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]

2. VA Station Name (City) and 3-Digit Station Number► **Louisville 603**

3. Protocol Title► **Force Feedback Redistribution & Eccentric-Focused Rehab post-SCI**

4. Animal Species covered by this ACORP► **domestic feline**

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.
► The mission of the Spinal Cord Injury & Disorders System of Care is to support, promote, and maintain the health, independence, quality of life, and productivity of veterans with spinal cord injury (SCI) throughout their lives. The current research proposal addresses these needs through basic animal studies which assess the effects of SCI and a novel rehabilitation strategy. Coordination of muscles in each leg and appropriate limb stiffness are critical for weight support and walking. Even though not directly damaged, the basic system responsible, lying partially in the muscle, becomes dysfunctional post-SCI. This study will increase understanding of, and test a new rehabilitation strategy designed to reverse, these changes and improve weight support and walking. The long term goal is enhanced recovery for those with SCIs. Knowledge gained also may be relevant for other neurological disorders with loss of connections and walking difficulty.

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.

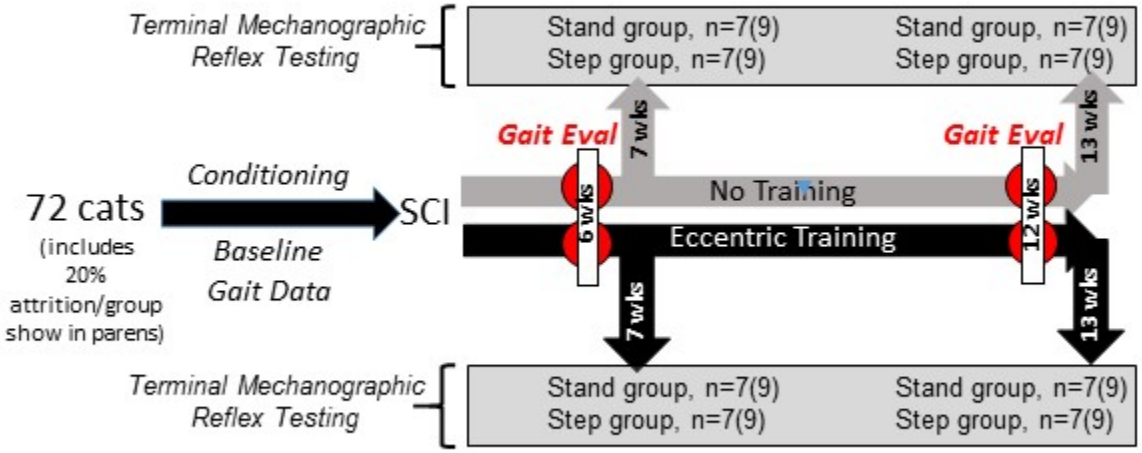
► A large number of individuals with incomplete SCIs (iSCI) show some preservation and/or recovery of sensory and motor function below the lesion. Some of these individuals regain some ability to walk and considerable data show that training can enhance basic walking patterns. However, sensory and motor deficits persist and walking often is typically accomplished using flexed lower extremities as if semi-crouching. Inadequate lower extremity strength has been identified as one of the greatest obstacles to walking recovery. Our preliminary data shows that muscles that straighten the leg (extensors) are actively involved in weight support and walking activities. These muscles are linked by many inhibitory connections that are important to the successful execution of a range of behaviors, including walking. These connections arise from specific sensors in muscles called Golgi tendon organs (GTOs). These GTOs are believed to control limb stiffness and promote coordination of

muscles within the leg during movement. The strength and distribution of these connections are flexible and can reconfigure based upon the demands of the task. Our preliminary data suggest that an injury which disrupts approximately half the spinal cord can immediately and persistently change the normal distribution of these connections and may be responsible for the diminished limb stiffness and poor weight acceptance seen in humans with spinal cord injuries. These findings provide new insight into potential mechanisms contributing to motor disruption and identify a new, potential rehabilitation strategy to target activity of these sensors. This strategy involves eccentric muscle activity, which means that a muscle increases its force while lengthening (stretching). This type of movement requires greater GTO activity than the strengthening approaches (increase force while shortening a muscle) typically used in rehabilitation. A simple way to increase eccentric activity is to walk downhill. The current study will determine if practicing (training) this type of activity will help normalize the inhibitory connections associated with the GTOs and promote better weight support and coordination of muscles within the legs while walking following spinal cord injury. Thus, an anticipated outcome is to understand if incorporating eccentric leg activities into a rehabilitation program will benefit humans with spinal cord injuries and greatly assist in recovering mobility, specifically walking.

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.

► ***Schematic of Overall Design and Groups***



The proposed studies are divided into two sets of experiments. The first set will carefully characterize the impact of a low thoracic hemi-section on gait kinematics during subphases where inhibitory force feedback is thought to be most active (Aim 1a, involves a No Training Group and periodic gait evaluations). These phases typically are associated with coordinated eccentric activity and/or weight acceptance/support. Pre- and post-SCI data, across time, will be compared. In the same animals, a comprehensive picture of GTO reflex (force-feedback) organization following SCI will be developed using a decerebrate preparation at two chronic time points (Aim 1b, involves No Training Group and Reflex Testing). Both standing and

walking preparations, in combination with mechanographic approaches will be used to test task-specificity of heterogenic force feedback responses in specific muscle combinations. These mechanographic studies will be done on a different protocol at GA Tech following transfer of animals to an approved IACUC at GA Tech (IACUC #[redacted]). Animals will be euthanized directly following the mechanographic data collection. In the second set of experiments, the basic experimental design is the same except that eccentric-focused training will be introduced at 2 wks post-SCI. This allows animals used in Aims 1a and 1b to serve as the controls for Aims 2a and 2b (involves Eccentric Trained Group). Eccentric training will use different 'downslope' gait tasks to more strongly activate force feedback circuitry. Gait kinematics will be carefully assessed during select subphases to test for training effects during different flat and downslope tasks (Aim 2a) and decerebrate, mechanographic studies used to characterize training effects on force dependent organization at the level of specific muscle combinations will be performed on a separate IACUC protocol following transfer to GA Tech. Histology will be completed in all animals to verify lesion characteristics. Behavioral data generated in the proposed work will be compared with an existing laboratory database in the [redacted] laboratory at GA Tech. The GA Tech database contains force feedback findings from control decerebrate preparations and thus will reduce the number of animals used as controls. Collectively, these studies will provide important information for evidence-based rehabilitation of motor skills by understanding the gait-associated impact of disrupting force feedback (Aim 1a), the extent of the disruption of this muscular control system within each limb following SCI (Aim 1b), and the potential to alter this disruption at the voluntary gait (Aim 2a) and basic reflex levels (2b) using a physical training approach.

8 intact cats (includes 20% attrition) → Normal controls for histology

8 additional animals will be used as normal controls across all aspects of the study. Unique controls are not needed for each aim and may be shared. Anatomical controls are necessary to provide normative comparisons for tissue volume and staining patterns as well as provide control tissue during staining procedures. Thus, 8 cats (7 plus ~10% attrition) will be used for this purpose. Despite the existence of a control decerebrate database, it is critical to periodically run controls to confirm that testing procedures and equipment are functioning properly. Thus, these histological controls also could be shared, by transfer to GA Tech, and serve as spinally-intact decerebrate controls if needed. In addition, it may become important to evaluate the reflex activity/involvement of additional muscles not well represented in the database, in which case the histological controls could be used first for mechanographic testing and then taken for histology.

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

► Based upon our preliminary results, we expect to see a large effect in behavioral outcomes measures between groups (control/pre-op and hemi-section). Significant differences in basic motor features across groups (although for locomotor features this will be pre-op versus post-op and then time post-injury) should be easily detected using a sample size of 8/group, with a power of 0.9 and an alpha level of p=0.05. We have found that 7 is a reasonable group size in

our prior studies to detect differences and thus have used that number in the proposed work. Our collective experiences across labs indicates an attrition rate of 15-20% across all procedures combined is reasonable for any problems encountered with lesion size, anesthesia complications, data acquisition, and/or tissue processing. This attrition rate (~20%; n=7/group + 20% (1.4) =9/group) has been considered and included in the total numbers of animals requested.

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.)

►Cats will be spayed. A spay is necessary for two primary reasons: first, to remove the effect of fluctuating hormone levels on central nervous system injury, and second, to prevent estrus related postural changes that interfere with locomotor function and, thus, data collection.
►Cats will undergo low spinal thoracic hemi-sections to allow testing of the effects of injury on gait/reflex function and to assess the effects of a potential rehabilitation strategy. This injury disrupts significant function by severing half of all ascending and descending connections, but allows spontaneous recovery of basic walking and bladder control providing a model in which treatments may be tested while minimizing the burden on the animal.
►Cats will be conditioned/trained to perform gait tasks pre-injury for food. Post-injury, cats will be divided into trained and non-trained groups. The untrained group will still be brought to the behavior room daily (Monday thru Friday), but will eat in a holding pen/behavior room. The trained group will eat while training. Food intake, weights and BCS of all animals will be carefully monitored to assure adequate daily food/caloric consumption. If cats do not consume their typical required daily intake during performance of their gait tasks, they will receivefood in the behavior room and/or in their home cage to ensure adequate nutrition.
►On non-training days, cats will eat in their home cages.
►Cats will have their hindquarters and a strip along their back shaved in preparation for collection of 3D kinematic/gait data capture. Shaving is necessary to allow placement of reflective markers which are tracked using a motion capture system.
►Cats will be transferred to a protocol at GA Tech IACUC #[redacted] to undergo terminal mechanographic testing (force responses to mechanical force generated by tendon stretches) after complete decerebration. Initial anesthesia is by inhalation of an isoflurane (5%) and oxygen mixture in an enclosed induction chamber. A tracheotomy will be performed and a surgical plane (lack of reflexes) maintained with isoflurane (1-4%). A surgical plane of anesthesia is continuously ensured by monitoring pedal reflex response and heart rate. An IV fluid line will be placed in the external jugular vein for fluids and end-tidal CO2, pulse rate and respiratory rate are monitored using a pulse-ox monitor (e.g. ear or tongue). A craniotomy will be performed under deep surgical anesthesia to remove the cerebral cortices and the brainstem will be transected, and all tissue rostral to the transection removed; after this procedure, the portions of the brain required for consciousness and the perception of pain are removed, thus rendering the animal permanently comatose and unable to feel any pain. For all terminal mechanographic experiments, a decerebration preparation will be used to assess reflex/Golgi Tendon Organ (GTO) circuitry organization. Several muscles of a single or both hind limbs will be separated and their tendons connected to sensors for mechanographic testing of individual muscles. After testing, all animals will be euthanized using a transcardial perfusion procedure.. This transfer will require transport of animals to GA Tech which will be done by the PI and/or research staff, and is part of an approved protocols at both institutions (GA Tech IACUC #[redacted]; UL IACUC #[redacted]).

