

Protocol #: [REDACTED]

Protocol Title: Afferent stimulation to evoke recto-colonic reflex for colonic motility **Error! Reference source not found.**

Principal Investigator: [REDACTED]

### Investigator Assurances

I agree to abide by the policies of the Louis Stokes Cleveland DVA Medical Center Institutional Animal Care and Use Committee (IACUC) and all applicable federal regulations.

I will adhere to the protocol as described and as modified.

I will submit any modifications of the protocol to the IACUC for review and approval before initiating them.

I will notify the IACUC of changes in the location of the animal research.

I will assist the IACUC in verifying compliance with the regulations.

I will notify the IACUC of any unexpected results that affect the welfare of the animals. I will report any unanticipated pain or distress, morbidity, or mortality to the attending veterinarian and the IACUC.

I understand and agree that emergency veterinary care, including euthanasia, will be administered to animals exhibiting unbearable pain distress or illness. Prior to any emergency treatment, the veterinary staff will make every effort to contact my representative or me.

I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. All other personnel involved in animal use in this project have been or will be trained in proper procedures relevant to this protocol, including but not limited to animal handling, administration of anesthetics and analgesics, aseptic technique, postoperative monitoring, and euthanasia. I will notify the IACUC when new employees are hired and will certify when their training to perform the relevant experimental procedures on live animals is complete.

I declare that the information provided in this protocol is accurate. If this project is to be funded, I certify that this protocol accurately describes all procedures in which I intend to involve laboratory animal subjects.

I declare that the studies described here do not unnecessarily duplicate previous work by others or by me.

[REDACTED]  
\_\_\_\_\_  
Signature of Principal Investigator

9/25/2015  
\_\_\_\_\_  
Date



**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] PhD
2. VA Station Name (City) and 3-Digit Station Number ▶ Louis Stokes Cleveland DVAMC (541)
3. Protocol Title ▶ Afferent stimulation to evoke recto-colonic reflex for colonic motility
4. Animal Species covered by this ACORP ▶ cat
5. Funding Source(s). Check each source that applies:
  - ▶ (X) Department of Veterans Affairs.
  - ▶ ( ) 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 ▶
    - (2) If approved by the R&D Committee, give the date of approval ▶
  - 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
      - ▶
    - (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
      - ▶
    - (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.
      - ▶

- 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 ▶  
 (2) IACUC approval number of other protocol ▶  
 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.

▶  
 Loss of bowel function is a major concern for veterans with central nervous disorders, such as spinal cord injury (SCI). Over 42,000 veterans have SCI, representing more than 15% of the total American population with SCI. Following SCI, the colon is often unable to evacuate stools via peristaltic propulsion. Losing bowel function can cause constipation, gastro-intestinal complications, and fecal incontinence, severely impacting health and quality of life.

The standard of care for individuals with neurogenic bowel dysfunction involves the design of a bowel program for predictable and effective elimination of the bowels. A bowel program includes diet, fluid intake, activity, and pharmaceutical or mechanical rectal stimulation. Approximately 80% of individuals with neurogenic bowel dysfunction use digital stimulation of the rectum to reflexively activate the colon, improve colonic motility, and facilitate bowel evacuation. However, the bowel routine can require over an hour to evacuate the bowels and this process often requires the assistance of a caregiver, which adds to the cost of care and reduces independence. Restoring bowel function is considered a high priority by individuals with paraplegia, but remains a critically unmet need requiring further development. The top priority for individuals with tetraplegia is reaching and hand function, but pelvic functions are still of high concern.

Digital rectal distension is the clinical standard for eliciting colon motility. The majority of individuals with neurogenic bowel dysfunction use digital rectal stimulation to mechanically distend the rectum and reflexively activate colon peristalsis, which loads stool into the rectum for bowel emptying. Digital rectal distension takes advantage of an existing reflex pathway affecting bowel function. The recto-colonic reflex has been well-studied in cats, dogs, and humans. Rectal distension activates mechanoreceptors innervating the rectum, which sends afferent information via small A $\delta$  and C fibers to the sacral spinal cord. This input modulates a spinal reflex pathway that activates motor efferent drive to the colon, causing coordinated peristalsis and increasing colonic motility. Digital rectal stimulation is effective, but it requires an individual, or a caregiver, to reach into the individual's rectum with gloved fingers. Even with digital rectal stimulation and other bowel program tools, the bowel routine still typically requires an hour or two to complete. Reliance on digital rectal stimulation is associated with lost independence, lost time, lost dignity,

and caregiver costs. Substituting rectal distension for a method that does not include these drawbacks would provide positive and social and psychological benefits.

We are currently developing an electrical stimulation approach to improve colonic motility as an alternative to digital rectal stimulation and taking advantage of the recto-colonic reflex. We collected preliminary evidence in acute spinally intact cats that electrical stimulation can evoke colonic activity and peristalsis. **The purpose of this study is to determine the effects of patterned electrical stimulation on colon motility after SCI**, which is a key step in translating the technology to clinical implementation. The results of the study will help us in a parallel study that we are conducting in humans with chronic SCI by identifying effective stimulation parameters and locations. Demonstration that our approach can produce clinically effective bowel emptying in these animals, whose colonic motility is slowed following SCI, would be a significant advance and give us insight on how to apply the approach to humans.

### 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 quantify the ability to electrically evoke colonic motility and empty the bowel; determine the neural interface design required for an implanted electrode; and validate a minimally invasive approach for future human subject testing. We will evoke colonic motility in chronic spinalized felines, the most appropriate preclinical large animal model, to restore defecation. This will allow direct evaluation of the preclinical potential by determining the ability to treat using this approach in the manner that we expect to treat humans.

**Aim 1** will determine the effect of electrical stimulation on colonic pressure (Aim 1.1) and colonic motility (Aim 1.2) in acute spinally intact cats. In two cohorts of animals, we will gain surgical access to the colon and its nerves. We will insert balloon catheters through the anus into the colon to record colonic pressure and motility. In one cohort of animals, we will measure the colon pressure in response to mechanical distension of the rectum and to electrical stimulation of nerves of the colon and rectum. In a second cohort, we will determine the dependence of colonic motility (movement of balloon catheters in colon) to varying stimulation parameters.

In **Aim 2** we will quantify the effect of electrical stimulation on colonic motility (Aim 2.1) and defecation (Aim 2.2) in chronic spinalized cats. We will implant stimulation electrodes and start with the electrical stimulation parameters that provided optimal colonic activity in Aim 1, and determine if electrical rectal stimulation can achieve colonic motility equivalent to mechanical rectal distension. Defecation will be measured by the elapsed time from the beginning of a stimulation session to complete bowel emptying in awake animals. Both implanted and rectal probe stimulation will be tested. These data will allow us to evaluate potential confounds of anesthesia and neural changes after SCI.

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.



**Aim 1: Determine the sensitivity of the recto-colonic reflex to electrical rectal stimulation parameters**

The objective of Aim 1 is to demonstrate that the recto-colonic reflex can be activated by electrical stimulation of the rectum and examine the sensitivity of reflex activation to input parameters. Based on

the recto-colonic reflex literature and our preliminary data using electrical afferent stimulation to modulate spinal reflex pathways, we **hypothesize** that electrically activating rectal afferents can evoke an excitatory recto-colonic reflex. This research aim is subdivided into two subaims 1.1 and 1.2 with different primary outcomes. The primary outcome of Aim 1.1 is **increased colonic pressure**, which provides a simple means of observing colon activity. If afferent stimulation evokes a recto-colonic reflex, then the smooth muscle of the colon will contract and increase colonic pressure. The primary outcome of Aim 1.2 is **increased colonic motility**, measured by peristaltic rate and direction, which is a clinically relevant measure of function. This subaim will also validate an approach using a small-diameter rectal probe electrode for non-invasive stimulation. Thus, Aim 1 will demonstrate the potential for this afferent stimulation approach to modulate the recto-colonic spinal reflex pathway and evoke colonic activity as measured by colon pressure and motility.

### **Aim 1.1 – Determine the effect of electrical rectal stimulation on colonic pressure**

Acute experiments lasting approximately one day will be conducted in 10 healthy cats of either gender that are neurologically intact. Animals will act as their own controls. Four stimulation paradigms will be tested on each animal, including 1) mechanical rectal distension (clinical standard), 2) electrical rectal stimulation, 3) ventral sacral root stimulation, and 4) electrical colon stimulation. The primary outcome variable is colonic pressure, which will be measured at baseline (without any stimulation) and in response to each stimulus paradigm. Pressures will be measured using three small balloon catheters – two in the colon and one in the rectum – inserted through the anus. Stimulation amplitude and frequency for each stimulus paradigm will be varied over a range that is informed by the literature. Each combination of stimulus parameters will be randomized and repeated at least five times. Colon activity, like bladder activity, may be affected by colon distension (e.g. the presence of a stool). Therefore, we will also adjust colon distension to approximate the presence of a stool and optimize colon excitability.

Electrical rectal stimulation will be applied and compared directly to rectal distension because we believe that both stimulus paradigms will evoke the same reflex and produce similar outcomes. Typically we find that electrical afferent stimulation is effective at amplitudes below 10 mA, which will define our range for stimulation amplitude. The literature has reported that rectal distension evokes mechanoreceptor afferent activity with firing rates of up to 40 Hz. Therefore, we will test frequencies in this range. Amplitude and frequency will be tested at several specific values within their test ranges and stimulus conditions will be randomized.

