

Use of Version 2 or 2a is OK until 1/1/05, then Version 3 must be used

Check all that apply:

☒ Maj. Surv. Surgery ☐ Primates ☐ Cat. E studies ☐ Haz. Agents ☒ Dogs, cats or pigs

ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP)

Version 3

Note: Use a separate form for each species. DO NOT include individual appendices if they are not relevant to the protocol being described. To check boxes, right click, choose properties, then click to check the box. Define all abbreviations the first time they are used. To add a row to a table, click inside one of the existing table cells, then select Table, Insert, Rows from the main menu of the program.

A. **ACORP Status.** Complete items A.1- A.8 below; then proceed to item B.

1. Name of Principal Investigator: Dr. [REDACTED] Ph.D.
2. VA Station Name and Number: Malcom Randall VA Medical Center, Gainesville, FL #573
3. Proposal Title: Training Effects on Recovery of Balance and Limb Accuracy in Cats Post-SCI
4. Animal Species covered by this ACORP (only one): Cat
5. Funding Source. Indicate the source(s) of funds that will be used to perform these animal procedures once approved by the VA IACUC:
 - ☒ Department of Veterans Affairs (could be either VA or NIH funds)
 - ☒ U. S. Public Health Service (e.g. NIH)
 - ☐ Private or Charitable Foundation. Identify:
 - ☐ University Departmental Funds. Identify University and Department:
 - ☐ Private Company. Identify:
 - ☐ Other. Identify:
6. Is this a new ACORP for a new project?
 - ☒ Yes. Proceed to item 7.
 - ☐ No. Answer A.6.a-d. below.
 - a. Indicate the status of this ACORP below:
 - ☐ This is an unchanged, approved ACORP intended for a new funding source.
 - ☐ This is a revised ACORP with a new funding source.
 - ☐ This is a revised ACORP that reflects changes or additional, new studies.
 - ☐ This ACORP is submitted as a three-year (3-year) renewal (see item d. below).
 - ☐ Other. Please specify:
 - b. Previous ACORP title:
 - c. Previous IACUC approval number (VA and affiliate, if applicable):
 - d. If this is a three-year renewal, provide a progress report describing work accomplished during the last approval period. Include the number of animals used, the objectives that were met and how the work proposed in this renewal extends the previous studies. If not applicable, go to item 7.
7. Do you plan on performing the animal procedures described in this form even if you do not receive intramural VA or extramural PHS, NSF, or other funding?
 - ☐ Yes.
 - ☒ No.
8. Indicate the type of animal use:
 - ☒ Research.
 - ☐ Teaching or Training.

- ☐ Testing.
- ☐ Sentinel animal use.
- ☐ Breeding and colony management only; no experimental procedures.
- ☐ Other. Please specify:

Proposal Overview

B. Lay Description. Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project might improve the health of people and/or other animals. A scientific abstract from a grant proposal is not acceptable. Once completed, proceed to item C.

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 SCI throughout their lives. The current research proposal addresses these needs through basic animal studies which assess the effects of two potential therapeutic treatments on re-growth of connections within the injured spinal cord and recovery of walking. The two therapeutic treatment approaches that will be assessed alone and in combination are: 1) motor training targeting features of locomotion required for stepping adaptations necessary for community walking; 2) intraspinal delivery of an enzyme that breaks down factors inhibiting growth in the injured spinal cord. These potential treatments also may be relevant for other neurologic disorders which result in loss of connections and walking difficulty.

C. Experimental Design.

1. Using non-technical (lay) language that a senior high school student would understand, describe the experimental design in no more than one or two paragraphs.

These studies will test the benefits of two potential therapies for spinal cord injury. These potential therapies are the use of training (rehabilitation approach) and application of an enzyme (chondroitinase abc) which breaks down growth inhibitory molecules that increase at the site of the spinal cord injury.

Animals used for these studies will be spayed and receive low thoracic spinal cord hemisections. All animals will be trained to perform a variety of behavioral tasks including crossing of a basic (wide, flat) runway and multiple skilled overground locomotor tasks (ladders, pegboards, etc) for food rewards. After baseline data on all tasks are collected, cats will receive hemisection injuries. Post-hemisection, they will be divided into a hemisection without chondroitinase abc (ch'ase) treatment group and a hemisection plus ch'ase treatment group as indicated in the table below. Cats will be further divided within these two large groups into smaller groups: skill-, basic- and un-trained groups. Approximately, seventeen weeks post-SCI, after completion of behavioral data collection and/or behavioral plateau, substances to trace connections (retrograde and anterograde tract tracers) will be placed into the spinal cords. These different tracing approaches must occur in different animals as introduction of one tracer can disrupt the transport/labeling of another tracer. Cats will be divided equally between retrograde and anterograde tracing studies for each training group. Based upon our experience, we will use the minimal group numbers necessary to show significant anatomical differences using tract tracing approaches. During all surgical procedures, cats will be deeply anesthetized and potent analgesics will be given post-operatively for 48 hours. Further, all cats will be sacrificed at the end of the study using anesthetic overdose and intracardial perfusion.

Hemisection, No Ch'ase treatment:	Hemisection + Ch'ase treatment:
Un-trained (n=12)	Un-trained (n=12)
Basic training (n=12)	Basic training (n=12)
Skilled training (n=12)	Skilled training (n=12)

2. In language scientific colleagues outside of your discipline would understand, describe the experimental design for the animal experiments planned, and the sequence of events to reveal what happens to the animals. Include all procedures and manipulations, and explain why they must be performed. Give your best estimate of how many animals will undergo the procedures or manipulations described. For complicated experimental designs, a flow chart, diagram, or table is strongly recommended to help the IACUC understand what is proposed. Do not describe the details of surgical procedures, monoclonal antibody production, or behavioral training here. Such details are requested later in appendices. Once completed, proceed to item D.

The experiments described in this proposal will address the potential benefits of a skill-training approach which targets several adaptive voluntary features of locomotion, as well as the effects of combining intraspinal ch'abc treatment with specific training on recovery. Although some studies, including our previous work, have treated with ch'abc on a background of what can be considered training, the benefits of repeated testing and/or conditioning paradigms to the reported "ch'abc mediated" recovery have not been addressed with the exception of a recent study in the rat following a C3 dorsal column cut. From a clinical perspective, it is critical to establish whether functional benefits can be obtained with ch'abc in the absence of training and if the combination of ch'abc and training is critical to the recovery of adaptive locomotor responses required during skilled forms of locomotion.

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.

For the experiments under Hypothesis 1, three groups of cats with low thoracic hemisections (hxs) will be studied. The first will be untrained. The second will undergo a basic locomotor training protocol in which cats will be exercised daily on a simple 30.5 cm wide x 4.5 m long horizontal runway. This daily conditioning approach was chosen as it is similar to the human condition of Brown-Sequard Syndrome (lateralized lesion similar to a hemisection) in which most individuals are performing basic walking each day. The third group will be skill-trained. In addition to crossing the simple runway, these cats will receive training on skilled locomotor tasks that require accurate limb trajectories, paw placements and significantly challenge balance. With the exception of the untrained group, cats will be extensively trained on their respective tasks and training time and/or repetitions will be controlled across these groups throughout the study. Pre-injury, all groups will receive training on basic and skilled tasks. Baseline performance data will be collected pre-injury on all cats on the trained basic and skilled tasks. In addition, two novel, untrained tasks requiring adaptations of the stepcycle also will be assessed. These tasks will be important in understanding transfer of adaptive features practiced on trained tasks to novel situations. Post-injury, all cats will be assessed periodically on these same tasks regardless of training group (un-, basic-, or skill-trained). Thus, some tasks post-injury will be trained, some untrained but familiar, and others novel depending on the training group. The early post-injury time frame for training and ch'abc delivery was chosen to parallel our published and current work. The early ch'abc delivery also parallels most of the studies conducted in the rodent. There are a variety of mechanisms that may enhance recovery in the acute setting that are not viable in the chronic lesion environment. For example, there is the potential to spare neurons that might undergo axotomy induced death and also preserve axonal populations or circuits that would otherwise undergo

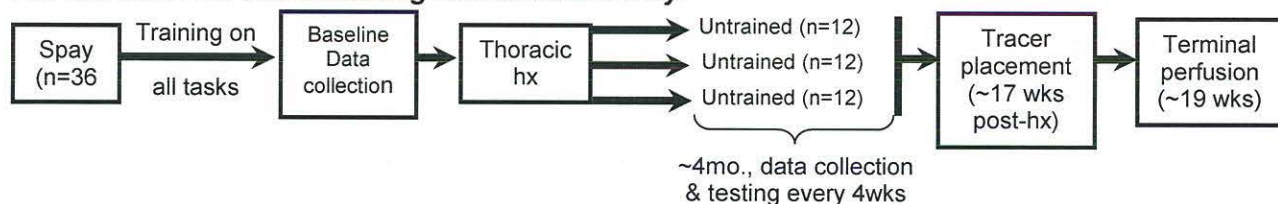
progressive degeneration. Successful outcomes with ch'abc and/or skill training would set the stage for future studies in the chronic injury setting. Our work, as well as that of others, shows that cats with low thoracic spinal hxs recover significant locomotor function but have persistent, quantifiable deficits. **Although cats quickly recover the ability to step on a treadmill and voluntarily cross a basic runway following hxs, they do not readily integrate the hindlimb ipsilateral to the lesion during tasks that require adaptation in limb trajectories such as ladder crossing. Typically cats with low thoracic hemisections will cross a ladder on 3 limbs.** The tests and assays used to define recovery will be critical in identifying the **adaptive** deficits. Basic locomotor rating scores are not sufficiently informative following hx injuries and deficits are only most obvious during more difficult tasks. Thus, for these studies, we have chosen a single simple task (crossing of a wide runway) as well as multiple skilled locomotor tasks that require different combinations of input from various levels of the neuraxis along with multiple quantitative analyses of specific locomotor features. The cat with a low thoracic hx provides an SCI model that is well-suited for testing the safety and efficacy of potential therapeutic agents.

Under Hypothesis 2, in addition to low thoracic hemisections, all cats will be treated with ch'abc for one month. Otherwise, the three groups and training are exactly the same as described above. At the end of the study, after the final data collection has been completed, all animals will undergo a final surgery for placement of tract tracer(s). Cats will survive for up to 2 weeks following tracer placement. All cats will be sacrificed by anesthetic overdose and transcardial perfusion.

These types of studies will help begin to identify what mechanisms may mediate recovery and how training approaches and/or ch'abc affect each individually or in combination. We propose to use a total of 80 cats for the entire 4 years of this project. Specifically, there will be 12 cats in each of the 6 training groups. Each training group will be further divided into anterograde (n=6) and retrograde (n=6) groups. A 10% attrition is included for tracer misplacement and lesion error (6 groups x 12/group + 10%x 72 =80).