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

► We have chosen to use the cat model due to our expertise in this model, the specificity of established locomotor tests in the cat and the existing literature on SCI, locomotion and GTO reflex activity in the cat. Our previous work and the existing literature give us interpretive power that is not possible in a less sentient species and allow us to directly compare any new work to our previous data. Further, mechanographic studies/approaches are established in the cat, but not the rodent.. Additionally, the cat is an important translational model for issues involving SCI in humans. These include its spinal size with respect to growth requirements for re-connectivity, elegant motor control, and influential work regarding task specific training.

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 ► **As the PI of the project, Dr.** [redacted] **will be involved with all aspects of the project. Her primary and most consistent contribution with the animal aspects of the study will be during surgeries. She has >25 years of experience with injections, perfusions, animal handling, spinal surgeries, spays, nursing care, behavioral training paradigms, locomotor recovery for spinal cord injured animals, tissue collection, tract tracing and histology. Initial training occurred at the Medical College of Pennsylvania as a graduate student and then U. of Florida and Malcolm Randall VAMC as a postdoctoral fellow. She has taken all the appropriate online CITI courses for VA animal training and IACUC modules since this program was initiated in 2006. She also has done all animal related training required by the affiliated Universities. Dr.** [redacted] **will continue to take all appropriate online course work at the VA as well as any required by the affiliated University, University of Louisville.**

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
Behavioral training & testing	**>25 years handling cats, using conditioning and training paradigms as well as collecting behavioral data.**
Injections and all aspects of nursing care	**>25 years handing spinally intact and spinal cord injured cats, giving SQ/IM/IP injections, manually evacuating bladder and bowel, skin checks**
Surgeries	**>25 years with spays and spinal surgeries.**
Terminal procedure	**>25 years performing intra- or trans-cardial perfusions and fresh tissue harvesting.**
Anesthesia	**>25 years with injectable and gas approaches including isoflurane**
Shaving cats	**>25 years shaving cat hindquarters and legs to allow marking/placement of reflective markers for gait capture**
Preparation for and Transport of SCI animals	**7 years of transporting cats with SCIs several times/year**

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

Name► [redacted]
Animal research experience ► [redacted] **will be involved with all aspects of the project. He has 22 years of experience with injections, perfusions, animal handling, spinal cord surgeries, nursing care, behavioral training paradigms, locomotor recovery for spinal cord injured animals, tissue collection and tract tracing placement. Initial training occurred at the University of Florida and the Malcom Randall VAMC. He has taken all the required animal training at the University of Louisville and the online training required by the VA (CITI).**

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Behavioral training & testing	**>22 years handling cats, using conditioning and training paradigms as well as collecting behavioral data.**
Injections and all aspects of nursing care	**>22 years handing spinally intact and spinal cord injured cats, giving SQ/IM/IP injections, manually evacuating bladder and bowel, skin checks**
Surgeries	**>22 years performing/assisting with spinal surgeries and assisting with spays.**
Terminal procedure	**>22 years performing intra- or trans-cardial perfusions.**
Anesthesia	**>22 years with injectable and gas approaches including isoflurane**
Shaving cats	**>22 years shaving cat hindquarters and legs to allow marking/placement of reflective markers for gait capture**
Preparation for, and Transport of SCI animals	**7 years of preparing (paperwork, carrier setup, vehicle setup, site coordination) for transport, communication during transport and review of transport processes.**

Name► [redacted]
Animal research experience ► [redacted] **will be involved with all aspects of the project. She has 3 years of experience with injections, perfusions, animal handling, assisting with spinal surgeries, nursing care, behavioral training paradigms, locomotor recovery for spinal cord injured animals, perfusions and tissue collection. Training has occurred at the University of Louisville and the Robley Rex VAMC. She has taken all the required animal training at the University of Louisville and the online training required by the VA (CITI).**

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Behavioral training & testing	**3 years handling cats, using conditioning and training paradigms as well as collecting behavioral data.**
Injections and all aspects of nursing care	**>3 years handing spinally intact and spinal cord injured cats, giving SQ/IM/IP injections, manually evacuating bladders, monitoring bowel function, skin checks**
Surgeries	**>3 years assisting with spinal surgeries and spays. She will be trained to perform independently by Dr.** [redacted] **and Mr.** [redacted]**.**

Terminal procedure	**>3 years performing assisting with and performing intra- or trans-cardial perfusions.**
Anesthesia	**>3 years with gas approaches including isoflurane**
Shaving cats	**>3 years shaving cat hindquarters and legs to allow marking/placement of reflective markers for gait capture**
Preparation for, and Transport of SCI animals	**She will be trained by Dr.** [redacted] **and Mr.** [redacted] **.**

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

Name►

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)

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

Name of Individual	Working with the VA IACUC	ORD web-based species specific course (Identify the species)	Any other training required locally (Identify the training)
[redacted]	**10/29/2014**	**Working with cats - 10/29/2014**	**Aseptic surgery – 5/11/2015**
[redacted]	**12/18/2014**	**Working with cats – 2/4/2015**	**Aseptic surgery – 5/11/2015**
[redacted]	**10/27/16**	**Working with cats – 8/8/16**	**Aseptic surgery – 8/8/16**

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"

► Transport - [redacted] **will train others on the team regarding transport of SCI animals. This will be done initially through discussion and review, co-transport and contact by phone during travel.**

Preparation for Transport – [redacted] **will train** [redacted] **in the paperwork and setup required for transport of animals. This will be done initially through discussion and review, co-setup and then monitoring before performing alone.**

Surgery – [redacted] **and** [redacted] **will train** [redacted] **on the aspects of surgery in which she is not yet independent. This training is already in progress but will continue by progressing through observation of the procedure, assistance with the procedure, monitoring while performing and finally independent performance.**

For all aspects of the study, no individual will be allowed to independently perform a procedure until the risk is not greater to the animal than that which is simply inherent to the procedure itself.

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]	**( )**	**(x) Univ. of Louisville**	**( )**	**yes**
[redacted]	**( )**	**(x) Univ. of Louisville**	**( )**	**yes**
[redacted]	**( )**	**(x) Univ. of Louisville**	**( )**	**yes**

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

► ( ) Yes. Describe them ►

► (**x**) 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
Cat	**Female**	**Adult**	[redacted] **(or any Class A, SPF cat source)**	**SPF**

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►**Spay, Spinal Cord Injury surgery (hemi-section), terminal transcardial perfusion**						
Species / Experimental Group / Procedure(s)	Year 1	Year 2	Year 3	Year 4	Year 5	**Category D TOTAL**
Cat, SPF/All groups/All procedures/adult; female	**19**	**18**	**19**	**16**		**72**
Cat, SPF/ terminal transcardial perfusion/adult; female	**2**	**2**	**2**	**2**		**8**

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**
Cat, SPF/All groups/All procedures/adult; female	**19**	**18**	**19**	**16**		**72**
Cat, SPF/ terminal transcardial perfusion/adult; female	**2**	**2**	**2**	**2**		**8**
						(combined total = 80)

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)
Spay ***See Appendix 5 for details.**	**At least every 6-12 hours for the first post- operative day. 1-2 times/day for the first week to monitor general behaviors and incision line.**	**Any of the individuals listed on the protocol.**	**<u>Pencillin G procaine benzathine</u>, ~100,000IU/kg, 1x pre- or peri-operatively; <u>-OR- Penicillin G, procaine (backup if above not available)</u> ~40,000 U/kg, IM, the day before, day of and day after surgery. If not given the day before surgery, it will be given for 7 days beginning on the day of surgery. <u>Atropine sulfate</u>, 0.04-0.06 mg/kg, SQ, pre-op only; <u>Acetylpromazine</u>, 0.1-0.2 mg/kg mg/kg, SQ pre-op only; <u>Isoflurane</u>, 1-5% by inhalation during prep and/or surgery; <u>Buprenorphine</u>, 0.01-0.05 mg/kg, SQ peri-operatively and then 2-3x/day (typically every 6-10 hours for first 24 hours and 8-12 hrs for remaining time out to 48 hours post-surgery).**

Spinal cord surgery (hemi-section of the spinal cord) *See Appendix 5 for details.	**At least every 6-12 hours for first 72 hours and until bladder function begins to return (usually ~2 days). At least daily after return of bladder function.**	**Any of the individuals listed on the protocol.**	**Pencillin G procaine benzathine, ~100,000IU/kg, 1x pre- or peri-operatively; -OR- Penicillin G, procaine (as backup if above not available) ~40,000 U/kg, IM, the day before, day of and day after surgery. If not given the day before surgery, it will be given for 7 days. Acetylpromazine, 0.1-0.2 mg/kg mg/kg, SQ pre-op only; Atropine sulfate, 0.04-0.06 mg/kg, SQ, per-op only; Acetylpromazine, 0.4-0.5 mg/kg, SQ pre-op only; Isoflurane, 1-5% by inhalation during surgery; Buprenorphine, 0.01-0.05 mg/kg, SQ every 6-12 hours for 48 hours.**

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► **Dr.** [redacted]
   Institutional affiliation► **Robley Rex VAMC and University of Louisville**
   email contact►[redacted]**;**[redacted]

   Name► **Dr.** [redacted]

Institutional affiliation► **University of Louisville**
email contact [redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted► **Dr.** [redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ► **10/31/16; 11/4/16; 12/20/16; 1/12-1/23/17**

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

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

a. Species	b. Type of housing*	c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)	e. Estimated maximum number of housing units needed at any one time
Cat	**House caging units appropriate for cats, and which allow joint housing/socialization**	**2-4**	**yes**	**18 Animals/yr will be housed at the Univ. of Louisville, RRC vivarium – requiring 6 quad pack cages (1 for change out during cage washing).**

*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:
► Standard, see below

** 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)
►

***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 below**	**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.
►Enrichment may include soft or blanket-style beds, resting boards and/or small soft cat toys. Post-injury, the resting boards are removed.

