and the battery charger coil, as stated in the main body of the protocol. The jackets are a standard approach and will not restrict the movements of the cat. At least two weeks before other procedures are conducted, study staff will put the jacket or harness onto the animal during daily enrichment periods. The animal will wear the jacket for a brief period, about a half hour, and each day gradually work up to wearing the jackets continuously. Animals will be rewarded with veterinary-approved cat treats while the jacket is being put on and periodically while the animal is wearing it. Study staff will also engage the animals in playing with cat toys to encourage the animals to move around and acclimate to the jacket.

**Day 14 follow-up at [redacted]: UroMOCA only**

Surgical Preparation: At least twelve hours prior to surgery, animals will begin fasting. The morning of the surgery, the cats will be transported to the [redacted] in approved private vehicles, according to the LSCVAMC ARF SOP. Buprenorphine and Robenacoxib will be administered the morning of surgery.

10–15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam andTorbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15minutes the animal remains bradycardic a dose of glycopyrrolate will be given.

A dose of Famotidine will be given during anesthesia to decrease nausea/inappetence.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

Animal will be placed in the supine position.

The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, end-tidal CO2, respiratory rate, non-invasive blood pressure, and temperature. Using these vital signs in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone,

as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels. Intraoperative monitoring will be recorded at regular intervals. (e.g., every 15 minutes). This record will become a part of the animal's medical record. Body temperature (38.5 degree Celsius) will be maintained using a hot-water circulated heating pad and drapes. Once physiological parameters have been stabilized a sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile site for catheterization. The animal will then be thoroughly draped with sterile drape. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures. Aseptic procedures by trained personnel will be utilized during the surgery. All tools will be steam sterilized prior to insertion. Drapes, caps, masks, gowns and sterile gloves will be utilized to maintain sterility.

Urodynamics

Animal will be transitioned to CRI propofol anesthesia and off isoflurane. An intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter. A urine sample will be collected and submitted for analysis, including culture and sensitivity. In addition to the intraurethral catheter for bladder pressure monitoring, an inflatable balloon catheter may be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with normal saline at known volumes from 5 to 50 mL. In addition to volume and pressure data from standard urodynamics, volume and pressure data from the UroMOCA will also be recorded as a function of infused bladder volume. Multiple urodynamics measurements will be done simultaneous with UroMOCA recordings to validate the UroMOCA and the automated analysis software.

A 3-D CT X-ray image will be taken to demonstrate the shape of the bladder and placement of the UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder, by infusion of contrast solution into the bladder.

Recovery

All catheters will be removed. Atipamezole may be given to reverse dexmedetomidine following the completion of surgery and the animal will be awoken. The cat will be observed after waking until ambulatory, and then it will be transported back to the VAMC. Cats will be observed frequently for bladder spasms, which indicate bladder pain or irritation, and other changes in animal behavior denoting bladder health and function.

For emergency overnight housing, the [redacted] has a small cat/dog recovery cage that can be utilized for an overnight stay and observation. The cage would be wheeled into a large, empty animal housing room where there is temperature, humidity and light control.

If additional pain management is needed - NSAID, Robenacoxib will be administrated, once a day for up to 3 doses total. Doses will be given if deemed necessary according to daily observations by veterinary/investigator staff.

**Ambulatory Testing – Day 15-30 at VAMC: UroMOCA only**

Animals will be monitored at least once per day for changes in behaviors, especially relating to bladder function, by study staff during daily enrichment periods and by staff at the Animal Research Facility. Notes will be made in the cat's medical record of positional changes, voiding, bladder spasms (see definition below), and other behaviors that occur during the observation and recording time. Investigators or study staff will conduct tests 3-5 times/week of the UroMOCA recharge, data transmission functions, collecting ambulatory bladder volume and pressure data. Data from the UroMOCA will be collected with an external antenna coil. This receiving antenna coil is either attached to a long cable and then to a data receiver circuit attached to a computer or attached directly to a little box that has the receiver circuit in it. If the receiving antenna coil is attached to the long cable, we will walk around holding the receiver antenna near the cat to get data. If the receiving antenna coil is directly attached to the small box, it will be powered by battery and have no cables attached. When used wirelessly, the antenna/box combo will be placed in a belt or backpack harness. None of these hinders the cat's movement. These data collection and recharge sessions are expected to last approximately 2−6 hours during animal enrichment in the animal facility.

A recharge coil may be built into a bedding unit for the cats for recharge while the cat is sleeping. All electronic components will be shielded from the cats.

**Device Extraction – 30 day Terminal surgery at** [redacted]**: UroMOCA only**

Surgical Preparation: 30 days after the initial surgery of the UroMOCA insertion, the cats will have the terminal procedure to remove the device.

At least twelve hours prior to surgery, animals will begin fasting. Buprenorphine will be given the morning of surgery and then at least 4 additional doses will be administrated (twice a day), and more if needed. Robenacoxib will also be administered in morning of surgery and at least 2 days post-op.

The morning of the surgery, the cats will be transported to the [redacted] in **approved** private vehicles, according to the LSCVAMC ARF SOP.

10−15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam and Torbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15 minutes the animal remains bradycardic, a dose of glycopyrrolate will be given. Cefazolin) and Baytril will be administered for pre−operative antibiotic prophylaxis. A dose of Famotidine will be given during anesthesia to decrease nausea/inappetence.

The front limbs will be shaven for placement of IV catheter. An intravenous cephalic vein catheter will be placed for IV access and the animal will be pre−oxygenated.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

All incision sites will be shaved. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, CO2, respiratory rate, non−invasive blood pressure, and temperature. Once physiological parameters have been stabilized a sterile scrub of 3−5 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. A lubricant gel will be applied to the eyes to keep them moist

throughout the procedures. Incision sites will be injected with Bupivacaine before incision.

The animal will be transitioned from isoflurane to CRI propofol for urodynamics testing and then transitioned back onto isoflurane.

Urodynamics

An intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter. A urine sample will be collected and submitted for analysis, including culture and sensitivity. In addition to the intraurethral catheter for bladder pressure monitoring, an inflatable balloon catheter may be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with normal saline at known volumes from 5 to 50 mL.

A 3-D CT X-ray image will be taken to demonstrate the shape and placement of the UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder by infusion of contrast solution into the bladder.

In addition to volume and pressure data from standard urodynamics, volume and pressure data from the UroMOCA will also be recorded as a function of infused bladder volume. Multiple urodynamic measurements will be done simultaneous with UroMOCA recordings to validate the UroMOCA and the automated analysis software. After the last urodynamics measurements, the UroMOCA will be removed (and examined for function in vitro) and the cat will be euthanized with an overdose of pentobarbital combination (Euthasol) 100mg/kg under deep anesthesia. Bladder will be harvested for histology.

**(SA2): Map patterned sacral root stimulation on to bladder and pelvic floor functions in awake, behaving cats**

**SA2 Summary**

1. **Day 0 (CCF):** Cystotomy with UroMOCA and electrode implantation; Urodynamics and Imaging
2. **Day 1-13 (VA) :** Ambulatory testing using UroMOCA and electrical stimulation to map bladder and pelvic floor functions
3. **Day 14 (CCF):** Follow up urodynamics, imaging and charging of UroMOCA
4. **Day 15-29/36 (VA):** Ambulatory testing using UroMOCA and electrical stimulation to map bladder and pelvic floor functions
5. **Day 30 or 37 (CCF):** Urodynamics, imaging – terminal procedure

**Day 0: Surgery at [redacted]: UroMOCA and electrodes**

Surgical Preparation: At least twelve hours prior to surgery, animals will begin fasting. Buprenorphine will be given the morning of surgery and then at least 4 additional doses will be administrated (twice a day), and more if needed. Robenacoxib will also be administered in morning of surgery and at least 2 days post-op.

The morning of the surgery, the cats will be transported to the [redacted] in **approved** private vehicles, according to the LSCVAMC ARF SOP.

10–15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam and Torbugesic (butorphanol tartrate) IM may be used. The

animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15 minutes the animal remains bradycardic, a dose of glycopyrrolate will be given. Cefazolin) and Baytril will be administered for pre-operative antibiotic prophylaxis. A dose of Famotidine will be given during anesthesia to decrease nausea/inappetence.

The front limbs will be shaven for placement of IV catheter. An intravenous cephalic vein catheter will be placed for IV access and the animal will be pre-oxygenated.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

The hind legs and the pelvic region, including the base of the tail and the lower abdomen will be shaven for cystotomy and the dorsal back will be shaved for the laminectomy. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, end-tidal CO2, respiratory rate, non-invasive blood pressure, and temperature. Using these vital signs in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels. Intraoperative monitoring will be recorded at regular intervals (e.g., every 15 minutes). This record will become a part of the animal's medical record. Body temperature (38.5 degree Celsius) will be maintained using a hot-water circulated heating pad and drapes. Once physiological parameters have been stabilized a sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures. Aseptic procedures by trained personnel will be utilized during the surgery. All tools will be steam sterilized and the UroMOCA and electrodes will be gas (ethylene oxide) sterilized prior to insertion.

Aseptic techniques will be used during surgery. The surgeon and assistant will scrub hands, wear clean scrubs, shoe covers, mask, bonnet, sterile gown and sterile gloves. Observers/anesthetist will wear clean scrubs, shoe covers, bonnet and mask. All participants will avoid touching non-sterile surfaces. Any instrument that touches outside the surgical field will be replaced and the hands of the surgeon and/or assistant will be re-gloved.

Urodynamics-Baseline

The first urodynamics measurement will be done prior to UroMOCA insertion to establish baseline bladder function for that cat. The animal will be transitioned to CRI propofol anesthesia, and off isoflurane. An intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter and a urine sample will be collected and submitted for analysis, including culture and sensitivity. In addition to the intraurethral catheter for bladder pressure monitoring, an inflatable balloon catheter may be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with saline at known volumes from 5 to 50 mL. A 3-D CT X-ray image will be taken to demonstrate the shape of the bladder, by infusion of contrast solution into the bladder.

Cystotomy, UroMOCA Implantation and Electrode and stimulation device implantation

The animal will be transitioned back onto isoflurane and bupivacaine (1mg/kg) will be administered at the incision site before and after incision. With the animal in supine position, a midline suprapubic incision will be made to expose the bladder and pelvic floor muscles. EMG electrode leads will be inserted into the pelvic floor muscles via laparotomy exposure. EMG electrode leads will then be tunneled under the skin to the dorsal surface of the animal and connected to an implantable stimulator via a dorsal skin incision at lumbosacral region. An intraurethral catheter will be inserted to maintain an empty bladder. The dome of the bladder will be incised and a UroMOCA will be placed into the bladder through this small, ~1cm incision, which will then be sutured closed with 4-0 synthtic, monofilament, absorbable suture; tapered needle swaged to suture and closed using Lembert suture pattern (continuous stich). Bladder will be filled with saline to check for leaks. The abdominal wall will also be sutured closed with the 4-0 absorbable suture, using a simple continuous pattern with gentle tissue handling. A simple interrupted pattern is recommended for abdominal wall closure in animals as they may lick at their incisions and a suture break will cause failure of the entire incision. The skin will then be closed using a continuous subcuticular pattern and then a loosely placed simple interrupted or simple cruciate pattern with 4-0 prolene,(non-absorbable, monofilament suture with a cutting needle). A 3-D CT X-ray image will be taken to demonstrate placement of the UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder, and lack of leakage through the bladder wall by infusion of contrast solution into the bladder.

After closure of the abdomen and X-Ray, the cat will be placed in the prone position. A sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish this second surgical incision site. The animal will then be thoroughly re-draped with sterile drape such that only the sterile incision site is exposed.

With the animal in a prone position, a dorsal midline sacral incision, approximately 5 cm in length, will be made to expose the sacral spine. A laminectomy will be performed to remove the bone of the spine to expose the sacral roots from S1 to S4. 4 nerve cuff electrodes will be implanted, including one each around the sacral roots S1 left side, S1 right side, S2 left side, and S2 right side. We are choosing left and right S1 and S2 sacral roots because urethral sphincter and bladder neurons predominantly run through these roots. Pelvic floor EMG will act as a proxy for urethral sphincter activity because it is significantly more feasible and practical to measure with the current tools available and is consistent with current clinical practice. We will also observe animal functions of bladder voiding versus defecation to distinguish between bowel and bladder emptying activity. All electrodes will be attached to two wireless implantable stimulation devices (StimPod, [redacted]). No wires or catheters will be exteriorized from the body, reducing the chance of infection and irritation. All stimulation parameters and data collection will be wireless. The muscles will be approximated with an absorbable suture. The skin will then be closed using with 4-0 prolene, non-absorbable, monofilament suture with a cutting needle, using a discontinuous suture pattern.

The skin sutures will be removed 10-14 days after the operation

Urodynamics-UroMOCA and neuromodulation/electrical stimulation

Animal will be transitioned to CRI propofol anesthesia and off isoflurane. In addition to volume and pressure data from standard urodynamics, volume and pressure data from the UroMOCA will also be recorded as a function of infused bladder volume. Multiple urodynamics measurements will be done

simultaneous with UroMOCA recordings to validate the UroMOCA and the automated analysis software. Up to four cystometrograms will be conducted to verify reflex lower urinary tract function, UroMOCA function, and responses to sacral root stimulation. Sacral root stimulation is expected to evoke bladder contractions and pelvic floor EMG; we will also observe for other activity, such as hindlimb movement.

Recovery

After UroMOCA insertion, electrode and stimulator implantation, confirmatory urodynamics, and 3-D CT imaging, all catheters will be removed. When the cats have become ambulatory, they will be transported back to the VA where the post-operative care will be provided until the next procedure. An Elizabethan collar will be placed to prevent licking of the incision. The Elizabethan collar may be removed intermittently while the cat is under direct observation. The cat will be observed after awakening and frequently afterward for bladder spasms, which indicate bladder pain or irritation, and other changes in animal behavior denoting bladder health and function.

Buprenorphine will be administered twice daily every (8-12 hours) as needed starting the first post-op day, to reduce post−operative pain, with additional doses if deemed necessary according to daily observations by veterinary/investigative staff. If additional pain management is needed after the surgery, a NSAID, Robenacoxib will be administrated, once a day (every 24 hours) for up to 3 days total. Acepromazine may be given as needed for excessive urinary bladder spasms. Doses will be given if deemed necessary according to daily observations by veterinary/investigator staff. The antibiotic Convenia will be given once after initial surgery, and additional with veterinary approval.