The basic flow of the experiments is presented below:

A. Flow chart for cats receiving hemisections-only:



B. Flow is identical for the 36 cats receiving hemisections + ch'abc except that cats will begin to receive c'abc at the time of injury and every other day for 4 weeks .

D. Describe the characteristics of the selected species, strain, stock, mutant, or breed that justify its use in the proposed study. Consider such characteristics as body size, species, strain, breed, availability, data from previous studies, and unique anatomic or physiologic features. Once completed, proceed to item E.

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 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. 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, our work on CS GAGs (the target of the potential therapeutic agent, Ch'ABC) suggests that sulfation patterns are more similar between cat

and human than rat and human, elegant motor control, and seminal work regarding task specific training. **Only female cats will be used due to greater ease with bladder care post-SCI. Their bladders are much easier to manually express and uric acid crystals do not pose the blockage risk seen with the male urethra. Further, due to their typically smaller size, they are much easier to handle and lift during behavioral training and filming sessions.**

Personnel

E. Give the names of all research staff expected to work with the animals in this study. For each person listed, describe their education, training, and experience with experimental animals in general AND describe their experience performing the exact procedures in the species described in this ACORP. This description must help IACUC members determine if all animal manipulations, including surgery, testing, and blood collection, are performed by individuals who are qualified to accomplish the procedures skillfully and humanely. A listing of academic degrees alone is not an adequate response. (Qualifications to perform euthanasia will be requested in item U.3. and need not be given here.) Once completed, proceed to item F.

██████████, Ph.D., Research Neurobiologist (VA) and Associate Professor (UF). As the PI of the project, Dr. ██████████ 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 20+ years experience with injections, perfusions, animal handling, spinal cord surgeries, 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. She has taken all the appropriate online UF and VA animal training and IACUC modules. She also has attended the Animal Awareness and Use Seminar at the University of Florida. The on-line modules completed at the University of Florida include: The Humane Care and Use of Laboratory Animals, Care and Use of the Rat, The Care and Use of the Cat, Aseptic Surgery of Rodents, Anesthesia and Analgesia of Rodents, Occupational Health and Safety, Working with the UF IACUC. She is registered with the University of Florida's EH&S for the animal contact program.

██████████, WOC (VA) and Laboratory Manager (UF). Mr. ██████████ will be involved with all aspects of the project including surgeries, animal care, training, perfusions, tissue harvesting, and post-mortem analyses. He has 16+ years of experience with rodent and cat surgeries, anesthesia, tissue collection, tract tracing, nursing/post-operative care, euthanasia, and behavioral training/locomotor evaluation. Part of his training occurred under Dr. ██████████. He is capable of performing all aspects of this project. He has taken all the appropriate online VA and UF animal training and IACUC modules. He also has attended the University of Florida Animal Awareness and Use Seminar. The specific online modules completed include: Laboratory Animals, Laboratory Rat, Laboratory Cat, Laboratory Mouse, Aseptic Surgery of Rodents, Anesthesia and Analgesia of Rodents, Working with the UF IACUC. He also has taken the Rodent Aseptic Surgical Techniques Workshop and Rodent Handling, Restraint, and Injection Techniques Workshop at UF. He is registered with the University of Florida's EH&S for the animal contact program.

██████████, B.S., WOC (VA) and Graduate Student (UF). Ms. ██████████ will be involved with all aspects of the project including surgeries, animal care, training, sacrifice, tissue harvesting, behavioral training/filming and post-mortem analyses. She has ~3 years of experience assisting with and performing cat surgery, anesthesia, tissue collection, tract tracing, nursing/post-operative care, euthanasia, and behavioral training/locomotor evaluation. Her training occurred under Dr. ██████████ and ██████████. She is capable of assisting with and performing all aspects of the protocol.

She has completed all the appropriate online courses at the University of Florida including "Working with the UF IACUC". She has also completed the appropriate VA animal training. She attended the "Animal Awareness Seminar" as part of her 1st year IDP course work. She is registered with the University of Florida's EH&S for the animal contact program.

F. If personnel do not have experience with the exact procedures described in this ACORP, how will they be trained, who will train them, and what are the training experiences or qualifications of the person(s) doing the training? If not applicable, enter "N/A". Once completed, proceed to item G. **Any new, less experience individuals that may be added to the protocol in the future will be supervised/trained by [REDACTED] and/or [REDACTED]. No one will be allowed to perform any procedure independently until it is deemed that her/his performance poses no additional risk to the animals than those inherent to the procedure itself or if the procedure were performed by an experienced individual.**

G. Occupational Safety and Health.

1. Have all personnel listed in item E. been enrolled in the Occupational Health and Safety Program for those with laboratory animal contact?

☐ Yes. Proceed to item G.2.

☒ No. If personnel have declined to participate, are enrolled in another equivalent program, or will enroll before studies commence, so indicate here and then proceed to item G.2.

The above research staff are registered with the University of Florida's EH&S and are approved to work with the species to be used in this protocol. This is appropriate as the animal procedures will occur at the University of Florida. [REDACTED] also is enrolled in the VA Occupational Health and Safety Program.

2. Are there any non-routine measures such as special vaccines or additional health screening techniques that would potentially benefit research, husbandry, or veterinary staff participating in or supporting this project? Routine measures included in the Occupational Health and Safety Program (vaccination for tetanus, rabies, and hepatitis B, and TB screening) need not be mentioned here.

☐ Yes. Describe them, then proceed to item H:

☒ No. Proceed to item H.

H. Complete the following table; then proceed to item I.

Strain, Stock, Mutant, or Breed	Gender	Age/Size	Source (Vendor)	Health Status*
Cat	Female	Adult	[REDACTED] (or any SPF cat source)	SPF

*For each strain, stock, mutant, or breed listed, provide information about the expected status of the animals:

-For rodents and rabbits, indicate specific-pathogen-free (SPF), gnotobiotic (germ-free or defined flora), conventional, feral, or other description.

-For dogs, cats, pigs, and other "large animals", indicate specific-pathogen-free (SPF), conditioned, conventional, feral, or other description.

-For non-human primates, indicate viral status (e.g., herpes B, SIV, etc.)

-Also indicate here if animals will be surgically altered by the vendor (e.g., ovariectomized rats).

I. Complete the tables below, assigning all requested animals by breed/strain/mutant to a USDA category of pain/distress. If you have difficulty determining the appropriate category, please contact the attending veterinarian or IACUC Chair for assistance. The same animal cannot be assigned to more than one USDA category. If several different procedures are planned, the animal should be placed in a category based on the most painful/distressful procedure. You are required by VA policy to describe planned procedures for the fourth and fifth years of a submitted VA grant even though,

under PHS policy, the IACUC must perform a new review three years after the initial approval date. Once completed, proceed to item J.

USDA Category B: List by year the number of animals that will be bred or purchased for breeding, but not used for experiments. This includes breeders, young that cannot be used because of improper genotype or gender, and any other animals that will not have any research procedures performed on them or participate in research studies. If numbers cannot be determined exactly, estimate as closely as possible. (Note: If tail snips are necessary for genotyping, this category is not appropriate.)

Breed/Strain/Mutant	Year 1	Year 2	Year 3	Year 4	Year 5

USDA Category C: List, by year the number of animals that will undergo procedures that involve no or only very brief pain or distress, with no need for or use of pain relieving drugs. Examples include observational studies, most intravenous and parenteral injections of non-irritating agents, most blood collections from peripheral vessels, and the collection of cells and/or tissues from animals after euthanasia has been performed.

Breed/Strain/Mutant	Year 1	Year 2	Year 3	Year 4	Year 5

USDA Category D: List by year the number of animals that will undergo procedures involving potential pain or distress that is relieved by appropriate anesthetics, sedatives, or analgesics. Examples include major and minor surgery performed under anesthesia (survival or non-survival), tissue or organ collections prior to euthanasia, painful procedures performed under anesthesia (such as retro-orbital blood collection in rodents), prolonged restraint accompanied by tranquilizers or sedatives, and experiments involving infectious or other hazardous materials in animals that have provisions for immediate euthanasia if they become sick to effectively prevent pain and/or suffering. If an endpoint is used that involves significant pain or distress, consideration should be given to putting animals into Category E.

Breed/Strain/Mutant	Year 1	Year 2	Year 3	Year 4	Year 5
Cat, SPF	20	20	20	20	

USDA Category E: List, by year, the number of animals that will undergo procedures in which pain or stress is NOT relieved with the use of anesthetics, analgesics, tranquilizers, or by euthanasia. Examples include studies in which animals are allowed to die without intervention (e.g. LD₅₀, mortality as an end-point), studies that allow endpoints that are painful or stressful, addictive drug withdrawals without treatment, pain research, and noxious stimulation.

Breed/Strain/Mutant	Year 1	Year 2	Year 3	Year 4	Year 5

TOTALS: Bring all totals for each year down, by breed/strain/mutant.

Breed/Strain/Mutant	Year 1	Year 2	Year 3	Year 4	Year 5
Cat, SPF	20	20	20	20	

J. **Description of USDA Category D and E procedures.** Are any USDA Category D or E studies planned?

☐ No. Proceed to item K.

☒ Yes. Complete items J.1. and J.2.; then proceed to item K.

1. List and describe all category D procedures by filling out the table below. If no category D

studies are proposed, enter "N/A" and proceed to item J.2. For any surgical procedures you will describe in Appendix 5, enter only a brief description in the "Procedure" column, then enter "See Appendix 5 for details."

Procedure	Frequency of monitoring after the procedure and how long animals will be monitored	Person(s) doing the monitoring	Analgesic, sedative, or anesthetic used, plus dose, route, and duration
Modified 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.	Penicillin G, procaine 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; Atropine sulfate , 0.04-0.06 mg/kg, SQ, pre-op only; Acetylpromazine , 0.1-0.2 mg/kg 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. Meloxicam , 0.2 mg/kg PO, first day, then 0.1 mg/kg SID for 2 additional days
Spinal cord lesion(hemisectomy)/port placement. *See Appendix 5 for details.	At least every 6-12 hours until bladder function begins to return (usually 1-2 days). Daily after return of bladder function.	Any of the individuals listed on the protocol.	Penicillin G, procaine 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.
Tract tracer placement *See Appendix 5 for details.	At least every 6-12 hours until bladder function begins to return (usually 1-2 days). Daily after return of bladder function.	Any of the individuals listed on the protocol.	Penicillin G, procaine 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; Atropine sulfate , 0.04-0.06 mg/kg, SQ, per-op only; Acetylpromazine , 0.1-0.2 mg/kg 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.
Intracardial perfusion	N/A as this is a terminal procedure	N/A as this is a terminal procedure	Sodium pentobarbital (~35 mg/kg, IP or IV initially) with one or more supplemental doses (25% of initial dose) as needed.