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

► ( ) This ACORP INCLUDES genetically modified animals.

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

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

► (**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.

►Due to disruption of motor and sensory function below the level of the spinal cord injury, it is important that the cage environment be modified to protect animals from subsequent injuries. These changes include:

- **Resting board removal so cats cannot rub laminectomy sites, jump off them and twist their backs or lie on their hard surface and cause peripheral nerve compression or skin ulcers.**
- **Thick bedding across entire cage bottom to prevent peripheral nerve compressions or skin ulcers. Cats are also observed at least 1-3x daily after injury and are repositioned by research staff to prevent skin irritation if unable to do so on their own immediately after surgery.**
- **Manual bladder expression 1-3x daily (based upon bladder fullness) by research staff until normal function returns.**

- **Cats that do not eat all of their food during behavior training will be provided additional food by the research staff in the behavioral room and/or home cage to ensure adequate nutrition.**
- **Small litter pans to discourage laying in them as the hard pan surface and/or litter may also cause nerve and skin compression and breakdown respectively.**

► ( ) 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.

► ( ) 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
		( )	( )
		( )	( )
		( )	( )

► (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**		
Research Resources Center animal facility at the University of Louisville.	(**x**) **Full accreditation**	( )**	**Research Resources Center animal facility at the University of Louisville.**	**Assigned by University of Louisville Research Resources (RRC) Vivarium (e.g.** [redacted] **)**
When transferred to GA Tech, animals will be housed at GA Tech facilities under a GA Tech IACUC protocol for typically 1-3 nights dependent upon number of animals transported.	( )	(**x**)** **This has been obtained-see attached waiver**	**GA Tech vivarium**	**Assigned by GA Tech animal facility veterinarian**

*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. **This has been obtained – see attached waiver.

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
Behavioral training and assessment, and feeding	**( )**	**(x)**	[redacted], **Univ of Louisville**	**(x) Cats will be transported the short distance in carrying cages from the housing rooms to the procedure room. All of this transport will occur within locked research buildings and will not go near patient areas.**	**( )**

Terminal transcardial Perfusion	( )	**(x)**	**[redacted] bldg. at Univ of Louisville. Core perfusion rooms (e.g. [redacted]) and designated perfusion areas (e.g. [redacted] hoods) will be used.**	**(x) Cats will be transported in carrying cages from the housing rooms to the procedure room. All of this transport will occur within locked research buildings and will use secure freight elevators-only.**	**( )**
Shaving	**( )**	**(x)**	**Research Resources Facility at Univ of Louisville (rms [redacted])**	**( )**	**(x)**
Spinal Cord Injury Surgery	**(x)**	**( )**	**Research Resources Facility at Univ of Louisville (USDA surgical suites Rm [redacted])**	**( )**	**(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")
Blood and tissue	**(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.)

► Animals will be euthanized prematurely if they exhibit life threatening or painful health problems that are refractory to treatment or morbidity that prevents assessment of gait. The spinal cord injuries they receive do not fall within the criteria for deciding to euthanize. Cats are observed after surgeries 1-3x/day by research and husbandry staff. Animals are respositioned to prevent skin irritation if unable to on their own immediately after surgery. Manual bladder expression is also performed. Fecal and/or urine soiling is prevented by frequent changing of bedding at times of daily observation. Most cats resume normal posture, behavioral patterns, such as purring, grooming, sleeping, social interaction with research and husbandry staff within 1-3 days. In the rare instance that a cat has an unacceptable lesion magnitude, and does not resume this type of normal cat behavior within that time frame, a veterinarian will be consulted and euthanasia considered.

In isolation, change from baseline weight is not a reasonable criteria for euthanizing. Because a cat may enter the animal facility weighing more than their ideal weight, it is important to use a body condition scale in addition to weight in monitoring their health status. Thus, If a cat becomes a 4 (underweight) on the on the 9 point Purina Body Condition Score and measures to increase weight are not quickly effective (i.e. greater food variety and/or special food such as a/d Hills urgent care diet), a veterinarian will be contacted. If weight loss progresses to a 3 (thin) a decision may be made to euthanize in consultation with the veterinarian. We believe it is extremely unlikely that any cats will progress to this point. We have used the low thoracic hemi-section model and locomotor training for 15 years without encountering significant weight loss/body condition scores that lead to euthanasia. However, endpoint criteria include the following rare instances: a cat has internal bleeding that cannot be stopped during/following a surgery or a cat is a risk for handling (i.e. bites) and staff cannot handle safely.

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

► (**x**) Some or all animals will NOT be euthanized 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.)

►The majority, if not all, of the animals on this protocol will be transferred to a protocol at GA Tech for a final, terminal mechanographic evaluation. At the end of that evaluation, the animals will be euthanized via transcardial perfusion on the GA Tech protocol.

► (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.)

►The animals which may be euthanized as part of the planned studies at the Louisville site include the normal controls (histology). Other animals that may undergo the following procedures include those that might be removed early from the study (see "T" above).

Check each method that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable
( )	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death► Method for verifying death► Secondary physical method►		( )	( )	( )
( )	Anesthetic overdose Agent► Dose► Route of administration►		( )	( )	( )
( )	Decapitation under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
( )	Exsanguination under anesthesia Agent► Dose► Route of administration►		( )	( )	( )
(x)	Other (Describe) ► **<u>Transcardial perfusion.</u> Cats initially will be deeply anesthetized using isoflurane and then sodium pentobarbital (35-60 mg/kg Nembutal, IP initially and supplements IV). IP is used to allow an overdose to be given while allowing the animal to remain viable for the heparin to circulate prior to perfusion procedure. The animal is deeply anesthetized on isoflurane when the IP injection is**		(x)	( )	( )

	given and as the drug takes effect. Further, the IP injection is given caudal and ipsilateral to the spinal lesion site where the animal has disrupted sensation. As needed, cats will be given one or more supplemental doses of sodium pentobarbital (25% of initial dose) to reach a deep plane of anesthesia. They will then be given 1 cc of 10% heparin (intravenous) followed ~20 minutes later by 1 cc of 1% sodium nitrite (intravenous). Immediately following administration of sodium nitrite, they will be perfused transcardially with 0.9% saline.				
( )	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:
   ►NA

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:
   ►NA

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.
   ► [redacted]**, Ph.D. She has >25 years of experience with euthanasia procedures, including transcardial perfusion as used in this protocol, in both rats and cats. Her initial training occurred at the Medical College of Pennsylvania where she received her Ph.D. Since that time she has continued to perform euthanasia using transcardial perfusion methods.**

   [redacted] **has >22 years of experience with euthanasia procedures, including transcardial perfusion, in both rats and cats. Part of his training occurred under Dr.** [redacted] **at the University of Florida and the Malcom Randall VAMC. Since that time he has continued to perform euthanasia using transcardial perfusion methods.**

   [redacted]**, has 3 years of experience with euthanasia procedures, including transcardial perfusion, in both rats and cats. Her training occurred under Dr.** [redacted] **and** [redacted] **at the University of Louisville and the Robley Rex VAMC. Since that time she has continued to perform euthanasia using transcardial perfusion methods.**

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.

► The carcass should be put in the refrigerator and a member of the [redacted] **lab contacted immediately.**

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

Name► [redacted]
Contact Information► [redacted]

Name► [redacted]
Contact Information► [redacted]

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

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

Name of Procedure	Identify Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP -- specify the Item letter(s)	Appendix 6
Spinal cord injury	**NA**	**C1., C2a.,C2b., C2c., E1., E2., J., T., V., W1., W2., Appendix 5**	()**
Spay	**NA**	**C2c., E1., E2., J., T., V., W1., W2., Appendix 5**	()**
Transcardial perfusion	**NA**	**C2c., Q., U., U3., V,**	( )**
Behavioral training	**NA**	**E1., E2., Q., V.**	(**X**)**
Special husbandry post-SCI	**NA**	**J., M.3., Appendix 6**	(**X**)
Transportation to GA Tech	**NA**	**Appendix 1**	

*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.
   ► Spay, Spinal Cord Injury

   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)
Pub Med	**10/25/16**	**1966 to present**	**yes**	**spinal cord injury (SCI), SCI models, Golgi Tendon Organ, reflex, regeneration, vestibulospinal tract, locomotion, rehabilitation, training, eccentric muscle activity, *in vivo,* and *in vitro.***	(x)	(x)	(x)	(x)
Current Contents	**10/25/16**	**Early 90's forward**	**yes**	**spinal cord injury (SCI), SCI models, Golgi Tendon Organ, reflex, regeneration, vestibulospinal tract, locomotion,**	(X)	(X)	(X)	(X)

				rehabilitation, training, eccentric muscle activity, *in vivo,* and *in vitro.*				
					( )	( )	( )	( )
					( )	( )	( )	( )

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.
   ► It is not possible to replace the *in vivo*, animal model because we are looking at a whole system response in which many factors are unknown. Thus, it is not possible to mimic the whole adult CNS and musculoskeletal system *in vitro* and computer modeling would be ineffective because of the myriad of unknowns in addition to the effects of our physical rehabilitation approach. This opinion is based upon 25 years of experience in the field of spinal cord injury research plus frequent careful scrutiny of the literature, including Pub Med (search terms include spinal cord injury, regeneration, plasticity, development, recovery, inhibitory factors, chondroitinase), scientific discussions with experts in the field, and attendance at scientific meetings.
   We have chosen to use the cat model due to our expertise in this model, the specificity of established locomotor tests in the cat and the existing literature on SCI, locomotion and reflex function in the cat. Our previous work and the existing literature give us interpretive power that is not possible in a less sentient species. Further, the cat has been/is an important translational model for issues involving SCI in humans.