Ventral sacral root electrical stimulation will activate purely motor efferents of the colon and will be used as a control for comparison to afferent-mediated increases in colonic pressures. Colon stimulation will be expected to activate both motor efferents and sensory afferents of the colon and might not produce the same pressures as ventral sacral root electrical stimulation. This approach will also provide a potential backup in Aim 2 if electrical rectal stimulation does not produce colonic motility sufficient for maintaining chronic spinalized cats. The two approaches of ventral sacral root stimulation and colon stimulation have been shown to increase colon activity (see Background). We will reproduce these results for comparison to electrical rectal stimulation.

After testing the four stimulation paradigms, the dorsal sacral roots S2 to S4 will be bilaterally transected to abolish sacral spinal reflexes of the rectum and colon, disrupting the flow of sensory information from the rectum to the spinal cord and ablating the spinal pathway for the recto-colonic reflex. The four stimulation paradigms will then be repeated to demonstrate which paradigms were primarily driven by spinal afferent-mediated pathways.

**Aim 1.2 – Determine the dependence of reflexively activated colonic motility on electrical rectal stimulation parameters**

The study design is similar to Aim 1.1. Acute experiments lasting approximately one day will be conducted in 10 healthy cats of either gender that are neurologically intact. Animals will act as their own controls. We will apply stimulation conditions, including rectal distension, electrical rectal and electrical colon stimulation, while measuring colonic activity. The infusion volumes of the two balloon catheters that we use for pressure recording will be varied to optimize colonic excitability. In addition, we will test stimulation using a minimally invasive thin rectal probe for electrical rectal stimulation, which will be inserted through the anus into the rectum and deliver electrical rectal stimulation on the inside surface of the rectum. The probe will also be able to provide colon stimulation. This minimally invasive probe will verify the effectiveness of a minimally invasive approach for future testing in humans. Use of a rectal probe electrode is meant to provide a minimally invasive means of testing the effectiveness of electrical rectal stimulation and provide a screening tool. Ultimately, future work will focus on developing an implantable system that does not require an individual to insert a probe into his rectum. Finally, we will measure external anal sphincter activity simultaneously with colon and rectal activity (EAS) using an electromyogram. These EAS data will determine the response of the EAS to the rectal stimulation or colon activity, and help us determine if an additional approach will need to be developed to inhibit the EAS and achieve complete bowel emptying.

**Aim 2: Determine the effect of electrical stimulation on colonic pressure, motility, and daily defecation in chronic spinalized cats**

Experiments will be conducted in 4 cats with chronic complete SCI. In a separate survival surgery **prior to spinalization**, electrodes will be implanted (using the electrode interface tested in Aim 1) to allow on-demand activation of colonic motility and defecation. Two weeks after the electrode implantation surgery, implants will be verified to be working by evoking reflex colonic activity via the implanted electrodes, then animals will be spinalized. The effectiveness of electrical rectal afferent stimulation to evoke reflexive **colonic activity (pressure and motility) and defecation** after chronic SCI will be quantified in biweekly acute testing sessions (Aim 2.1). These data will allow direct comparison to Aim 1 and verify effectiveness of reflex activation of **colonic pressure** (the primary outcome measure for Aim 2.1). The ability to use electrical rectal afferent stimulation as the primary clinical bowel routine for cats with chronic SCI will be determined in Aim 2.2. These data will determine the clinical effectiveness of a bowel pacemaker for evoking defecation in chronic SCI cats. **Defecation time** (the primary outcome measure) will be determined for randomized daily treatments of control (no stimulation), mechanical rectal stimulation, and electrical stimulation (implanted and rectal probe). A terminal experiment under chloralose anesthesia will be conducted to allow direct comparison to data from Aim 1. We have utilized a very similar study design approach to evaluate the efficacy of implanted electrodes on bladder function in chronic SCI cats.

Week -2	Week 0	Weeks 1-2	Weeks 3-8: Randomized Daily Stimulation Testing	
Electrode Implant	SCI	SCI stability	Biweekly testing sessions	Terminal Procedure

**Aim 2.1 – Determine the effect of electrical rectal stimulation on colonic pressure and motility in chronic spinalized cats**

This subaim will determine if the recto-colonic reflex is activated in chronic SCI animals as it was in intact animals during biweekly testing sessions under anesthesia. These data will help determine if SCI or anesthesia are factors in evoking the recto-colonic reflex electrically.

*Biweekly Testing Sessions:* We will conduct biweekly experiments in the chronic spinalized cats under propofol anesthesia to measure recruitment of the recto-colonic reflex in response to mechanical and electrical stimulation (as in Aim 1). Testing sessions will occur at weeks 4, 6, and 8, after animals have healed from SCI. Cats will be lightly anesthetized with propofol and testing sessions will last for approximately 4 hours each. Although anesthetics including propofol can suppress spinal reflexes, we have previously demonstrated that spinal reflexes can be elicited in chronic SCI cats under closely monitored light propofol anesthesia. Colonic motility will be measured as in Aim 1 using two balloon catheters inserted through the anus into the colon and rectum and measuring (1) the colonic pressures evoked by electrical stimulation and (2) the rate at which tonic waves propagate between balloons. If balloons are defecated, then defecation rate will also be measured. These data will be compared to data collected in Aim 1 in the intact cats. We will also determine the threshold for electrical activation of the EAS and unintended structures, such as the sciatic nerve.

**Aim 2.2 – Determine the ability of chronic electrical rectal stimulation to produce repeated, clinically effective defecation in spinalized cats**

This subaim will demonstrate that we can maintain a chronic spinalized animal with electrical stimulation. In humans, routine bowel care typically involves mechanical stimulation every 15-20 minutes, with each stimulus lasting approximately 30 seconds. The bowel care can take 30-120 minutes to achieve complete bowel emptying, which is determined by the lack of feces loading into the rectum and the presence of mucus.

*Daily Stimulation Testing:* Four treatment conditions (mechanical, implanted and minimally invasive electrical, and no stimulation) will be tested to measure their effects on defecation time, defined as the time from stimulation onset to complete bowel emptying. Treatment conditions will be randomized and applied daily. Animals will have their bladders expressed twice daily. During the first care visit, before the bladder is expressed, stimulation will be applied and defecation time recorded. If defecation is not completed within two hours, animals will be given a water enema to flush the bowels clean. In addition, during twice daily animal care, cages will be checked for feces, and feces will be weighed to determine mass and frequency of defecation to determine if defecation is altered following spinalization and if it is restored following stimulation.

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

►  
Aim 1.1 (acute, spinally intact cats): The primary outcome measure is colonic pressure. Based on the literature, we expect to achieve colonic pressures of up to 10 cmH<sub>2</sub>O over baseline in response to stimulation, with a standard deviation of 10 cmH<sub>2</sub>O. Using a t-test to compare treatment groups, 7 animals is sufficient to achieve a power of 0.8. We are asking for an additional 3 animals per Aim because not all animals demonstrate reflex activity.

Aim 1.2 (acute, spinally intact cats): The justification for the number and neurological state of animals is the same as in Aim 1.1. Surgical and experimental manipulations are very similar between Aims 1.1 and 1.2. However, unlike striated muscle responses or even bladder responses to stimulation, the colon moves and responds very slowly, on the order of minutes to tens of minutes, which will likely require the two separate cohorts for experiments for Aims 1.1 and 1.2 due to the amount of time required for an experiment (approximately 18-20 hours). We will make efforts to have some overlap between the cohorts in each aim, using animals for both aims, but pilot experiments suggest that this is not feasible because it took approximately 14 hours to collect data for one Aim. Using a single animal to collect data for two aims simultaneously could take two days and we do not have the support to conduct experiments of that length at this time.

Aims 2.1 and 2.2 (chronic, spinalized cats): Chronic experiments will be conducted in 6 spinalized female cats. The same cohort of cats will be used in both Aim 2.1 and Aim 2.2. Chronic SCI cats are the most appropriate large animal model, the "gold standard", for preclinical evaluation. Females are typically utilized for chronic SCI studies because bladder expression (part of twice daily SCI animal care) is easier and less traumatic in females. We expect that data collection will require approximately 8 weeks of chronic SCI. Individual animals may be maintained for up to 4 weeks longer to obtain statistically significant measures if effect sizes or variances change substantially. Results will be compared to results from Aim 1. We expect a rise in colonic pressure from approximately 10 to 30 cmH<sub>2</sub>O in response to stimulation, with a standard deviation of 10 cmH<sub>2</sub>O. We are able to collect sufficient data to achieve statistical significance based on both the number of animals and the number of testing sessions per animal. Only 4 animals are needed in Aim 2 because data is collected over several sessions for each animal, as opposed to data collected during a single session per animal in Aim 1. Therefore, 3 biweekly testing sessions each for 4 animals should be sufficient to achieve statistical significance. We are requesting 2 additional animals on this protocol because not all animals will be appropriate to advance to the chronic spinalization phase or may not demonstrate spinal reflex recovery following spinalization.

Aim: Experiment	Number of Animals
Aim 1.1: Colonic pressure in response to acute electrical rectal stimulation in intact cats	10
Aim 1.2: Colonic motility in response to acute electrical rectal stimulation in intact cats	10
Aim 2: Colonic pressure, motility, and defecation in chronic spinalized cats	6

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.)



#### **Implantation of Electrodes (surgical)**

General anesthesia induced with ketamine and maintained with isoflurane (1-3%). A cephalic catheter will be placed for IV access. 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, SPO<sub>2</sub>, CO<sub>2</sub>, 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 fine wire electrode (or nerve cuff electrode, if appropriate) will be implanted on the target nerve. A midline incision of the lower abdomen will be made to expose the abdominal cavity and the branch of the pelvic splanchnic nerve innervating the distal colon or rectum. A stimulating electrode will be implanted on the nerve branch and secured in place by suturing the electrode lead wire to the serosal surface of the colon and to the abdominal wall muscles. The electrode wires will be tunneled up to the back where they will exit through a small midline incision in the upper thoracic region of the back. The electrode lead wires will be secured where they exit the skin and fastened to a connector. The surgical sites will be closed. This small connector will be covered with a jacket to prevent damage to the animal or the connector from the animal scratching or moving against objects, such as the cage. Following surgery, animals will be kept under constant supervision until they are sternal. Buprenorphine will be administered twice daily for 3 days post operatively for analgesia. They will be housed and under the care of the veterinarians and

experienced technicians of the Cleveland VA animal facility. The skin sutures will be removed seven-ten days after operation.