2. Each year a report describing and justifying all category E procedures must be submitted by each facility to the USDA and the VA. If no category E studies are proposed, enter "N/A" and proceed to item K. Otherwise, describe each category E procedure, and justify completely why pain or distress relief cannot be provided for each procedure. If the species is covered by USDA regulations, your description will be used in the USDA annual report. If animals will be allowed to experience natural death as a result of experimental procedures (e.g. infectious disease or oncology studies), or an endpoint is used that allows the animals to experience significant pain or distress, you must justify why an alternate endpoint (such as weight loss, clinical signs, tumor size, etc.) prior to death or pain or distress can not be used. If animals will undergo category D procedures as well, describe them in item J.1. above.

N/A

K. **Justification for number of animals requested and group sizes.** Describe how the estimated number of animals needed for the experiments was determined. When appropriate, provide the number and type of experimental and control groups in each experiment, the number of experiments planned, and the number of animals in each group. The ILAR *Guide* states that whenever possible, the number of animals requested should be justified statistically. A power analysis is strongly encouraged to justify group sizes when appropriate. Once completed, proceed to item L.

The number of animals in each group is based upon completed retrograde tracing studies showing that 6 cats/group is sufficient to show significant changes in several of the pathways to be studied. Thus, each of the 6 “training” groups will have 12 animals each (6 for retrograde and 6 for anterograde tracing studies). When a 10% attrition (for tracing misplacement, lesion error, etc.) is added to the 72 animals across groups, the total number of animals to be used is 80 ($6 \times 12 + 10\%(72)$).

L. **Laboratory Animal Veterinary Support.** Complete items L.1-L.3, then proceed to item M.

1. Give the name of the laboratory animal veterinarian responsible for providing adequate care to the animals that will be used and their institutional affiliation:

The University of Florida Animal Care Services Veterinarians will be available, as necessary, to care for the animals in the studies. These veterinarians include Drs. [REDACTED], [REDACTED] and [REDACTED].

2. VA Policy requires that a laboratory animal veterinarian be consulted during the planning stages of any procedure involving laboratory animals, before IACUC review. Give the name of the laboratory animal veterinarian consulted during the planning of procedures involving animals. As an alternative to an actual meeting, the veterinarian may perform a pre-review of the ACORP and provide comments to the PI so that the ACORP may be revised prior to IACUC review:

Dr. [REDACTED] – initial review on 05/10/10

Dr. [REDACTED] – review of revisions on 06/30/10

3. Give the date of the veterinary consultation (meeting date, or date written comments were provided by the veterinarian to the PI):

May 10, 2010 – Dr. [REDACTED]'s written comments provided to PI by IACUC staff

M. **Husbandry.**

1. Caging needs. To help the animal care staff with caging needs, please indicate the type of caging that you will need; then go on to item M.2:

- ☐ Gnotobiotic (germ-free and defined flora) isolators
- ☐ Biohazard or other special hazard containment caging
- ☐ Sterile rodent microisolator caging, with filtered cage top
- ☐ Non-sterile rodent microisolator caging, with filtered cage top
- ☐ Standard rodent shoebox caging with no filter top
- ☐ Standard non-rodent caging, appropriate for species
- ☒ Other. Describe: solid floor cat caging.

2. The ILAR *Guide for the Care and Use of Laboratory Animals* states that consideration should be given to housing social animals in groups whenever possible. Will social animals be housed singly?

- ☐ Yes. Complete item M.3.
- ☒ No. Proceed to item M.4.
- ☐ Not Applicable; the species involved is not a social animal. Proceed to item M.4.

3. Please provide a justification for housing social animals singly; then proceed to item M.4.

Cats will be housed in joint cages unless there is a specific situation that warrants single housing. These situations would include:

- 1) a cat that does not get along well with other cats (i.e. fights).**
- 2) a medical condition that warrants single housing (i.e. acute post-op period).**
- 3) an unequal number of cats (our cages only hold 2 cats/cage).**

4. The ILAR *Guide for the Care and Use of Laboratory Animals* recommends the use of contact bedding (i.e., shoebox or microisolator cages) instead of wire mesh floors for housing rodents. Will rodents be housed on suspended wire mesh floors or other flooring in which the animals do not rest on bedding?

- ☒ Not applicable; this ACORP does not describe rodent use. Proceed to item M.6.
- ☐ No. All rodents will be housed in shoebox or other caging in which the animals rest directly on bedding. Proceed to item M.6.
- ☐ Yes. Proceed to item M.5.

5. Why is caging with wire mesh flooring necessary?

NA

6. Indicate the appropriate response below:

- ☒ This ACORP does not address the use of dogs, primates, or genetically engineered or modified (e.g. transgenic/knockout/knockin) animals. Proceed to item M.7.
- ☐ This ACORP addresses the use of dogs. Answer item M.6.a. below, and M.6.c if applicable.
- ☐ This ACORP addresses the use of primates. Answer item M.6.b. below, and item M.6.c. if applicable.
- ☐ This ACORP addresses the use of genetically engineered or modified animals. Answer item M.6.c. below.

- a. Is there any scientific justification for excluding the dogs in this study from the institutional dog exercise plan required by USDA?

- ☐ No. Proceed to item M.7.
- ☐ Yes. Provide a scientific justification for excluding the dogs; then proceed to item M.7:

- b. Is there any scientific justification for excluding the primates from the institutional primate psychological enrichment plan required by USDA?

- ☐ No. Proceed to item M.7.
- ☐ Yes. Provide a scientific justification for excluding the primates; then proceed to item M.7.:

- c. Do the genetically engineered or modified animals exhibit any characteristic clinical signs or abnormal behavior related to their genotype?

- ☐ No. Proceed to item M.7.
- ☐ Yes. Describe here, then proceed to item M.7.:

7. Will any cannulae, acrylic implants, venous catheters, or other similar medical devices be implanted into an animal such that the device extends chronically through the skin?

- ☒ No. Proceed to item N.
- ☐ Yes. Explain what implantation and wound management measures will be taken to minimize the chances of chronic infections around the device(s) where they penetrate the skin (then proceed to item N):

N. Housing Sites.

1. Will all animals purchased with VA or VA Foundation funds be housed only in VA facilities?
 - ☐ Yes. Proceed to item N.2.
 - ☒ No. Complete and attach ACORP Appendix 1, "Use Of a Non-VA Facility to House Animals Purchased with VA or VA Research And Education Corporation Funds", then go to item N.2.

2. Give the location(s), inside or outside of the animal facility, where animals will be housed permanently or temporarily, then proceed to item O:

Cats will be housed in the [REDACTED] animal facility. The [REDACTED] is where the PI's behavioral laboratory is located. There are no cat training and filming facilities available at the VA.

O. **Antibody Production.** Will animals be used to produce monoclonal or polyclonal antibodies, or will existing hybridoma cell lines be injected into animals to harvest antibody?

- ☒ No. Proceed to item P.
- ☐ Yes. Complete and attach Appendix 2, "Antibody Production;" then proceed to item P.

P. **Test Substances.** Will test substances be administered to animals? For the purposes of this question, test substances are defined as materials administered to animals. This includes, but is not limited to, radioisotopes, toxins, antigen, pharmacological agents, infectious agents, carcinogens or mutagens, biomaterials, prosthetic devices, and cells, tissues, or body fluids. (Note: The following substances do not need to be entered in Appendix 3 unless they are hazardous: routine pre- or post-operative drugs described in the Surgery Appendix [Appendix 5], antigens, adjuvants, hybridomas described in the Antibody Production Appendix [Appendix 2], and euthanasia agents entered in item U, Euthanasia.)

- ☐ No. Proceed to item Q.
- ☒ Yes. Complete and attach Appendix 3, "Test Substances;" then proceed to item Q.

Q. **Location of procedures.** Complete the table below, indicating where all non-surgical procedures and manipulations will be performed. The IACUC must be aware of all procedures performed outside of the animal facility. To help the IACUC track the sites of animal use outside the animal facility, give the location of any laboratory or other areas outside of the animal facility in which animals will be manipulated in any way. Be sure to include the sites of procedures such as radiography, fluoroscopy, computed axial tomography (CT), or magnetic resonance imaging (MRI) that may be performed outside the animal facility.

Non-surgical Procedure	Building and Room Number	Method of discreet transport, if required through <u>non-research</u> areas (enter N/A if not applicable)*
Euthanasia	[REDACTED]	Cats will be transported in carrying cages. They will not go through patient areas.
Chondroitinase ABC injections	[REDACTED]	Cats will not be transported out of animal facility.
Behavioral training	Cat behavior room in the [REDACTED] animal facility ([REDACTED])	Cats will not be transported out of animal facility.

*Describe how animals will be transported to and from these sites. Transportation must be in accordance with the *Guide*, USDA regulations, and PHS policy in climate-controlled vehicles and sanitizable transport cages when appropriate. Such transport must be discreet such that

hospital staff and patients are not aware of the transport, and are not exposed to allergens and/or body fluids from the transported animal(s). Once completed, proceed to item R.

R. Body Fluid, Tissue, and Device Collection.

1. Will any body fluids, tissues, or devices be collected from animals AFTER euthanasia?

☐ No. Proceed to item R.2.

☒ Yes. List the fluids, tissues, and/or devices here; then proceed to item R.2.:

CNS, PNS, blood, port

2. Will any body fluids, tissues, or implanted devices or materials be collected from animals BEFORE euthanasia?

☒ No. Proceed to item S.

☐ Yes. Proceed to item R.3.

3. Is collection in live animals limited to blood collection associated with antibody production?

☐ No. Complete and attach Appendix 4, "Antemortem Specimen Collection." Then proceed to item S. If the body fluid, tissues, or devices are collected as a surgical procedure, please be sure to also describe these collections as part of the surgical protocol in Appendix 5, "Surgery."

☐ Yes. Because blood collection associated with antibody collection is already described in Appendix 2, "Antibody Production", DO NOT complete Appendix 4, "Antemortem Specimen Collection." Proceed to item S.

- S. Surgery.** Will survival or non-survival surgery be performed?

☐ No. Proceed to item T.

☒ Yes. Complete and attach Appendix 5, "Surgery;" then proceed to item T.

T. Endpoint Criteria. What specific endpoint criteria will be used for determining when sick animals, both on and off study, will be euthanatized or otherwise removed from a study? Examples of appropriate criteria that should be considered include a weight loss limit as a percentage of initial or expected body weight, allowable durations of anorexia, allowable tumor size or total tumor burden expressed as a percentage of body weight, the presence of health problems refractory to medical intervention, and severe psychological disturbances. Other criteria appropriate for the species under consideration should also be considered. When complete, proceed to item U.:

Animals will be euthanized prematurely if they exhibit life threatening or painful health problems that are refractory to medical treatment. The spinal cord injuries they receive do not fall within the criteria for deciding to euthanize.