The cat will be group housed in the cat room but will be isolated in a cage (cage rested) in the room for two weeks after surgery. The cat will be brought out under observation for Interim/Ambulatory Testing during that time. After that time the cat will no longer need the cage and will be able to socialize with the other cats. Stiches will be removed at 10−14 days post-op.

**Ambulatory Testing –Day 1-13 at VAMC: UroMOCA and electrodes**

Animals will be monitored at least once per day for changes in behaviors, especially relating to bladder function, by study staff during daily enrichment periods and by staff at the Animal Research Facility. The cat will be crate rested for the first two weeks so the frequency of using the litterbox, and amount/frequency of input/output can be recorded by the ARF staff and lab staff. Notes will be made in the cat's medical record of positional changes, voiding, bladder spasms (see definition below), and other behaviors that occur during the observation and recording time. Investigators or study staff will conduct tests 3-5 times/week of the UroMOCA recharge, data transmission functions, collecting ambulatory bladder volume and pressure data along with stimulation and EMG data. Data from the UroMOCA will be collected with an external antenna coil. This receiving antenna coil is either attached to a long cable and then to a data receiver circuit attached to a computer or attached directly to a little box that has the receiver circuit in it. If the receiving antenna coil is attached to the long cable, we will walk around holding the receiver antenna near the cat to get data. If the receiving antenna coil is directly attached to the small box, it will be powered by battery and have no cables attached. When used wirelessly, the antenna/box combo will be placed in a belt or backpack harness. The wireless stimulator may also be placed in this belt.

Bladder spasms: In the absence of an urodynamics procedure or the bladder device in place, bladder spasms will be identified as urinary incontinence episodes or other significant phenotypical changes to the animal's normal bladder emptying habits. Normal habits will be established during the acclimation period for at least one week before any procedures are conducted. Animal behaviors will be observed by study staff during daily enrichment periods and by staff at the VAMC Animal Research Facility.

We plan to conduct preliminary experiments testing sacral root stimulation in awake, behaving animals to (1) map spontaneous bladder and pelvic floor activity to bladder state during normal physiological bladder states of filling and emptying; (2) map low frequency sacral root stimulation to bladder and pelvic floor activity; and (3) map kilohertz frequency sacral root stimulation to bladder and pelvic floor activity. Bladder state (i.e. bladder filling or bladder emptying) will be an independent variable that may affect outcomes.

(1) map spontaneous bladder and pelvic floor activity to bladder state during normal physiological bladder states of filling and emptying

Map bladder and pelvic floor activity to different bladder states and animal behaviors, including bladder storage (filling) and emptying. We will measure bladder pressure, bladder volume, and pelvic floor EMG when the animal is at rest during filling and when the animal is using a litter box to empty its bladder. We will monitor animal behaviors, including rest; movement or play as during enrichment; vocalization or other signs of discomfort or distress; seeking litter box; and squatting in litter box and actively emptying bladder or bowel contents. These behaviors will act both as secondary measures for (2) and (3), and as indications of bladder state (filling or emptying). We will characterize the bladder pressure and pelvic floor EMG during these two bladder states for comparison to standard urodynamics data, which includes anesthesia and non-physiological fast bladder filling. These data will inform the stimulation trigger events for milestones (2) and (3) by providing more informed definitions of bladder state (filling or emptying) that include bladder pressure and volume and pelvic floor activity.

(2) map low frequency sacral root stimulation to bladder and pelvic floor activity

Map sacral root stimulation to bladder and pelvic floor activity during bladder storage and emptying states. We will measure recruitment of bladder pressure, pelvic floor EMG, thresholds for limb movement, and animal behaviors (comfort) as a function of stimulation amplitude and bladder state (filling or emptying). During each of the two bladder states, stimulation will be applied at each of the 4 electrode sites in turn, randomized. For bladder filling, we will wait at least one hour after the animal used the litter box to empty and verify that the animal is in a state of rest. For bladder emptying, stimulation will be applied when the animal climbs into the litter box, but before the animal assumes a posture for emptying. Stimulation will be charge-balanced biphasic square wave pulses, 20 Hz, 0.2 ms pulse width, less than 30 s in duration per bout of stimulation with at least a minute of rest in between stimuli, and amplitude = 0-1 mA. These parameters are based on typical sacral root stimulation parameters for evoking bladder contractions. Stimulation will last until maximal bladder pressure is achieved and emptying is occurring, or stopped after 30 s, whichever comes first. Stimulation

amplitudes will be randomized in this range and we will characterize the relationships between stimulation amplitude and response amplitudes of bladder pressure and pelvic floor EMG. We hypothesize that stimulation at each root will result in increased bladder pressure, pelvic floor EMG, and sacral root ENG, regardless of electrode site, and amplitudes of responses will correlate with stimulation amplitude.

(3) map kilohertz frequency sacral root stimulation to bladder and pelvic floor activity.

Map kilohertz frequency stimulation of the sacral roots to bladder and pelvic floor activity during bladder storage and emptying states. We will measure bladder pressure and pelvic floor EMG and observe limb function and animal behaviors as a function of sacral root block and bladder state (filling or emptying). Stimulation will be applied on all 4 sacral root electrode sites simultaneously as 10 kHz, 10Vpp, sinusoidal waveforms, lasting 30s in duration, which is the typical duration of a cat emptying their bladder. Stimulation will be applied during filling and emptying states as described above. We hypothesize that kilohertz stimulation will block sacral root conduction, significantly reducing bladder pressure and pelvic floor EMG regardless of bladder state (filling or emptying). We are particularly interested in the efficiency (i.e. time course and completeness of block) of kilohertz frequency stimulation and its potential for use in conjunction with other neurostimulation approaches to improve bladder emptying without unwanted side effects.

**Day 14 follow-up at [redacted]: UroMOCA and electrodes**

Surgical Preparation: At least twelve hours prior to surgery, animals will begin fasting.

The morning of the surgery, the cats will be transported to the [redacted] in approved private vehicles, according to the LSCVAMC ARF SOP. Buprenorphine and Robenacoxib will be administered the morning of surgery.

10–15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam andTorbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15minutes the animal remains bradycardic a dose of glycopyrrolate will be given.

A dose of Famotidine will be given during anesthesia to decrease nausea/inappetence.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

Animal will be placed in the supine position.

The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, end–tidal CO2, respiratory rate, non–invasive blood pressure, and temperature. Using these vital signs in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels. Intraoperative monitoring will be recorded at regular intervals. (e.g., every 15 minutes). This record will

become a part of the animal's medical record. Body temperature (38.5 degree Celsius) will be maintained using a hot-water circulated heating pad and drapes. Once physiological parameters have been stabilized a sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile site for catheterization. The animal will then be thoroughly draped with sterile drape. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures. Aseptic procedures by trained personnel will be utilized during the surgery. All tools will be steam sterilized prior to insertion. Drapes, caps, masks, gowns and sterile gloves will be utilized to maintain sterility.

Urodynamics

Animal will be transitioned to CRI propofol anesthesia, and off isoflurane. An intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter. A urine sample will be collected and submitted for analysis, including culture and sensitivity. In addition to the intraurethral catheter for bladder pressure monitoring, an inflatable balloon catheter will be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with normal saline at known volumes from 5 to 50 mL. In addition to volume and pressure data from standard urodynamics, volume and pressure data from the UroMOCA will also be recorded as a function of infused bladder volume. Multiple urodynamics measurements will be done simultaneous with UroMOCA recordings to validate the UroMOCA and the automated analysis software.

A 3-D CT X-ray image will be taken to demonstrate the shape of the bladder and placement of the UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder, by infusion of contrast solution into the bladder.

Recovery

All catheters will be removed. Atipamezole may be given to reverse dexmedetomidine following the completion of surgery and the animal will be awoken. The cat will be observed after waking until ambulatory, and then it will be transported back to the VAMC. The cats will be observed daily for bladder spasms, which indicate bladder pain or irritation, and other changes in animal behavior denoting bladder health and function.

For emergency overnight housing, the [redacted] has a small cat/dog recovery cage that can be utilized for an overnight stay and observation. The cage would be wheeled into a large, empty animal housing room where there is temperature, humidity and light control.

If additional pain management is needed - NSAID, Robenacoxib will be administrated, once a day for up to 3 doses total. Doses will be given if deemed necessary according to daily observations by veterinary/investigator staff.

**Day 15-30 at the VAMC Animal Research Facility: UroMOCA and electrodes**

Interim/Ambulatory Testing

Same as Day 1-13

**Device Extraction – Terminal surgery at** [redacted]**: UroMOCA and electrodes**

Surgical Preparation: 30-37 days after the initial surgery of the UroMOCA insertion, the cats will have the terminal procedure to remove the device.

At least twelve hours prior to surgery, animals will begin fasting.

The morning of the surgery, the cats will be transported to the [redacted] in approved private vehicles, according to the LSCVAMC ARF SOP.

10−15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam (5mg/ml) and Torbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15minutes the animal remains bradycardic a dose of glycopyrrolate will be given.

The front limbs will be shaven for placement of IV catheter. An intravenous cephalic vein catheter will be placed for IV access and the animal will be pre−oxygenated.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

All incision sites will be shaved. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, CO2, respiratory rate, non−invasive blood pressure, and temperature. Once physiological parameters have been stabilized a sterile scrub of 3−5 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. A lubricant gel will be applied to the eyes to keep them moist
throughout the procedures. Incision site will be injected with Bupivacaine before incision.

The animal will be transitioned from isoflurane to CRI propofol for urodynamics testing and then transitioned back onto isoflurane.

Urodynamics

An intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter. A urine sample will be collected and submitted for analysis, including culture and sensitivity. In addition to the intraurethral catheter for bladder pressure monitoring, an inflatable balloon catheter will be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with normal saline at known volumes from 5 to 50 mL.

A 3-D CT X-ray image will be taken to demonstrate the shape and placement of the UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder by infusion of contrast solution into the bladder.

In addition to volume and pressure data from standard urodynamics, volume and pressure data from the UroMOCA will also be recorded as a function of infused bladder volume. Multiple urodynamics measurements will be done simultaneous with UroMOCA recordings to validate the UroMOCA and the automated analysis software. After the last urodynamics measurements, the UroMOCA will be removed (and examined for function in vitro) and the cat will be euthanized with an overdose of pentobarbital combination (Euthasol) 100mg/kg under deep anesthesia. Bladder will be harvested for histology.

**Photography**

During surgery the animal will be partially draped and we will take photographs all through the surgery for documentation. We need to photograph key aspects of the anatomy and surgery to document our methods and to allow for retrospective identification of anatomical landmarks. Most photographs will only be seen by the study team. Some select photographs will be published in research journals. Surgery photographs will be cropped to prevent animal identification.

We will also be using a FLIR camera for thermal imaging. This will allow us to see how warm the recharge coil gets, and if the animal's skin gets warm from the coil. The FLIR thermal imager we will use needs to be connected to a smart phone for it to work.

We will also be filming the room with a GoPro camera when the cat is wearing the jacket and coil. This way we can be out of the room, and then go back to the timestamp on the camera and correlate it with data -such as when the cat uses a litterbox.

All collected materials will be stored on an encrypted USB-key or laptop prior to upload to the [redacted] research drive. This network drive is password protected and only accessible to [redacted] employees.

| Study design for the chronic feline experiments | | | | | | |
|---|---|---|---|---|---|---|
| | **Days -5-0** | **Day 0** (at CCF) | **Days 1-13** | **Day 14** (at CCF) | **Days 15-29** | **Day 30-37** (at CCF) |
| **SA1 UroMOCA implanted (n = 3)** | **Animal Observation** | **UroMOCA Implant and Initial test** | **Daily observations, ambulatory UroMOCA charging & testing 3-5 days /week** | **Interim test** | **Daily observations, ambulatory UroMOCA charging & testing 3-5 days /week** | **Terminal test & UroMOCA extraction** |

| **SA2 UroMOCA and electrodes implanted (n = 6)** | **Animal Observation** | **UroMOCA and electrode implant and Initial test** | **Daily observations, ambulatory UroMOCA charging & testing (days 1-13;3-5 days /week); electrode testing (After 7 days;3-5 days /week)** | **Interim test** | **Daily observations, ambulatory UroMOCA charging & testing; electrode testing 3-5 days /week** | **Terminal test & UroMOCA extraction** |
|---|---|---|---|---|---|---|

D. **Species. Justify the choice of species for this protocol.**

► Cats are the most suitable animal model for this study because they are an appropriate and common animal model for experiments to understand the control of LUT (lower urinary tract) function by the peripheral nervous system. They are the smallest animal that controls its bladder in a similar way as humans. The goal of this project is to develop and validate a new tool to provide a biomarker of lower urinary tract function and use this tool in neurophysiology experiments of the lower urinary tract in healthy and disease states. Though bladders from larger animals, such as pigs, are appropriate for research in biomechanical properties, felines are the preferred animal model for studies in the neural control of the lower urinary tract, because the neurophysiology of the lower urinary tract has already been validated for felines. In addition, experiments exploring the neurophysiology of the lower urinary tract would be unreasonably laborious and expensive with a larger animal model, which is another reason why felines are used. Rodents are not always considered appropriate animal models because of their size and they do not exhibit the same behaviors and control of the lower urinary tract, suggesting that neural circuits may not be as well conserved to humans as is seen in a cat model.

## Personnel

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.)

1. PI

Name► [redacted], Ph.D.
Animal research experience ►21 years' experience with survival surgery and experimental procedures on the bladder in small animals: rats, mice, cats, and rabbits. [redacted] has implanted bladder devices in cats, dogs, and approximately 8 calves. Dr. [redacted] will provide project oversight and coordination. Dr. [redacted] will supervise the study design, data analysis and all procedures of this project.

Qualifications to perform specific procedures

| Specific procedure(s) that the PI will perform personally | Experience with each procedure in the species described in this ACORP |
|---|---|

| | |
|---|---|
| UroMOCA insertion | [redacted] will not be assisting with the surgery. |
| Urodynamics | 3 years' experience in cat |
| Ambulatory Testing | 3 years' experience |

Co-PI

Name►[redacted], Ph.D.
Animal research experience ►10+ years of experience in animal surgery and electrophysiology experiments in cats, rats, and mice. [redacted] has established laboratories for research focused on understanding the neural control of bladder and bowel function and developing approaches to restore function lost to neural disease or trauma. With [redacted] expertise in neuroscience and animal research, particularly with the feline LUT model, [redacted] will oversee UroMOCA design and testing activities throughout the study. [redacted] specializes in neural stimulation approaches to restore pelvic functions, including bladder function. [redacted] has extensive knowledge of the anatomy and function of the neural control circuitry of the lower urinary tract.