### **Spinal Cord Transection (surgical)**

In a separate surgery, which will occur two weeks following the initial implant surgery to allow for extended recovery and testing of the electrode implant, the animal will be spinalized. The same induction and surgical preparation procedures will be used as is listed in the Implantation of Electrodes Procedure. Using aseptic methods, a laminectomy will be made at the lumbo-thoracic junction to expose the thoracic spinal cord (T10-T12). The dura will be opened, local anesthetic (1% Marcaine) will be applied to the cord, and the cord will be elevated slightly within the spinal canal and transected under magnification with a surgical microscope. Hemostasis will be ensured using a fine tip thermal cauterizer. Gelfoam will be packed between the caudal and rostral ends of the transection to minimize bleeding. The dura will be closed and checked for cerebrospinal fluid leakage. Hemostasis of the cut bone ends will be improved by applying sterile bone wax. The surgical wound will be repaired in layers and bandaged. Post-operative care will be the same as with the electrode implantation.

### **Biweekly Testing Sessions for Spinalized Animals (nonsurgical)**

Stimulation prior to spinal transection will determine the status of the implanted electrodes and if the animal has the appropriate responses to be considered for spinalization. In addition, every 2 weeks after spinalization, the cats will undergo an experimental test under anesthesia. Anesthesia will be induced with ketamine (30 mg/kg IM) and maintained with Propofol (0.3 mg/kg/min IV). In our previous experience with chronic spinalized cats and routine testing sessions, we found that propofol was the preferred anesthetic for induction and maintenance of an anesthetic plane over ketamine for the animal to easily go down and recover on a regular basis while maintaining our ability to efficiently collect data on spinal reflex-drive functions. A cephalic catheter will be placed for access to a vein. The cats will then be intubated with the appropriate size endotracheal tube and the sacral dermatome (upper thigh) area will be shaved. The cat will then be placed on a monitoring system for such vitals as heart rate, pulse oxygenation, and expired CO<sub>2</sub>, and will be maintained on continuous Propofol IV (0.3 mg/kg/min) for the duration of the testing session, which should last approximately 4 hours. Chloralose is the preferred anesthetic because it does not suppress spinal reflex pathways as other anesthetics do. However, it would not be feasible to use chloralose for a 4-hour testing session every two weeks because it is a strong, long-lasting drug. A light dose of propofol is more practical for biweekly testing because it is sufficient to keep the animal still without significantly altering reflex responses. Then when the testing session is finished we can stop IV flow of propofol and the animal will recover quickly and safely. Three balloon catheters will be inserted through the anus into the colon to measure colonic and rectal pressures. Small needle electrodes will be placed around the anus to measure external anal sphincter electromyograms. Testing will involve delivering electrical stimulation to the already-implanted electrodes while pressure and motility responses, and external anal sphincter responses, are recorded. Instrumentation, including balloon catheters, endotracheal tube, cephalic catheter (if used), and external anal sphincter electromyogram electrodes, will be removed and the animal will be monitored until she is sternal.

### **Daily Stimulation Testing (nonsurgical)**

Four treatment conditions (mechanical stimulation, implanted or minimally invasive electrical stimulation, and no stimulation) will be tested to measure their effects on defecation time, defined as the time from stimulation onset to complete bowel emptying. Treatment conditions will be randomized and applied daily. Animals will have their bladders expressed twice daily. During the first care visit, before the bladder is expressed, stimulation will be applied and defecation time recorded. If defecation is not completed within two hours, animals will be given a water enema to flush the bowels clean. This daily testing is meant to maintain the animal's bowels and will not involve surgery, anesthesia, or drugs.

### **Terminal Procedure (surgical)**

Animals in Aim 1 will only undergo a terminal experiment. Animals in Aim 2 will undergo a terminal experiment either after the Biweekly Testing Sessions if they advanced to the spinalization phase, or after the Pre-spinalization Implant Testing Session if they were not appropriate to advance to the chronic spinalization phase.

General anesthesia induced with ketamine and maintained with isoflurane (1-3%). All surgical manipulations will be performed under isoflurane anesthesia. A cephalic catheter will be placed for IV access. The animal will then be intubated to assist in respiration. All incision sites will be shaved and aseptically prepared with 3-5 alternating rotations of isopropyl alcohol and betadine. The animal will be attached to probes connecting to a monitoring system, which monitors ECG, heart rate, SPO<sub>2</sub>, CO<sub>2</sub>, respiratory rate, non-invasive blood pressure, and temperature. An intraurethral catheter will be placed to keep the bladder empty. A small lateral incision at the neck will expose the carotid artery and an arterial catheter will be placed to measure continuous invasive arterial blood pressure.

With the animal in a prone position, a midline sacral incision will be made to expose the sacral spine. A laminectomy will remove the bone of the spine to expose the sacral roots from S2 to S4. A nerve cuff electrode will be implanted around the sacral root S2 for activating motor efferents to the colon later. The electrode leads will be secured to the skin and the surgical site closed with sterile suture. The animal will be transitioned to the supine position and a midline suprapubic incision will be made to expose the colon, rectum, and its nerves. The colon will be emptied of stool by flushing with saline via the anus. Three balloon catheters - 2 in the colon and 1 in the rectum - will be inserted through the anus into the colon to measure colon and rectal pressures and small needle electrodes will be placed around the anus to measure external anal sphincter electromyograms. The animal will then be transitioned from isoflurane to chloralose anesthesia gradually over the course of an hour. The anesthetic is switched because isoflurane is known to suppress spinal reflexes, which we are studying, and chloralose does not have this effect. We typically use chloralose anesthetic for our preclinical bladder experiments because sacral reflexes remain intact and other anesthetics can suppress these reflexes and this chloralose-anesthetized cat preparation has been used by others for studying bowel reflexes. Buprenorphine will be administered as an analgesic to supplement chloralose.

Testing will include mechanical or electrical stimulation with varying stimulus parameters. After completing stimulation testing, the sacral dorsal roots will be transected, removing sensory input to the spinal cord, and stimulation testing will be repeated.

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

► Cats are chosen as the animal model for bowel function because this is the lowest animal that controls its bowels with behaviors similar to humans and will fit our instrumentation. The literature demonstrates a history of use of cats for bowel function experiments and the recto-colonic reflex has been demonstrated in cats. Female cats are chosen for the chronic studies in Aim 2 because bladder expression (part of twice daily SCI animal care) is easier and less traumatic in female cats.

### 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], PhD

Animal research experience ► 8+ years of experience in animal surgery and electrophysiology experiments in cats, rats, and mice.

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
Electrode Implantation	8+ years of experience
Spinalization	1 year of experience
Biweekly Test Sessions	1 year of experience
Daily Stimulation	1 year of experience
Terminal Procedure	8+ years of experience

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

Name ► [REDACTED], PhD

Animal research experience ► 20+ years of experience with animal surgery and electrophysiology in cats and other species (including rats, mice, and dogs).

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Electrode Implantation	20+ years of experience
Spinalization	10+ years of experience
Biweekly Test Sessions	10+ years of experience
Daily Stimulation	10+ years of experience
Terminal Procedure	20+ years of experience

Name ► [REDACTED]

Animal research experience ► 6 months of experience with animal surgery and electrophysiology 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
Terminal Procedure	6 months of experience supporting terminal procedure (anesthesia, animal monitoring, animal handling).

Name ▶ [REDACTED]

Animal research experience ▶ 6 months of experience with animal surgery and electrophysiology 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
Electrode Implantation	to be trained
Spinalization	to be trained
Biweekly Test Sessions	to be trained
Daily Stimulation	to be trained
Terminal Procedure	6 months of experience supporting terminal procedure (anesthesia, animal monitoring, animal handling).

Name ▶ [REDACTED]

Animal research experience ▶ None.

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Electrode Implantation	to be trained
Spinalization	to be trained
Biweekly Test Sessions	to be trained
Daily Stimulation	to be trained
Terminal Procedure	to be trained

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

Name ▶ [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)
Induction and animal monitoring during surgical procedures	BS from University of Akron and 5 years exp working in veterinary offices doing surgeries, and animal care
Animal husbandry	BS from University of Akron and 5 years exp working in veterinary offices doing surgeries, and animal care

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)

<p>[REDACTED], PhD</p>	<p>8/10/15</p>	<p>Occupational Health VA 7/29/15</p> <p>VA ARF Orientation 7/29/15</p> <p>Waste Anesthetic Gas Policy 7/29/15</p> <p>Working with Cats in Research Settings 7/29/15</p>	<p>Safety Training 6/17/15</p> <p>Facility ARF Orientation Please contact Dr. [REDACTED] to verify</p> <p>Facility hand on training with vet Please contact Dr. [REDACTED] to verify</p>
<p>[REDACTED], PhD</p>	<p>12/26/14</p>	<p>Occupational Health VA 6/17/15</p> <p>VA ARF Orientation 6/17/15</p> <p>Waste Anesthetic Gas Policy Please complete</p> <p>Working with Cats in Research Settings 12/26/14</p>	<p>Safety Training 4/27/15</p> <p>Facility ARF Orientation Please contact Dr. [REDACTED] to verify</p> <p>Facility hand on training with vet Please contact Dr. [REDACTED] to verify</p>
<p>[REDACTED]</p>	<p>5/16/14</p>	<p>Occupational Health VA Please complete</p> <p>VA ARF Orientation Please complete</p> <p>Waste Anesthetic Gas Policy Please complete</p> <p>Working with Cats in Research Settings Please complete</p>	<p>Safety Training 5/4/15</p> <p>Facility ARF Orientation Please schedule with Dr. [REDACTED]</p> <p>Facility hand on training with vet Please schedule with Dr. [REDACTED]</p>