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, a veterinarian will be contacted and measures taken to increase weight. If weight loss progresses to a 3 (thin) a decision to euthanize will be made 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 hemisection model and locomotor training for 6 years without encountering weight loss/body condition score that lead to euthanasia.

In the rare instance that a cat has internal bleeding that cannot be stopped during/following a spay procedure, the cat will be euthanized. In the rare instance that a cat has an unacceptable lesion magnitude or is a risk for handling (i.e. bites), the choice may be made to euthanize.

U. **Euthanasia.** Will animals be euthanatized as part of the planned studies?

- ☐ No. Describe the final disposition of the animals here, then proceed to item U.4:
☒ Yes. Complete items U.1. - U.4. below; then proceed to item V.

1. Describe the exact method of euthanasia for each animal used. Include the agents used, dose (as applicable), and route of administration:

Fixation via intracardial perfusion. Cats will be deeply anesthetized with sodium pentobarbital (~35 mg/kg, IP or IV). 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 followed by a fixation (i.e. 4% paraformaldehyde and/or 1.25%-4% glutaraldehyde in a buffer solution (pH 7.4)). It is critical that the heart continue beating until the perfusion of fluids has begun to prevent collection of blood in the vascular system which interferes with histological procedures. The fixed tissue of interest (including spinal cord and brains) will be removed after perfusion.

2. Are all euthanasia methods acceptable according to the latest report of the AVMA Panel on Euthanasia? (if you are unsure how to answer, contact your veterinarian or IACUC for guidance)

- ☒ Yes. Proceed to item U.3.
☐ No. Justify any method that is not considered "acceptable" by the latest report of the AVMA Panel on Euthanasia, then proceed to item U.3.:

3. List the personnel who will perform euthanasia and indicate their training and experience with the method of euthanasia and the species involved. If personnel are not yet trained, indicate so and explain how they will be trained before performing euthanasia themselves.

██████████, Ph.D. She has >20 years of experience with euthanasia procedures 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 both intracardial fixation methods as well as methods in which fresh tissue is harvested.

██████████ has >16 years of experience with euthanasia procedures in both rats and cats. His training occurred under Drs. ██████████ and ██████████ at the Gainesville VAMC and the University of Florida in intracardial fixation methods as well as methods in which fresh tissue is harvested.

██████████, has >3 years of experience with euthanasia procedures in cats. Her training occurred under Dr. ██████████ and ██████████ at the University of Florida. She is well versed in intracardial fixation methods as well as methods in which fresh tissue is harvested.

4. If the animal care staff find an animal dead, how should the carcass be handled (e.g. refrigerated or frozen), and should a member of your staff be contacted immediately?

The carcass should be put in the refrigerator and a member of the ██████████ lab contacted immediately.

V. **Special Procedures.** Are any experimental procedures or special husbandry procedures planned that are NOT described in the local standard operating procedures (SOP) manual or elsewhere in this ACORP? Special procedures can include special restraint practices (including non-human primate

chaining), special animal health monitoring, special diets, caging, environmental control, exercise, environmental enrichment, means of identification, use of noxious stimuli, forced exercise, or behavioral manipulation.

- ☒ Yes. Complete and attach Appendix 6, "Special Husbandry and Procedures;" then proceed to item W.
☐ No. Proceed to item W.

W. Consideration of Alternatives and the Prevention of Unnecessary Duplication. Complete items W.1 through W.5 below; then proceed to item X. Keep copies of computer database search results in your files to demonstrate your compliance with the law if regulatory authorities or the IACUC should choose to audit your project.

1. Investigators must consider less painful or less stressful alternatives to procedures, and provide assurance that proposed research does not unnecessarily duplicate previous work. You should perform one or more database searches to meet these mandates unless compelling justifications can be made without doing so. Complete the table below for each database search you conduct to answer items W.2 through W.5 below. You must provide complete information in the first four columns of the table to comply with USDA Policy #12.

Name of the database (s)	Date search was performed	Period (years) covered by each search	Key words and/or search strategy used	Indicate below for which alternative mandate each search was conducted by placing an "X" in the proper column			
				Computer models or <i>in vitro</i> techniques (item W.2)	Use of less-sentient species (item W.3)	Use of less stressful model or methods, or fewer animals (item W.4)	Lack of unnecessary duplication (item W.5)
Pub Med	4/16/10	1966 to present	spinal cord injury (SCI), SCI models, proteoglycans, regeneration, chondroitin sulfate, glycosaminoglycans, proteases, drug delivery, locomotion, <i>in vivo</i> , and <i>in vitro</i> .	X	X	X	X
Current Contents	4/16/10	Early 90's-2009	spinal cord injury (SCI), SCI models, proteoglycans, regeneration, chondroitin sulfate, glycosamin	X	X	X	X

			oglycans, proteases, drug delivery, locomotion, <i>in vivo</i> , and <i>in vitro</i> .				

2. Could any of the animal procedures described in this ACORP be replaced by computer models or *in vitro* techniques? Indicate below if such replacement is or is not possible, and provide a narrative on how you came to your conclusion:

No, it is not possible because we are looking at a whole system in which many factors are unknown. Thus, it is not possible to mimic the whole adult CNS system *in vitro* and computer modeling would be ineffective because of the myriad of unknowns in addition to the effects of our enzyme of interest. This opinion is based upon 20+ years of experience in the field of spinal cord injury research plus frequent careful scrutiny of the literature, including Medline and 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.

3. Could a smaller, less sentient mammalian species or a non-mammalian species (e.g. fish, invertebrates) substitute for the mammals in any of the experiments planned? Indicate below if such substitution is or is not possible and provide a narrative on how you came to your conclusion:

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. Additionally, the cat is an important translational model for issues involving SCI in humans.

4. Could a different animal model or different animal procedure that involves 1) less distress, pain, or suffering, or 2) fewer animals substitute for any proposed animal model or animal procedure planned? Indicate below if such replacement is or is not possible, and provide a narrative on how you came to your conclusion:

No. The basic studies have been done in rats. Due to the important findings in the rat, it is now critical to move onto a more translational model. By studying the cat, we can analyze recovery of locomotor function in greater detail than can be done in the rodent. This detail is important in understanding how treatment promotes recovery and how chondroitinase abc may work in humans.

5. Does the proposed research unnecessarily duplicate previous work? Indicate below if the proposed work unnecessarily duplicates previous work and provide a narrative on how you came to your conclusion:

It does not duplicate previous work. There is no indication of duplicate or parallel studies in the literature.

X. Other Regulatory Considerations. Complete items X.1, X.2, and X.3 below; then proceed to item Y.

1. **Controlled drugs.**

a. Will all drugs used in animals and classified as controlled substances by the DEA be stored in a double-locked cabinet, and be accessible only to authorized personnel in accordance with VA policy?

- ☐ Not applicable- no controlled drugs will be used. Proceed to item X.2.
☐ No. Please explain here, then go to item X.1.b.:
☒ Yes. Complete item X.1.b.

b. List the controlled substances that will be used in animals for this project here, and include the building and room number where they will be stored, then go to item X.1.c.:

Sodium Pentobarbital and Buprenorphine will be used in this project. Both will be stored in the [REDACTED] laboratory at the University of Florida, [REDACTED]. Except when in use, the drugs will be kept in a double lock box bolted to an inner wall of Room [REDACTED].

c. To comply with VA pharmacy policies, all controlled substances used on VA property must be ordered through and received by the local VA pharmacy prior to issue for research use. Will the use of all controlled substances comply with these VA pharmacy policies?

- ☐ No. Please explain (then proceed to item X.2):
☒ Yes. Proceed to item X.2.

2. Will any human patient procedural areas be used for these animal studies?

- ☒ No. Proceed to item X.3.
☐ Yes. Complete and attach Appendix 7, "Request to Use Patient Procedural Area;" then proceed to item X.3.

3. Will an explosive anesthetic or other explosive agent be used in any portion of these animal studies?

- ☒ No. Proceed to item Y.
☐ Yes. Complete and attach Appendix 8, "Request to Use Explosive Agent;" then proceed to item Y.

Y. Appendices. Please indicate which of the following Appendices are completed and attached. Do not attach blank appendices which are not applicable to this ACORP. Check with your IACUC to see if an optional Appendix 9, "Additional Local Information", is required.

- ☒ Appendix 1, "Use of a Non-VA Facility to House Animals Purchased with VA or VA Research and Education Corporation Funds" (ref item N)
☐ Appendix 2, "Antibody Production" (ref item O)
☒ Appendix 3, "Test Substances" (ref item P)
☐ Appendix 4, "Antemortem Specimen Collection" (ref item R)
☒ Appendix 5, "Surgery" (ref item S)
☒ Appendix 6, "Special Husbandry and Procedures" (ref item V)
☐ Appendix 7, "Request to Use Patient Care Procedural Areas for Animal Studies" (ref item X)
☐ Appendix 8, "Request to Use Explosive Agent in the Animal Facility or in Animals" (ref item X)
☐ Appendix 9, "Additional Local Information"

Z. Certifications. Important: If this ACORP will be submitted to VA Central Office for Just-In-Time approval prior to receiving VA funding, the signatures of the Principal Investigator(s), IACUC Chair and veterinarian must appear below in items Z.1 and Z.3. The requirement for an R&D Committee Chair signature and the requirement that signatures be less than a year old have been dropped.

1. Certification by Principal Investigator(s).

To the best of my knowledge, I certify that the information provided in this Animal Component of Research Protocol (ACORP) is complete and accurate. I understand that IACUC approval is valid for one year only, that approval must be renewed annually, that every third year the IACUC must perform a new review of my protocol, and that I might be required to complete a newer version of the ACORP and provide additional information at the time of the triennial review. I also understand that IACUC approval must be obtained before I:

- Use additional animal species, increase the number of animals used, or increase the number of procedures performed on individual animals;
- Change procedures in any way that might increase the pain/distress category in which the animals are placed, or might otherwise be considered a significant departure from the written protocol;
- Perform additional procedures not described in this ACORP;
- Allow other investigators to use these animals on other protocols, or use these animals on another of my IACUC-approved protocols.

I further certify that:

- No personnel will perform any animal procedures until they have been approved by the IACUC. When new or additional personnel become involved in these studies, I will submit their qualifications, training, and experience to the IACUC and seek IACUC approval before they are involved in animal studies;
- I will ensure that all personnel are enrolled in an institutional Occupational Health and Safety Program prior to their contact with animals, or have declined in writing to participate, if allowed by local policy;
- I will provide my after-hours telephone numbers to the animal care staff in case of emergency.