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.
   By making multiple assessments across 2 laboratories through transfer of animals at the end of the study to GA Tech, we have reduced animal numbers. Further, by comparing data obtained to data in an existing data base of control force feedback data, we reduced the requirement for testing of non-SCI controls.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
   ►This study uses a partial spinal cord injury versus one of greater magnitude. We have upgraded to a more sophisticated 3D motion analyses system which captures all aspects of the animals at once and requires fewer task completions to provide sufficient data for analyses. We have incorporated the use of a penicillin formulation that requires a single vs multiple injections.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
   ► There are no indications of duplicate or parallel studies in the literature. Further, we have no indication of duplicate or parallel studies from discussions with our colleagues or through attendance at professional meetings.

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 Phar-macy	Non-VA
Sodium Pentobarbital (e.g. Nembutal)	**(x)**	( )*	**Personnel listed on this project**	( )	**(x**)**	**(x)**	( )
Buprenorphine	**(x)**	( )*	**Personnel listed on this project**	( )	**(x**)**	**(x)**	( )
	( )	( )*		( )	( )	( )	( )

****Note that there is a current MOU regarding shared space between the VA and UoL –Our DEA controlled substances are inspected monthly by the VA.**

*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:

► ( ) Some controlled substances will 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.

► (**x**) 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"
► ( ) 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			
M.1			
M.2			
U.4.a			
U.4.b			
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
[redacted]	[redacted]	02/16/2017

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], **D.V.M., M.S., DACLAM**	[redacted]	02/16/2017
Name of IACUC Chair	Signature	Date
[redacted], **PhD**	[redacted]	02/16/2017

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], **Ph. D.**	[redacted]	02/16/2017
Name of Institutional Veterinarian	Signature	Date
[redacted], **D.V.M., M.S., DACLAM**	[redacted]	02/16/2017

Name of IACUC Chair	Signature	Date
[redacted], **Ph.D.**	[redacted]	02/16/2017

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], **PhD**	[redacted]	02/16/2017

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] **Ph.D.**	[redacted]	02/16/2017

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

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

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

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

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

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

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

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

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

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

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

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

I further certify that:

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

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

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

Name of IACUC Chair	Signature	Date

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

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

ACORP Appendix 1

USE OF A NON-VA FACILITY TO HOUSE ANIMALS PURCHASED WITH VA OR VA RESEARCH AND EDUCATION CORPORATION FUNDS

Non-VA Facility Name	Is this facility accredited by AAALAC?	
	Yes	No*
University of Louisville	☒	☐
Georgia Institute of Technology (GA Tech)	☐	☒
	☐	☐

*According to VHA Handbook 1200.7, "Use of Animals in Research", paragraph 7.f., all VA animal facilities and affiliate or other animal facilities that house animals purchased with VA (including VA Research and Education Corporation) funds must be accredited by AAALAC. Under exceptional circumstances, a waiver may be requested in writing from the CRADO (Chief Research and Development Officer) or designee through the CVMO (Chief Veterinary Medical Officer). See Appendix A of VHA Handbook 1200.7 for information on how to contact the CVMO.

Non-VA Facility Location and Specified Housing Rooms:

University of Louisville Research Resource Center vivaria are located on the Medical Campus at the corner of [redacted] and [redacted] in [redacted]. Cats will be housed within [redacted].

The GA Tech Vivarium is located on the GA Tech Campus in Atlanta, GA. Cats will be housed in one of the feline designated housing rooms. They will only be housed at this facility once the animals have been transferred to an existing IACUC protocol at GA Tech

Transfer of animals to GA Tech:

The majority of the study will be conducted in the [redacted] laboratory in Louisville, KY (chronic injuries, eccentric training, kinematic analyses of gait and histology), the custom equipment designed and built by Dr. [redacted] for the mechanographic approaches is located in his lab at GA Tech. However, it is a reasonable 6 – 6 ½ hour drive (Louisville facility to Atlanta facility). Dr. [redacted] and/or another member of her team will make this this trip with cats and may be present during mechanographic data collections in the [redacted] lab. Dr. [redacted] is already on an IACUC protocol at GA Tech, has made this trip in the past multiple times, done acute lesions during the mechanographic procedures, and set up perfusion facilities in the [redacted] lab to preserve and collect tissues following the experiments so that histological assessments may be done. Other [redacted] lab members may share these roles in the future as part of this project. Mr. [redacted] already is appropriately trained. This arrangement, in addition to Dr. [redacted] hands on approach, willingness to travel to Louisville, and Skype meetings, have fostered a close collaborative relationship between the two laboratories. Also critical is that this arrangement allows animals to be transported in a manner that decreases potential stresses associated with transport, keeps them in excellent health, and under appropriate care during the trip. There are no apparent adverse effects of the travel on the animals. Combined expertise across the two laboratories greatly enhances the quality, uniqueness and potential positive outcomes of the proposed studies.

Typically one to three cats at a time will be transported to GA Tech to undergo terminal experiments using the mechanographic methods described in Dr. [redacted] GA Tech IACUC protocol ([redacted]). The

cats will be placed in standard cat carriers which have appropriate cushion bottoms to protect these SCI animals from peripheral nerve compressions and/or skin ulcers. An absorbent pad is used in lieu of a standard litter pan within the carrier. All personal vehicle(s) used are inspected by the UL IACUC for adequacy of transport. This includes both Dr. [redacted]'s and Mr. [redacted]'s, climate controlled vehicle(s). Once the animals are at GA Tech, they are transferred to Dr. [redacted] approved IACUC protocol # [redacted] (contact for additional information - [redacted] IACUC compliance officer at GA Tech**)** for terminal procedures as described in an approved protocol at the respective institution. Prior to transporting any animals to GA Tech, appropriate USDA forms for transporting and transferring ownership of the cats to GA Tech will be completed and approved. Food and water for 24 hours, in case of an emergency, are carried in the transport vehicle. The benefit of transportation by Dr. [redacted], Mr. [redacted], and/or another appropriately trained member of her group is quick (direct) transport, appropriate/ knowledgeable handling of spinal cord injured animals and continuity of handling during transfer (Dr. [redacted] currently is approved to handle animals at GA Tech and other members of her lab group will be added for at GA Tech as appropriate).

ACORP APPENDIX 3
BIOSAFETY
VERSION 4

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

1. **Summary of <u>All</u> Materials Administered to Animals on this Protocol.** Complete the table below for <u>all</u> materials to be administered to any animal on this protocol, indicating the nature of the material by marking EVERY box that applies, and indicating the BSL number for any infectious agents:
   *indicates VA pharmacy is typical 'vendor' for identified drug and others are listed simply as alternatives if VA pharmacy cannot provide for some reason.

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
Acepromazine (not DEA controlled)	**Medical/veterinary vendor/pharmacy (e.g. Patterson Veterinary Supply, Henry Schein Animal Health, Midwest Veterinary Supply, Zoopharm)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )
Atropine (not DEA controlled)	***VA Pharmacy Medical/veterinary vendor/pharmacy (see options above)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )
isoflurane (not DEA controlled)	***VA Pharmacy Medical/veterinary vendor/pharmacy (see options above)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )
Saline or Lactated Ringers Solution (not DEA controlled)	**Medical/veterinary vendor/pharmacy (e.g. Patterson Veterinary Supply, Henry Schein Animal Health, Midwest Veterinary Supply, Zoopharm)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )

buprenorphine	***VA Pharmacy Medical/veterinary vendor/pharmacy (see above options plus Akorn Opthalmic and Parenteral Health Care & Zoopharm)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )
Sodium pentobarbital (e.g. Nembutal)	***VA Pharmacy Medical/veterinary vendor/pharmacy (e.g. Akorn Ophthalmic and Parenteral Health Care)**	( )	( )BSL_	( )	( )	( )	( )	(**x**)
Penicillin G procaine (not DEA controlled)	**Medical/veterinary vendor/pharmacy (see options above)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )
Penicillin G procaine and benzathine (not DEA controlled)	**Medical/veterinary vendor/pharmacy (see options above)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )
Heparin (not DEA controlled)	***VA Pharmacy Medical/veterinary vendor/pharmacy (e.g. Akorn Ophthalmic and Parenteral Health Care)**	( )	( )BSL_	( )	( )	( )	(**x**)	( )
Sodium nitrite (not DEA controlled)	***VA Pharmacy Medical/veterinary vendor/pharmacy (e.g. Akorn Ophthalmic and Parenteral Health Care)**	( )	( )BSL_	( )	( )	( )	( )	(**x**)
Saline (not DEA controlled)	**Medical/veterinary vendor/pharmacy (e.g. Patterson Veterinary Supply, Henry Schein Animal Health, Midwest Veterinary Supply, Zoopharm). Given as initial flush for transcardial perfusion**	( )	( )BSL_	( )	( )	( )	( )	(**x**)

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

Material* (Identify the specific agent, device, strain, construct, isotope, etc.)	**Dose** (e.g., mg/kg, CFU, PFU, number of cells, mCi) and **Volume** (ml)	**Diluent* or Vehicle***	**Route** of admin	**Frequency or duration** of admin	**Reason** for Administration and **Expected Effects**	Location of **Further Details** in this ACORP (specify "Main Body" or "App #", and identify the Item)	Administration Under **Anesthesia, sedation, or tranquilization** (Y/N)
Acepromazine	**0.1-0.2mg/kg (<.5m)**	**NA**	**SQ**	**1x**	**sedation and prevention of potential tachycardia during spinal injury**		**N**
Atropine	**0.04-0.06 mg/kg (<.5ml)**	**NA**	**SQ**	**1x**	**control excess salivation/secretions**		**N**
isoflurane	**1-5%**	**Oxygen**	**inhalation**	**During prep and throughout surgery** **During shaving prior to filming**	**anesthesia**		**N**
buprenorphine	**0.01-0.05 mg/kg (~0.1-0.6mls)**	**NA**	**SQ**	**Peri-operative and then for 48 hours. For first day (24 hrs) ~every 6-10 hrs. For second day,~every 8-12 hours**	**analgesia**		**Y,N**