[REDACTED]	4/4/15	Occupational Health VA 4/3/15  VA ARF Orientation 4/3/15  Waste Anesthetic Gas Policy Please complete  Working with Cats in Research Settings 4/3/15	Safety Training 5/15/15  Facility ARF Orientation Please schedule with Dr. [REDACTED]  Facility hand on training with vet Please schedule with Dr. [REDACTED]
[REDACTED]	Please complete	Occupational Health VA Please complete  VA ARF Orientation Please complete  Waste Anesthetic Gas Policy Please complete  Working with Cats in Research Settings Please complete	Safety Training 1/9/15  Facility ARF Orientation Please schedule with Dr. [REDACTED]  Facility hand on training with vet Please schedule with Dr. [REDACTED]

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"

▶ [REDACTED], [REDACTED], and [REDACTED] are students working with Drs. [REDACTED] and [REDACTED]. Ms. [REDACTED] and Ms. [REDACTED] have completed relevant animal training to handle and monitor animals and have begun training with Drs. [REDACTED] and [REDACTED] on surgical procedures relevant to the terminal procedure. Drs. [REDACTED] and [REDACTED] will continue to train them and Mr. [REDACTED] for the terminal procedure. The chronic study, which includes the other procedures, is planned to start after acute studies have been completed. During that time Ms. [REDACTED] and Mr. [REDACTED] will be trained by Drs. [REDACTED] and [REDACTED] on procedures relevant to the chronic study (Ms. [REDACTED] is expected to have graduated before that time and will not receive that training).

**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], PhD	(X)	( )	( )	
[REDACTED], PhD	(X)	( )	( )	
[REDACTED]	( ) Please complete	( )	( )	
[REDACTED]	( ) Please complete	( )	( )	
[REDACTED]	( ) Please complete	( )	( )	

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 ▶ Special precautions in the event of the occurrence of a cat bite include notification of Personnel Health and the VA veterinary staff. The animal that caused the bite must be quarantined for 10 days. If the animal dies during this time or exhibits signs associated with rabies, the animal must be euthanized and the head shipped for rabies testing. If rabies testing is positive, or the carcass is discarded prior to testing, the bitten individual must undergo rabies post exposure treatment by Personnel Health.

▶ ( ) 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
<b>Domestic short-haired cat</b>	<b>M/F</b>	<b>6-12 months; 3.5-5.0 kg</b>	[REDACTED]	<b>SPF, purpose-bred</b>

- 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
<b>cat</b>	<b>10</b>	<b>10</b>	<b>3</b>	<b>3</b>		<b>16</b>

**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
<b>cat</b>	<b>10</b>	<b>10</b>	<b>3</b>	<b>3</b>		<b>16</b>

- 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)
<b>Electrode Implantation</b>	<b>Daily for the first 3 days and then weekly</b>	[REDACTED], [REDACTED],	<b>See Appendix 5 for details</b>
<b>Spinalization</b>	<b>Twice daily for the entire duration</b>	[REDACTED], [REDACTED],	<b>See Appendix 5 for details</b>
<b>Biweekly Testing</b>	<b>Entire procedure</b>	[REDACTED], [REDACTED],	<b>See Appendix 5 for details</b>
<b>Terminal Procedure</b>	<b>Entire procedure</b>	[REDACTED], [REDACTED],	<b>See Appendix 5 for details</b>

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], DVM, DACLAM

Institutional affiliation ▶ LSCDVAMC

email contact ▶ [REDACTED], [REDACTED]

2. Veterinary consultation during the planning of this protocol.

Name of the laboratory animal veterinarian consulted ▶ [REDACTED]

Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ▶ 8/25/15

M. **Husbandry.** As a reference for the animal husbandry staff, 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 <i>Guide</i> and USDA regulations? (yes/no***)	e. Estimated maximum number of housing units needed at any one time
cat	Group housed	8/room	Yes	1-2
cat	Single housing in the post-operative period for 2 weeks	1	Yes	1-2

\*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:

► Cages for spinalized cats will not be equipped with a litter pan or resting board as these cats will not be able to use them.

\*\* 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 will only be singly housed during the 2-week recovery period following spinalization to allow for safe conditions for healing (see Appendix 9). Otherwise, animals will be kept in social housing in accordance with the *Guide*.

\*\*\*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
cat	Cages for spinalized cats will not be equipped with a litter pan or resting board as these cats will not be able to use them.	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.

► Animals with spinal transections will not have intact motor or sensory function of the hindquarters. Therefore, resting boards will not be placed in their cages because the animals may develop pressure

sores from them. Similarly litter boxes will not be placed because the animals will not use them and they may develop pressure sores from resting against the edge of a litter box (see Appendix 9).

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.



- ▶ (X) 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.



The fine wires leading to the electrodes will cross the skin at the connector. The site will be inspected daily during the first week, then weekly, and again daily during the SCI phase. The site will be bandaged initially. If needed, surgical Dacron will be added to the subcutaneous exit site to promote tissue ingrowth. The wires are very fine and flexible; therefore, exit site "pistoning" is not expected as a complication. Research personnel will be responsible for bandage and wound care, and post-op care.

We prefer and intend to house animals socially. However, due to the electrode wire exit site, the use of jackets or single housing may be required if animals cause problems for the electrode wire exit site. In general, animals will be housed single after the procedures until they are recovered enough for group housing.

- ▶ (X) 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.



Once cats have received the spinal transection they will require special housing. Resting boards, part of enrichment housing, may be removed if the cats are becoming caught under them. Litter boxes may be removed or modified so the cats do not unknowingly rest on rim of a litter box and create pressure sores. Since the cats will not be able to urinate on their own, and will be unaware of when they are defecating, the litter boxes will not be necessary. Cardboard sheets should be placed on the bottoms of the cages to prevent sores and legs being caught in the grates. This will also allow for quick cleanup of soiled cages.

- ▶ ( ) 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	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
Electrode Implantation	(X)	( )	ARF surgery [REDACTED]	( )	(X)
Spinalization	(X)	( )	ARF surgery [REDACTED]	( )	(X)
Biweekly Test Sessions	( )	(X)	PI Lab	(X)	( )
Daily Stimulation	( )	(X)	Animal housing room	( )	(X)
Terminal Procedure	(X)	( )	PI Lab	(X)	( )

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")
Implanted electrodes	(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.

In cases of severe and untreatable sores and self-mutilation, animals will be euthanized.

Weight loss after SCI is normal and expected due to hindlimb muscle wasting and bone loss from disuse of paralyzed limbs (estimated 10%); however, this weight loss will be extended over a several week period. Acute weight loss (defined as more than 10% of an animal's weight over a one or two week period) will be taken as a serious health issue, and will be discussed with the veterinary staff as grounds for euthanasia.

If the spinal transection is not 100% complete and the animal shows signs of remaining sensation, we will not automatically consider the animal for termination (since partial SCI can still result in chronic colonic dysfunction, bladder and urethral spasticity), unless the animal shows indication of intractable pain or co-morbidities already described here. In addition, if an incomplete transection results in the animal being able to feel electrical stimulation and that stimulation causes pain or distress, then we will not perform stimulus testing without anaesthesia. Pain or distress will be determined by behavioral responses to pinching and electrical stimulation, such as vocalization, ears drawn back, or withdrawal. If the animal, whether with an incomplete or complete SCI, suffers chronic, unmanageable pain or distress, it 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 <sub>2</sub> from a compressed gas tank Duration of exposure after apparent clinical death ► Method for verifying death ► Secondary physical method ►		( )	( )	( )

(X)	Anesthetic overdose Agent ▶ Euthasol Dose ▶ 100 mg/kg Route of administration ▶ IV	cat	(X)	( )	( )
( )	Decapitation under anesthesia Agent ▶ Dose ▶ Route of administration ▶		( )	( )	( )
( )	Exsanguination under anesthesia Agent ▶ Dose ▶ Route of administration ▶		( )	( )	( )
( )	Other (Describe) ▶		( )	( )	( )
( )	Other (Describe) ▶		( )	( )	( )

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.  
▶  
 ██████████ and ██████████ 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 electrodes 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], [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
Biweekly Testing Sessions		Items:	(X)**
Daily Stimulation Testing		Items:	(X)**
		Items:	( )**
		Items:	( )**

\*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.

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.

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)
Pubmed	8/14/15		terminal procedure, spinalization, electrode implant, biweekly testing	Bowel function, colonic motility, spinal cord injury, digital rectal stimulation, nerve stimulation, computational model, cat	(X)	(X)	(X)	(X)
ALTBIB	8/14/15		terminal procedure, spinalization, electrode implant, biweekly testing	Bowel function, colonic motility, spinal cord injury, digital rectal stimulation, nerve stimulation, computational model, cat	(X)	(X)	(X)	(X)
Web of Science	8/14/15		terminal procedure, spinalization, electrode implant, biweekly testing	Bowel function, colonic motility, spinal cord injury, digital rectal stimulation, nerve stimulation, computational model, cat	(X)	(X)	(X)	(X)

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, dogs, and pigs are typically used for bowel function research, including research focusing on neurophysiology and electrophysiology. Smaller animals, such as rodents, may be appropriate for some neurophysiology studies, but the reflex pathways that we are studying have not been demonstrated in rodents. In addition, rodents do not exhibit the same bowel and storage defecation behaviors that larger animals do. No computer models have been found via searches or scientific conferences. There are presently no computer models that adequately represent the rectal afferent reflex pathways involved in these studies. The physiology of control of bowel function is insufficiently understood to allow for adequate computer simulation and the system is too complex for in vitro or tissue culture studies. Indeed, this work attempts to provide the characterization and refinement of our understanding of these reflexes.