Name of Principal Investigator(s)	Signature/	Date
Dr. [REDACTED]	[REDACTED]	9.3.10

2. Minority Opinions (For IACUC Use). IACUC members must be given the opportunity to submit minority opinions on this form. Enter any written minority opinions here (or attach separate pages labeled "IACUC Minority Opinion"). If there are no minority opinions, leave this space blank:

3. Approval Signatures. To the best of their abilities, the undersigned verify that the IACUC has evaluated the care and use of the animals described in 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, and find the use of animals described in this ACORP to be appropriate.

Name of Attending Veterinarian (VMO or VMC)	Signature	Date

Name of Attending Veterinarian (VMO or VMC)	Signature	Date
[REDACTED], DVM	[REDACTED]	9/28/10
Name of IACUC Chair	Signature	Date
[REDACTED], PhD	[REDACTED]	9/28/10

ACORP Appendix 1

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

VERSION 3

1. Indicate which non-VA facilities will house animals purchased with VA or VA Research and Education Corporation funds for this project, and give the current AAALAC International accreditation status for each. Be sure to consider affiliated institutions and contract facilities that purchase and house animals on your behalf to make custom antibodies or other biological products. Consult with your veterinarian or IACUC to determine which institutions must be entered. USDA policies and PHS policy clarifications may also be helpful. Once completed, proceed to item 2.

Non-VA Facility Name	Is this facility accredited by AAALAC?	
	Yes	No*
University of Florida, [REDACTED]	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	<input type="checkbox"/>	<input type="checkbox"/>
	<input type="checkbox"/>	<input type="checkbox"/>

*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.

2. In what non-VA building(s) and room(s) will the animals be housed?

University of Florida, [REDACTED], [REDACTED] floor, room [REDACTED].

3. Return to item N.2. on the ACORP.

ACORP Appendix 3

TEST SUBSTANCES

Version 3

1. **Toxic Agents.** Will toxic chemicals, toxic pharmacologic agents, known or suspected mutagens, carcinogens, teratogens, DNA-binding, or other similar agents be used in animals?

☒ No. Proceed to item 2.

☐ Yes. Complete items 1.a, 1.b, 1.c, and 1.d, then proceed to item 2.

a. Table of toxic agents:

Agent	Diluent	Route of admin.	Dose (e.g. mg/kg) and Volume (ml)	Frequency and duration of administration	Reason for admin., and expected effects

b. Indicate which of the above agents, if any, are known or suspected mutagens, carcinogens, or teratogens:

c. Are any of the agents above on the CDC/USDA list of "select agents" that might have bioterrorism? Check the appropriate response below and proceed to item 1.d.

☐ No.

☐ Yes, but agent(s) will be used in quantities that fall below minimums specified by "select agent" legislation, and thus these agents are not covered by "select agent" legislation.

☐ Yes. Ask your research office to contact the VACO Chief Biosafety Officer for further instructions as soon as possible. You will have to obtain a CDC and/or USDA license and VACO approval before beginning your studies with this agent.

d. Will the animals be anesthetized or sedated when these agents are administered?

☐ No. Proceed to item 2.

☐ Yes. Detail the method of anesthetic, sedative, or tranquilizer administration including agent, dose and volume, and route; then proceed to item 2:

2. **Infectious Agents.** Will bacteria (including rickettsia), viruses, fungi, protozoa, prions, or other infectious agents be used in animals? If the agent will have a radioactive label added, also complete item 4 below. Likewise, if the infectious agent contains recombinant nucleic acid, fill out item 6 below for the agent as well.

☒ No. Proceed to item 3.

☐ Yes. Complete items 2.a, 2.b, 2.c, and 2.d; then proceed to item 3.

a. Complete the table below:

Agent and strain or construct	CDC Biosafety Level of agent (BSL1, 2, 3, 4)	Route of admin.	Dose (e.g. CFU, PFU) and volume administered (ml)	Frequency of administration

b. Has an antibiogram, anti-viral drug sensitivity screen, or other appropriate drug sensitivity panel been determined for the agent(s) listed to assist physicians in selecting proper therapy if an inadvertent human infection occurs?

c. Will the animals be anesthetized or sedated when these agents are administered?

☐ No. Proceed to item 2.d.

☐ Yes. Detail the method of anesthetic, sedative, or tranquilizer administration including agent, dose and volume, and route; then proceed to item 2.d:

d. Are any of the agents on the CDC/USDA list of "select agents" that might have bioterrorism uses? Check the appropriate response below and proceed to item 3.

☐ No.

☐ Yes. Ask your research office to contact the VACO Chief Biosafety Officer for further instructions as soon as possible. You will have to obtain a CDC and/or USDA license and VACO approval before beginning your studies with this agent.

3. **Biological Materials.** Will serum, cell lines, tissue, nucleic acid or other biological materials be administered to animals? If any of the agents are radioactive or will have a radioactive label added, also complete item 4 for that agent.

☐ No. Proceed to item 4.

☒ Yes. Complete items 3.a., 3.b., and 3.c.; then proceed to item 4.

a. Table of biological materials:

Material (e.g. fluid, cells, tissues)	Diluent	Source (e.g. vendor, other animals, colleague)	Route of admin.	Dose (e.g. ml/kg, mg/kg, cells/kg) and volume (ml)	Freq. and duration of admin.	Reason for admin., and expected effects
chondroitinase abc (bacterially derived, purified enzyme)	Saline	Seikagaku Corporation, Sigma, and/or Acorda Therapeutics	Intra-theccal/spinal	25-50µls of 1U/200µls	Every other day	To cleave inhibitory CS- GAG chains in the CNS scar. It is believed that this cleavage will enhance axonal growth.
FluoroGold	Saline	Fluorochrom e, LLC	Intra-spinal injections	1-3 µls of 1-10% soln injected.	once	Label neurons
Biotinylated Dextran Amine (BDA)	2.5% triton	Invitrogen	Intra-spinal injections	0.1-3 µl of ~5% injected.	once	Label neurons
Horseradish peroxidase (unconjugated or conjugated to wheat germ agglutinin)	Saline	Sigma	Intra-spinal injections or superficial placement using gelfoam	1-3 µls of 1-10% soln injected	once	Label neurons

Fast Blue	Saline	Sigma	Intra-spinal injections or superficial placement using gelfoam	1-3 μ ls of ~2% soln injected.	Once	Label neurons
Diamindino Yellow	Saline	Sigma	Intra-spinal injections or superficial placement using gelfoam	1-3 μ ls of ~3% soln injected.	Once	Label neurons

b. Will the animals be anesthetized or sedated when these agents are administered?

☐ No. Proceed to item 4.

☒ Yes. Detail the method of anesthetic, sedative, or tranquilizer administration including agent, dose and volume, and route; then proceed to item 4:

For chase or control treatments, cats will be anesthetized and maintained on 1-5% isoflurane by inhalation. 25-50 microliters of enzyme solution will be injected slowly into the subcutaneous port across 5 minutes. Immediately following completion of injection, the cats will be removed from anesthesia. It is critical that the animals not move during the injection period. We expect the animal to be anesthetized less than 10 minutes.

Tracer injections will be made during a surgical procedures. Thus, the animals will be under a surgical plane of anesthesia.

c. How will these materials be screened to make sure they do not harbor infectious agents that could infect other laboratory animals or people?

The enzyme and/or tracers are purified by the companies selling them. There is no indication that they harbors any infectious agents that pose a hazard to laboratory animals or humans.

4. **Radioactive Agents.** Will radioactive compounds or agents be administered to animals?

☒ No. Proceed to item 5.

☐ Yes. Complete items 4.a., 4.b., and 4.c.; then proceed to item 5.

a. Table of radioactive agents:

Radioactive Agent (include isotope)	Diluent	Agent dose (mg/kg) and Vol. (ml)	Activity (e.g. mCi/kg)	Route of admin.	Frequency and duration of admin.	Reason for admin., and expected effects

b. Which investigator has been given permission by the Radiation Safety Committee or equivalent committee to utilize the isotope(s) indicated above?

- c. Will the animals be anesthetized or sedated when these agents are administered?
- ☐ No. Proceed to item 5.
- ☐ Yes. Detail the method of anesthetic, sedative, or tranquilizer administration including agent, dose and volume, and route; then proceed to item 5:

5. **Other Agents.** Will other substances not listed previously in this appendix be administered to animals? Do not include anesthetics/analgesics/sedatives you will describe elsewhere in the ACORP as part of surgery and postoperative care.

- ☒ No. Proceed to item 6.
- ☐ Yes. Complete items 5.a. and 5.b.; then proceed to item 6.

a. Table of other agents:

Agent	Diluent	Agent dose (e.g. mg/kg) and Vol. (ml)	Route of admin.	Frequency and duration of admin.	Reason for admin., and expected effects

- b. Will the animals be anesthetized or sedated when these agents are administered?
- ☐ No. Proceed to item 6.
- ☐ Yes. Detail the method of anesthetic, sedative, or tranquilizer administration including agent, dose and volume, and route; then proceed to item 6:

6. **Recombinant nucleic acid and recombinant infectious agents.**

- a. Do any of the agents noted above in items 1-5 above have recombinant nucleic acid in them?
- ☒ No. Proceed to item 7.
- ☐ Yes. Answer item 6.b.

b. Are the recombinant constructs exempt from the animal research guidelines included in the latest version of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* publication?

- ☐ No. You must conduct the animal experiments involving recombinant nucleic acid according to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. Consult with your Biosafety Committee and veterinarian to make sure you comply. Go to item 7.
- ☐ Yes. Go to item 7

7. **Pain or Distress.** Will animals potentially experience pain and/or distress as a result of the administration of agents listed above in items 1, 2, 3, 4, 5, or 6?

- ☒ No. Proceed to item 8.
- ☐ Yes. Describe the nature of the pain and/or distress that animals might experience and describe measures that will be taken to alleviate any pain and/or distress here, then proceed to item 8:

8. **Hazardous/Toxic Agents.** Are any of the agents listed above in items 1-6 hazardous or toxic to humans or animals, or covered by the *NIH Guidelines for Recombinant DNA and Gene Transfer*?

- ☒ No. **You have completed this appendix; no further information is required in this appendix. Go to item Q on the ACORP. YOU DO NOT NEED TO GET SIGNATURES IN ITEM 9. BELOW!**

- ☐ Yes. Complete items 8.a., 8.b., and 9; then return to item Q on the ACORP.

a. Table of hazardous agents, committee approvals, and personnel exposed:

Toxic or hazardous agent(s) from items 1-5 above, or non-exempt agent(s) from item 6.	Safety, biosafety, or radiation safety committee that has approved the use of this hazardous agent	Indicate whether VA or affiliate committee	List all animal facility staff who will come in contact with animals given these agents or with contaminated bedding, cages, or other items.

b. Detail how the individuals listed in the table above (item 8.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:

9. **Signatures.** By our signatures, we certify that:

a. Before any animal experiments involving the agents listed in item 8.a. are performed, SOPs designed to protect all animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and the IACUC; and

b. All staff that might be exposed to these agents will be informed of possible risks and will be properly trained to follow the SOPs to minimize the risk of exposure. As is appropriate, concurrence signatures from biosafety or radiation safety personnel are also required as shown.