Sodium pentobarbital (e.g. Nembutal)	**35-60 mg/kg (~.5-1.5mls)**	**NA**	**IP, IV**	**1x + supplement if needed**	**anesthesia**		**Y**
Penicillin G procaine	**40,000 IU/kg (<.6ml)**	**NA**	**IM**	**Peri—operatively and then 1x/day for 3-7 days**	**to prevent infection**		**Y,N**
Penicillin G procaine and benzathine	**1,000,000 IU/kg (<.6 mls)**	**NA**	**IM**	**1x**	**to prevent infection**		**Y**
Saline or Lactated Ringers Solution (IV bag)	**~3ml/kg/hr**	**NA**	**IV**	**Throughout surgery**	**hydration**		**Y**
Heparin	**1000 IU (~1ml)**	**NA**	**IV**	**1x**	**blood thinner to allow better vessel clearing during terminal transcardial perfusion**		**Y**
Sodium nitrite	**1% (0.5-1ml)**	**Saline**	**IV or left ventricle**	**1x**	**vasodilator to allow better vessel clearing during terminal transcardial perfusion**		**Y**
Saline (0.9%)	**~200mls**	**NA**	**Left ventricle/ transcardial**	**1x**	**Initial flush of blood from heart/ cardio-vascular system**		**Y**

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

►

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

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

► **Isoflurane used during surgery will be preceded by acepromazine (0.1-0.2mg/kg, <.5mls), SQ)**

- **Lactated Ringers or Saline Solution will be given during surgery while under isoflurane anesthesia (1-4%, inhalation).**

- **Penicillin may be given after sedation with isoflurane (1-4%, inhalation) during surgical prep or at the end of surgery while still anesthetized. However, Penicillin administered on non-surgery days will not be given under sedation.**

- **Sodium Pentabarbital given IP will be done under isoflurane. If given IV initially, will not require anesthesia.**

- **Heparin given IV will be done under Nembutal (>35mg/kg, IP or IV, ~1/2ml/kg)**

- **Sodium Nitrite given IV will be done under Nembutal (>35mg/kg, IP or IV, ~1/2ml/kg)**

- **Saline via transcardial delivery will be done under Nembutal ((>35mg/kg, IP or IV, ~1/2ml/kg)**

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.

► These drugs are all delivered either SQ or IM as quick injections and cause no, little or only momentary pain. Animals will only require gentle restraint.

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 *NIH Guidelines for Research Involving Recombinant DNA Molecules*	Exempt
	( )	( )
	( )	( )
	( )	( )
	( )	( )
	( )	( )
	( )	( )

9. **Potential for Pain or Distress**. Complete the table below for each of the agents listed in Item 1, above, that is expected to have potentially painful or distressing effects on the animals (see ACORP App. 3 Instructions, for details).

Name of Agent	Nature of Potential Pain/Distress	Measures to Alleviate Pain/Distress

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.
    ►

    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	**Spay**	( )	( )	(**x**)	(**x**)*
2	**Spinal Cord Injury**	( )	( )	(**x**)	(**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:
► **Purpose bred cats will be spayed by the vendor prior to purchase, arrival at the animal facility, and placement on the protocol. However, if a cat cannot be spayed prior to arrival, it will be spayed prior to enrollment. A spay is necessary for two main reasons: first, to remove the effect of fluctuating hormone levels on magnitude of central nervous system injury, and to prevent estrus related postural changes that interfere with locomotor function.**
The overall goal of this project is to identify an approach which enhances recovery of motor function following spinal cord injury. Hence, we need to generate a spinal cord injury in order to determine ways to restore motor function and control.

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
►**There will be a minimum of 2 weeks between any surgical procedures. This minimum time frame was chosen to allow recovery from the spay procedure prior to the spinal cord injury. Typically, however, the time frame is anticipated to be between 4 and 8 weeks to allow for conditioning and collection of baseline data prior to injury. Cats will be maintained 7-13 weeks after injury before terminal procedure.**

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 ► **Spay procedure: When possible, cats will be spayed by the vendor as it is less costly than performing ourselves. Cats not spayed by the vendor will undergo ovariohysterectomy after acclimation at the home facility. A skin incision between the level of the umbilicus and the pubic symphysis will be made followed by muscle incision along the linea alba tendon. The uterine horns will be located as they extend from behind the bladder. The horns will be followed rostrally to identify the ovaries. The horns and ovaries will then be gently separated from fascia.**

Hemostats will be used to clamp off the blood flow at the level of each ovary as well as at the level of the uterine cervix. Absorbable sterile suture (2-0 or 3-0) will be used to tightly tie off the vessels at each level. The entire uterus and ovaries will then be removed and the abdominal cavity closed in layers. The muscle will be closed using a 2-0 or 3-0 absorbable monofilament (preferable) or braided sterile suture. Subcutaneous tissues will be closed using a 2-0 or 3-0 absorbable monofilament (preferable) or braided sterile suture. The skin also will be sutured with a 2-0 or 3-0 absorbable monofilament preferable) or braided sterile suture. An inverted, interrupted stitch (subcuticular) will be used on the skin so that stitch removal is not necessary. An adhesive (e.g. medical cyanoacrylate glues which include MediBond, MediCryl, PeriAcryl, GluStitch, Xoin, Gesika, VetGlu, Vetbond, LiquiVet, Indermil, LiquiBand, Histoacryl, IFABond) may also be used during skin closing (e.g. to help seal the skin opening over the last stitch).

Surgery 2 ► **Spinal Hemisection:** **The T9/10 spinal level is determined by palpation of bony landmarks including the last rib, dorsal spinal processes and the iliac crests. The skin is then slit longitudinally and the muscle bluntly separated from the dorsal and lateral processes as well as the laminae. A bilateral laminectomy is then performed to expose the underlying T9/10 spinal level. The dura slit longitudinally and the dorsal columns and dorsal root entry zones visualized to identify the spinal midline. Half of the spinal cord will be completely severed using iridectomy scissors. Any fibers adhering to the ventral or lateral dura will be gently lifted with suction and cut with iridectomy scissors. The dura may be left open or closed with 10-0 or 8-0 suture. This choice is based upon any spinal swelling and/or integrity of the dura. Durafilm and gelfoam will be placed on top of the dura. The muscle will be closed using a 2-0 or 3-0 absorbable monofilament (preferable) or braided sterile suture. Subcutaneous tissues will be closed using a 2-0 or 3-0 absorbable monofilament (preferable) or braided sterile suture. The skin also will be sutured with a 2-0 or 3-0 absorbable, monofilament preferable) or braided sterile suture. An inverted, interrupted stitch (subcuticular) will be used on the skin so that stitch removal is not necessary. An adhesive (e.g. medical cyanoacrylate glues which include MediBond, MediCryl, PeriAcryl, GluStitch, Xoin, Gesika, VetGlu, Vetbond, LiquiVet, Indermil, LiquiBand, Histoacryl, IFABond) may also be used during skin closing (e.g. to help seal the skin opening over the last stitch).**

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**	**(x)**	**(x)**	**(x)**	**(x) Pre-op prep & post-op recovery**
[redacted]	**1,2**	**(x)**	**(x)**	**(x)**	**(x) Pre-op prep & post-op recovery**
[redacted]	**1,2**	**(x)**	**(x)**	**(x)**	**(x) Pre-op prep & post-op recovery**
		( )	( )	( )	( )

		( )	( )	( )	( )

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

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
Research Resources Center Vivarium	**USDA species surgical suites (Rm#s [redacted])**	**1,2**	**(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 hrs**	( ) --	**(x) – cephalic vein**	( ) --
2	**(x) -- <12 hours**	( ) --	**(x) – cephalic vein**	( ) --
4	( ) --	( ) --	( ) --	( ) --

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

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Penicillin G procaine and benzathine	**1,2**	**100,000 IU/kgBW**	**IM**	**1X**	**Pre- or peri-op**
Penicillin G, procaine	**1,2**	**40,000 U/kg BW**	**IM**	**1X**	**The day before, of and post-surgery OR 7 days, including day of surgery, if not given the day prior to surgery OR for suspected UTI (alternative to Pen G procaine and benzathine if not available)**
Atropine	**1,2,3**	**0.04-0.06 mg/kg**	**SC**	**1X**	**immediate**
Acepromazine	**1,2,3**	**0.1-0.2 mg/kg**	**SC**	**1X**	**immediate**
Isoflurane	**1,2,3**	**1-5%; Inhalation**	**During induction and throughout surgery**	**During induction and throughout surgery**	**immediate**

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 ► **Prior to taking the animal to the surgery area, the surgical site will be identifed by palpating the pelvis and the ribs and visualizing the umbilicus. All hair within 2 cm of an imaginary line between the umbilicus and the pubic symphysis (surgical site) will be removed with clippers. Any remaining loose hair will be wiped away or removed with a vacuum. The surgical site will be scrubbed with a surgical scrub solution (such as Betadine or Hibiclens) using sterile gauze, swabs or sponges. A circular motion will be used such that cleaning progresses from the center of the surgical site outward. The scrubbed area will be rinsed with 70% alcohol or sterile H20 using a sterile gauze or swab. This procedure will be repeated again 3x once the animal is placed on the surgical table. Following preparation of the surgical site, a sterile drape will be placed over the abdomen such that the prepared surgical site-only is exposed by an opening in the drape. If necessary, additional drapes/towels will be used so that the animal's entire body is covered to maintain a sterile field.**

Surgery 2 ► **Prior to taking the animal to the surgery area, the surgical site will be identified by palpating the pelvis, ribs and vertebra to localize the dorsal T9 and T10 processes. All hair within 2 cm of an imaginary line between T7 and T12 will be removed with clippers. Any remaining loose hair will be wiped away or removed with a vacuum. The surgical site will be**

scrubbed with a surgical scrub solution (such as Betadine or Hibiclens) using sterile gauze, swabs or sponges. A circular motion will be used such that cleaning progresses from the center of the surgical site outward. The scrubbed area will be rinsed with 70% alcohol using a sterile gauze or swab. This procedure will be repeated again 3x once the animal is placed on the surgical table. Following preparation of the surgical site, a sterile drape will be placed over the back such that the prepared surgical site-only is exposed by an opening in the drape. If necessary, additional drapes/towels will be used so that the animal's entire body is covered to maintain a sterile field.

6. **Intra-operative management.**

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

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
isoflurane	**( )***	**1,2,3**	**1-5%**	**inhalation**	**throughout surgery**
Saline/ringers/like solution	**( )***	**1,2,3**	**3-10 ml/kg/hr**	**IV**	**throughout surgery**
	( )*				

   * 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.
   ►NA

   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 are on a heating pad (e.g. circulating water blanket, bair hugger) during pre-surgical procedures. If a cat's body temperature should happen to drop significantly below 99 (in the mid-low 90's), heated rolled towels/wraps and/or saline bags covered with a wrap will be placed under the sterile drapes next to the animal's body.