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.



Bowel function is a very slow process, which limits our ability to collect data in a feasible amount of time, which is why we split Aim 1 into two sub-aims. Where possible, tests and outcome measures from Aims 1.1 and 1.2 will be conducted to further minimize the number of animals used. The chronic study was designed based on our experience and the literature to achieve statistically significant data in few animals by extending the length of the chronic study and thus record more data. The length of the chronic study, however, does not increase pain or discomfort to the animals. A power analysis was conducted to determine the smallest number of animals needed to achieve these research objectives. The effect sizes were based on outcomes reported in the literature for mechanical stimulation of the reflex that we are studying with electrical stimulation, and the sample sizes are similar as those in other animal studies for bowel function, both acute and chronic.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.



Searches showed that the type of surgical anesthesia used for our procedures is the most normal and safest for cats. We have planned out our pain management to include fentanyl transdermal patches, which will deliver long lasting pain medication with little manipulation of the animal (as opposed to injectable opioids) following surgical procedures. We have performed a similar study in chronic spinalized cats and have modified this protocol from what we have learned. Finally, we will continue to contact other investigators conducting similar studies to search for best practices to maintain the chronic SCI animals.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.



This work does not unnecessarily duplicate previous work. We found fewer than a dozen journal articles reporting on stimulation (mechanical or electrical) to promote defecation in animals and all but three of those papers were published before the year 2000. Most of them explored mechanical distension of the rectum to promote defecation, which we hope to evoke electrically with a similar mechanism. One paper examined the neural pathways of the recto-colonic reflex and defecation in response to mechanical and electrical stimulation (de Groat and Krier 1978), but did not focus on the effects of varying electrical stimulation parameters to evoke defecation reflexes. A few papers tested electrical stimulation to evoke defecation. However, they all used different electrical stimulation approaches, these approaches were not practical for clinical translation, and they did not take advantage of the neural pathways elucidated by de Groat and others. Therefore, the work planned in this protocol will make an important impact by identifying a practical, reliable approach for restoring

bowel function, which can be translated clinically. 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. Indeed, we spoke with Dr. de Groat regarding his earlier work and discussed the impact of our research aims.

**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
Ketamine	(X)	( )*	[REDACTED]	(X)	( )	(X)	( )
Fentanyl	(X)	( )*	[REDACTED]	(X)	( )	(X)	( )
Buprenorphine	(X)	( )*	[REDACTED]	(X)	( )	(X)	( )
Euthasol (phenytoin + pentobarbital)	(X)	( )*	[REDACTED]	(X)	( )	(X)	( )
Propofol	(X)	( )*	[REDACTED]	(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 be used on VA property, and all of these will be obtained through the local VA pharmacy.

▶ ( ) 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"
- ▶ ( ) 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"
- ▶ (X) 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	[REDACTED] to complete		
M.1	Animal care and use program SOP's	5.1	2/2014
M.2	Animal care and use program SOP's	5.2	2/2014
U.4.a	Animal care and use program SOP's	5.35.2	2/2014

U.4.b	Animal care and use program SOP's	6.4	2/2014
V			

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
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[REDACTED]	[REDACTED]	9/25/2015

**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:
  - ▶ ( ) 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]	10-9-15
Name of IACUC Chair	Signature	Date
[REDACTED]	[REDACTED]	9/24/15

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
[REDACTED]	[REDACTED]	9/25/2015
Name of Institutional Veterinarian	Signature	Date
[REDACTED]	[REDACTED]	10/19/15
Name of IACUC Chair	Signature	Date
[REDACTED]	[REDACTED]	9/24/15

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]	9/28/15

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]	9/25/05

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

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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 All Materials Administered to Animals on this Protocol.** Complete the table below for all 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
Ketamine	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Isoflurane	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Chloralose	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Buprenorphine	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Propofol	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Duragesic patch (Fentanyl)	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Marcaine (bupivacaine)	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Cephazolin (antibiotic)	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Baytril (antibiotic) (enrofloxacin)	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Metacam (meloxicam)	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Gel Foam	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )
Bone Wax	VA Pharmacy	( )	( ) BSL_	( )	( )	( )	(X)	( )

Euthasol (pentobarbital + phenytoin)	VA Pharmacy	( )	( )BSL_	( )	( )	( )	( )	(X)
Electrode implants	[REDACTED] Lab	( )	( )BSL_	( )	( )	( )	( )	( )

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)
Ketamine	30 mg/kg (1 mL)	Sterile saline	IM	Induction	Anesthetic	Main Body	N
Isoflurane	1-3%	Vaporized	Inhalant	Maintenance	Anesthetic	Main Body	Y
Chloralose*	85 mg/kg (30 mL), 10 mg/kg/hr (5 mL)	Sterile saline	IV	Every 4 hours	Anesthetic, for Terminal Procedure testing	Main Body	Y
Propofol	0.3 mg/kg/min (0.1 mL)	Sterile saline	IV	Maintenance	Anesthetic	Main Body	Y
Buprenorphine	0.01 mg/kg (0.2 mL)	Sterile saline	IV, IM, or SQ	Every 12 hours during surgery with chloralose	Analgesic	Main Body	Y
Duragesic patch (Fentanyl)	25 mcg patch	N/A	Topical	12 hours prior to surgery	Analgesic	App 5	N
Metacam (meloxicam)	0.1 mg/kg (0.25 mL)	Sterile saline	SQ	Pre-op; Post-op SID, 5 days	Analgesic	App 5	N

Marcaine (bupivacaine)	1.25 mg/kg (1 mL)	Sterile saline	SQ	Once, Before electrode implant or spinalization	Analgesic	App 5	Y
Cephazolin	25 mg/kg (0.5 mL)	Sterile saline	SQ	Pre-op; Post-op BID, 7-10 days	Antibiotic	App 5	N
Baytril (antibiotic) (enrofloxacin)	5 mg/kg (1 mL)	Sterile saline	SQ	Pre-op; Post-op SID, 7-10 days	Antibiotic	App 5	N
Gel Foam	One application, (5 cc)	Solid	Surface application	Once, during Spinalization	Minimize bleeding at spinal cord	App 5	Y
Bone Wax	One application, (5 cc)	Solid	Surface application	Once, during Spinalization	Minimize bleed of spine (bone)	App 5	Y
Euthasol (pentobarbital + phenytoin)	100 mg/kg (2 mL)	Sterile saline	IV	Endpoint	Euthanasia	Main Body	Y
Electrode implants	1 electrode	electrode	Extra-neural	Implantation procedure	Neural Interface	App 5	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.)

► Chloralose is used because isoflurane is known to suppress spinal reflexes, which we are studying, and chloralose does not have this effect. We typically use chloralose anesthetic for our preclinical bladder experiments because sacral reflexes remain intact and other anesthetics can suppress these reflexes and this chloralose-anesthetized cat preparation has been used by others for studying bowel reflexes. Chloralose will be mixed in sterile saline (for injection) in a clean beaker. The beaker will be covered and placed on a heater/stirrer with a magnetic stirrer because mixing chloralose requires low heat and constant mixing. When a dose of chloralose needs to be administered, the appropriate volume of chloralose will be drawn into a sterile syringe and allowed to cool to body temperature before injecting chloralose intravenously. An inline filter will be placed between the syringe and needle for additional filter sterilization.

**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):

►  
 Cats will be anesthetized with 30 mg/kg ketamine by IM injection. Then, electrodes will be implanted under 1-3% vaporized, inhaled isoflurane maintenance anesthesia.

Under 1-3% vaporized isoflurane anesthesia during the Spinalization procedure, Marcaine is applied as an analgesic on the spinal cord before it is transected, Gel foam is applied to minimize bleeding at the spinal cord transection site and Bone wax is applied to minimize bleeding on the cut ends of bone of the spine.

During the Terminal Procedure, Buprenorphine (0.01 mg/kg IV) is given as an analgesic in combination with chloralose anesthesia (10 mg/kg/hr IV). Buprenorphine (0.01 mg/kg IM) is also provided for post-operative analgesia without anesthesia.

At the end of the Terminal Procedure, under 1-3% vaporized isoflurane anesthesia, Euthasol (100 mg/kg IV) will be administered to euthanize the animal.

- 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)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(Yes/No)	( )	( )	( )**
		(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 <i>NIH Guidelines for Research Involving Recombinant DNA Molecules</i>	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
Implanted electrodes	Surgical dissection	General anesthesia and post-operative analgesia listed in Appendix 5.

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 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	Electrode Implantation	( )	( )	(X)	(X)*
2	Spinalization	( )	( )	(X)	(X)*
3	Terminal Procedure (expose abdominal cavity, spinal cord)	(X)	( )	( )	( )*
4		( )	( )	( )	( )*

\*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:



We believe that the approach of separating the surgical procedures is justified and we have successfully made the case during both NIH and VA grant reviews. We chose this study design to minimize the number of spinalized animals required to perform the study since spinalization is the greatest source of potential animal morbidity. Using only one procedure to both implant electrodes and spinalized animals would result in some spinalized animals being unsuccessful due to ineffective sensory afferent stimulation or implant problems. By only spinalizing those animals that successfully demonstrate reflex activation of the bowel in response to sensory afferent stimulation, we reduce the number of animals that need to be spinalized.