Principal Investigator(s)	Signature(s)	Date
██████████, Ph.D.	██████████	9.3.10
Institutional Veterinarian	Signature	Date
██████████, DVM	██████████	9/27/10
Biosafety Officer or Chair, Research Safety or Biosafety Committee (typed)	Signature	Date
██████████, Ph.D.	██████████	09/24/10
Radiation Safety Officer, or Chair, Radiation Safety or Isotope Committee (typed)	Signature	Date
	N/A	
IACUC Chair (typed)	Signature	Date
██████████, Ph.D.	██████████	9/24/10

ACORP Appendix 4

ANTEMORTEM SPECIMEN COLLECTION

VERSION 3

1. **Blood Collection.** Will blood be collected from live animals (anesthetized or awake) as a part of this proposal?

☒ No. Proceed to item 3.

☐ Yes, but all collections are described in Appendix 2, "Antibody Production", so no further information need be provided here; proceed to item 3.

☐ Yes. Complete the table below; then proceed to item 2.

Site and Method of blood collection	Amount of blood collected, expressed as volume (ml) and % of body weight (assume 1 ml of blood weighs 1 gram)	Number of blood collections	Interval between collections

2. **Use of Anesthetics, Tranquilizers, or Analgesics for Blood Collection.** Will anesthetics, tranquilizers, or analgesics be used to prevent pain or stress during collection of blood described in item 1 above?

☐ No. Justify the omission of pain-relieving agents (either scientifically or because the collection method involves no or momentary pain) and completely describe the physical restraint that will be used during collection here:

☐ Yes. Complete the following table, then proceed to item 3.

Anesthetic, tranquilizer, or analgesic agent	Dose (mg/kg) & volume (ml)	Route	Frequency

3. **Other Tissue Collection.** Will other body fluids (e.g. cerebrospinal fluid, peritoneal fluid, urine) or tissues be collected from live animals (awake or anesthetized) as a part of this protocol?

☐ No. Proceed to item 5.

☒ Yes. Complete the following table; then proceed to item 4.

****We will not remove any tissues prior to euthanasia. However, we will invade the subcutaneous space shortly before euthanasia, when the animal is deeply anesthetized to recover the port system that was used to deliver enzyme.**

Tissue or fluid collected	Site & method of collection	Amount (g) or volume (ml)	Number of collections	Interval between collections

Port and partial tubing	The port system is positioned subcutaneously. Once the animal is deeply anesthetized and within minutes of terminal perfusion, we will expose the port by opening the skin with a scalpel. The port and partial tubing will be removed by severing the sutures and fascia holding it in place.	NA	1x – just prior to intracardial perfusion which is the euthanasia procedure used.	NA

4. **Use of Anesthetics, Tranquilizers, or Analgesics for Collection of Fluids or Tissues.** Will anesthetics, tranquilizers, or analgesics be used to prevent pain or stress during collection of body fluids or tissues described in item 3 above?

☐ No. Justify the omission of pain-relieving agents (either scientifically or because the collection method involves no or momentary pain) and completely describe the physical restraint that will be used during collection here, then go to item 5:

☒ Yes. Complete the following table, then go to item 5.

Anesthetic, tranquilizer, or analgesic agent	Dose (mg/kg) & volume (ml)	Route	Frequency
Sodium pentobarbital	25-35 mg/kg. If necessary, cats will be given a supplemental dose of sodium pentobarbital (25% of initial dose)	IP or IV	1x

5. Proceed to item S on the ACORP.

ACORP Appendix 5

SURGERY

VERSION 3.1

1. **Surgery Classification.** The *Guide* defines a major survival surgery as a surgery in which a major body cavity is penetrated and exposed or surgery in which substantial impairment of physical or physiological functions is produced. Examples of such surgeries provided in the *Guide* include laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation.

a. Will major survival surgery be performed on any animal as part of the proposed experimental plan?

- ☐ No. Proceed to item 2.
☒ Yes. Proceed to item 1.b. below.

b. Will more than one major survival surgery be performed on any animal as part of the proposed experimental plan?

- ☐ No. Proceed to item 2.
☒ Yes. Complete item 1.c. and 1.d. below.

c. Provide a complete scientific justification for performing more than one major survival surgery on individual animals:

Cats in this study may undergo as many as three major survival surgeries. These surgical procedures are spay, spinal cord injury (with or without port placement), and tract tracer placement:

Spay

Cats deemed to have an appropriate temperament for behavioral studies may be spayed. The 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.

Spinal Cord Injury (with or without port placement)

The overall goal of this project is to determine approaches which enhance axonal connectivity and promote recovery of voluntary locomotor function following spinal cord injury. Hence, we need to generate the spinal cord injury in order to determine ways to re-establish motor function and control. Ports must be placed in those cats to receive ch'abc treatment so that "fresh" enzyme may be delivered. Our prior studies show that this enzyme does not remain viable at body temperature and must be redelivered ~every 24-48 hours.

Tract Tracing

In order to determine what cells respond to treatment, it is necessary to use tract tracing techniques. This will allow us to do critical analyses that are not possible with any other technique. These analyses include identification of the neuronal populations responding (by identification of the nuclei/level of the neural axis where the labeled neurons are located), as well as determine the percentage of neurons and or axons contributing to these responses. This information is qualitatively and quantitatively different than that which can be obtained from immunohistochemical studies.

d. Give the interval(s) between the multiple surgeries, and the rationale for choosing the interval(s) here, then proceed to item 2:

- Spay will occur at least 3-4 weeks prior to any other surgical procedures. The 3-4

weeks will allow adequate healing time for the surgical openings in the muscle and skin.

- Spinal Cord Injury (with or without port placement) will typically occur ~2 months after the spay procedure. This timeframe will allow healing of the spay-related incisions, as well as collection of baseline locomotor data post-spay.

- The tract tracer will be injected ~2 weeks prior to sacrifice. Typically, this will be ~17 weeks after the spinal cord injury with or without port placement. However, if a cat is not behavioral material, the tracer could be placed during the same procedure in which the lesion is made to determine an acute baseline for neuronal counts.

2. **Description of Procedure(s).** Describe the surgical procedure(s) in enough detail so that the IACUC reviewers can determine what procedure(s) are actually being performed. If several different surgeries are being performed, be sure to describe each one. When finished, proceed to item 3:

General comments: For these studies, purpose-bred, adult female cats weighing ~3-4 kg will be used. All surgical procedures (i.e., spay, spinal injury (with or without port placement) and tracer placement) will be carried out under strict sterile conditions and on a circulating water heating pad. Cats will receive penicillin G, procaine (40,000U/kg IM) the day prior to surgery, the day of surgery, and the day after surgery. Preceding all surgical procedures, cats will be given atropine sulfate (0.04-0.06 mg/kg SQ) and acetylpromazine (0.1-0.2 mg/kg SQ) to control salivation and to sedate the cat prior to being anesthetized. Using a mixture of oxygen and isoflurane (2-5%) the cat will be anesthetized by inhalation of gases in an enclosed chamber which will be attached to the gas scavenging system. The surgical area will be shaved and the cat will then be intubated and hooked up to the anesthetic gases (1-5% oxygen and isoflurane mixture) for the duration of the surgical procedure. An IV line will be set in a limb vessel to allow delivery of fluids (like saline or Lactated Ringers) during the surgical procedure. The surgical area will be cleaned 3x using 70% alcohol followed by a betadine solution. The surgical plane of anesthesia will be monitored by respiration, heart rate and the absence of reflex responses. The percent of gas anesthesia will be adjusted as needed to maintain the appropriate level of anesthesia (surgical plane). Throughout the surgery, the animal lies on a circulating, water, heating pad and body temperature, respiration, and expired CO₂ are continuously monitored. All surgical procedures are performed in a sterile environment using strict sterile technique. Following surgery, if needed due to problems with an IV line or intake, ~20 cc of sterile saline, ringers, or like solution will be given SQ. If this is quickly absorbed, another ~20 cc will be given to prevent dehydration as a result of fluids lost and/or not taken in during, or acutely following, surgery. All cats will be observed closely for signs of post-operative pain, infection and regulation of body temperature.

After all surgical procedures, cats will recover in a warm environment (i.e. in an intensive care incubator unit or in their cages on circulating water pads) for several hours to overnight. All cats will be closely monitored by laboratory personnel. Immediately following surgery the cats will be given an initial dose of buprenorphine (0.01-0.05mg/kg, SQ). Additional doses of buprenorphine (0.01-0.05 mg/kg, SQ) will be given subcutaneously every 6-12 hours for 48 hours. If the animal shows any sign of discomfort after 48 hours, treatment will be extended for an additional day. Treatment for pain behaviors that persist beyond this point will be determined in consultation with a veterinarian.

Spay procedure: When possible, cats will be spayed by the vendor as it is less costly than performing ourselves. All cats will undergo a ovariohysterectomy. A skin incision between the level of the umbilicus and the pubic symphysis will be made followed by muscle incision along the linea alba. The entire uterus along with ovaries will then be removed and the abdominal cavity closed in layers. **This will be done by making two ligatures with absorbable suture above each ovary and at the cervix to effectively tie off the vessels at each site. Once secure ligatures are made, the ovaries and uterus will be removed by cutting between the ligatures at each site. The abdominal cavity will be**

closed in layers using absorbable suture. The skin will be sutured using an inverted stitch so that suture removal is not needed. The cat will be allowed to recover for several weeks prior to any other surgical procedure. Cats are spayed for two reasons: 1) to remove the potential for hormone-related effects on injury magnitude; and 2) to prevent interruptions in behavioral training or data collection due to the postural changes associated with estrus. It is necessary to perform this surgical procedure separate from the spinal cord hx and port implantation surgery so that we can train the cats and collect baseline data for locomotor function after the cat has recovered from the spay procedure.

Hemisection injury without port placement: Muscle will be cut next to the dorsal spinal processes between ~T8-11 and the muscle retracted laterally. A bilateral laminectomy will be performed to expose the underlying T9/10 spinal level. The dura will be slit longitudinally and the dorsal columns and dorsal root entry zones visualized to identify the spinal midline. The left 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. Durafilm and gelfoam will be placed on top of the dura and the back closed in three layers (muscle, subcutaneous, skin) using absorbable sutures. Typically interrupted stitches will be used on each layer and inverted stitches on the skin so that suture removal is not required.