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| UroMOCA insertion | Has 3+ years' experience |
| Laminectomy | Has 10+ years' experience in rats and cats |
| Urodynamics | Has 5+ years' experience |
| Ambulatory Testing | Has 5+ years' of experience with cat ambulatory testing |

2. Other research personnel (copy the lines below for each individual)

Name►[redacted], M.D.
Animal research experience ►[redacted] M.D. is an experienced urological surgeon and will be performing the implantation of a wireless bladder pressure sensor. Dr. [redacted] has extensive experience performing the following procedures in mice: anesthesia, injection, and non-viability study of distributions of lymphocytes for cancer treatment. [redacted] has 3 years of experience performing cystoscopic and suprapubic implantation of the device prototype into calf bladders along with cystoscopic implantation in pig bladders in vitro.

Qualifications to perform specific procedures

| Specific procedure(s) that the PI will perform personally | Experience with each procedure in the species described in this ACORP |
|---|---|
| UroMOCA insertion | Dr. [redacted] is an experienced urological surgeon who has also performed the suprabubic device implantation in calves. He will be advised on any cat-specific topics by the [redacted] vet, Dr. [redacted]. |
| Laminectomy | N/A |

| | |
|---|---|
| Urodynamics | Dr. [redacted] is an experienced urological surgeon who has also performed urodynamics in calves. [redacted] will be advised on any cat-specific topics by the [redacted] vet, Dr. [redacted] |
| Ambulatory Testing | Will not be responsible for ambulatory testing |

Name▶ [redacted], PhD.
Animal research experience ▶ Has 3 years of experience with calves and 3 years with cat experiments. [redacted] has performed urodynamics and pre and post op care of the animals.

Qualifications to perform specific procedures

| Specific procedure(s) that the PI will perform personally | Experience with each procedure in the species described in this ACORP |
|---|---|
| UroMOCA insertion | N/A |
| Laminectomy | Will not be performing laminectomy |
| Urodynamics | 3+ years' experience with cat urodynamics |
| Ambulatory Testing | 3 years' experience |

Name▶ [redacted]
Animal research experience ▶ [redacted] is trained on mouse handling and breeding and has been working with mice and rats for 10 years. [redacted] is surgically trained and has done over 200 vaginal surgeries, 500 thoracotomies, 1000 IP and SQ injections and 200 saphenous and tail vein blood collections in rodents. [redacted] has performed 25 pig surgeries and helped familiarize others with pre- and post-operative care.
[redacted] has experience working with 7 cats. [redacted] will be assisting during surgery and pre–and post op.

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| UroMOCA insertion | N/A |
| Laminectomy | N/A |
| Urodynamics | 3 years' experience |
| Ambulatory Testing | 3 years' experience |

Name▶ [redacted]
Animal research experience ▶ A research technician with 15 years of research animal handling experience. Mr. [redacted] will perform pre-surgery anesthesia, post-operative care and analgesia, and assist on urodynamic testing. [redacted] has 8 years of experience assisting with urodynamic procedures in rats and mice. [redacted] has 3 years of experience with CT fluoroscopy, urodynamics and ambulatory monitoring of calves. [redacted] also has 3+ years' experience with cat procedures including ambulatory testing.

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| UroMOCA insertion | N/A |
| Laminectomy | N/A |
| Urodynamics | 3 years' experience |
| Ambulatory Testing | 3 years' experience |
| | |

Name▶[redacted], MD
Animal research experience ▶ Over 5 years' experience with aseptic survival surgery and handling of rats and 1 month experience with cats surgeries. Dr.[redacted]will perform UroMOCA insertion and laminectomies along with assisting in electrode placement, post-operative care and analgesia, and assist on urodynamic testing.

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| UroMOCA insertion | Has experience on 1 successful cat surgery/UroMOCA implantation |
| Laminectomy | To be trained |
| Urodynamics | Has experience on 1 successful cat experiment (Multiple rounds of urodynamics) |
| Ambulatory Testing | To be trained |

Name▶[redacted]
Animal research experience ▶ [redacted]is an electrical engineer that won't be performing any surgeries.[redacted]will assist in urodynamics and ambulatory testing.[redacted]has 2 months' experience in ambulatory testing in cats

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| UroMOCA insertion | N/A |
| Laminectomy | N/A |
| Urodynamics | 6 months' experience |
| Ambulatory Testing | 2 months' experience |

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual)

Name▶[redacted]– Vet tech with [redacted]department at [redacted]

Qualifications to perform specific support procedures in the animals on this protocol

| Specific support procedure(s) assigned to this individual | Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training) |
|---|---|
| Will lead in preparing the animals for surgery, anesthesia induction, monitoring and recovery. | |

Name▶[redacted] Staff

Qualifications to perform specific support procedures in the animals on this protocol

| Specific support procedure(s) assigned to this individual | Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training) |
|---|---|
| [redacted]staff may assist with anesthesia induction, monitoring and recovery if required. | |

Name▶ARF (VA) Staff

Qualifications to perform specific support procedures in the animals on this protocol

| Specific support procedure(s) assigned to this individual | Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training) |
|---|---|
| ARF staff may assist with administering drugs including analgesics and antibiotics and with animal monitoring. | |

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course

| Name of Individual | Working with the VA IACUC | ORD web-based species specific course (Identify the species) | Any other training required locally (Identify the training) |
|---|---|---|---|
| [redacted] Ph.D. | | CITI training-cat | Hands on cat training |
| [redacted], PhD | | CITI training-cat | Hands on cat training |
| [redacted] M.D. | | CITI training-cat | Hands on cat training |
| [redacted] M.D. | | CITI training-cat | Hands on cat training |
| [redacted], PhD. | | CITI training-cat | Hands on cat training |
| [redacted] | | CITI training-cat | Hands on cat training |
| [redacted] M.S. | | CITI training-cat | Hands on cat training |
| [redacted] | | CITI training-cat | Hands on cat training |

Please enter completion dates for all listed above.

F. **Training to be provided.** List here each procedure in Item E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, enter "N/A"
▶Dr. [redacted] and Dr. [redacted] will train appropriate staff in device implantation and urodynamics. Dr. [redacted] will train appropriate staff on laminectomy and electrode placement. [redacted] veterinarian, Dr. [redacted] and VA veterinarian, Dr. [redacted] will provide insight for the device implantation. Dr. [redacted] will train all the necessary staff in ambulatory testing.

G. **Occupational Health and Safety.**

1. Complete one line in the table below for each of the personnel identified in Item E:

| Name | Enrollment in OHSP | | Declined optional services | Current on Interactions with OHSP? (yes/no) |
|---|---|---|---|---|
| | VA program | Equivalent Alternate Program – identify the program | | |
| [redacted] Ph.D. | (x) | ( ) | ( ) | Yes |
| [redacted] PhD | (x) | ( ) | ( ) | Yes |
| [redacted] M.D. | (x) | ( ) | ( ) | Yes |
| [redacted] | (x) | ( ) | ( ) | Yes |
| [redacted] PhD. | (x) | ( ) | ( ) | Yes |
| [redacted] | (x) | ( ) | ( ) | Yes |
| [redacted] | (X) | ( ) | ( ) | Yes |
| [redacted] | (x) | ( ) | ( ) | Yes |
| [redacted] | (X) | ( ) | ( ) | Yes |

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?

▶ (**X**) Yes. Describe them ▶ In spite of the fact that these purpose-bred and indoor-housed cats are unlikely to be exposed to rabies, the guidelines on cat bites are rather strict and mandated at the state level. All study staff should be aware of cat bite protocols to prevent rabies. All skin – penetrating cat bites must be reported to Personnel Health and to the ARF Veterinarian and Supervisor so that the mandated 10-day quarantine/observation period may be instituted.

▶ ( ) No.

## Animals Requested

H. **Animals to be Used.** Complete the following table, listing the animals on separate lines according to any specific features that are required for the study (see ACORP Instructions, for guidance, including specific

terminology recommended for the "Health Status" column):

| Description (include the species and any other special features not shown elsewhere in this table) | Gender | Age/Size on Receipt | Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony) | Health Status |
|---|---|---|---|---|
| Domestic short-haired cat | M/F | >6 months; 3.5-5.0 kg | [redacted] | SPF, purpose-bred, rabies vaccinated by vendor |

I. **Numbers of animals requested.** See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

**USDA Category B**

| Procedures ▶ | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedures(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | **Category B TOTAL** |
| | | | | | | |

**USDA Category C**

| Procedures ▶ | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | **Category C TOTAL** |
| | | | | | | |

**USDA Category D**

| Procedures ▶ | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | **Category D TOTAL** |
| UroMOCA insertion - chronic | 3 | | | | | 3 |
| UroMOCA and electrodes - chronic | | 6 | | | | 6 |

**USDA Category E**

| Procedures ▶ | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | **Category E TOTAL** |
| | | | | | | |

**TOTALS over all Categories**

| Species / Experimental Group /Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | **GRAND TOTAL** |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| | 3 | 6 | | | | 9 |

J. **Management of USDA Category D procedures**. Indicate which statement below applies, and provide the information requested.

► ( ) This protocol does NOT include any Category D procedures.

► (**X**) This protocol INCLUDES Category D procedures. List each Category D procedure and provide the information requested. (For surgical procedures described in Appendix 5, only identify the procedure(s) and enter "See Appendix 5 for details.)

| Procedure | Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery) | Person(s) responsible for the monitoring | Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered) |
|---|---|---|---|
| UroMOCA insertion - chronic | See Appendix 5 for details | | |
| UroMOCA and electrodes - | See Appendix 5 for details | | |
| Urodynamics and CT fluoroscopy | See Appendix 6 for details | | |

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

► (X) This protocol does NOT include any Category E procedures

► ( ) This protocol INCLUDES Category E procedures. Identify each Category E procedure included in this ACORP and justify scientifically why the pain or distress cannot be relieved.

►

## Veterinary Care and Husbandry

L. **Veterinary Support**.

1. Identify the laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

Name► [redacted] D.V.M., Consulting Veterinarian
Institutional affiliation► LSCDVAMC
email contact► [redacted]@va.gov, [redacted]@gmail.com

Name► [redacted], D.V.M
Institutional affiliation► [redacted]

email contact▶[redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted▶
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ▶[redacted]11.3.2019

M. **Husbandry.** As a reference <u>for the animal husbandry staff</u>, summarize here the husbandry requirements of the animals on this protocol. (Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals. Use Appendix 9 to document any aspects of the husbandry that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

1. Caging needs. Complete the table below to describe the housing that will have to be accommodated by the housing sites for this protocol:

| a. Species | b. Type of housing* | c. Number of individuals per housing unit** | d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***) | e. Estimated maximum number of housing units needed at any one time |
|---|---|---|---|---|
| cat | Group housed | 8/room | Yes | 1 room |
| cat | Singly housed in 4x6x3ft cage for up to 14 days post device insertion | 1 | yes | 1 |

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:
▶
** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a justification if any animals will be housed singly (if species is not considered "social", then so note)
▶ Cats are generally considered to be solitary animals but are group housed when compatible. After implant surgery, the cat will be cage rested for first two weeks per standard veterinary laparotomy post op care. This will prevent jumping and injury to the surgical site.
***Use Appendix 9 to document "departures" from the standards in the *Guide*.

2. Enrichment. Complete the table below to indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required (See ACORP Instructions, for more information on enrichment requirements. Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards in the *Guide*.):

| a. Species | b. Description of Enrichment* | c. Frequency |
|---|---|---|
| cat | Standard (see SOP) | daily |

*If enrichment will be provided according to a local SOP, enter "standard (see SOP)" and enter the SOP into the table in Item Y. If the local standard enrichment is not described in a SOP, enter "standard, see below", and describe the standard species-specific enrichment here.
►

3. Customized routine husbandry. Check all of the statements below that apply to the animals on this protocol, and provide instructions to the animal husbandry staff with regard to any customized routine husbandry needed.

► ( ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals,and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.
►

► ( ) Devices that extend chronically through the skin WILL be implanted into some or all animals on this protocol. Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.
►

► ( ) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.
►

► ( ) This ACORP does NOT include use of any animals that will require customized routine husbandry.

N. **Housing Sites**. Document in the tables below each location where animals on this protocol may be housed.

► (X) Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU.

| Building | Room number | Inside of VMU? | |
|---|---|---|---|
| | | Yes | No |
| LSCDVAMC ARF | Cat room TBD | (X) | ( ) |

► ( ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table.

| Name of Non-VA Facility | Is this facility accredited by AAALAC? | | Building | Room Number |
|---|---|---|---|---|
| | Yes -- enter status* | No** | | |
| | ( ) | ( )** | | |

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

## Special Features

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".

► (X) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

► (**X**) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".

► ( ) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Q. **Locations of procedures.** Complete the table below, listing the location(s), inside or outside of the animal facility, for each of the procedures to be performed on animals on this protocol.

| Procedure | Surgical? | | Bldg/Room Number | Requires transport through non-research areas? | |
|---|---|---|---|---|---|
| | Yes | No | | Yes – describe method of discreet transport | No |
| Surgical Prep | ( ) | (X) | [redacted] | (X) Animals will be placed in travel cages with a blanket over them and transported to a car using a cart. They will then be transported to the [redacted], where the cage will be placed under another blanket and taken into [redacted] on a cart. Personal vehicle transport will conform to VA IACUC approved SOP. Two employees from Dr. [redacted]s lab and at least one member of the ARF will be approved for transport in their private vehicles prior to the first transport. | ( ) |
| UroMOCA insertion | (X) | ( ) | [redacted] | (X) Same as above | ( ) |

| | | | | | |
|---|---|---|---|---|---|
| Electrode implantation | (X) | ( ) | [redacted] | (X) Same as above | **( )** |
| Urodynamics and CT imaging | ( ) | (X) | [redacted] | (X) Same as above | **( )** |
| Ambulatory Testing | ( ) | (X) | LSCDVAMC ARF | (X) Testing will be done in ARF housing room. | **(X)** |
| Euthanasia | (X) | ( ) | [redacted] | (X) Animals will be placed in travel cages with a blanket over them and transported to a car using a cart. They will then be transported to the [redacted] where the cage will be placed under another blanket and taken into [redacted] on a cart. Personal vehicle transport will conform to VA IACUC approved SOP. Two employees from Dr. [redacted]s lab and at least one member of the ARF will be approved for transport in their private vehicles prior to the first transport. | ( ) |

R. **Body Fluid, Tissue, and Device Collection.** List each body fluid, tissue, or device to be collected, and complete the table below to indicate the nature of the collection. Check the relevant Appendices in Item Y, below, and complete and attach them, as shown in the column headings.

| Body Fluid, Tissue, or Device to be Collected | Collected AFTER Euthanasia | Collected BEFORE Euthanasia | | |
|---|---|---|---|---|
| | | Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production") | Collected as Part of a Surgical Procedure (Appendix 5, "Surgery") | Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection") |
| Urethra, bladder and surrounding tissue | (X) | ( ) | ( ) | ( ) |
| Blood | (X) | ( ) | ( ) | ( ) |
| Wireless device - UroMOCA | (X) | ( ) | ( ) | ( ) |
| Urine | ( ) | ( ) | ( ) | (X) |

S. **Surgery.** Does this protocol include any surgical procedure(s)?

► (X) Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".