   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.
   ► At least 2-3 vitals are recorded every 15-20 minutes to ensure and document a surgical plane of anesthesia. Vitals, may include respiratory rate, heart rate, blood pressure, core body temperature, expired CO2. At least one vital is monitored continually throughout survival surgery. Ear and pedal reflexes may also be used to assure a surgical depth of anesthesia.

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

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

Surgery	Survival Period	Measures for Maintaining Sterility

# (see Item 1)		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
1	**7-13 weeks**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	**( )***
2	**7-13 weeks**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	**(x)**	**( )***
		( )	( )	( )	( )	( )	( )	( )	( )*
		( )	( )	( )	( )	( )	( )	( )	( )*

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

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

Surgery 1 ► **Cats are typically recovered in a 'climate controlled' recovery unit. If one of these is not available, cats are recovered on heating pads (e.g. circulating warm water pads) in housing cages. If body temperature is <97-98 degree F and/or does not rapidly recover to normal levels, a Bair hugger and/or warm blankets/towels are placed in the recovery unit/cage with the animal. If the animal appears dehydrated, 20 cc of fluid (i.e. saline, ringer/like solution) may be given SQ. If this is quickly absorbed, additional fluids may be given.**

Surgery 2 ► **Cats are typically recovered in a 'climate controlled' recovery unit. If one of these is not available, cats are recovered on heating pads (e.g. circulating warm water pads) in housing cages. If body temperature is <97-98 degree F and/or does not rapidly recover to normal levels, a Behr hugger and/or warm blankets/towels are placed in the recovery unit/cage with the animal. If the animal appears dehydrated, 20 cc of fluid (i.e. saline, ringer/like solution) may be given SQ. If this is quickly absorbed, additional fluids may be given.**

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**	**0.01-0.05 mg/kg (~.01-.05mls)**	**SQ**	**2-3x/day**	**2 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:
►NA

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)

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

(1) Immediate post-operative monitoring **– Animals are recovered in a warm environment (e.g. temperature controlled recovery units or units with circulating water pads, Bair hugger or warm blankets). Each is monitored continuously until able to maintain a sternal posture and core body temperature has reached at least 99°F.**

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1, 2	**continuously**	**~30-90 minutes**	[redacted], [redacted], [redacted] **or veterinary staff**

(2) Post-operative monitoring after the immediate post-operative period - **Following all surgeries, animals will be checked at least every 6-12 hours for the first 72 hours post-op. Within 2-4 hours of surgery, cats are offered and eating wet food and drinking water ad lib following this type of surgery. Palatable as well as high protein wet foods suitable for post-surgical animals may be used. Dry food is not reintroduced for several days as the wet food helps with hydration. Within this timeframe, cats are sitting up, moving around the cage and repositioning themselves. If we see the involved limb positioned oddly, we will reposition. General positioning or turning, however, is not needed as this injury model allows general body movement and repositioning as soon as anesthesia wears off. In combination with the use of our thick foam cushions, this is adequate to allow the cat to be comfortable and prevents skin breakdown and peripheral nerve compression. If at the end of 72 hours, bladder function has recovered and no pain behaviors are apparent, animals still will be checked 1-2x daily. If bladder function has shown no or only partial recovery, monitoring will continue at least every 6-12 hours until this issue is resolved. Cats typically have a bowel movement within 2-4 day of surgery. Their bowels are lightly palpated daily to assure there is no hard fecal matter blockage or problem. Mineral oil may be mixed into the wet food to facilitate bowel movements and a warm sudsy enema will be used if no bowel movement occurs and/or the bowel has firm/hard fecal matter. As animal begin to have bowel movements they are often soft and may not occur in the litter pan. Thus, the cage floor/cushion is typically covered with an absorbent pad (chuck) which can be easily removed and replaced so that they animal does not sit or lay in fecal matter. Any fecal matter on the cat will be washed off – however, this typically is not required with this injury model. In the rare event that pain appears beyond 72 hrs post-surgery when buprenorphine has been discontinued, buprenorphine will be extended for 24 additional hours. Veterinary staff will be contacted if pain behaviors are seen beyond this timeframe and/or if an animal**

does not seem to respond adequately to buprenorphine. During general handling in the immediate, post-immediate, and chronic post-operative periods, cats will receive social interaction (e.g. ear rubs, head scratches). Their skin integrity, hair quality, cleanliness, responsiveness to human and other animal presence, other general behaviors and general body positions/warmth/movements will be assessed to assure general health and welfare. One-two days after surgery, cats typically seek attention and/or exhibit purring, grooming, sleeping, and other typical cat behavior. Within 2-3 days post-operatively, cats can turn/reposition themselves in the cage without assistance. Within 1-3 days post-operatively, most cats are capable of standing independently and can take at least several steps.

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1,2	**~hourly (minimum)**	**Intermediate post-op period (ranges from 1-3 hrs post)**	[redacted], [redacted], [redacted] **or veterinary staff**
1,2	**Every 6-12 hrs (minimum)**	**3 days**	[redacted], [redacted], [redacted] **or veterinary staff**
1,2	**2x daily (minimum)**	**2 weeks**	[redacted], [redacted], [redacted] **and/or animal care staff**

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 ► **Constipation may be a result of almost any surgical procedure due to anesthetics and analgesics. Although this has not been a complication we have needed to treat post-spay, if it were to present we would use food supplements (e.g. mineral oil, laxatone, etc) and/or an enema if required (see greater detail below under surgery 2).**
A potential complication is herniation. We have not had this occur, but if it appeared to present we would consult with the veterinarian, request imaging to verify, and then proceed with veterinary approval/assistance to surgery to resolve.
A potential complication may be thrombosis. In this case, we would consult with the veterinarian to corroborate interpretation and euthanize.
A complication of spay may be internal bleeding. This is a rare occurrence (1x/~25 years). If signs of persistent internal bleeding (low body temp, lethargic, apparent blood pooling in abdomen) are present, we would consult with the veterinarian and intervene with treatment/surgical intervention or euthanasia.

Surgery 2 ► **During the acute post-operative period, bladder emptying will be disrupted and manual bladder expression (accomplished by applying gentle, but firm pressure to the bladder through the abdominal wall) will be done by the research team. Typically bladder function recovers within 48 hours following the SCI procedures outlined in this protocol. If a cat should present with blood in its urine post-SCI, Penicillin will be started (7 days;Penicillin G, 40,000 U/mg, IM) as a prophylatic step to prevent infection and a**

veterinarian notified. If blood persists or a persistent infection develops, additional treatment will be determined in consultation with a veterinarian.

Following injury, bowel function becomes sluggish due to the surgical anesthesia and use of buprenorphine. This sometimes leads to constipation. Thus, bowel movements are monitored to verify if evacuation is occuring and dietary supplements may be used to promote motility and/or prophylactically (e.g. mineral oil, laxatone). Ample water intake also will be encouraged by mixing water with wet food. If constipation should develop and adequate bowel movement has not been seen by 5-7 days post-op, an enema may be done. Typically this is done using a lubricated Tom cat catheter and a 50-150 ml syringe filled with warm (~body temp) water and a very small amount of gentle soap (>1%, e.g. Dove). The catheter is inserted into the anus up to several inches and the solution gently pushed in until there is reflux. This usually produces results within minutes. Fiber supplements, like Metamucil, are not preferred due to the cat's relatively low water intake and the potential for binding. A cat may be allowed to go >7 days post-surgery without a bowel movement, IF upon palpation the bowel does not seem to be filled with hard stool, there is no pain with palpation and the cat seems to be generally active and eating normally. This approach prevents us from giving an enema to a cat that had an undocumented bowel movement – meaning that someone cleaned the cage/litter pan and did not know, or forgot, to document the stool in the chart and/or that the cat is eating feces. Once the first bowel movement is produced, cats typically defecate effectively on their own. If a constipation persists and does not resolve naturally or with intervention, the veterinarian will be consulted to determine additional steps.

Occassionally, food intake is inadequate post-op in some cats without any evidence of pain. Thus, a high protein, high caloric, palitable post-surgical diet (e.g. Hill's prescription diet, a/d –urgent care) may be incorporated into feeding for several days to increase protein and calorie intake.

Rarely will a cat present with pain after 72 hours when pain medication is discontinued. Signs of distress/pain may be indicated by excessive vocalization, struggling when handled, guarding, lethargy, hiding, abnormal grooming, or poor appetite. If the animal shows any of these or other signs of discomfort or pain after 72 hours, analgesia will be extended for an additional day. If pain does not resolve, treatment will be discussed and determined in consultation with a veterinarian.

In >15 yrs of using buprenorphine we have seen 1-2 cats that did not appear to receive adequate analgesic benefits from this drug. If we appear to have such a cat, we will contact the veterinarian and determine an alternative approach for mitigating surgically-related pain.

Sensation is partially disrupted below the lesion. Thus, cats are housed on thick soft bedding to prevent skin ulcers or peripheral nerve compressions. Similar bedding also is used in carrying cages which transport them between the housing and behavior room. Further, smaller litter pans adequate for use but not to comfortably lay in are used for similar reasons.

Cats may lose weight following spinal cord injury or become plump post-injury, particularly if in an unexercised group. Weights, body condition scores and diets will be closely monitored and diet adjusted to maintain the health of the cats. Although we have never had to euthanize a cat due to low weight or body condition score, if an animal were to fall below a 4/4+ and did not respond to changes in diet, it would be considered for, and/or the veterinarian consulted regarding the choice to move to, euthanasia.

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

Surgery 1 ► **Irreparable internal bleeding, thrombosis resulting in loss of motor function.**

Surgery 2 ► **Body condition score of 4/4- that is not responding to intervention/dietary change.**

(3) In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)
►Drugs that alter inflammatory processes (i.e. steriods, NSAIDs) should only be used topically in SCI animals. Systemic treatment may change the impact of the CNS injury - particuarly if given during the acute injury period. Drugs that directly affect the CNS should be avoided - other than those already used, or similar to those used, in the course of the study (i.e. buprenorphine, anesthetising agents). These drugs, however, are not an issue with regards to an acutely spayed animal or any animal pre-SCI.