Hemisection injury with port placement: The hemisection will be performed as described above. In addition, a micro-implantable infusion port will be placed subcutaneously and lateral to the vertebral column. The port is small with an overall body diameter of ~1cm and a reservoir of ~10 microliters. The body of the port and tubing are made of biocompatible materials and may include metals and silicone, polyurethane, and CBAS™-coated polyurethane. The port will be secured to underlying muscle using vet bond and suture. The small, flexible delivery tube from the port will be secured to the muscle at several intervals with absorbable or nylon suture and the final length secured to the lamina immediately caudal to the lesion using vet bond. The delivery tube end will be placed into the lesion site, the port filled, 1 dose of ch'abc given (25-50 uls of 0.5 U/1ml saline or buffer), and the dura sutured. Durafilm and gelfoam will be placed on top of the dura and the back closed in layers as described above. The port can be palpated easily through the skin and solution is delivered using SQ injections into the port.

Neuronal Tracer Injections: All cats will undergo a surgery ~13 days prior to their sacrifice. They will be reanesthetized, a laminectomy will be performed to remove the reformed bone, and the sutured dura re-slit using an 11-scalpel blade. A few microliters of a neuronal tracer (see Appendix 3) will be injected into the injured spinal cord. The dura, muscle, subcutaneous layer and skin will be closed as described above.

3. Provide the names of the personnel who will perform the surgery; then proceed to item 4. Note that the surgical experience of each person involved in surgery should be listed in item E of the ACORP:

Any of the personnel listed in the ACORP may perform the survival surgeries ([REDACTED], [REDACTED], [REDACTED]).

4. Provide the names of the personnel who will perform the anesthetic induction and monitor the animal during surgery here, then proceed to item 5:

Same as above under #3.

5. Provide the building and room number(s) where the surgical procedure(s) will be performed. A

dedicated surgical facility must be used for major survival surgeries on non-rodent species (the definition of a major survival surgery is provided in item 1 above). If allowed by local policy, non-survival surgery on non-rodent species and survival surgery on rodent species may be performed in a procedure room or laboratory. Then proceed to item 6:

A designated surgical suite () for large animal surgeries within the animal facility on the floor of the will be used.

6. Pre-operative procedures. Pre-operative procedures should include all preparations of the animal(s) for surgery. Check and describe which of the following procedures will be performed. Then proceed to item 7.

☒ Fasting (rarely used in rodents or rabbits). Indicate the length of the fasting period:

>12 hours

☐ Withhold water. Indicate the length of time that water will be withheld:

☒ Catheter placement. Indicate the site(s) in which venous catheter(s) will be placed for vascular access during surgery:

Forelimb

☐ Other. Describe other pre-operative procedures:

7. Pre-operative medications. Complete the following table. Include any antibiotics, sedatives, or tranquilizers, and the anesthetic agent(s) that will be used to induce anesthesia prior to surgical site preparation; then proceed to item 8.

Agent	Dose (mg/kg) & volume (ml)	Route	Frequency (e.g. times/day)	Duration (e.g. days)
Penicillin	Penicillin G, procaine 40,000 U/kg BW	IM	1X	The day before, of and post-surgery OR 7 days if not given the day prior to surgery
Atropine sulfate	0.04-0.06 mg/kg	SQ	1X	Once, prior to surgery
Acetylpromazine	0.1-0.2 mg/kg	SQ	1X	Once, prior to surgery
Isoflurane	1-5%	Inhalation	During induction and throughout surgery	During induction and throughout surgery

8. Preparation of the surgical site. Detail how the surgical site(s) will be prepared prior to surgery. Include details of hair-clipping, skin disinfection, and the use of surgical drapes. Then proceed to item 9:

Prior to taking the animal to the surgery area, all hair within 2 cm of the 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 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.

9. **Intraoperative medications.** Complete the following table including any anesthetic agents, paralyzing agents, fluids, or other pharmaceuticals that will be administered to the animal during surgery. Also include experimental pharmaceuticals. Then proceed to item 10.

Agent	Dose (mg/kg) & volume (ml)	Route	Frequency
isoflurane	1-5%	inhalation	throughout surgery
Saline/ringers/like solution	5-10 ml/kg (~1drip/10 sec)	IV	throughout surgery

10. **Paralyzing agents.** Are any of the above medications considered paralyzing agents?

☒ No. Proceed to item 11.

☐ Yes. **Very Important!!** Federal regulations prohibit the use of paralytics (neuromuscular blocking agents) for surgery unless other appropriate anesthetic agents are used to induce a surgical plane of anesthesia. Paralytics do not provide any pain relief; therefore, animals are unable to respond physically to pain because motor reflexes are paralyzed. Justify the use of these agents and indicate how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain. Then proceed to item 11:

11. **Physical support.** Describe any physical methods used to support patients during surgery (e.g. heating pads, blankets, etc.); then proceed to item 12:

Cats are on a heating pad (typically a circulating water blanket) throughout any of the described surgical procedures. If a cat's body temperature should happen to drop significantly (in the mid-low 90's), heated saline bags covered with a wrap will be placed under the sterile drapes next to the animal's body.

12. **Intra-operative monitoring.** Describe methods used to monitor the state of anesthesia and general well-being of the animal during surgery. Then proceed to item 13:

Respiration, heart rate, core body temperature, expired CO₂ are monitored continually throughout survival surgery.

13. Will the animals regain consciousness following surgery?

☐ No. You have completed this appendix. ***No further information is required in this appendix. Return to item T on the ACORP itself.***

☒ Yes. Proceed to item 14.

14. **Survival surgery considerations and post-operative care.** Complete items 14.a-f. below. Then proceed to item 15.

a. How long will the animal(s) survive after surgery? (If multiple surgeries are planned, answer for the last surgery before euthanasia):

- Up to 6-12 months post-spay (dependent upon time required for training behaviors initially).
- Up to ~5 months post-spinal cord injury.
- 13 days after the neuronal tracer injections.

- b. Is the room where the procedures will be performed (listed in item 5 above) suitable for sterile/aseptic surgery?

Yes.

- c. Indicate which of the following procedures will be used to maintain a sterile field during surgery:

- ☒ Sterile instruments.
☒ Surgeon cap.
☒ Sterile gloves.
☒ Surgeon scrub.
☒ Sterile drapes.
☒ Sterile gown.
☒ Face mask.
☐ Other. Describe:

- c. List any methods used to support the patients in the immediate post-operative period (e.g., heating pads, blankets, fluids, etc.):

Cats are typically recovered in a climate controlled recovery unit. If one of these is not available, cats are recovered on heating pads (typically circulating warm water pads) in housing cages. If body temperature does not rapidly recover to normal levels, 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) will be give SQ. If this is quickly absorbed, additional fluids may be given.

- e. Unless scientifically or otherwise justified to the IACUC's satisfaction, you are obligated to routinely provide post-operative pain relief for all vertebrate animals undergoing survival surgery. Do you plan to use analgesics to provide postoperative pain relief to the animals following surgery?

☐ No. Provide a justification for not using postoperative analgesics here:

☒ Yes. Complete the following table listing post-operative analgesics agent(s) that will be used after surgery to control pain:

Agent	Dose (mg/kg) & Volume (ml)	Route	Frequency (e.g. times/day)	Duration (e.g. days)
buprenorphine	0.01-0.05mg/kg	SQ	2-3x/day (every 6-12 hours)	2 days
meloxicam	0.2 mg/kg first day, then 0.1 mg/kg SID for 2 additional days	PO	Once for 3 days.	3 days

- f. Complete the following table for other medications (such as fluids, antibiotics, anti-coagulants, and other pharmacological agents) that will be administered post-operatively.

Agent	Dose (mg/kg) & Volume (ml)	Route	Frequency (e.g. times/day)	Duration (e.g. days)
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Penicillin	Penicillin G, procaine 40,000 U/kg BW	IM	once	The day before, of and post-surgery – OR- 7 days if not given the day prior to surgery

15. Frequency and Responsibility for post-operative care. Complete items 15.a. -15.c. below, then proceed to item 16. The names and after-hours telephone (or other contact) numbers of the personnel listed below must be provided to the VMU staff in case of an emergency.

- a. Give the frequency of postoperative monitoring and how long the monitoring will continue:

Following all surgeries, animals will be checked at least every 6-12 hours for the first 48 hours post-op. If at the end of 48 hours, bladder function has recovered and no pain behaviors are apparent, animals will be checked 1-2x daily. If bladder function has shown no recovery, monitoring will continue at least every 6-12 hours until this issue is resolved. In the rare event that pain appears to persist beyond 48 hrs post-surgery, buprenorphine will be extended for 24 additional hours. Veterinary staff will be contacted if pain behaviors extend beyond this timeframe.

- b. Who will be responsible for post-operative care until the animal can ambulate without danger to itself?

██████████, Ph.D., ██████████, ██████████.

- c. Who will be responsible for post-operative care thereafter (including after-hours, weekends, and holidays)?

██████████, Ph.D., ██████████, ██████████.

16. Post-operative complications. Complete items 16.a. - d. below; then proceed to item 17.

- a. Describe any possible or expected post-operative complications and what will be done if these complications arise:

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. If a cat should present blood in its urine, Penicillin will be started (7 days; Penicillin G, 40,000 U/mg, IM) and a veterinarian notified. If blood persists or a persistent infection develops, additional treatment will be determined in consultation with a veterinarian. **Cats will be monitored for bowel movements and their colons palpated for retained feces daily during the early post-op period. Laxatone and/or a fiber supplement may be added to the diet to encourage defecation. If feces are not produced within a few days and the colon is firm with feces, a warm water sudsy enema will be used. Once the first bowel movement is produced, cats defecate effectively on their own. Following the acute post-op period, cats will be checked on at least once a day to observe their general health (including alertness,**

movement, and skin integrity). In our experience, cats have tolerated hemisection lesions well and do not usually present with health problems. All cats will be given buprenorphine (0.01-0.05 mg/kg, SQ, every 6-12 hrs) for the first 48 hours post-op. Rarely will a cat will have pain that presents past 48 hours. 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 48 hours, buprenorphine treatment will be extended an additional day. If pain does not resolve, treatment will be discussed and determined in consultation with a veterinarian. **The ability to integrate weight support with the limb caudal and ipsilateral to the SCI during ambulation recovers during the first week. As this ability recovers, extra care is given to guarding the cats during crossing of the runway and if needed, their tails are firmly grasped at the base during this early training period. Sensation is partially disrupted below the lesion. Thus, cats are housed on thick soft bedding to prevent any skin ulcers or peripheral nerve compressions. This same sort of bedding is also used in the carrying cages which transport them between the housing and behavior room. Following a hx, cats do not show self mutilation.**

- b. Provide criteria by which a decision to euthanize a surgical patient post-operatively will be made:

Animals will be euthanized prematurely if they exhibit life threatening or painful health problems that are refractory to medical treatment. The intended spinal cord injury to be used in this study does not fall into this category.