► ( ) NO animals on this protocol will undergo surgery.

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►Signs of wound or urinary tract infections will be discussed with the veterinary staff. It will be investigated by urine culture and treated by systemic antibiotics. If wound infection and urinary tract infection persist and the animal is non−responsive to treatment, is debilitated and fails to recover, the experiments will be terminated and the animal will be euthanized. Signs of wound infection will be discussed with the veterinarian. If a wound infection persists and the animal is non−responsive to treatment, is debilitated, and fails to recover, the experiments will be terminated and the animal will be euthanized. In addition, weight loss greater than 15% or intractable pain or distress determined by behavioral responses, including vocalization, ears drawn back, or withdrawal, will be determined as cause for euthanasia. In cases of severe and untreatable sores and self-mutilation, animals will be euthanized

U. **Termination or removal from the protocol**. Complete each of the following that applies:

► ( ) Some or all animals will NOT be euthanatized on this protocol. Describe the disposition of these animals. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these methods of disposition. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►

► (X) Some or all animals MAY be euthanatized as part of the planned studies. Complete the table below to describe the exact method(s) of euthanasia to be used. (Use Appendix 9 to document any departures from the standards in the *Guide* represented by these methods. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

| Check each method that may be used on this protocol | Method of Euthanasia | Species | AVMA Classification | | |
|---|---|---|---|---|---|
| | | | Acceptable | Conditionally Acceptable | Unacceptable |
| ( ) | $CO_2$ from a compressed gas tank<br>Duration of exposure after apparent clinical death►<br>Method for verifying death►<br>Secondary physical method► | | ( ) | ( ) | ( ) |

| | | | | | |
|---|---|---|---|---|---|
| (X) | Anesthetic overdose<br>Agent► Pentobarbital or pentobarbital combination<br>Dose► 100mg/kg<br>Route of administration► IV<br>Death will be confirmed as cessation of vital signs, including heart rate (via ECG), breathing (end-tidal $CO_2$), zero blood pressure, and zero pulse oxygenation. The animal will be sedated under anesthesia at the time of IV pentobarbital injection | | (X) | ( ) | ( ) |
| ( ) | Decapitation under anesthesia<br>Agent►<br>Dose►<br>Route of administration► | | ( ) | ( ) | ( ) |
| ( ) | Exsanguination under anesthesia<br>Agent►<br>Dose►<br>Route of administration► | | ( ) | ( ) | ( ) |

1. For each of the methods above that is designated as "Conditionally Acceptable" by the AVMA, describe how the conditions for acceptability will be met:
   ►

2. For each of the methods above that is designated as "Unacceptable" by the AVMA, give the scientific reason(s) that justify this deviation from the AVMA Guidelines:
   ►

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.

   ► The BRU/AFIC staff will perform euthanasia. They have years of experience performing euthanasia in cats using the methods listed in this protocol.

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
      ► Investigative staff should be contacted immediately. The animal should be refrigerated for later collection of implanted device by investigative staff.

   b. Describe how the PI's staff should be contacted.
      ► (X) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
      Name► [redacted]
      Contact Information► [redacted]
      Name► [redacted]

Contact Information►[redacted]

► ( ) There is no need to contact the PI's staff immediately. Describe the routine notification procedures that will be followed. If the routine notification procedures are described in a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
►

V. **Special Procedures.** List each special procedure (including special husbandry and other special procedures) that is a part of this protocol, and specify where the details of the procedure are documented. See ACORP Instructions, for examples.

| Name of Procedure | Identify Where the Details of the Procedure are Documented | | |
|---|---|---|---|
| | SOP (title or ID number)* | Other Items in this ACORP -- specify the Item letter(s) | Appendix 6 |
| Urodynamics and CT fluoroscopy | | Items: | (X)** |
| Ambulatory Testing UroMOCA only | | Items: | (X)** |
| Ambulatory Testing UroMOCA and electrodes | | Items: | (X)** |

*If any special procedure is detailed in a SOP, identify the SOP and enter the information requested about the SOP in the table in Item Y.

**If any special procedure is detailed in Appendix 6, check "Appendix 6" in Item Y, below, and complete and attach Appendix 6.

(Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

W. **Consideration of Alternatives and Prevention of Unnecessary Duplication.** These are important to minimizing the harm/benefit to be derived from the work.

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ►surgery, laparotomy, cystotomy, electrode implantation, laminectomy

   Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.

Literature search was done with help of CCF Librarian with access to eSirius.

| Name of the database | Date of search | Period of years covered by the search | Potentially painful or distressing procedures addressed | Key words and/or search strategy used | Indicate which mandate each search addressed | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Replacement of animals (item W.2) | Reduction in numbers of animals used (item W.3) | Refinement to minimize pain or distress (item W.4) | Lack of unnecessary duplication (item W.5) |
| Medline | 10-22-19 | 1946 to date | Cystotomy, contrast media, implant, distress, pain | Urinary bladder, pressure, urodynamics, biomechanic, wireless, catheter free,implants | (X) | (X) | (X) | ( ) |
| Medline | 11-21-19 | 1946 to date | | Urinary bladder, pressure, urodynamics, biomechanic, wireless, catheter free,implants, cats, surgery, cystotomy, | ( ) | ( ) | ( ) | (X) |
| Agricola | 10-22-19 | 1946-date | Cystotomy, contrast media, implant, distress, pain | Urinary bladder, pressure, urodynamics, biomechanic, wireless, catheter free,implants | (X) | (X) | (X) | ( ) |
| Medline | 11-21-19 | 1946 to date | | Urinary bladder, pressure, urodynamics, biomechanic, wireless, catheter free,implants, cats, surgery, cystotomy | ( ) | ( ) | ( ) | (X) |
| Pubmed | 11-21-19 | | electrode implant | Bladder function, spinal cord injury, nerve stimulation, computational model, cat, surgery, laparotomy, cystotomy, electrode implantation, laminectomy | (X) | (X) | (X) | (X) |
| ALTBIB | 11-21-19 | | electrode | Bladder function, | (X) | (X) | (X) | (X) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | implant, biweekly testing | nerve stimulation, computational model, cat, surgery, laparotomy, cystotomy, electrode implantation, laminectomy | | | | |

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.
► Cats are the most suitable animal model for this study because they are an appropriate and common animal model for experiments to understand the control of LUT function by the peripheral nervous system. They are the smallest animal that controls its bladder in a similar way as humans and the smallest animal that will still provide viable test results for a device that will eventually be scaled up for human use.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.
►We plan to use 9 animals. The number of animals proposed is the minimum number needed to get meaningful results from the experiments. An explanation for each experimental group was performed and documented in Item C.2.b.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
►Less painful alternatives were not found. Non-painful euthanasia will be done at the end of the experiment. One article was on the impact of surgical timing and intervention. We will be using a surgeon that has performed many human and animal surgeries and is very efficient to so our surgeries are as fast as possible to minimize the surgical time. The protocol written by our previous surgeon has been optimized to utilize the safest and most common surgical anesthesia and procedures.
General anesthesia, local anesthesia and perioperative analgesia will be used to limit pain.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
►There was no duplication of the study. One of the hits was an article on work from Dr. Damaser's group. May of the articles were different pressure measurements in humans, but not with any device that has similar capabilities as ours. There were two devices that can measure pressure that there was a reference to, but there is not device out there that can measure volume as ours can. We routinely conduct literature searches, speak with colleagues at national meetings, and obtain peer-review opinions through grant and manuscript submission, which have not identified previous work that would be duplicated.

X. **Other Regulatory Considerations.**

1. **Controlled drugs.**

a. Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions, for explanations about the information requested.

| Controlled substances | Storage | | Personnel Authorized to Access | Location for Use | | Procurement | |
|---|---|---|---|---|---|---|---|
| | Double-locked | Not Double-locked* | | VA Property | Not on VA Property | VA Pharmacy | Non-VA |
| pentobarbital sodium and phenytoin sodium | (X) | ( )* | [redacted] Personnel | ( ) | (X) | ( ) | (X) |
| propofol | (X) | ( )* | [redacted] Personnel | ( ) | (X) | ( ) | (X) |
| buprenorphine | (X) | ( )* | VA ARF staff, [redacted] The controlled substances will be stored in the Pyxis drug dispensing machine | (X) | ( ) | (X) | ( ) |
| Ketamine | (X) | ( )* | [redacted] Personnel | ( ) | (X) | ( ) | (X) |
| midazolam | (X) | ( )* | [redacted] Personnel | ( ) | (X) | ( ) | (X) |
| butorphanol | (X) | ( )* | [redacted] Personnel | ( ) | (X) | ( ) | (X) |

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary.
▶

b. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

▶ (**X**) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy. (Buprenorphine)

▶ (**X**) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.

▶ ( ) Other. Explain▶

2. **Human patient care equipment or procedural areas**. Does this protocol involve use of any human patient care equipment or procedural areas?

► ( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".

► (X) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

3. **Explosive agents**. Does this protocol involve use of any explosive agent?

► ( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".

► (X) No explosive agent(s) will be used as part of this protocol.

Y. **Summary of Attachments.** To assist the reviewers, summarize here which of the following apply to this ACORP.

**Appendices.** Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

► ( ) Appendix 1, "Additional Local Information"
► ( ) Appendix 2, "Antibody Production"
► (X) Appendix 3, "Biosafety"
► (X) Appendix 4, "Ante-mortem Specimen Collection"
► (X) Appendix 5, "Surgery"
► (X) Appendix 6, "Special Husbandry and Procedures"
► ( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
► ( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
► ( ) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"

**Standard Operating Procedures (SOPs).** List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

| Item | SOP | | Approval Date |
|---|---|---|---|
| | Title | ID | |
| C.2.c | N/A | | |
| M.1 | Cat Husbandry | 13 | 3/16/2019 |
| M.2 | Cat Enrichment | 14 | 3/16/2019 |
| U.4.a | N/A | | |
| U.4.b | N/A | | |
| V | Photography Policy | | 9/20/2018 |

Z. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol. Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

   a. **Certification by Principal Investigator(s):**

   I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

   I understand that further IACUC approval must be secured before any of the following may be implemented:

   - Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
   - Changing any procedure in any way that has the potential to increase the pain/distress category to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
   - Performing any additional procedures not already described in this ACORP;
   - Use of any of these animals on other protocols, or by other investigators.

   I further certify that:

   - No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC. When new or additional personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
   - I will provide my after-hours contact information to the animal care staff for use in case of emergency.

| Name(s) of Principal Investigator(s) | Signature | Date |
|---|---|---|
| [redacted] | [redacted] | 12/20/19 |
| [redacted] | | 1/9/202. |

   b. **Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:

► ( X ) No minority opinions were submitted by any IACUC participant for inclusion.

► ( ) Minority opinions submitted by IACUC participants are copied here
►

► ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages► )

| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
|---|---|---|
| [redacted] | [redacted] | 12/16/19 |
| Name of IACUC Vive-Chair | Signatu[redacted] | Date |
| [redacted] | [redacted] | 12/18/19 |

2. **Appendix 2. Antibody Production.** No signatures required.

3. **Appendix 3. Biosafety.**

   a. **Certification by PI(s) and IACUC Officials:**

   We certify that:

   - Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;

   - All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|

| | | |
|---|---|---|
| | | |
| | | |
| Name of Institutional Veterinarian | Signature | Date |
| [redacted] | [redacted] | 12/16/19 |
| Name of IACUC Vice-Chair | Signature | Date |
| [redacted] | [redacted] | 12/16/19 |

b. **Certification by Biosafety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "toxic", "infectious", "biological", or "contains recombinant nucleic acid";
- The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;
- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

| Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee | Signature | Date |
|---|---|---|
| [redacted] | [redacted] | 12/17/19 |

c. **Certification by Radiation Safety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "radioactive";
- The use of each radioactive agent is further documented as required in Items 7 and 10.a of Appendix 3;
- The use of each radioactive agent has been approved by the appropriate committee(s), as shown in Item 10.a of Appendix 3.

| Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee | Signature | Date |
|---|---|---|
| | | |

4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.

5. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:

   - To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
   - The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
   - The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
   - The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
   - Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:

     - Identification of each animal such that care for individual animals can be documented.
     - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;
     - Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
     - Daily records covering at least the period defined as "post-operative" by local policy.
     - The signature or initials of the person making each entry.

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| [redacted] | [redacted] | 12/20/19 |
| [redacted] | [redacted] | 1/9/2020 |

6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.

7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies.**

a. **Certification by the Principal Investigator(s).** I certify that, to the best of my knowledge, the information provided in Appendix 7 of this ACORP is complete and accurate, and the use of patient care equipment or areas for these animal studies will be as described.

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| | | |
| | | |

b. **Certification by the officials responsible for the use of any human patient care equipment in animal procedural areas.** Each of the following must sign to indicate that they have granted approval for the human patient care equipment to be moved to the VMU or other animal procedural area to be used on animals and then returned to the human patient care area, as described in Appendix 7. Leave this section blank, if not applicable.

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |
| Name of the Manager of the Human Patient Care Equipment | Signature | Date |
| | | |

c. **Certification by the officials responsible for the use of the equipment in human patient care areas for these animal studies.** Each of the following must sign to indicate that they have granted approval for animals to be transported into human patient care areas for study or treatment, as described in Appendix 7. Leave this section blank, if not applicable.

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |
| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
| | | |
| Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment | Signature | Date |

| | | |
|---|---|---|
| Name of ACOS for R&D | Signature | Date |
| | | |
| Name of Chief of Staff | Signature | Date |
| | | |
| Name of Director or CEO of the Facility (Hospital or Clinic) | Signature | Date |
| | | |

8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals.**

   a. **Certification by the Principal Investigator(s).**

   I certify that, to the best of my knowledge, the information provided in Appendix 8 of this Animal Component of Research Protocol (ACORP) is complete and accurate, and the use of explosive agents in these animal studies will be as described.