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	**Clinical charts for each animal are kept in the animal facility within, or on a counter or wall adjacent to, the animal's housing room.**	**Research staff ([redacted], [redacted], [redacted]) and animal care staff (veterinarian, vet techs and general animal techs).**	(x)	(x)
2	**Clinical charts for each animal are kept in the animal facility within, or on a counter or wall adjacent to, the animal's housing room.**	**Research staff ([redacted], [redacted], [redacted]) and animal care staff (veterinarian, vet techs and general animal techs).**	(x)	(x)

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

ACORP APPENDIX 6
SPECIAL HUSBANDRY AND PROCEDURES
VERSION 4

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

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

Special Procedure		Features							
Number	Brief Description	Husbandry	Restraint	Noxious Stimuli	Exercise	Behavioral Conditioning	Irradiation	Imaging	Other**
1	**Housing Environment Modifications to protect SCI cats from common morbidities including peripheral nerve compression, skin ulcers and further damage to their spinal cords. Cats will also be repositioned if unable to do so when observed 1-3x/daily by research staff to prevent skin irritation. Fecal and/or urine soiling will be prevented by frequent changing of bedding material as needed by research and/or husbandry staff.**	(**x**)	( )	( )	( )	( )	( )	( )	( )
2	**Stimulation to promote bladder and bowel emptying, including manual expression by research staff 1-3x/daily post-SCI as needed.**	(**x**)	( )	( )	( )	( )	( )	( )	( )
3	**Behavioral conditioning, training & food intake. Although food is an essential component of positive feedback for behavioral training, if all food is not eaten, the balance of the cat's daily food requirement will be supplied in a bowl in the behavior room and/or home cage to ensure adequate nutrition.**	(**x**)	( )	( )	( )	(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.
►

a. on the animal:

Special Procedure 1 ► **Housing Environment Modifications:**

1) **Immediately following spinal cord injury, the cats must be housed on a thick bedding of shredded newspaper (6-8") or thick foam (i.e. 4" egg crater memory foam). This bedding must be maintained for the rest of the study to prevent skin ulcers and peripheral nerve compression. If sensory testing of the hindlimbs by the P.I. should show that sensation has 'fully' recovered (due to a treatment) then consideration may be given to reducing or removing the padding.**

2) **Resting boards must be removed permanently from cages of spinal cord injured cats. This must be done to prevent cats from 1) rubbing their incision sites which could further damage their spinal cords; 2) from jumping or rolling off of them and potentially hurting themselves; and 3) lying/sitting for extended periods on a the hard board surface on a sensory compromised limb/hindquarters which is likely to cause skin breakdown and/or nerve compession. Any other item(s) that may encourage activity likely to lead to morbidity cannot be kept in the cage.**

3) **Small litter pans must be used in the cages of spinal cord injured animals. These pans must be small enough to prevent the animal from laying in the pan. Like resting boards (above), the hard pan and litter can cause potential peripheral nerve compression and/or skin breakdown.**

Special Procedure 2 ► **Stimulation to promote bladder and bowel emptying:**
During the acute post-operative period and anytime that there is an acute health concern, cats will be monitored and provided with nursing care a minimum of 2-3x daily. During the acute post-operative period this may include manual bladder expression (accomplished by applying gentle, but firm pressure to the bladder through the abdominal wall). Typically bladder function recovers within 24-48 hours following the procedures outlined in this protocol. Cats may also experience constipation post surgery due to sluggish bowels as a result of anesthesia and spinal cord injury. To prevent and/or relieve constipation, mineral oil or another oral laxative (i.e. laxatone) may be given/mixed into their food and/or their anal opening stimulated with warm water or manually by general rubbing. If a reasonable (size and hardness) bowel movement does not occur within 5-7 days post-op, a warm, sudsy enema or suppository may be used. Typically, bowel movements are seen within 2-5 days post-op and these measures are atypical with the form of SCI used in this study.

Special Procedure 3 ► **Behavioral conditioning, training & food intake:**
Cats are trained daily (~5 times/week) to walk on a treadmill, cross a variety of runways/gait tasks and have reflexes tested. Animals will be trained to perform these tasks for food rewards (food rewards consist of a palatable nutritionally complete diet along with treats). Animals' food preferences as well as nutritional content will be taken into consideration. All tasks are easily achieved physically by spinally intact animals and initial conditioning is simply to establish routine and consistency in performance (behavioral conditioning). The primarily focus is on learning to stay on the runway (not jump off, height is not challenging) and cross smoothly (not stop, not run and to cross when cued). Learning may need to be graded (e.g. reward initially for partial crossing – food at different points along the runway- and ultimately for full crossings-only). Initially the trainer may walk beside the cat to encourage performance. Following SCI, training does not resume for ~2 weeks. This delay is to mimic what happens clinically in rehabilitation, allow general surgical recovery, as well as some recovery of hind limb movement prior to re-initiating training. Following injury, the cats are already conditioned to the task, but

trainers may walk beside cats to provide cues and prevent injury by intercepting a slip, fall or jump. Our prior experience and the published literature shows that following a thoracic hemi-section, cats typically recover basic stepping movements within 1-2 weeks with or without training. During our training approach, physical cuing (i.e. flipping of paw into dorsiflexion to allow plantar versus dorsal stepping, provision of a physical stop/boundary via a trainer's hand to prevent excessive lateral trunk/pelvic deviation, by animal guarding (hands hovering or body block edge of runway) to prevent falling, patterning/moving the limb through appropriate range, etc.) may be provided to enhance performance and/or training. For those animals who may require greater assistance to allow practice and/or task accomplishment, weight support and/or balance may be assisted by gently holding the base of the tail supporting the abdomen by hand or by using a towel/cloth as a sling during walking. As recovery progresses, cues are continuously decreased to promote recovery and independence. All animals are closely monitored during each task to prevent any potential injury.

Animals in the eccentric-focused training groups typically will be trained 5days/week with the goal of performing at least 200 steps on the decline treadmill (e.g. 10^{o}; 0.5m/s) and ~200-400 steps on a (decline) ramp (e.g. 10^{o} or 26^{o}) and/or (down) stairs daily. Initially, this training may be divided into two sessions/day and fewer steps may be accomplished. As the animal's stamina gradually increases, the number of steps will be increased. The flat treadmill and runway (12" wide by 12' long surface/ramp) may be used more during the acute recovery period, but the goal is to integrate these tasks only a few times/week with the main training occurring on the declining surface tasks as time increases post-injury.

On training days, animals typically receive their food in the behavior room; and on non-training days (i.e. weekends) they will be fed in their cages. However, water will be available to them at all times in their cages (*ad lib).*The amount of food an animal receives daily will be based on the cat's body condition, weight gain/loss, and energy requirements. The goal is to provide the average caloric intake needed to meet an adult cat's requirements. The approach that will be used to determine each cat's caloric intake needs will be based upon feline dietary energy requirements (RER) and was provided to us by veterinarians working with our prior studies. For example, adult spayed cats require a typical caloric (kcal) average of 1.2 x RER/day. If a cat is overweight, the typical calculation changes to 0.8 x RER. Thus, although access to food will have a time restriction, the quantity (aka caloric value) provided will be appropriate for an adult cat and thus is not restricted. . Further, if we have an underweight cat whose body condition does not begin to adjust towards normal after a week of increased food we will try different/additional palatable food types, higher caloric foods, and/or contact a veterinarian. If we have a cat that does not seem to care much for the general cat food used, we also will present this cat with additional dietary options.

During training and at all points post-injury, cats will be weighed weekly using a calibrated scale to monitor weight. Typically weights will be taken in the middle of the training week. In addition to weekly weight, a body condition score will be used to monitor overall general health.

Cats with SCIs typically, like humans, experience some muscle atrophy below the level of the lesion. Thus, using a body condition scoring system, such as the Purina 9 point scale, are applied by focusing above the level of the injury with an understanding that scoring may be affected below the injury by muscle atrophy which is a normal event following spinal cord injury.

<u>9 Point BCS Scale</u>

Too Thin
1 Ribs visible on shorthaired cats; no palpable fat; severe abdominal tuck; lumbar vertebrae and wings of ilia easily palpated.
2 Ribs easily visible on shorthaired cats; lumbar vertebrae obvious with minimal muscle mass; pronounced abdominal tuck; no palpable fat.
3 Ribs easily palpable with minimal fat covering; lumbar vertebrae obvious; obvious waist behind ribs; minimal abdominal fat.
4 Ribs palpable with minimal fat covering; noticeable waist behind ribs; slight abdominal tuck; abdominal fat pad absent.
Ideal
5 Well-proportioned; observe waist behind ribs; ribs palpable with slight fat covering; abdominal fat pad minimal.
Too Heavy
6 Ribs palpable with slight excess fat covering; waist and abdominal fat pad distinguishable but not obvious; abdominal tuck absent.
7 Ribs not easily palpated with moderate fat covering; waist poorly discernible; obvious rounding of abdomen; moderate abdominal fat pad.
8 Ribs not palpable with excess fat covering; waist absent; obvious rounding of abdomen with prominent abdominal fat pad; fat deposits present over lumbar area.
9 Ribs not palpable under heavy fat cover; heavy fat deposits over lumbar area, face and limbs; distention of abdomen with no waist; extensive abdominal fat deposits.

The treadmill and runways are composed of metal and wood. All wood surfaces are protected by several coats of paint over which a water resistant sealer typically has been used. Most runway surfaces are covered with an indoor-outdoor plastic type carpet/AstroTurf or rubber surface that is removable and washable. The equipment we use is not suitable for total disinfection. Thus, 1) our equipment disinfection procedures have been developed with guidance of veterinarians in prior studies and 2) SOPs for these procedures are kept in our behavior room. In general, the runways are vacuumed and maintained free of gross debris and any food or spattered food removed using water during training. Runways with indoor/outdoor plastic carpet, rubber matting on them (i.e. the runways, steps) also may have these surface coverings removed and these can be cleaned periodically in several ways beyond wiping with water or cleansers - for example AstroTurf type rugs or rubber surfaces, when loosely rolled fit within a standard washing machine.