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



The interval will be long enough to determine that sensory afferent stimulation effectively produces bladder contractions in that animal, which we estimate to be approximately 2 weeks. This time will also allow complete recovery from the initial surgery.

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 ► Electrode Implantation**

Twelve hours prior to surgery, animals will begin fasting and a Fentanyl patch will be applied. One-two hours pre-operatively, Metacam (0.1 mg/kg, SQ) will be administered to enhance post-operative analgesia, and Cephazolin (25 mg/kg, SQ) and Baytril (5 mg/kg, SQ) will be administered for pre-

operative antibiotic prophylaxis. General anesthesia induced with ketamine (30 mg/kg, IM) and maintained with isoflurane (1-3%). A cephalic catheter will be placed for IV access. 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, SPO<sub>2</sub>, CO<sub>2</sub>, 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. Incision sites will be injected with Marcaine before incision. A small midline abdominal incision will expose the abdominal cavity, colon, and peripheral nerves of the colon. A fine wire electrode (or nerve cuff electrode, if appropriate) will be implanted on the target colonic or rectal afferent nerve located on the ventral surface of the distal colon. Implantation of the electrode is not expected to interfere with other anatomical structures. The electrode wires will be tunneled up to the back and secured where they exit the skin to a connector at a second incision site. The surgical wounds will be closed. This small connector will be covered with a jacket to prevent damage to the animal or the connector from the animal scratching or moving against objects, such as the cage. The purpose of the jacket is to cover the small connector and protect it. It will be worn for as long as the animal has the connector – from electrode implant to terminal procedure, up to 16 weeks. Following surgery, animals will be kept under constant supervision until they are sternal. Buprenorphine (0.01 mg/kg, SQ) will be administered twice daily for 3 days and Metacam (0.1 mg/kg SQ) will be administered once daily for 5 days post operatively for analgesia. Cephazolin (25 mg/kg, SQ) and Baytril (5 mg/kg, SQ) will be administered twice daily for 7-10 days as an antibiotic. They will be housed and under the care of the veterinarians and experienced technicians of the Cleveland VA animal facility. The skin sutures will be removed seven days after operation.

#### Surgery 2 ► Spinalization

Following an extended recovery and testing period from the initial electrode implant surgery (which will last approximately 2 weeks), the animal will be spinalized. Twelve hours prior to surgery, animals will begin fasting and a Fentanyl patch will be applied. The same pre-operative, induction, and surgical preparation procedures will be used as is listed in the Electrode Implantation Procedure. Using aseptic methods, a laminectomy will be made at the lumbo-thoracic junction to expose the thoracic spinal cord (T10-T12). The dura will be opened, local anesthetic (Marcaine) will be applied to the cord, and the cord will be elevated slightly within the spinal canal and transected under magnification with a surgical microscope. Hemostasis will be ensured using a fine tip thermal cauterizer. Gelfoam will be packed between the caudal and rostral ends of the transection to minimize bleeding. The dura will be closed and checked for cerebrospinal fluid leakage. Hemostasis of the cut bone ends will be improved by applying sterile bone wax. The surgical wound will be repaired in layers and bandaged. Post-operative care will be the same as with the electrode implantation.

#### Surgery 3 ► Terminal Procedure

Animals in Aim 1 will only undergo a terminal experiment. Animals in Aim 2 will undergo a terminal experiment either after the Biweekly Testing Sessions if they advanced to the spinalization phase, or after the Pre-spinalization Implant Testing Session if they were not appropriate to advance to the chronic spinalization phase.

General anesthesia induced with ketamine and maintained with isoflurane (1-3%). All surgical manipulations will be performed under isoflurane anesthesia. A cephalic catheter will be placed for IV access. 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, SPO<sub>2</sub>, CO<sub>2</sub>, respiratory rate, non-invasive blood pressure, and temperature. An intraurethral catheter will be placed to keep the bladder empty. A small lateral incision at the neck will expose the carotid

artery and an arterial catheter will be placed to measure continuous invasive arterial blood pressure.

With the animal in a prone position, a midline sacral incision will be made to expose the sacral spine. A laminectomy will remove the bone of the spine to expose the sacral roots from S2 to S4. A nerve cuff electrode will be implanted around the sacral root S2 for activating motor efferents to the colon later. The electrode leads will be secured to the skin and the surgical site closed with sterile suture. The animal will be transitioned to the supine position and a midline suprapubic incision will be made to expose the colon, rectum, and its nerves. The colon will be emptied of stool by flushing with saline via the anus. Three balloon catheters will be inserted through the anus into the colon to measure colon pressures and small needle electrodes will be placed around the anus to measure external anal sphincter electromyograms. The animal will then be transitioned from isoflurane to chloralose anesthesia gradually over the course of an hour. The anesthetic is switched because isoflurane is known to suppress spinal reflexes, which we are studying, and chloralose does not have this effect. We typically use chloralose anesthetic for our preclinical bladder experiments because sacral reflexes remain intact and other anesthetics can suppress these reflexes and this chloralose-anesthetized cat preparation has been used by others for studying bowel reflexes. Buprenorphine will be administered as an analgesic to supplement chloralose.

Testing will include mechanical or electrical stimulation with varying stimulus parameters. After completing stimulation testing, the sacral dorsal roots will be transected, removing sensory input to the spinal cord, and stimulation testing will be repeated.

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)	(X)	( )
[REDACTED]	1, 2, 3	(X)	(X)	(X)	( )
[REDACTED]	3	( )	(X)	(X)	( )
[REDACTED]	1, 2, 3	(X)	(X)	(X)	( )
[REDACTED]	1, 2, 3	( )	(X)	(X)	( )

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

Building	Room	Surgery	Type of Space
----------	------	---------	---------------

	Number	#(s) (see Item 1)	Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
LSCDVAMC ARF	ARF surgery	1, 2, 3	(X)	( )*	( )*
LSCDVAMC	TBD Lab	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 hours	( ) --	(X) – cephalic vein	(X) – Metacam, Baytril, Cephazolin
2	(X) – 12 hours	( ) --	(X) -- cephalic vein	(X) -- Metacam, Baytril, Cephazolin
3	(X) – 12 hours	( ) --	(X) -- cephalic vein	(X) -- empty bowel
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 prior to preparation of the surgical site on the animal.

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Fentanyl patch	1, 2	Patch	topical	once	12 hours pre-op
Metacam	1, 2	0.1 mg/kg (0.25 mL)	SQ	Once	1-2 hours pre-op
Cephazolin	1, 2	25 mg/kg (0.5 mL)	SQ	Once	1-2 hours pre-op

Baytril	1, 2	5 mg/kg (1 mL)	SQ	Once	1-2 hours pre-op
Ketamine	1, 2, 3	30 mg/kg (1 mL)	IM	once	Induction

- 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 ► Electrode Implantation**

Incision sites include the lower abdominal midline and the back between the shoulder blades. Areas will be shaven and cleaned with 3 cycles of betadine and alcohol scrubs, then injected with Marcaine. Prior to the first incision the animal will be draped on all sides with sterile surgical drape such that only shaved and scrubbed areas of the body are exposed.

**Surgery 2 ► Spinalization**

The incision site will be the middle of the back at the lower thoracic region. The area will be shaven and cleaned with betadine and alcohol, then injected with Marcaine. Prior to the first incision the animal will be draped on all sides with sterile surgical drape such that only shaved and scrubbed areas of the body are exposed.

**Surgery 3 ► Terminal Procedure**

Incision sites include the lower abdominal midline and the lower back at the sacral region. Areas will be shaven and cleaned with betadine and alcohol.

**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
<b>Isoflurane</b>	( )*	<b>1, 2, 3</b>	<b>1-3%</b>	<b>Inhalant</b>	<b>Maintenance</b>
<b>Chloralose</b>	( )*	<b>3</b>	85 mg/kg (30 mL), 10 mg/kg/hr (5 mL)	<b>IV</b>	<b>Every 4 hours</b>
<b>Buprenorphine</b>	( )*	<b>3</b>	0.01 mg/kg (0.2 mL)	<b>IV</b>	<b>Every 12 hours</b>
<b>Marcaine</b>	( )*	<b>1, 2</b>	1.25 mg/kg (1 mL)	<b>Topical/SQ</b>	<b>Once</b>
<b>Euthasol</b>	( )*	<b>3</b>	100 mg/kg (2 mL)	<b>IV</b>	<b>Once</b>

\* 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.).

►  
 Cats will be intubated with the appropriate size tracheal tube to keep airways open. Our anesthesia machine will supply ventilation in cases of depressed respiration. Cats will also receive a cephalic vein catheter to allow access for IV administration of emergency drugs if necessary and also to deliver IV fluids of either lactated ringers or sodium chloride with dextrose and sodium bicarbonate added. The cats will also be connected to a dextrose/saline drop at 10ml/kg/hr. Cats will be placed on a water circulating heating pad to help maintain core body temperature of 38.5 degrees C. 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.

►  
 Cats will be ventilated throughout surgery at 10-12 bpm on an anesthesia and ventilating machine. We will monitor ECG, heart rate, respiratory rate, expired CO2, SPO2, noninvasive blood pressure or arterial blood pressure, and temperature. Using these vital signs in conjunction with reflex responses such as eye blink, toe punch, 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.

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 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
2	8-12 weeks	(X)	(X)	(X)	(X)	(X)	(X)	(X)	( )*
		( )	( )	( )	( )	( )	( )	( )	( )*
		( )	( )	( )	( )	( )	( )	( )	( )*

\* Describe any "other" measures to be taken to maintain sterility during surgery.