   I further certify that:

   - Procedures involving explosive agent(s) will be performed within a properly operating, ventilated safety hood;
   - All electrical equipment operating when explosive agent(s) are in use will be positioned and powered outside of the hood;
   - Once the seal is broken on any containers of explosive agents, they will be kept in a safety hood throughout use, stored in an explosion-proof refrigerator or other approved storage area, and discarded properly once completely emptied;
   - Proper procedures will be used for safe and appropriate disposal of items (including animal carcasses) that may contain residual traces of the explosive agent(s).

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| | | |
| | | |

b. **Certification by the officials responsible for overseeing the use of explosive agent(s) in this protocol.** Each of the following must sign to verify that they or the committee they represent have granted approval.

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |
| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
| | | |
| Name of Safety/Biosafety Officer for the Facility | Signature | Date |
| | | |
| Name of ACOS for R&D | Signature | Date |
| | | |
| Name of VISN Regional Safety Officer | Signature | Date |
| | | |

9. **Departures from "Must" and "Should" Standards in the *Guide*.** No signatures required.

**ACORP APPENDIX 3**
**BIOSAFETY**
**VERSION 4**

See ACORP App. 3 Instructions, for more detailed explanations of the information requested.

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:

| **Material** (Identify the specific agent, device, strain, construct, isotope, etc.) | **Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors) | **Nature of Material** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Toxic Agent (Item 4) | Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4) | Biological Agent (Item 6) | Radioactive Agent (Item 7) | Contains Recombinant Nucleic Acid (Item 8) | Routine Pre- or Post-Procedural Drug | Euthanasia agent |
| Visipaque (320mg/ml) Radio-opaque solution (same solution used clinically for bladder imaging) – Administered at [redacted] | VA Pharmacy | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | ( ) |
| UroMOCA device- Administered at [redacted] | Manufactured in-house and [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | ( ) |
| Sacral Nerve Cuff electrodes | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | ( ) |
| Pelvic Floor EMG electrodes | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | ( ) |
| Stimulator | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | ( ) |
| Bladder catheter to measure pressure – only during surgery | [redacted]or Fisher Scientific | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | ( ) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Bupivicaine- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Buprenorphine- Administered at [redacted] and/or LSCVAMC | VA Pharmacy and/or [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Cefazolin- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Dexmedetomidine- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Enrofloxacin (Baytril) - Administered at [redacted] and/or LSCVAMC | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Isoflurane- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Pentobarbitol + phenytoin/ Euthasol- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | (X) |
| Propofol- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| glycopyrrolate- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Robenacoxib- Administered at [redacted] | [redacted] and/or VA Pharmacy | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Betadine- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Atipamezole- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Ketamine- Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Convenia (cefovecin sodium)- Administered at [redacted] and/or LSCVAMC | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Torbugesic (butorphanol tartrate) Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Famotidine Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Midazolam Administered at [redacted] | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Lactated ringers IV | [redacted] | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| acepromazine | VA Pharmacy | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |

2. **Summary of How Materials will be Administered.** Complete the table below for each of the materials shown in the table in Item 1 above:

| **Material*** (Identify the specific agent, device, strain, construct, isotope, etc.) | **Dose** (e.g., mg/kg, CFU, PFU, number of cells, mCi) and Volume (ml) | **Diluent* or Vehicle*** | **Route** of admin | **Frequency or duration** of admin | **Reason** for Administration and **Expected Effects** | Location of **Further Details** in this ACORP (specify "Main Body" or "App #", and identify the Item) | Administration Under **Anesthesia, sedation, or tranquilization** (Y/N) |
|---|---|---|---|---|---|---|---|
| Visipaque Radio-opaque solution (same solution used clinically for bladder imaging) | 25ml | Sterile saline | Transurethral through catheter | Once per surgery | For CT scan | App 5 | Y |
| UroMOCA device | 1 | N/A | Cystotomy | Once | Measure pressure and volume | Main Body, App5 | Y |
| Sacral Nerve Cuff electrodes | 4 | N/A | 5 cm dorsal midline sacral incision | Once | Needed for electrical stimulation | Main Body, App5 | Y |
| Pelvic Floor EMG electrodes | 2 | N/A | 5 cm dorsal midline sacral incision | Once | Needed to detect muscle changes in response to electrical stimulation | Main Body, App5 | Y |
| Stimulator | 1 | N/A | 5 cm dorsal midline sacral incision | Once | Used for electrical stimulation | Main Body, App5 | Y |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Bladder catheter to measure pressure | 1 | N/A | Cystotomy | Once | Measure pressure | Main Body, App5, Amendment | Y |
| Acepromazine (10 mg/ml) | 0.05mg/kg (0.02ml-0.025ml) | N/A | IM or SQ | As needed | sedative medication for excessive urinary bladder spasms | Main Body, App5 | N |
| Atipamezole (5mg/ml) | 0.2 mg/kg (0.14-0.20ml) | Sterile water | IV/IM | End of procedure | Helps reverse sedation caused by dexmedetomidine | Main Body, App5 | Y |
| Bupivicaine (5mg/ml) | 1.25 Mg/kg (0.88-1.25ml) Total dose will not exceed 2mg/kg per cat | Sterile saline | SQ | Once at surgery and prior to waking the cat up | Analgesics/sedatives | Main Body, App5 | Y |
| Buprenorphine (0.3mg/ml) | 0.01−0.02 Mg/kg (0.12-0.33ml) | Sterile saline | IM;IV; SQ | One dose IM, then one dose IV for terminal surgeries; 2-3 times per day for survival surgeries | Analgesics/sedatives | Main Body, App5 | N |
| Torbugesic (butorphanol tartrate):10mg/ml | 0.1−0.4 mg/Kg (0.035-.2ml) | | IM | Once per surgery | Anesthetic | Main Body, App5 | N |
| Cefazolin (100mg/ml) | 25 Mg/kg (0.88-1.25ml) | Sterile saline | IV | 1 Dose | Anti−infective | Main Body, App5 | N |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Convenia (cefovecin sodium) 80mg/ml stock | 8mg/kg (0.34-0.5ml) | Sterile water | SQ | Once post op, then once every 14 days or per veterinary instruction | Antibiotic | Main Body, App5 | N |
| Dexmedetomidine (100ug/ml) | 0.005mg/kg (0.18-0.25ml) | | IM;SQ | Once | Analgesics/sedatives | Main Body, App5 | N |
| Dexmeditomidine/ ketamine/butorphanol (Torbugesic) Mix* | Dexmeditomidine: 11.1ug/kg (0.1ml) Ketamine: 2.2mg/kg(0.2ml) butorphanol: 0.22mg/kg mixed with dexdom(0.1ml) | | IM | Once | Analgesics/sedatives | Main Body, App5 | N |
| Midazolam/ Torbugesic (butorphanol tartrate) Mix** | Midazolam: 0.1-0.2mg/kg(5mg/ml Butorphanol: 0.22mg/kg mixed with dexdom(0.1ml) | | IM | Once | Analgesics/sedatives | Main Body, App5 | N |
| Enrofloxacin (Baytril) (22.7 mg/ml) | 5mg/kg (0.77-1.10ml) | Sterile saline | SQ | 1 Dose | Anti-infective | Main Body, App5 | N |
| Famotidine (10 mg/ml) | 0.2 mg/kg | | IM or SQ | Once per surgery if needed | decrease nausea/inappetence | Main Body, App5 | Y |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Glycopyrrolate (0.2mg/ml) | 0.01 mg/kg IM (0.18-0.25ml) or 0.005mg/kg IV (0 .09-0.13ml) | Sterile water | IM or IV | If needed | To reverse dexmedetomidine in case of bradycardia (slowing of heart rate) | Main Body, App5 | Y |
| Isoflurane | To Effect 1−3 % | Vaporized | Inhalation | Once Per Surgery | Anesthetic | Main Body, App5 | N |
| Ketamine (100mg/ml) | 30mg/kg (1.0-1.5ml) | Sterile water | IM | Once | Anesthetic | Main Body, App5 | N |
| Midazolam (5mg/ml) | 0.1−0.2 mg/kg (.07ml-0.14ml) | | IM | Once per surgery | Anesthetic | Main Body, App5 | N |
| Pentobarbitol + phenytoin/ Euthasol (390mg/ml and 10 mg/ml) | 100mg/kg (0.8-1.25ml) | Sterile saline | IV | Once | Anesthetic/Euthanasia agent | Main Body, App5 | Y |
| Propofol (Bolus) (10 mg/ml) | 1-6 Mg/kg (0.35-3.00ml) or to effect | Sterile saline | IV | 1 Dose | Anesthetic to assist in intubation | Main Body, App5 | Y |
| Propofol (CRI) (10 mg/ml) | 0.3 Mg/kg/min (0.1ml/min) | Sterile saline | IV | During Urodynamics | Anesthetic | Main Body, App5 | Y |
| Robenacoxib (20mg/ml) | 2mg/kg (0.35-0.50ml) | Sterile water | SQ | Once/day ; Given at surgery, and every 24 hours up to 3 more doses | Minimize inflammation (NSAID) | Main Body, App5 | Y |
| Betadine (10% povidone-iodine) | | | | Before incision | Antiseptic | Main Body, App5 | Y |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Lactated ringers IV | 3ml/kg/hr | | IV | Continuous | Hydration during surgery | Main Body, App5 | Y |

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade. Check on-line for formulations that are FDA approved for administration to humans (http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm) or animals (http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847). Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, explain here why the use of a non-pharmaceutical grade formulation is necessary, and describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)
▶

3. **Anesthesia, Sedation, or Tranquilization.** Complete 3.a. and 3.b. below:

a. For each material with "Y" entered in the last column of the table in Item 2 above, describe the anesthesia, sedation, or tranquilization to be used, identifying the anesthetic, sedative, or chemical tranquilizer, and detailing the dose, volume, and route of administration (Make sure that these agents are also included in Item 1 of this appendix, as materials to be administered):

▶ Dexmedetomidine (0.005mg/kg) will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of 0.1−0.2 mg/kg Midazolam (5mg/ml) and 0.1−0.4mg/Kg Torbugesic (butorphanol tartrate) IM may be used.

Anesthesia will be maintained with isoflurane by inhalation to effect or propofol IV CRI.

b. For each material with "N" entered in the last column of the table in Item 2 above, explain why no anesthesia, sedation, or tranquilization is necessary, or can be provided, and describe any alternate methods of restraint that will be used.

▶The delivery of these materials does not present additional pain or distress and are used to provide antibiotic or analgesic treatment.

4. **Toxic Agents.** Complete the table below for each of the materials listed as a "toxic agent" in the table in Item 1 above, checking the all of the properties that apply (see ACORP App. 3 Instructions, for details).

| Name of Toxic Agent | a. Mutagen | b. Carcinogen | c. Teratogen | d. Select Agent? | | | e. Other – specify toxic properties |
|---|---|---|---|---|---|---|---|
| | | | | Not a Select Agent | Select Agent Used in Sub-threshold Quantities | Select Agent that Requires Registration/Approval | |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |

*For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ▶

Registered with CDC or USDA ▶
Registration Number ▶
Registration Date ▶
Expiration Date of Registration ▶

Name of official who granted approval on behalf of VACO▶
Date of approval▶

5. **Infectious Agents.** Complete the table below for each of the materials listed as an "infectious agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

| Name and BSL Number of Infectious Agent | a. ABSL Number * | b. Drug Sensitivity Panel Available? (Describe) | c. Select Agent? | | |
|---|---|---|---|---|---|
| | | | Not a Select Agent | Select Agent used in Sub-threshold quantities | Select Agent that Requires Registration/Approval |
| | | (Yes/No) | ( ) | ( ) | ( )** |

*Complete the following for each agent for which the ABSL Number given is less than the BSL Number shown (copy the lines below for each agent):

Name of agent ▶
Justification for applying ABSL measures that are less protective than those recommended ▶

**For each "select agent" that requires registration/approval (copy the lines below for each agent):

Name of agent ▶

Registered with CDC or USDA ▶
Registration Number ▶
Registration Date ▶
Expiration Date of Registration ▶

Name of official who granted approval on behalf of VACO▶
Date of approval▶

6. **Biological Agents.** Complete the table below for each of the materials listed as a "biological agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

| Name of Biological Agent | Screening for Infectious Agents |
|---|---|
| | |

7. **Radioactive Agents.** Complete the table below for each of the agents listed as a "radioactive agent" in the table in Item 1 above (see ACORP App. 3 Instructions, for details).

| Name of Radioactive Agent (specify the isotope) | Authorized Individual | Approving Committee or Official |
|---|---|---|
| | | |

8. **Agents Containing Recombinant Nucleic Acid.** For each of the materials checked in the table in Item 1, above, as "contains recombinant nucleic acid", indicate which of the conditions applies (see ACORP App. 3 Instructions, for details).

| Name of Agent that Contains Recombinant Nucleic Acid | Subject to the *NIH Guidelines for Research Involving Recombinant DNA Molecules* | Exempt |
|---|---|---|
| | ( ) | ( ) |

9. **Potential for Pain or Distress**. Complete the table below for each of the agents listed in Item 1, above,

that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3 Instructions, for details).

| Name of Agent | Nature of Potential Pain/Distress | Measures to Alleviate Pain/Distress |
|---|---|---|
| UroMOCA Device | Surgical dissection | Intraoperative anesthesia/analgesia, post-operative analgesia. |
| Sacral Nerve Cuff electrodes | Surgical dissection, irritation to nerve | Intraoperative anesthesia/analgesia, post-operative analgesia. |
| Pelvic Floor EMG electrodes | Surgical dissection | Intraoperative anesthesia/analgesia, post-operative analgesia. |
| Stimulator | Surgical dissection | Intraoperative anesthesia/analgesia, post-operative analgesia. |

10. **Protection of Animal Facility Staff from Hazardous Materials.** Complete Items 10.a and 10.b, below, for each of the agents listed in the table in Item 1, above, as "toxic", "infectious", "biological", "radioactive", or "contains recombinant nucleic acid" (detailed in Items 4 – 8). This item specifically addresses members of the animal facility staff; protection of the research staff from each of these agents must be addressed in Item G of the main body of the ACORP. See ACORP App.3 Instructions, for details.

    a. Complete the table below.

| Name of Hazardous Agent | Approving Committee or Official | Institution (VA or affiliate) | Names of Animal Facility Staff Members at Risk |
|---|---|---|---|
| | | | |

    b. Detail how the individuals listed in the table above (Item 10.a.) have been (or will be) informed of the possible risks of exposure, and have been (or will be) trained to avoid exposure to these agents.