Special Procedure 4 ►

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

Special Procedure 1 ► **Removal of resting boards. Following spinal cord injury, sensation is lost below the level of the spinal cord lesion at least acutely. Due to this loss, the animal may not perceive the sensory input that cues animals (including humans) to reposition themselves to prevent ulcers and nerve compression on hard or firm surfaces. Additionally, following a hemisection, coordination is compromised and a cat will more easily roll off a resting board. Thus, the cage bottom is covered with a thick cushion and the resting board removed. Although it is likely that sensory input recovers in the affected hindlimb and hindquarters, we err on the side of caution and maintain the cushion within the cage for the duration of the study after injury. Animals are also manually respositioned by the research staff 1-3x/day if unable to perform this task alone by the research staff to prevent any possible skin irritation.**

Special Procedure 2 ► **Manual bladder/bowel expression. Following spinal cord injury voluntary**

bladder function typically is compromised and voiding ineffective at emptying part or all of the bladder contents for 24-72 hours, and in some cases longer. If the bladder becomes overly full and continues to fill, there is risk of bladder rupture. If a bladder frequently becomes very full without emptying, there is an increased risk of kidney problems and bladder infections. Thus, we assure that these situations do not occur by checking bladder size by manual palpation, watching for soiled cage pads/cushions/litter pans and assisting with emptying until voiding becomes sufficiently effective. Cats may also experience constipation post-surgery due to sluggish bowels as a result of anesthesia and spinal cord injury. Fecal and/or urine soiling is prevented by frequent changing of bedding by husbandry and/or research staff.

Special Procedure 3 ► **Behavioral conditioning, training & food intake.**
Conditioning locomotor performance to a food reward enhances the researcher's ability to accurately assess recovery and ability. It decreases the impact of other motivation-related factors (or lack of). It sets performance expectations (i.e. the cat knows its routine is to go from one end of a walkway to the other and/or perform stepping on the treadmill). It also allows us to closely monitor individual food intake and ensure they are adequately fed, particularly post-injury. Post SCI, animals may also be supported by the research staff to ensure that animals do not fall when conditioning on the treadmill.

Special Procedure 4 ►

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		
3		
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	Expected Potential Pain and/or Distress	
	No	Yes

Item 1)		Description	To Be Relieved	Not to Be Relieved
1	( )	**Skin irritation/ulcers/injury/falling**	(**x**)[a]	( )[b]
2	( )	**Constipation/bladder infection/distention**	(**x**)[a]	( )[b]
3	( )	**Injury post SCI during behavioral training/falling from treadmill**	(**x**)[a]	( )[b]
4	( )		( )[a]	( )[b]

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

Procedure Number (see Item 1)	Agent	Dose (mg/kg) & vol (ml)	Route of admin	Freq of admin (times/day)	Duration of admin (days post-procedure)
1					
2					
3					
4					

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

Special Procedure 1 ► **Resting boards are removed from cages post-SCI and the cage bottom is covered with a thick cushion and the resting board removed permanently. Although it is likely that sensory input recovers in the affected hind limb and hindquarters, we err on the side of caution and maintain the cushion within the cage for the duration of the study after injury. Animals may also be manually repositioned by the research staff 1-3x/day by the research staff to prevent any possible skin irritation. Injury has never occurred, however, we hope to prevent any pain or distress by taking these described measures.**

Special Procedure 2 ► **Cats will be monitored and provided with nursing care a minimum of 2-3x daily during the acute post-operative period. This may include manual bladder expression (accomplished by applying gentle, but firm pressure to the bladder through the abdominal wall). Typically, bladder function recovers within 24-48 hours following the procedures outlined in this protocol. Antibiotics are administered prophylactically at time of surgery to prevent infections. Cats may also experience constipation post-surgery due to sluggish bowels as a result of anesthesia and spinal cord injury. To prevent and/or relieve constipation, mineral oil or another oral laxative may be given/mixed into their food and/or their anal opening stimulated with warm water or manually by general rubbing. If a reasonable (size and hardness) bowel movement does not occur within 5-7 days post-op, ~~a~~ an enema or suppository may be used. Typically, bowel movements are seen within 2-5 days post-op and these measures are atypical with the form of SCI used in this study. Injury has never occurred, however, we hope to prevent any pain or distress by taking these described measures.**

Special Procedure 3 ► **Following injury, the cats are already conditioned to the task, but trainers**

may walk beside cats to provide cues and prevent injury by intercepting a slip, fall or jump. Our prior experience and the published literature shows that following a lateral thoracic hemi-section, cats typically recover basic stepping movements within 1-2 weeks with or without training. During our training approach, physical cuing (i.e. flipping of paw into dorsiflexion to allow plantar versus dorsal stepping, provision of a physical stop/boundary via a trainer's hand to prevent excessive lateral trunk/pelvic deviation, by animal guarding (hands hovering or body block edge of runway) to prevent falling, patterning/moving the limb through appropriate range, etc.) may be provided to enhance performance and/or training. For those animals who may require greater assistance to allow practice and/or task accomplishment, weight support and/or balance may be assisted by gently and firmly gripping the base of the tail supporting the abdomen by hand or by using a towel as a sling during walking. Support also may be given by cradling the ventral trunk in the trainer's hands. As recovery progresses, cues are continuously decreased to promote recovery and independence. At a minimum all animals are closely monitored during each task to prevent any potential injury. Injury has never occurred, however, we hope to prevent any pain or distress by taking these described measures.

Special Procedure 4 ►

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 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

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	**Observation 2-3x/daily for 2-5 days post-operatively by research and/or husbandry staff and then 1-2x/daily**	**If a cat does not resume normal cat behavior, has severe skin ulcers, extreme weight loss and/or post-surgical sepsis, a veterinarian will be consulted and euthanasia considered.**
2	**Observation 2-3x/daily for 2-5 days post-operatively by research and/or husbandry staff and then 1-2x/daily**	**If a cat does not resume normal cat behavior, has severe skin ulcers, extreme weight loss and/or post-surgical sepsis, a veterinarian will be consulted and euthanasia considered.**

3	**Observation 2-3x/daily for 2-5 days post-operatively by research and/or husbandry staff and then 1-2x/daily**	**If a cat does not resume normal cat behavior, has severe skin ulcers, extreme weight loss and/or post-surgical sepsis, a veterinarian will be consulted and euthanasia considered.**
4		

RE: exemption for non-AAALAC facility

Fallon, Michael DVM PhD [Michael.Fallon@va.gov]

To:
[redacted]

Cc:
[redacted]

Tuesday, May 24, 2016 12:34 PM

Dr. [redacted]- This email message serves to notify you that your VA project has been given a waiver against VHA Handbook 1200.07 to perform part of your animal research at Georgia Tech University, which is not currently accredited by AALAC, International. Please attach this email to your ACORP when it is uploaded into the JIT system for pre-funding clearance.

Mike

Michael T. Fallon, DVM, PhD, DACLAM, CPIA
Chief Veterinary Medical Officer
ORD, VHA

Mailstop 508/151V; Room 4A106
Atlanta VA Medical Center
1670 Clairmont Road
Decatur, GA 30033
Michael.fallon@va.gov
404-732-5471

Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
[redacted]	Louisville, KY-603	MERIT/Summer 2016	Force Feedback Redistribution & Eccentric-Focused Rehab Post-SCI

	SCORE	DESCRIPTION	ACTION NEEDED BY IACUC
●	0	No concerns noted. Any comments provided are for information only.	*None*. No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
○	1	Some concerns noted.	*The IACUC must review the **level 1** 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).* No further correspondence with the CVMO is needed; the ACORP(s) is(are) cleared and represent(s) no bar to funding the application.
○	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.	*A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. Upload the following at https://vaww.gateway.research.va.gov: (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. *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(s).*
○	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.	*A response to each of the **level 3** concerns listed below must be reviewed and cleared by the CVMO* before work can resume and funding can be released. (If unusual circumstances dictate that work should continue despite concerns, notify the CVMO immediately.) *A response to each of the **level 2** concerns noted below must be reviewed and cleared by the CVMO* before funding can be released. For **level 2 and 3** concerns, upload the following at https://vaww.gateway.research.va.gov : (1) a memo addressing the concerns, signed by the PI, veterinarian, and IACUC Chair; and (2) (a) revised ACORP(s) approved by the IACUC. *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(s).*

(cont.)

The ACORP for Dr. [redacted] has received an overall score of 0, which means that it is cleared and represents no bar to funding the application. No concerns were identified during the review of the final protocol. The investigator, the Attending Veterinarian, and the IACUC(s) are commended for their dedication and commitment to humane animal care and use.

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 (hemisection) to improve understanding and evaluate a novel rehabilitation strategy to improve muscle function and improve weight support and walking. The investigator is commended for her dedication to conducting this study as humanely as possible and still achieve the scientific objectives. This ACORP was originally uploaded on 12/12/16 and subsequent to the initial review, the grant sections were requested. Although, the experimental plan was clear; the invasive nature of this study requires rigorous attention to detail to ensure the procedures utilized were not misinterpreted. The reviewer's comments were provided in a JIT note dated 12/14/16 (shown below) and the reviewer discussed these issues via a telephone conversation with Dr. [redacted]. Drs. [redacted], [redacted], and [redacted] (the reviewer) worked together to revise the protocol to address the concerns raised in the 12/14/16 JIT note. As a result of this extensive interaction, no concerns were identified in the final ACORP uploaded on 2/24/17. No further correspondence with the CVMO is needed; the ACORP is cleared and represents no bar to funding the application.
	12/14/16 JIT Note: Thank you for the grant sections, the information provided was helpful. After reviewing both the ACORP and the grant sections, the Office of the CVMO is recommending the following revisions: (1) the lay summary is too technical, please express in lay terms that the average person could understand. (2) explain how the eccentric training of the SCI cats is actually accomplished during their training (use of sling or other apparatus). (3) clarify the two types of training tasks, (4) include a description of the studies conducted at Georgia Tech, (5) elaborate on the once a day feeding of the cats during training, and (6) elaborate further on nursing care and the clinical presentation of the cats (function ability). Dr. [redacted] should advise and assist Dr. [redacted] in revising the ACORP; please upload the new version when available. Thank you, [redacted], DVM

(cont.)

(cont.)