►

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

**Surgery 1 ► Electrode Implantation**

Monitoring will be continuous until endotracheal extubation. Subsequently, the animals will be monitored closely (at least every 30 minutes) until they are in sternal recumbency. They will be kept in a recovery cage with blankets and a water circulating heating pad until they are ready for transport (which will not occur until sternal recumbency). They will retain the endotracheal tube until

appropriate breathing rates have been reestablished and until signs of swallowing or chewing occur and will retain an IV drip until sternal recumbency. Post-operative monitoring will be documented and maintained as part of the animal's medical record.

**Surgery 2 ► Spinalization**

**Same as Surgery 1:** The animals will be monitored closely until they are in sternal recumbency. They will be kept in a recovery cage with blankets and a water circulating heating pad until they are ready for transport (which will not occur until sternal recumbency). They will retain the endotracheal tube until appropriate breathing rates have been reestablished and until signs of swallowing or chewing occur and will retain an IV drip until sternal recumbency.

- 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	Buprenorphine	0.01 mg/kg	IM	BID	3 days
1, 2	Metacam	0.1 mg/kg	SQ	SID	5 days

\*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	Cephazolin	25 mg/kg	SQ	BID	7-10 days
1, 2	Baytril	5 mg/kg	SQ	SID	7-10 days

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

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)

1, 2	Continuous	until sternal	[REDACTED], [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	Twice daily	Until endpoint	[REDACTED], [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 ▶ Electrode Implantation**

The most likely consequences include nerve damage due to improper electrode implantation and infection. The electrode leads will be secured at the exit site and tacked down subcutaneously at implant. The animals will wear a jacket to protect the exit site and prevent the animal from playing with them. If any irritation around lead site is observed, it will be cleaned thoroughly and bandaged to promote healing. If nerve damage occurs and appears to be significant enough to affect the animal's health or the experiment, the animal will not advance to the chronic spinalization phase, but will undergo the terminal procedure. Following the initial surgery, initial antibiotics will be administered for infection. If an infection develops, the veterinarian will be consulted.

**Surgery 2 ▶ Spinalization**

Most complications will be **due to the hind limb paralysis**. We may see:

- Pressure sores
- Self-mutilation
- Depression
- Weight loss due to muscle mass loss

Following SCI, we may also see autonomic complications, such as:

- Constipation
- Urinary tract infection
- Urine and fecal soiling/scalding

Autonomic Dysreflexia (AD) -rapid increase in blood pressure above site of injury as a mechanism to alert brain of irritation of some kind below level of injury (e.g. extended bladder or bowel, etc.) In humans, AD can cause headaches, nausea, vomiting and sweating.

Following spinalization surgery, we will be using a detailed score sheet daily that will watch for all conditions listed below. Proactivity is the best defense against such complications.

**Urine and fecal soiling/scalding** - We have purchased feline shampoo and feline wipes for bathing purposes. Cats will receive a "sponge bath" with wipes daily or as often as needed to keep genital area clean and dry. If soiling is extensive, they will receive a full water and shampoo bath.

**Pressure sores-** The cat cage and lounge area will be supplied with cardboard and cloth bedding to soften the resting areas for the cats. Any sores observed will be treated with ointment and bandaged until healed.

**Self-mutilation and depression due to paralysis of hind limbs** - The first defense against self-mutilation is a happy, well-adjusted cat. We have put in place an extensive conditioning and environmental enrichment process well before the cats are chosen for spinal transection. Cats will be given multiple hours a day of playtime with investigators as well as the other cats in the room. This will encourage them to return to an active and healthy mental state, which historically has helped prevent animals from self-mutilating. In the case of self-mutilation we will treat the areas with ointment and bandaging to allow healing. We will also place e-collars on the cats to prevent access to mutilated areas. We will consider drug options for subduing anxiety or relieving pain if that appears to be the cause of the mutilation attempts. If after consulting with a veterinarian it is determined that self-mutilation is resulting from unmanageable pain, the animal will be euthanized.

**AD (autonomic dysreflexia)** – AD is the rapid increase in blood pressure in response to a physiological event or irritation and acts as a mechanism to alert the brain of this event. In humans with SCI, AD is usually caused by bowel obstructions, infections, extended bladders, pressure sores, and injuries. In these experiments, AD is primarily addressed by using a lower-level SCI since AD is more common in cervical level injuries. AD will also be minimized by using regular bladder and bowel management to prevent over-distension. In addition, the animal will be checked daily for development of any skin problems that could lead to pressure sores that could contribute to AD, as well as injuries that could lead to AD. We will also look for signs of irritability, altered mood, elevated temperature, and energy and activity level.

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

**Surgery 1 ► Electrode Implantation**

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.

**Surgery 2 ► Spinalization**

Signs of wound or urinary tract infections will be discussed. 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. In cases of severe and untreatable sores and self-mutilation, animals will be euthanized.

Weight loss after SCI is normal and expected due to hind limb muscle wasting and bone loss from disuse of paralyzed limbs (estimated 10%); however, this weight loss will be extended over a several week period. Acute weight loss (defined as more than 10% of an animal's weight over a one or two week period) will be taken as a serious health issue, and will be discussed with the veterinary staff as grounds for euthanasia.

If the spinal transection is not 100% complete and the animal shows signs of remaining sensation, we will not automatically consider the animal for termination (since partial SCI can still result in chronic bladder and urethral spasticity), unless the animal shows indication of intractable pain or co-morbidities already described here. In addition, if an incomplete transection results in the animal being able to feel electrical stimulation and that stimulation causes pain or distress, then we will not perform stimulus testing without anaesthesia. Pain or distress will be determined by behavioral responses to pinching and electrical stimulation, such as vocalization, ears drawn back, or withdrawal.

- (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 euthanized instead.)

► There are no drugs that should not be used in an emergency medical situation for the care of these animals.

- 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	Animal housing room	[REDACTED]	(X)	( )
2	Animal housing room	[REDACTED]	(X)	( )
3			( )	( )
4			( )	( )

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	Biweekly Testing Sessions	(X)	( )	( )	( )	( )	( )	( )	(X)
2	Daily Stimulation Testing	(X)	( )	( )	( )	( )	( )	( )	(X)
3	Daily baths, bladder care, etc.	(X)	( )	( )	( )	( )	( )	( )	( )
4		( )	( )	( )	( )	( )	( )	( )	( )

\*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.

► Electrical stimulation via implanted electrodes will be administered. For Biweekly Testing, animals will be anesthetized with propofol for up to 4 hours and instrumented for measuring colonic pressures and anal sphincter electromyograms. No surgeries are conducted as part of these special procedures.

- 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 ► Biweekly Testing Sessions**

Anesthesia will be induced with ketamine (30 mg/kg IM) and maintained with Propofol (0.3 mg/kg IV). A cephalic catheter will be placed for access to a vein. The cats will then be intubated with the appropriate size endotracheal tube and the sacral dermatome (upper thigh) area will be shaved. The cat will then be placed on a monitoring system for such vitals as heart rate, pulse oxygenation, and expired CO<sub>2</sub>, and will be maintained on Propofol (0.3 mg/kg/min, IV) for the duration of the testing session, which should last approximately 4 hours. Chloralose is the preferred anesthetic because it does not suppress spinal reflex pathways as other anesthetics do. However, it would not be feasible to use chloralose for a 4-hour testing session every two weeks because it is a strong, long-lasting drug. A light dose of propofol is more practical for biweekly testing because it is sufficient to keep the animal still without significantly altering reflex responses. Then when the

testing session is finished we can stop IV flow of propofol and the animal will recover quickly and safely. Three balloon catheters will be inserted through the anus into the colon to measure colonic and rectal pressures. Small needle electrodes will be placed around the anus to measure external anal sphincter electromyograms. Testing will involve delivering electrical stimulation to the already-implanted electrodes while pressure and motility responses, and external anal sphincter responses, are recorded. In addition, a small plug electrode will be inserted into through the anus into the rectum to test an alternative, minimally invasive electrical stimulation approach. Instrumentation, including balloon catheters, endotracheal tube, cephalic catheter (if used), and external anal sphincter electromyogram electrodes, will be removed and the animal will be monitored until she is sternal.

**Special Procedure 2 ► Daily Stimulation Testing**

Animals will have their bladders expressed twice daily as part of their routine care visits. During the first care visit of each day, before the bladder is expressed, electrical stimulation via the implanted electrodes will be applied and defecation time recorded. If defecation is not completed within two hours, animals will be given a water enema to flush the bowels clean.

**Special Procedure 3 ► Daily bath, bladder care, etc. (special husbandry)**

Following spinalization, animals will require additional care due to their loss of motor and sensory function of their hindquarters. Twice daily, their bladders will be manually expressed for emptying. Animals will be cleaned when they have bowel movements or once daily, whichever is more frequent. Resting boards will be removed from cages to prevent pressure sores and litter boxes will be removed because the animals will not use them and may develop pressure sores from resting against them.

**Special Procedure 4 ►**

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

**Special Procedure 1 ► Biweekly Testing Sessions**

Stimulation prior to spinal transection will determine the status of the implanted electrodes and if the animal has the appropriate responses to be considered for spinalization. In addition, every 2 weeks after spinalization, the cats will undergo an experimental test under anesthesia to determine the responses of the chronic spinalized animal's colon to electrical stimulation for comparison with testing performed in acute intact animals. Data demonstrating an effect in a chronic, spinalized animal preparation is critical to advancing to clinical translation for use by humans with SCI.

**Special Procedure 2 ► Daily Stimulation Testing**

Four treatment conditions (mechanical stimulation, implanted or minimally invasive electrical stimulation, and no stimulation) will be tested to measure their effects on defecation time, defined as the time from stimulation onset to complete bowel emptying. Treatment conditions will be randomized and applied daily. This daily testing is meant to maintain the animal's bowels and will not involve surgery, anesthesia, or drugs.