    ▶

11. **Signatures.** Provide the applicable signatures on the signature pages (Item Z.3) of the main body of this ACORP

## ACORP Appendix 4
### ANTEMORTEM SPECIMEN COLLECTION
### VERSION 4

See ACORP App. 4 Instructions, for more detailed explanations of the information requested.

1. **Summary.** Complete the table below for each specimen to be collected from a live animal on this protocol (see ACORP App. 4 Instructions, for details).

| Specimen Collected | Site and Method of Collection | Anesthesia (Yes/No) | Amount Collected Each Time | Volume Replacement (Yes/No/NA) | Total Number of Collections per Animal | Time Intervals Between Successive Collections |
|---|---|---|---|---|---|---|
| Urine | Through Catheter | Yes | 1-40 ml | No | 3 | 2 weeks |

2. **Use of Anesthetics, Tranquilizers, or Analgesics.**

   a. For each specimen described in Item 1, above, as being collected WITHOUT anesthesia, complete Items 2.a(1) and 2.a(2), below:

      (1) Explain why no measures will be taken to prevent pain (e.g., because of scientific requirements described here, or because the collection method involves no more than minor or momentary pain).
      ▶

      (2) Completely describe any method of physical restraint that may be used.
      ▶

   b. For each specimen described in Item 1, above, as being collected WITH anesthesia, complete the following table:

| Anesthetic, tranquilizer, or analgesic agent | Dose (mg/kg) and volume (ml) | Route of administration | Frequency of administration |
|---|---|---|---|
| Isoflurane | 1-3% with oxygen | Inhalation | Continuous |

3. **Volume Replacement for Fluid Collections.**

   a. For each fluid specimen described in Item 1, above, for which NO volume replacement will be provided, explain why not.
   ▶Urine does not need to be replaced into the bladder. For urodynamics we need to start with an empty bladder.

   b. For each fluid specimen described in Item 1, above, for which volume replacement WILL be provided, describe the replacement fluids that will be administered (including their composition, volume, and route of administration).
   ▶

4. **Monitoring the animals.** Detail how the animals will be monitored after collection of specimens to ensure that they recover appropriately (see ACORP App. 4 Instructions, for details).
►Removal of urine does not require any special monitoring.

**ACORP Appendix 5**
**SURGERY**
**VERSION 4**

See ACORP App. 5 Instructions, for more detailed explanations of the information requested.

1. **Surgery Classification.** Complete the table below for each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). See ACORP App. 5 Instructions, for details.

| Surgery | | Terminal | Survival | | |
|---|---|---|---|---|---|
| # | Description (specify the species, if ACORP covers more than one) | | Minor | Major | One of Multiple* |
| 1 | UroMOCA implant (cystotomy)-chronic | ( ) | ( ) | (X) | ( )* |
| 2 | UroMOCA, electrodes and stimulator implantation - chronic | ( ) | ( ) | (X) | ( )* |
| 3 | Device Extraction | (X) | ( ) | ( ) | ( )* |

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:

►

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):

►

2. **Description of Surgeries.** Describe each surgery listed in Item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

**Surgery 1** ► *For all drug dose information, see Biosafety Appendix 3, table 2*

Surgical Preparation: At least twelve hours prior to surgery, animals will begin fasting.

The morning of the surgery, the cats will be transported to the [redacted]n approved private vehicles, according to the LSCVAMC ARF SOP. Buprenorphine will be given pre-operatively and then at least 4 additional doses will be administrated (twice a day), and more if needed.

Robenacoxib will also be administered the morning of surgery for multimodal analgesia and at least 2 days post-op.

10–15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam and Torbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15 minutes the animal remains bradycardic, a dose of glycopyrrolate will be given. Cefazolin) and Baytril will be administered for pre-operative antibiotic prophylaxis. A dose of Famotidine will be given during anesthesia to decrease nausea/inappetence.

The front limbs will be shaven for placement of IV catheter. An intravenous cephalic vein catheter will be placed for IV access during pre-operative preparation.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

The hind legs and the pelvic region, including the base of the tail and the lower abdomen will be shaven for cystotomy. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, end-tidal CO2, respiratory rate, non-invasive blood pressure, and temperature. Using these vital signs in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels. Intraoperative monitoring will be recorded at regular intervals (e.g., every 15 minutes). This record will become a part of the animal's medical record. Body temperature (38.5 degree Celsius) will be maintained using a hot-water circulated heating pad and drapes. Once physiological parameters have been stabilized a sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures. All tools will be steam sterilized and the UroMOCA will be gas (ethylene oxide) sterilized prior to insertion. Aseptic procedures by trained personnel will be utilized during the surgery. Aseptic techniques will be used during surgery. The surgeon and assistant will scrub hands, wear clean scrubs, shoe covers, mask, bonnet, sterile gown and sterile gloves. Observers/anesthetist will wear clean scrubs, shoe covers, bonnet and mask. All participants will avoid touching non-sterile surfaces. Any instrument that touches outside the surgical field will be replaced and the hands of the surgeon and/or assistant will be re-gloved.

Bupivacaine (1mg/kg) will be administered at the incision site before and after incision. With the animal in supine position, a midline suprapubic incision will be made to expose the bladder. The dome of the bladder will be incised and a UroMOCA will be placed into the bladder through this small, approximately 1 cm incision. The bladder incision will then be sutured closed with 4-0 synthetic, monofilament, absorbable suture; tapered needle swaged to suture and closed using Lembert suture pattern (continuous stich). Bladder will be filled with saline to check for leaks. The abdominal wall will also be sutured closed with the 4-0 absorbable suture, using a simple continuous pattern with gentle tissue handling. Simple interrupted is recommended. The skin will then be closed using a continuous subcuticular pattern and then a loosely placed simple interrupted or simple cruciate pattern with 4-0 prolene,(non-absorbable, monofilament suture with a cutting needle). The skin sutures will be removed 10-14 days after the operation. A 3-D CT X-ray image will be taken to demonstrate placement of the

UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder, and lack of leakage through the bladder wall by infusion of contrast solution into the bladder.

Proper anesthesia will be maintained by Monitoring the vital signs ECG, heart rate, respiratory rate, expired CO2, SPO2, noninvasive blood pressure or arterial blood pressure, and temperature in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels.

After UroMOCA insertion, confirmatory urodynamics and 3−D CT imaging, all catheters will be removed and the animal will be awoken. When the cats have become ambulatory, they will be transported back to the VA where the post-operative care will be provided until the next procedure. The cat will be observed after awakening and daily afterward for bladder spasms by study staff or animal facility staff, which indicate bladder pain or irritation, and other changes in animal behavior denoting bladder health and function.

Buprenorphine will be administered twice daily every (8-12 hours) as needed starting the first post-op day, to reduce post−operative pain, with additional doses if deemed necessary according to daily observations by veterinary/investigative staff. If additional pain management is needed after the surgery, a NSAID, Robenacoxib will be administrated, once a day (every 24 hours) for up to 3 days total. Acepromazine may be given as needed for excessive urinary bladder spasms. Doses will be given if deemed necessary according to daily observations by veterinary/investigator staff. The antibiotic Convenia will be given once after initial surgery, and additional with veterinary approval.

**Surgery 2▶** *For all drug dose information, see Biosafety Appendix 3, table 2*

Surgical Preparation: At least twelve hours prior to surgery, animals will begin fasting. Buprenorphine will be given the morning of surgery and then at least 4 additional doses will be administrated (twice a day), and more if needed.

Robenacoxib will also be administered the morning of surgery for multimodal analgesia and at least 2 days post-op.

The morning of the surgery, the cats will be transported to the [redacted] in approved private vehicles, according to the LSCVAMC ARF SOP.

10−15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam and Torbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15 minutes the animal remains bradycardic, a dose of glycopyrrolate will be given. Cefazolin and Baytril will be administered for pre−operative antibiotic prophylaxis. A dose of Famotidine will be given during anesthesia to decrease nausea/inappetence.

The front limbs will be shaven for placement of IV catheter. An intravenous cephalic vein catheter will be placed for IV access during pre-operative preparation.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

The hind legs and the pelvic region, including the base of the tail and the lower abdomen will be shaven for cystotomy and the dorsal back will be shaved for the laminectomy. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, end−tidal CO2, respiratory rate, non−invasive blood pressure, and temperature. Using these vital signs in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels. Intraoperative monitoring will be recorded at regular intervals (e.g., every 15 minutes). This record will become a part of the animal's medical record. Body temperature (38.5 degree Celsius) will be maintained using a hot−water circulated heating pad and drapes. Once physiological parameters have been stabilized a sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures. All tools will be steam sterilized and the UroMOCA and electrodes will be gas (ethylene oxide) sterilized prior to insertion.

Aseptic techniques will be used during surgery. The surgeon and assistant will scrub hands, wear clean scrubs, shoe covers, mask, bonnet, sterile gown and sterile gloves. Observers/anesthetist will wear clean scrubs, shoe covers, bonnet and mask. All participants will avoid touching non-sterile surfaces. Any instrument that touches outside the surgical field will be replaced and the hands of the surgeon and/or assistant will be re-gloved.

Bupivacaine (1mg/kg) will be administered at the incision site before and after incision. An intraurethral catheter will be inserted to maintain an empty bladder. With the animal in supine position, a midline suprapubic incision will be made to expose the bladder and pelvic floor muscles. EMG electrode leads will be inserted into the pelvic floor muscles via laparotomy exposure. EMG electrode leads will then be tunneled under the skin to the dorsal surface of the animal and connected to an implantable stimulator via a dorsal skin incision at lumbosacral region. The dome of the bladder will be incised and a UroMOCA will be placed into the bladder through this small incision, which will then be sutured closed with 4-0 synthtic, monofilament, absorbable suture; tapered needle swaged to suture and closed using Lembert suture pattern (continuous stich). Bladder will be filled with saline to check for leaks. The abdominal wall will also be sutured closed with the 4-0 absorbable suture, using a simple continuous pattern with gentle tissue handling. The skin will then be closed using a continuous subcuticular pattern and then a loosely placed simple interrupted or simple cruciate pattern with 4-0 prolene,(non-absorbable, monofilament suture with a cutting needle).

After closure of the abdomen, the cat will be placed in the prone position. A sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish this second surgical incision site. The animal will then be thoroughly re-draped with sterile drape such that only the sterile incision site is exposed.

With the animal in a prone position, a dorsal midline sacral incision, approximately 5 cm in length, will be made to expose the sacral spine. A laminectomy will be performed to remove the bone of the spine to expose the sacral roots from S1 to S4. 4 nerve cuff electrodes will be implanted, including one each around the sacral roots S1 left side, S1 right side, S2 left side, and S2 right side. We are choosing left and right S1 and S2 sacral roots because urethral sphincter and bladder neurons predominantly run through these roots. Pelvic floor EMG will act as a proxy for urethral sphincter activity because it is significantly more feasible and practical to measure with the current tools available and is consistent with current clinical practice. We will also observe animal functions of bladder voiding versus defecation to distinguish between bowel and bladder emptying activity. All electrodes will be

attached to two wireless implantable stimulation devices (StimPod, [redacted]. No wires or catheters will be exteriorized from the body, reducing the chance of infection and irritation. All stimulation parameters and data collection will be wireless.

The skin will then be closed using with 4-0 prolene, non-absorbable, monofilament suture with a cutting needle, using a discontinuous suture pattern. A 3-D CT X-ray image will be taken to demonstrate placement of the UroMOCA in the bladder, size of the UroMOCA compared to capacity of the bladder, and lack of leakage through the bladder wall by infusion of contrast solution into the bladder.

After UroMOCA insertion, electrode and stimulator implantation, confirmatory urodynamics, and 3-D CT imaging, all catheters will be removed. When the cats have become ambulatory, they will be transported back to the VA where the post-operative care will be provided until the next procedure. The cat will be observed after awakening and daily afterward for bladder spasms, which indicate bladder pain or irritation, and other changes in animal behavior denoting bladder health and function.

Buprenorphine will be administered twice daily every (8-12 hours) as needed starting the first post-op day, to reduce post−operative pain, with additional doses if deemed necessary according to daily observations by veterinary/investigative staff. If additional pain management is needed after the surgery, a NSAID, Robenacoxib will be administrated, once a day (every 24 hours) for up to 3 days total. Acepromazine may be given as needed for excessive urinary bladder spasms. Doses will be given if deemed necessary according to daily observations by veterinary/investigator staff. The antibiotic Convenia will be given once after initial surgery, and additional with veterinary approval.

The cat will be group housed in the cat room but will be isolated in a cage (cage rested) in the room for two weeks after surgery. The cat will be brought out under observation for Interim/Ambulatory Testing during that time. After that time the cat will no longer need the cage and will be able to socialize with the other cats. Stiches will be removed at 10−14 days post-op.

**Surgery 3 ▶**

30-37 days after the initial surgery of the UroMOCA insertion, the cats will have the terminal procedure to remove the device.

At least twelve hours prior to surgery, animals will begin fasting.

The morning of the surgery, the cats will be transported to the [redacted] in approved private vehicles, according to the LSCVAMC ARF SOP.

10−15 minutes prior to placing IV catheter, dexmedetomidine will be administered. Alternately, a mix of 0.1mL dexmedetomidine, 0.1mL Torbugesic (butorphanol tartrate) and 0.2mL Ketamine (per 4.5 kg cat) IM or a mix of Midazolam (5mg/ml) and Torbugesic (butorphanol tartrate) IM may be used. The animal will be given the reversal agent Atipamezole, if the animal becomes bradycardic after the hypertensive phase of dexmedetomidine. If after 15minutes the animal remains bradycardic a dose of glycopyrrolate will be given.

The front limbs will be shaven for placement of IV catheter. An intravenous cephalic vein catheter will be placed for IV access and the animal will be pre−oxygenated.

General anesthesia will be induced by isoflurane. CRI Propofol may be administered IV to effect. If additional anesthetic is needed, a bolus of propofol may be used. The animal will then be intubated to assist in respiration.

All incision sites will be shaved. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO2, CO2, respiratory rate, non−invasive blood pressure, and temperature. Once physiological parameters have been stabilized a sterile scrub of 3−5 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures. Incision sites will be injected with Bupivacaine before incision.

With the animal in supine position, a midline suprapubic incision will be made to expose the bladder. The bladder will be incised and the UroMOCA will be removed (and examined for function in vitro) and the cat will be euthanized with an overdose of pentobarbital combination (Euthasol) 100mg/kg under deep anesthesia. The bladder will be harvested for histology.

3. **Personnel.** Complete the table below for each individual who will be involved in any of the surgeries on this protocol.

| Name | Surgery #(s) (see Item 1) | Role in Surgery | | | |
|---|---|---|---|---|---|
| | | Surgeon | Assistant | Manage Anesthesia | Other (describe) |
| [redacted] | 1,2,3 | ( ) | ( ) | (X) | (X) Animal preparation including anesthesia and pre- and post-op medication |
| [redacted] Staff | 1,2,3 | ( ) | ( ) | (X) | ( ) |
| [redacted] Ph.D. | 1,2,3 | ( ) | (X) | ( ) | ( ) |
| [redacted], PhD | 1,2,3 | (X) | (X) | ( ) | ( ) |
| [redacted], M.D. | 1,2,3 | (X) | (X) | ( ) | ( ) |
| [redacted], M.D. | 1,2,3 | (X) | (X) | ( ) | ( ) |
| [redacted], PhD. | 1,2,3 | ( ) | (X) | ( ) | ( ) |
| [redacted] | 1,2,3 | ( ) | (X) | ( ) | ( ) |
| [redacted] | 1,2,3 | ( ) | (X) | ( ) | ( ) |
| [redacted] | 1,2,3 | ( ) | (X) | ( ) | ( ) |

4. **Location of surgery.** Complete the table below for each location where surgery on this protocol will be performed.

| Building | | | Type of Space |
|---|---|---|---|

| | Room Number | Surgery #(s) (see Item 1) | Dedicated Surgical Facility | Other Dedicated Surgical Space | Other Space not Dedicated to Surgery |
|---|---|---|---|---|---|
| [redacted] Building | [redacted] | 1,2,3 | (X) | ( )* | ( )* |

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
▶

5. **Pre-operative protocol.**

a. **Pre-operative procedures.** Complete the table below for each pre-operative procedure that will be performed to prepare the animal(s) for surgery.

| Surgery #(s) (see Item 1) | Fast (Specify Duration) | Withhold Water (Specify Duration) | Place Intravenous Catheter(s) (Specify Site(s)) | Other – Describe |
|---|---|---|---|---|
| 1 | (X) –12 hrs | ( ) -- | (X) -- cephalic catheter (leg) | ( ) -- |
| 2 | (X) –12 hrs | ( ) -- | (X) -- cephalic catheter (leg) | ( ) -- |
| 3 | (X) –12 hrs | ( ) -- | (X) -- cephalic catheter (leg) | ( ) -- |
| 4 | ( ) -- | ( ) -- | ( ) -- | ( ) -- |

b. **Pre-operative medications.** Complete the table below. Include agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered <u>prior</u> to preparation of the surgical site on the animal.

| Agent | Surgery #(s) (see Item 1) | Dose (mg/kg) & volume (ml) -The volume is calculated based on 3.5-5kg weight | Route of administration | Frequency of administration (e.g., times/day) | Pre-operative period of treatment (e.g., immediate, or # of days) |
|---|---|---|---|---|---|
| Cefazolin (100 mg/ml) | 1,2 | 25 mg/kg (0.88ml-1.25ml) | IV | once | immediate |

| | | | | | |
|---|---|---|---|---|---|
| Glycopyrrolate (0.2mg/ml) | 1,2 | 0.01mg/kg (0.2-0.25mL; SQ/IM) or 0.005mg/kg (0.09-0.1mL; IV) | SQ, IM, IV | If necessary | immediate |
| Enrofloxacin (Baytril; 22.7 mg/ml | 1,2 | 5 mg/kg (0.77-1.10ml) | SQ | once | immediate |
| Propofol – Bolus (10 mg/ml) | 1,2,3 | 1-6mg/kg (0.35-3.00ml) | IV | once | immediate |
| Propofol (CRI) (10 mg/ml) | 1,2,3 | 0.3 Mg/kg/min (0.1ml/min | IV | As needed | immediate |
| Robenacoxib (20 mg/ml) | 1,2,3 | 2 mg/kg (0.35-0.5ml) | SQ | once | 1-2 hours |
| Buprenorphine (0.3 mg/ml) | 1,2,3 | 0.01–0.02 mg/kg (0.12-0.33ml) | SQ | Once/route | Immediate |
| Ketamine (100mg/ml) | 1,2,3 | 30mg/kg (1.0-1.5ml) | IM | Once | 10–15 minutes prior to placing IV catheter |
| Torbugesic (butorphanol tartrate; 10 mg/ml | 1,2,3 | 0.1–0.4mg/Kg (0.035ml-0.2ml) | IM | Once | 10–15 minutes prior to placing IV catheter |
| Midazolam (5 mg/ml) | 1,2,3 | 0.1–0.2 mg/kg (0.07ml-0.2ml) | IM | Once | 10–15 minutes prior to placing IV catheter |
| Atipamezole (5 mg/ml) | 1,2,3 | 0.2mg/kg (0.14ml-0.2mL) | IM | Once | If necessary |
| Famotidine (10 mg/ml) | 1,2,3 | 0.2 mg/kg (0.07ml-0.1mL) | IM;IV;SQ | Once | immediate |
| Dexmedetomidine (100 ug/ml) | 1,2,3 | 0.005mg/kg (0.18-0.25ml) | IM;SQ | once | 10–15 minutes prior to placing IV catheter |
| Dexmeditomidine/ketamine/butorphanol (Torbugesic) Mix* | 1,2,3 | Dexmeditomidine: 11.1ug/kg(0.1ml) Ketamine: 2.2mg/kg(0.2ml) butorphanol: 0.22mg/kg mixed with dexdom(0.1ml) | IM | Once | If necessary |

| | | | | | |
|---|---|---|---|---|---|
| Midazolam/ Torbugesic (butorphanol tartrate) Mix** | 1,2,3 | Midazolam: 0.1-0.2mg/kg(5mg/ml Butorphanol: 0.22mg/kg mixed with dexdom(0.1ml) | IM | Once | If necessary |
| Isoflurane | 1,2,3 | To Effect 1-3 % | Inhalation | Once | Immediate |
| Lactated Ringers | 1,2,3 | 3ml/kg/hr (maintenance rate) | IV | Once | Continuous |

*May be used in place of dexmedetomidine or Midazolam/ Torbugesic (butorphanol tartrate) Mix
** May be used in place of dexmedetomidine or Dexmeditomidine/ketamine/butorphanol (Torbugesic)Mix
Mix

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ▶ The hind legs and the pelvic region, including the base of the tail and the lower abdomen will be shaven for cystotomy. A midline suprapubic incision will be made to expose the bladder. All incision sites will be shaved. A sterile scrub of 3-5 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. Incision sites will be injected with Bupivacaine (1.25 mg/kg, SQ) before incision.

Aseptic procedures by trained personnel will be utilized during the surgery. All tools will be steam sterilized and the UroMOCA will be gas (ethylene oxide) sterilized prior to insertion.

Surgery 2 ▶ The hind legs and the pelvic region, including the base of the tail and the lower abdomen will be shaven for cystotomy and the dorsal back will be shaved for the laminectomy. A midline suprapubic incision will be made to expose the bladder. All incision sites will be shaved. A sterile scrub of 3-5 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. Incision sites will be injected with Bupivacaine (1.25 mg/kg, SQ) before and after incision.

After closure of the abdomen, the cat will be placed in the prone position. A sterile scrub of 3 rotations of Betadine and Isopropyl Alcohol will be given to establish this second surgical incision site. The animal will then be thoroughly re-draped with sterile drape such that only the sterile incision site is exposed.

Aseptic procedures by trained personnel will be utilized during the surgery. All tools will be steam sterilized and the UroMOCA and electrodes will be gas (ethylene oxide) sterilized prior to insertion. Drapes, sterile gloves will be utilized to maintain sterility.

Surgery 3 ▶ A midline suprapubic incision will be made to expose the bladder. All incision sites will be shaved. A sterile scrub of 3−5 rotations of Betadine and Isopropyl Alcohol will be given to establish a sterile incision site. The animal will then be thoroughly draped with sterile drape such that only the sterile incision site is exposed. Incision sites will be injected with Bupivacaine (1.25 mg/kg, SQ) before and after incision. Clean technique will be used for this nonsurvival procedure.

6. **Intra-operative management.**

a. **Intra-operative medications.** Complete the table below for each agent that will be administered to the animal during surgery.

| Agent | Paralytic* | Surgery #(s) (see Item 1) | Dose (mg/kg) & volume (ml) | Route of administration | Frequency of dosing |
|---|---|---|---|---|---|
| Isoflurane | ( )* | 1,2,3 | 1-3% with oxygen | Inhalation via face mask | Continuous |
| Euthasol end-stage (390 mg/ml and 10 mg/ml) | ( )* | 3 | 100mg/kg (0.88-1.25ml) | IV | once |
| Bupivacaine (5mg/ml) | ( )* | 1,2,3 | 1.25mg/kg (0.88-1.25ml) | SQ | Once |
| Buprenorphine (0.3 mg/ml) | ( )* | 1,2,3 | 0.01−0.02 mg/kg (0.12-0.33ml) | ~~IV~~ | Once |
| Propofol - CRI (10 mg/ml) | ( )* | 1,2,3 | 0.3 Mg/kg/min (0.1ml/min) | IV | To effect |
| Lactated ringers IV | ( )* | 1,2,3 | 0.3mg/kg/hr (maintenance rate) | IV | Continuous |

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.
▶

b. Intra-operative physical support. For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).

▶For all surgeries, body temperature (38.5 degree Celsius) will be maintained using a hot−water circulated heating pad and drapes. A lubricant gel will be applied to the eyes to keep them moist throughout the procedures.

c. Intra-operative monitoring. Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.

▶For all surgeries, there will be monitoring of the vital signs: ECG, heart rate, respiratory rate, expired CO2, SPO2, noninvasive blood pressure or arterial blood pressure, and temperature in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels.

Intra-operative assessment and vital signs will be documented every 15 minutes during anesthesia.

7. Survival surgery considerations. For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a. – 7.g.

a. Complete the table below for each survival surgery listed in Item 1, above.

| Surgery # (see Item 1) | Survival Period | Measures for Maintaining Sterility | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sterile Instruments | Surgical Cap | Sterile Gloves | Surgical Scrub | Sterile Drapes | Sterile Gown | Face Mask | Other* |
| 1,2 | 30 - 37days | (X) | (X) | (X) | (X) | (X) | (X) | (X) | (X)* |

* Describe any "other" measures to be taken to maintain sterility during surgery.
▶ The surgeon will don clean scrubs and scrub hands

b. For each surgery, describe the immediate post-operative support to be provided to the animals.

The cats will be closely monitored until they are ambulatory. Animals will be monitored daily for changes in behaviors, especially relating to bladder function. Normal, daily observations of the cats will be conducted by the veterinary staff as part of standard operating procedures and will be recorded as part of the standard records. The investigators will also observe the cat daily post-operatively. Investigator observations will be recorded on a distress scoring sheet and maintained in the study records.

c. Post-operative analgesia. Complete the table below for each surgery listed in item 1, above.

| Surgery # (see Item 1) | Agent* | Dose (mg/kg) & Volume (ml) | Route of Administration | Frequency of Dosing (e.g., times/day) | Period of treatment (e.g. days) |
|---|---|---|---|---|---|
| 1,2 | Robenacoxib (20 mg/ml) | 2mg/kg (0.35-0.50ml) | SQ | once per day | 3 days post-op |

| | | | | | |
|---|---|---|---|---|---|
| 1,2 | Buprenorphine (0.3 mg/ml) | 0.01–0.02 Mg/kg (0.12-0.33ml) | SQ | BID (twice daily) as needed | Starting 1st post-op |

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:
►

d. Other post-operative medications. Complete the following table to describe all other medications that will be administered as part of post-operative care.

| Surgery # (see Item 1) | Medication | Dose (mg/kg) & Volume (ml) | Route of Administration | Frequency of dosing (e.g. times/day) | Period of treatment (e.g. days) |
|---|---|---|---|---|---|
| 1,2 | Convenia (cefovecin sodium; 80 mg/ml) | 8mg/kg (0.34-0.5ml) | SQ | Once post op, additional with veterinary consultation | Cefazolin is given pre-op |

e. Post-operative monitoring. <u>After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency</u>.

(1) Immediate post-operative monitoring

| Surgery # (see Item 1) | Frequency of Monitoring | Duration at this Frequency | Name(s) of Responsible Individual(s) |
|---|---|---|---|
| 1,2 | Animals will be kept under constant direct supervision | Until extubated | [redacted] staff |
| 1,2 | Animals will be kept under continuous supervision | Until they are ambulatory | [redacted] |

(2) Post-operative monitoring after the immediate post-operative period

| Surgery # (see Item 1) | Frequency of Monitoring | Duration at this Frequency | Name(s) of Responsible Individual(s) |
|---|---|---|---|
| 1,2 | Daily | 30 – 37 days | [redacted] |

f. Post-operative consequences and complications.

(1) For each surgery, describe any common or expected post-operative consequences or complications that may arise and what will be done to address them.

Surgery 1,2 ▶ The cats are expected to fully recover to and maintain physiologic status following surgery. The UroMOCA device is not expected to produce any persistent or significant pain and/or distress to the cat. No wires will be exteriorized from the body, reducing the chance of infection and irritation. We will monitor the cats for any signs of pain, including reluctance to move, papillary dilation and hiding. Cats in this study may exhibit increased urinary frequency and discomfort for up to 2 weeks. Pain on urination may be manifested by urinating outside the litter box. Serosanguinous coloration of urine up may be seen up to 10 days, and ~3-5% of body weight loss due to being refined in a cage for 14 days may also be seen. Any signs of wound or urinary tract infections (straining to urinate, blood in urine, discomfort on bladder palpation) will be discussed with the veterinary staff. It will be investigated by urine culture and treated by systemic antibiotics. If wound infection and urinary tract infection persist and the animal is non-responsive to treatment, is debilitated and fails to recover, the experiments will be terminated and the animal will be euthanized. Signs of wound infection will be discussed with the veterinarian. If a wound infection persists and the animal is non-responsive to treatment, is debilitated, and fails to recover, the experiments will be terminated and the animal will be euthanized. In addition, weight loss greater than 15% or intractable pain or distress determined by behavioral responses, including vocalization, ears drawn back, or withdrawal, will be determined as cause for euthanasia.

(2) List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1,2▶ Endpoint criteria have been defined to determine when animals, either on or off study, will be euthanatized or otherwise removed from the study. The five areas of observation are body weight, physical appearance, measurable clinical signs, unprovoked behavior, and response to stimulus. The following indicates the observations that will be made in each of these categories and criteria that would warrant consideration of humane euthanasia. Indications that are specific to this project include intractable infections.
Normal, daily observations of the calves will be conducted by the veterinary staff as part of standard operating procedures and will be recorded as part of the standard records. The investigators will

also observe the cat daily post-operatively. Investigator observations will be recorded on a distress scoring sheet and maintained in the study records.