**Special Procedure 3 ► Daily bath, bladder care, etc. (special husbandry)**

These cats will have hind limb paralysis and loss of sensation in the hindquarters, so they will need to be observed closely for sores, self-mutilation, and overall emotional and physical health. They will not necessarily be able to manage their bladder on their own or attend to grooming of the insensate region.

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]
4		

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	( )	There may be discomfort from the placement of balloon catheters and EMG electrodes. In addition, animals may not be ambulatory during testing. Therefore, procedures will be conducted under propofol anesthesia.	(X) <sup>a</sup>	( ) <sup>b</sup>
2	(X)	Cats may receive an incomplete spinal cord transection that may result in neuropathic pain or distress. An incomplete spinal lesion in itself is not a problem for the study if the animal still develops neurogenic bowel dysfunction. However, if it results in pain that cannot be relieved, then the animal will be euthanized.	( ) <sup>a</sup>	( ) <sup>b</sup>
3	(X)	Regular handling to clean the animal or empty the bladder is not expected to cause harm or distress. It will serve as augmented husbandry and enrichment.	( ) <sup>a</sup>	( ) <sup>b</sup>
4	( )		( ) <sup>a</sup>	( ) <sup>b</sup>

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	Ketamine	Induction: 30 mg/kg (1 mL)	IM	Once for Induction	Once every 2 weeks
1	Propofol	Maintenance: 0.3 mg/kg/min (0.1 mL/min)	IV	Maintain anesthesia	4 hours every 2 weeks

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

Special Procedure 1 ►

Lubricant will be used to insert balloon catheters. The balloon, when inflated, is approximately the size of a cat stool.

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

Special Procedure 1 ►

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	ECG, heart rate, respiratory rate, expired CO <sub>2</sub> , SPO <sub>2</sub> , noninvasive blood pressure, and temperature; reflex responses such as eye blink, toe punch, and jaw tone, as well as capillary refill time	A testing session will last no longer than 4 hours. Then we will allow the animal to regain consciousness while monitored.
2	Animals will be normal behaving and monitored by visual inspection, looking for signs of pain or distress such as vocalization, ears back, or withdrawal	If stimulation causes pain or distress, we will stop stimulation.
3	Animals will be normal behaving and monitored by visual inspection, looking for signs of pain or distress such as vocalization, ears back, or withdrawal	If bladder and bowel care cause pain or distress, we will consult a veterinarian for potential reasons and interventions.

**ACORP Appendix 9**  
**DEPARTURES FROM “MUST” AND “SHOULD” STANDARDS IN THE GUIDE (2011)**  
**VERSION 4**

See ACORP App. 9 Instructions, for more detailed explanations of the information requested.

For each IACUC-approved “departure” of this protocol from a “Must” or “Should” standard in the *Guide*, provide the following information. (Consult the IACUC or the Attending Veterinarian for help in determining whether any “departures” are involved.):

Copy the lines below for each departure.

**Removing resting boards and litter boxes from cages**

Briefly summarize the “Must” or “Should” standard, and provide the number(s) of the page(s) on which it appears in the *Guide*

- ▶ The Guide’s section on Microenvironment in Chapter 3 (page 50) states “All animals should be housed under conditions that provide sufficient space as well as supplementary structures and resources required to meet physical, physiologic, and behavioral needs”. For cats, these structures and resources would include a resting board or perch, and a litter pan.

Describe the specific alternate standard(s) that will be met on this protocol, and how they will be monitored.

- ▶ Resting boards and litter pans will be removed from cages for animals that have had a spinal cord lesion.

Provide the scientific, veterinary medical, or animal welfare considerations that justify this departure

- ▶ The animals will not make use of resting boards or litter pans after spinalization. Without sensation or control of their lower extremities, animals will soil themselves without using the litter pan, and may develop pressure ulcers on their hind-quarters from the resting boards. Twice daily animal care will include checking for pressure ulcers, manual bladder emptying, and cleaning of the animal if the animal has soiled itself.

**Singly housed animals**

Briefly summarize the “Must” or “Should” standard, and provide the number(s) of the page(s) on which it appears in the *Guide*

- ▶ The Guide’s section on Behavioral and Social Management in Chapter 3 (page 64) states “Appropriate social interactions among members of the same species (conspecifics) are essential to normal development and well-being... When selecting a suitable social environment, attention should be given to whether the animals are naturally territorial or communal and whether they should be housed singly, in pairs, or in groups. An understanding of species-typical natural social behavior (e.g., natural social composition, population density, ability to disperse, familiarity, and social ranking) is key to successful social housing.” For cats, group housing is preferred for behavioral and social management.

Describe the specific alternate standard(s) that will be met on this protocol, and how they will be monitored.

- ▶ Animals will only be singly housed during the 2-week recovery period following spinalization to allow for safe conditions for healing. Otherwise, animals will be kept in social housing in accordance with the Guide.

Provide the scientific, veterinary medical, or animal welfare considerations that justify this departure

- ▶ Spinal transection leaves the animal extremely physically vulnerable during the post-op period while the animal is healing, particularly the spine. The animal will be housed singly to receive special post-op care and to avoid trauma from physical interactions with other animals.

## Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
██████████	Cleveland, OH-541	MERIT/Summer 2015	Afferent Stimulation to Evoke Recto- colonic Reflex for Colonic Motility

SCORE	DESCRIPTION	ACTION NEEDED BY IACUC
○ 0	No concerns noted. Any comments provided are for information only.	<i>None.</i> No further correspondence with the CVMO is needed; <u>the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.</u>
● 1	Some concerns noted.	<i>The IACUC must review the <b>level 1</b> concerns listed below and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).</i> No further correspondence with the CVMO is needed; <u>the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.</u>
○ 2	Concerns are noted that must be addressed by the local IACUC and PI before funding can occur, but work described in the ACORP(s) may continue.	<i>A response to each of the <b>level 2</b> concerns noted below must be reviewed and cleared by the CVMO <u>before funding can be released.</u></i> Upload the following at <a href="https://vaww.gateway.research.va.gov">https://vaww.gateway.research.va.gov</a> : (1) a memo addressing the concerns, dated and signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. <i>The IACUC must review each of the <b>level 1</b> concerns listed and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).</i>
○ 3	Significant concerns are noted that must be addressed by the local IACUC and PI before funding can occur, and work described in the ACORP(s) listed below must cease immediately.	<i>A response to each of the <b>level 3</b> concerns listed below must be reviewed and cleared by the CVMO <u>before work can resume and funding can be released.</u></i> (If unusual circumstances dictate that work should continue despite concerns, notify the CVMO immediately.) <i>A response to each of the <b>level 2</b> concerns noted below must be reviewed and cleared by the CVMO <u>before funding can be released.</u></i> For <b>level 2 and 3</b> concerns, upload the following at <a href="https://vaww.gateway.research.va.gov">https://vaww.gateway.research.va.gov</a> : (1) a memo addressing the concerns, signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. <i>The IACUC must review each of the <b>level 1</b> concerns listed and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s).</i>

(cont.)

The ACORP for Dr. [REDACTED] has received an overall score of 1, which means that it is cleared and represents no bar to funding the application, although some concerns were raised, as shown below.

Please note that a separate score is shown for each of the individual concerns (shown in parentheses under the Item number to which each of the individual concerns refers), to assist you in interpreting the review. An explanation of each of the levels of concern is shown above, in the chart on the previous page. The IACUC must review each of the **level 1** concerns listed and decide what response is needed. This action must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP, but no further correspondence with the CVMO is needed.

In case of questions about this review, please contact Dr. [REDACTED], Assistant Chief Veterinary Medical Officer at [REDACTED] or [REDACTED].

### REVIEWER FEEDBACK

ACORP Item number(s) (score)	Comments/Concerns
ACORP (cat)	This ACORP uses a feline model of spinal cord injury to determine the effects of patterned electrical stimulation on colon motility in order to produce clinically effective bowel emptying. The experimental plan is well written with a clear rationale for the proposed procedures. Some concerns were identified.
Item C.2, U, and Appendix 5 (1)	Item C.2 and Appendix 5 describe a terminal surgical procedure but do not indicate that the cat is euthanized with Euthasol following stimulation testing after sacral dorsal root transection or how death is confirmed in item U. Please reconcile.
Items G and H (0)	The cats used are purpose bred and vaccinated against rabies by the vendor; it might be worthwhile to include this information in item G.2.
Item T and Appendix 5 (1)	Since weight loss is expected as a consequence of spinal cord injury, how frequently are the cats weighed?  Please elaborate further on the nursing care plan and the environmental enrichment plan for the cats with spinal cord injuries. Are the cats turned multiple times per day to minimize pressure sores? Is petroleum jelly used as a barrier to protect skin from urine scald?
Appendix 9 (1)	An Appendix 9 is not needed for removing resting boards and litter boxes from the cages of spinal cord injured cats because other measures (cardboard mats, soft bedding, nursing care, social enrichment, etc.) are provided to meet the physical, physiological, and behavioral needs of these cats. No deviation from the <i>Guide</i> has occurred.  Likewise, single housing of these cats is not an approved departure, rather it is a specifically established exception to the <i>Guide</i> based on the following statement on pg. 51:

(cont.)

“Social should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility (see also section on Behavioral and Social Management).” Specifically established exceptions have no reporting requirements; Appendix 9 is not needed.

For additional information on *Guide* Deviations and Departures please see:

- VA Animal Research Training Exercises (optional) – a number of the exercises specifically discuss deviations and departures.  
[http://www.research.va.gov/programs/animal\\_research/required\\_training.cfm](http://www.research.va.gov/programs/animal_research/required_training.cfm)
- OLAW Departures from the *Guide*  
<http://grants.nih.gov/grants/olaw/departures.htm>