**Criteria for humane euthanasia**

| Category | Observation | Humane Endpoint Criteria |
|---|---|---|
| Body Weight | Body Weight | • >15% weight loss over a 7-day period. |
| | Food/water consumption | • Anorexia for >72 hr |
| Appearance | Coat condition | • Poor coat condition, absence of grooming |
| | Posture | • Persistent 'hang dog' posture |
| Clinical Signs | Respiration | • Persistently Labored |
| | Tremors | • Continuous tremor |
| | Convulsions | • > 10 min in duration |
| | Prostration | • > 2 hr |
| | Infection | • Intractable infection that is not resolved by removal of the implanted components and antibiotic treatment. |
| | Dehiscence | • Chronic, open sores or incisions that will not heal |
| | Failure to urinate | • Urethral obstruction that cannot be relieved |
| | Painful Urination | • Painful urination not relieved by medication |
| | Uroabdomen | • Urine leakage into the abdomen |
| Unprovoked Behavior | Socialization | • No peer interaction |
| | Vocalizaton | • Pesistent pain vocalization which cannot be mitigated |
| | Self-Mutilation | • Chewing, scratching, or other means that cannot be prevented with an Elizabethian collar or other similar measure, resulting in mutilation or open wounds. |
| Response to stimulus | Provoked behavior | • Unresponsive to extraneous activity or stimulation |

(3) In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)

▶None

g. Maintenance of post-surgical medical records. Complete the table below for each surgery, specifying where the records will held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

| Surgery # (see Item 1) | Location of Records | Name(s) of Individual(s) Responsible for Maintaining Written Records | Research Personnel | Veterinary Staff |
|---|---|---|---|---|
| 1 | VA Room [redacted] and in cat's medical records in housing room. | [redacted] ARF Staff | (X) | (X) |
| 2 | VA Room [redacted] and in cat's medical records in housing room. | [redacted], ARF Staff | (X) | (X) |
| 3 | VA Room [redacted] | [redacted] | (X) | ( ) |

8. **Certification.** The PI must sign the certification statement in Item Z.5 of the main body of the ACORP

**ACORP APPENDIX 6**
**SPECIAL HUSBANDRY AND PROCEDURES**
**VERSION 4**

See ACORP App. 6 Instructions, for more detailed explanations of the information requested.

1. **Description of Procedures.** Complete the table below for each procedure listed in Item V of the main body of the ACORP that is not detailed in a SOP or in another item or Appendix of the ACORP. For each special procedure, check all features that apply.

| Special Procedure | | Features | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Brief Description | Husbandry | Restraint | Noxious Stimuli | Exercise | Behavioral Conditioning | Irradiation | Imaging | Other** |
| 1 | Urodynamics and CT fluoroscopy | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | (X) | ( ) |
| 2 | Ambulatory Testing with UroMOCA only | ( ) | ( ) | ( ) | ( ) | (X) | ( ) | ( ) | ( ) |
| 3 | Ambulatory Testing with UroMOCA and electrodes | ( ) | ( ) | ( ) | ( ) | (X) | ( ) | ( ) | ( ) |

*Husbandry refers to all aspects of care related to the maintenance of the animals, including (but not limited to) provision of an appropriate diet, access to water, control of environmental conditions, and the selection of primary and secondary enclosures.

**Describe any "Other" features that are involved.
►

a. Provide a complete description of each special procedure listed above, including the duration of the procedure, how frequently it will be repeated in any one animal, and any effects it is expected to have on the animal:

**Special Procedure 1** ► Urodynamics and CT fluoroscopy

Under general anesthesia (see appendix 5): An intraurethral catheter will be inserted via the urethra into the bladder for bladder filling/emptying and for pressure monitoring. The bladder will be emptied via the intraurethral catheter. In addition to the intraurethral catheter for bladder pressure monitoring (Fig. 1), an inflatable balloon catheter will be inserted into the rectum to estimate abdominal pressure since it is used to distinguish between bladder pressure events and other abdominal activity. Measurement of abdominal pressure allows us to validate our automated algorithm for data analysis. The bladder will be infused with normal saline at known volumes from 5 to 50 mL. Volume data from the UroMOCA and pressure data from the UroMOCA, intraurethral catheter, and rectal balloon catheter will be recorded as a function of infused bladder volume. The first urodynamics measurement will be done prior to UroMOCA insertion to establish baseline bladder function for that cat.

**Special Procedure 2** ▶ Ambulatory Testing with UroMOCA only

Animals will be monitored at least once per day for changes in behaviors, especially relating to bladder function by study staff or ARF staff. Notes will be made in the cat's medical record of positional changes, voiding, bladder spasms, and other behaviors that occur during the daily observation. 3-5 days/week, investigators or study staff will conduct tests of the UroMOCA recharge and data transmission functions, collecting ambulatory bladder volume and pressure data. Data from the UroMOCA will be collected with an external antenna coil. This receiving antenna coil is either attached to a long cable and then to a data receiver circuit attached to a computer or attached directly to a little box that has the receiver circuit in it. If the receiving antenna coil is attached to the long cable, we will walk around holding the receiver antenna near the cat to get data. If the receiving antenna coil is directly attached to the small box, it will be powered by battery and have no cables attached. When used wirelessly, the antenna/box combo will be placed in a belt or backpack harness. A recharge coil may also be placed in this harness. An alternate method of recharging would be to integrate the recharge coil into a cat bed or mat that the animal sleeps on. These data collection and recharge sessions are expected to last approximately 2-6 hours during animal enrichment in the VA animal research facility. We may record video of the room while the cat is wearing the backpack so that we can record the timepoints when animal does certain actions, such as eating, jumping and using litterbox.

**Special Procedure 3** ▶ Ambulatory Testing with UroMOCA and electrodes

Animals will be monitored at least once per day for changes in behaviors, especially relating to bladder function by study staff or ARF staff. After allowing for recovery from the initial implant surgery, we will begin recording bladder and pelvic floor data in response to sacral root stimulation protocols. 3-5 days/week, investigators or study staff will conduct tests of the recharge and data transmission functions of implanted devices and collect ambulatory bladder volume and pressure data in response to electrical stimulation paradigms. Data from the UroMOCA will be collected with an external antenna coil. This receiving antenna coil is either attached to a long cable and then to a data receiver circuit attached to a computer or attached directly to a little box that has the receiver circuit in it. If the receiving antenna coil is attached to the long cable, we will walk around holding the receiver antenna near the cat to get data. If the receiving antenna coil is directly attached to the small box, it will be powered by battery and have no cables attached. When used wirelessly, the receiving antenna/box combo will be placed in a belt or backpack harness. Along with the receiving antenna/box combo, a recharge coil and/or a wireless stimulator/data collector may be placed in the backpack. The Data collection and recharge sessions are expected to last approximately 2-6 hours during animal enrichment in the animal facility and will be coupled with animal enrichment activities. An alternate method of recharging would be to integrate the recharge coil into a cat bed or mat that the animal sleeps on. We will measure bladder pressure, bladder volume, and pelvic floor EMG when the animal is at rest during filling and when the animal is using a litter box to empty its bladder. We will monitor animal behaviors, including rest; movement or play as during enrichment; vocalization or other signs of discomfort or distress; seeking litter box; and squatting in litter box and actively emptying bladder or bowel contents. We will characterize the bladder pressure and pelvic floor EMG during these two bladder states for comparison to standard urodynamics data, which includes anesthesia and nonphysiological fast bladder filling.

We will measure recruitment of bladder pressure, pelvic floor EMG, thresholds for limb movement, and animal behaviors (comfort) as a function of stimulation amplitude and bladder state (filling or emptying). During each of the two bladder states, stimulation will be applied at each of the 4 electrode sites in turn, randomized. For bladder filling, we will wait at least one hour after the animal used the litter box to empty and verify that the animal is in a state of rest. For bladder emptying, stimulation will be applied when the animal climbs into the litter box, but before the animal assumes a posture for emptying. We hypothesize that stimulation at each root will result in increased bladder pressure, pelvic floor EMG, and sacral root ENG, regardless of electrode site, and amplitudes of responses will correlate with stimulation amplitude.

Finally, we will measure bladder pressure and pelvic floor EMG and observe limb function and animal behaviors as a function of sacral root block and bladder state (filling or emptying). Stimulation will be applied during filling and emptying states as described above. We hypothesize that kilohertz stimulation will block sacral root conduction, significantly reducing bladder pressure and pelvic floor EMG regardless of bladder state (filling or emptying). We are particularly interested in the efficiency (i.e. time course and completeness of block) of kilohertz frequency stimulation and its potential for use in conjunction with other neurostimulation approaches to improve bladder emptying without unwanted side effects.

Recording sessions will be conducted at least 3 times per week and will last for at least 2 hours per session under normal physiological conditions. After at least 2 weeks of data recording in awake, behaving animals, we will conduct a terminal experiment, during which we will retest sacral root stimulation while the animal is anesthetized with isoflurane and then with CRI propofol to allow for reflex activity. We will also conduct standard catheter-based urodynamics. From initial implant until terminal experiment, devices will be regularly awoken, tested, and recharged to assure consistent function of instrumentation. We may record video of the room while the cat is wearing the backpack so that we can record the timepoints when animal does certain actions, such as eating, jumping and using litterbox.

The cat may wear the backpack harness at all times if it helps the cat get accustomed to the backpack. If the cat is accustomed to backpack harness, then it will only wear the backpack harness during ambulatory testing periods.

b. Explain why each of these special procedures is necessary:

**Special Procedure 1** ▶ The purpose of this study is to develop, test, and validate a new tool to measure bladder pressure and volume. We will be testing to see if the UroMOCA device provides us with the same results/data as the traditional catheter based urodynamic testing of bladder pressure and volume.

**Special Procedure 2** ▶ The ambulatory testing will be to validate that the UroMOCA is well accepted by the cat. We will also be refining the for wireless charging and receiving pressure and volume readings from the device.

**Special Procedure 3** ▶ Studies of the neural mechanisms underlying control of the lower urinary tract are usually conducted in acute experiments under anesthesia and with bladder measurement methods that include a catheter and faster-than-physiological retrograde bladder filling, all of which can significantly affect the behavior of neural circuits and organ function. In this procedure, we will

conduct neurostimulation experiments in awake, behaving animals to generate new neural mapping data of the lower urinary tract and test hypotheses that are relevant to efforts for translating neurostimulation approaches for bladder function.

2. **Personnel.** Complete the table below for each special procedure listed in Item 1, above. Identify the individual(s) who will be responsible for carrying out the procedures, and those who will be responsible for monitoring the condition of the animals during and after the procedures. After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency.

| Procedure Number (see Item 1) | Responsible Individual(s) | |
|---|---|---|
| | Carrying Out Procedure | Monitoring the Animals |
| 1 | [redacted] | [redacted] |
| 2 | [redacted] | [redacted] |
| 3 | [redacted] | [redacted] |
| 1-3 | Study Staff | Study Staff and ARF Staff |

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so, describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

| Procedure Number (see Item 1) | Expected Potential Pain and/or Distress | | | |
|---|---|---|---|---|
| | No | Yes | | |
| | | Description | To Be Relieved | Not to Be Relieved |
| 1 | ( ) | Urodynamics | (X)[a] | ( )[b] |
| 2 | (X) | Ambulatory Testing with UroMOCA only | ( )[a] | ( )[b] |
| 3 | (X) | Ambulatory Testing with UroMOCA and electrodes | ( )[a] | ( )[b] |

a. For each procedure for which potential pain and/or distress is expected, but WILL be prevented or alleviated by administration of the analgesic(s) or stress-relieving agents, complete the table below:

| Procedure Number (see Item 1) | Agent | Dose (mg/kg) & vol (ml) | Route of admin | Freq of admin (times/day) | Duration of admin (days post-procedure) |
|---|---|---|---|---|---|
| 1 | This procedure performed under anesthesia. See Appendix 5 for details on induction and maintenance. | | | | During procedure |

Describe any non-pharmacological measures to be taken to address the potential pain and/or distress:

Special Procedure 1 ▶

Special Procedure 2 ▶

Special Procedure 3 ▶

b. For each procedure for which potential pain and/or distress is expected and <u>will NOT be prevented or alleviated</u>, provide the scientific justification for this:

Special Procedure 1 ▶

Special Procedure 2 ▶

Special Procedure 3 ▶

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the special procedures, and list the criteria that will be used to determine when individual animals will be removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

| Procedure Number (see Item 1) | Monitoring Methods | Endpoint Criteria |
|---|---|---|

| | | |
|---|---|---|
| 1 | Electrocardiogram heart rate, non-invasive blood pressure, blood oxygenation, end-title CO2, and body temperature will be monitored. Using these vital signs in conjunction with reflex responses such as eye blink, toe pinch, and jaw tone, as well as capillary refill time, we will continuously monitor anesthetic and physiologic levels. Cats will be closely monitor until they are ambulatory. Animals will be monitored daily for changes in behaviors, especially relating to bladder function. Normal, daily observations of the cats will be conducted by the veterinary staff as part of standard operating procedures and will be recorded as part of the standard records. The investigators will also observe the cat daily post-operatively. Investigator observations will be recorded on a distress scoring sheet and maintained in the study records. | >15% weight loss over a 7-day period<br><br>Intractable infection that is not resolved by removal of the implanted components and antibiotic treatment.<br><br>Chewing, scratching, or other means that cannot be prevented with an Elizabethan collar or other similar measure, resulting in mutilation or open wounds. |
| 2 | During the wireless ambulatory testing with the backpacks, the cats will be checked every 1-2 hours to make sure the backpack is still in place and that there are no other problems. | >15% weight loss over a 7-day period<br><br>Intractable infection that is not resolved by removal of the implanted components and antibiotic treatment.<br><br>Chewing, scratching, or other means that cannot be prevented with an Elizabethian collar or other similar measure, resulting in mutilation or open wounds. |
| 3 | During the wireless ambulatory testing with the backpacks, the cats will be checked every 1-2 hours to make sure the backpack is still in place and that there are no other problems. | >15% weight loss over a 7-day period<br><br>Intractable infection that is not resolved by removal of the implanted components and antibiotic treatment.<br><br>Chewing, scratching, or other means that cannot be prevented with an Elizabethian collar or other similar measure, resulting in mutilation or open wounds. |