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, a veterinarian will be contacted and measures taken to increase weight. If weight loss progresses to a 3 (thin) a decision to euthanize will be made 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 hemisection model and locomotor training for 6 years without encountering weight loss/body condition score that lead to euthanasia.

In the rare instance that a cat has internal bleeding that cannot be stopped during/following a spay procedure, the cat will be euthanized. In the rare instance that a cat has an unacceptable lesion magnitude or is a risk for handling (i.e. bites), the choice may be made to euthanize early.

- c. In case there is an emergency medical situation and you or your staff cannot be reached, identify drugs or classes of drugs that should not be used as part of the treatment plan:

Drugs that alter inflammatory processes (i.e. steroids) should only be used topically. Systemic steroid treatment may change the impact of the CNS injury - particularly if given during the acute period. Drugs that directly affect the CNS should not be used - other than those already used in the course of the study (i.e. buprenorphine, anesthetizing agents).

- d. In the event that emergency euthanasia must be performed or an animal is unexpectedly found dead, how should the carcass be handled?

The carcass should be placed in the refrigerator. The ACS should then call someone in the [REDACTED] lab regarding the carcass.

17. Responsibility for maintaining animal post-surgical medical records. Who will be

ACORP Appendix 6
SPECIAL HUSBANDRY AND PROCEDURES
VERSION 3

1. **Special Husbandry.** Are special husbandry practices required for this protocol that are not described in the local Standard Operating Procedures (SOP) manual? Examples of special husbandry practices include temperature extremes, food or water deprivation, dietary manipulations, calorie restrictions, special housing/caging, modified light cycle, special health monitoring, and unusual means of identification.

- ☐ No. Proceed to item 2.
☒ Yes. Complete items 1.a. and 1.b.; then proceed to item 2.

- a. Provide a complete description of all non-standard practices or procedures. Make sure that the frequency and duration of these practices or procedures are stated:

Procedures associated with housing:

- 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 crate foam). This bedding must be maintained for the rest of the study to prevent skin ulcers and peripheral nerve compression.
- 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; and 2) keep cats from jumping or rolling off of them and potentially hurting themselves. If sensory testing of the hindlimbs by the P.I. shows that sensation has recovered (due to a treatment) then consideration may be given to reducing or removing the padding. **See #4 below for comments on sensory testing.**
- 3) 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.
- 4) If sensory testing of the hindlimbs by the P.I. shows that sensation has recovered (due to a treatment) then consideration may be given to reducing or removing the padding. **Sensation will be generally checked by response to pinch. If this response appears voluntary, additional sensory testing may be done including use of Von Frey filaments. If there is any question regarding sensory integrity, the conservative decision to continue to protect through surface padding and resting board removal will be made.**

Procedures associated with training/behavioral conditioning:

Cats are trained daily (~5 times/week) to walk on a treadmill, cross a variety of runways 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). Thus, on training days, animals typically will be fed only 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*) and on non-training days, food also will be available throughout the day in their cages. During training, the cats will receive food for performance on the overground runways (typically on the platforms at the end of the runways). If sufficient training has been accomplished for the day and

the cat has not received an appropriate caloric intake for the day, the cat will be given additional food in a bowl on the floor or on one of the training apparatuses. If a cat does not perform tasks sufficiently (meaning a reasonable number of repetitions for those tasks that it is capable of) during a training session, it will not receive additional food at that time, but will be brought back to the behavior room later during the day for another training period and additional food.

The longest interval between training sessions would not exceed 24 hours as training occurs a minimum of 1x/day during training days. Thus, cats would eat at least 1x/day. During non-training days, food is available in the cat's home cage. Typically the amount of food eaten during training and ad lib in the cat's cages are similar.

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 our ACS veterinarians. For example, adult spayed cats require a typical caloric (kcal) average of $1.2 \times \text{RER/day}$. If a cat is overweight, the typical calculation changes to $0.8 \times \text{RER}$. 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 and/or contact an ACS 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 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. An optimal body condition of the cats will have the ribs, lumbar vertebrae, pelvic bones and other bony structures easily palpable with slight fat cover, concave abdominal tuck, smooth hourglass shape to waist, abdominal fat pad minimal (for example see Purina body condition chart, <http://placervillevet.com/feline%20body%20condition.htm>). This would typically be a 5 on the 9 point scale. We use the 9 point scale (vs 5 pt) for greater accuracy and an example of it is pasted below and comes from <http://www.purina.com/cats/health/BodyCondition.aspx?print=1>. (pictures available on website, but will not import into this document). For cats that come in with a body condition score of 5, a target and floor weight will be established following the general rule of no more than a 15% weight loss. For those cats that come in below or above a 5, initial target and floor weights will identified and re-evaluated weekly as cats move towards the body condition score of 5. Once a body condition score of 5 is achieved, the 15% weight loss criteria will be used as a floor weight.

Understanding Your Cat's Body Condition

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.

Weights and body scores will be documented in each cat's behavior records in the behavior room. For cats with problem weights or condition scores, weekly documentation also will be made in the clinical records as requested by ACS veterinarians.

Because food intake and weight are carefully monitored, there should not be any adverse side effects from our food conditioned training paradigm. In fact the training (physical exercise) that these cats receive puts them in better health than cats that do not receive training. If any problems do arise, a veterinarian will be contacted. Cats in the untrained group also will receive their food in the behavior room on training days and have their intake monitored. They will not perform the training tasks, but instead will be fed in an enclosed area on the floor or on the ends of the runways. The time in the behavior room will be controlled so that it is similar to that of the trained group.

Cats also are trained to be quiet/still when held by, or standing beside, the trainer for testing of hindlimb reflexes (eg. withdrawal to pinch, placing response, positive support response). This happens very quickly and is associated with the comfort level of the cat with the trainer rather than any motor ability/skill.

The runways are composed of metal and wood. All wood surfaces are protected by several coats of paint over which a water resistant sealer has been used. Most runway surfaces are covered with an indoor-outdoor plastic type carpet that is removable. The equipment we use is not suitable for total disinfection and the veterinary staff is in agreement with this. Thus, 1) our equipment disinfection procedures have been developed with guidance of an Animal Care Services veterinarian at UF 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 on them (i.e. the flat runways) also may have the rugs removed and these can be cleaned periodically in several ways beyond wiping with water or cleansers - for example these rugs, when loosely rolled fit within a standard washing machine. Cleansers used on runways to wipe up spills or clean the surface include commercially available cleaners such as Top Job or Lysol. After cleaning with these cleansers, the surfaces will be rinsed multiple times with clear water.

A variety of runways will be used to train and/or test the cats' locomotor skills. These include a basic (12" wide, flat or inclined) runway, and multiple skilled overground locomotor tasks (narrow beam (2" width), ladders, pegboards, runway with obstacle(s) requiring change in limb trajectories to step over, etc). The spacing of rungs, pegs and obstacles, as well as heights/widths of obstacles, may be varied to present novel situations for testing and assessment of limb trajectory modifications not specifically practice. Trainers will assist as necessary during crossing. At each end of a runway task is a platform which the cat may stand or sit on. Food rewards are given on these platforms.

b. Justify the use of these non-standard practices or procedures:

Following spinal cord injury, sensation is lost below the level of the spinal cord lesion. Due to this loss, the animal will not perceive the sensory input that cues animals (including humans) to reposition themselves to prevent ulcers and nerve compression on hard or firm surfaces. This will be particularly true with respect to the hindquarter/hindlimb ipsilateral to the lesion. Both the cage bottom and the resting boards are hard surfaces that may cause ulceration or nerve compression. Further, if cats rub their backs on the resting boards over the surgical site they could cause undesired spinal damage and pain. Additionally, following a hemisection, coordination is compromised and a cat will more easily roll off or make a poor jump coming off of the resting board. Either of these could cause the cat to get hurt.

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).

2. **Other Procedures.** Are other procedures such as prolonged physical restraint, use of noxious stimuli, forced exercise, behavioral manipulations, total or partial body irradiation, radiography or other imaging studies planned but not described elsewhere in the ACORP?

☒ No. Proceed to item 3.

☐ Yes. Complete items 2.a. and 2.b.; then proceed to item 3.

a. Check all of the following procedures that are proposed:

☐ Prolonged physical restraint, including chairing.

☐ Noxious stimuli.

☐ Forced exercise.

☐ Behavioral manipulations.

☐ Other. Describe:

b. Describe each procedure and the expected outcome(s) in detail. Make sure that the frequency, duration, and interval between repeated manipulations are described:

3. In the table below, identify who will perform the procedures and practices listed in items 1 and 2 and who will be responsible for monitoring the condition of these animals. After-hours telephone (or other contact) numbers of the personnel listed must be provided to the veterinary staff in case of an emergency.

Person	Role (performing procedure/ monitoring)	Office Phone	Pager or cell phone	After-hours contact number	E-mail address
Dr. [REDACTED]		[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]		[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]		[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

4. Do these practices or procedures have the potential to cause more than momentary pain and/or discomfort?

☒ No. You have completed this appendix; ***no further information is required in this***

appendix. Go to item W on the ACORP.

☐ Yes. Describe the potential pain and/or discomfort here; then proceed to item 5:

5. Will pain or stress-relieving agents be administered to the animals that experience pain and/or discomfort? Then proceed to item 6.

☐ No. Provide a scientific justification for not using pain or stress relieving agents here:


☐ Yes. Fill out the table below.

Agent	Dose (mg/kg) & volume (ml)	Route	Frequency (e.g. times/day)	Duration (e.g. days)

6. Describe the methods used to monitor the condition of the animals during and after the procedures and the criteria that will be used to remove individual animals from these procedures should pain or suffering be present:

7. Proceed to item W on the ACORP.

Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
	Gainesville, FL #573	MERIT/Spring 2010	Training Effects on Recovery of Balance and Limb Accuracy in Cats Post-SCI

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

(cont.)

The ACORP for Dr. [REDACTED] has received an overall score of 0. No further correspondence with the CVMO is needed; the ACORP is approved and represent(s) no bar to funding the application.

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)	<p>This ACORP uses a cat model of Spinal Cord Injury (SCI) to investigate whether rehabilitative training and enzyme therapy will enhance re-growth of connections within injured spinal cords and lead to the recovery of walking ability. The cats utilized in this study will undergo multiple survival surgeries, one of which will result in physical impairment. Several aspects of this application are commendable including the use of the body scoring system, the attention to pre- and post-operative care, the sanitation of the locomotor training equipment and housing modifications. Unfortunately, critical components of this study lack necessary detail and are summarized below.</p> <p><u>Documents (request for significant change, memorandum, revised ACORP and appendices) were received on 9/29/10. The investigator has clearly and concisely addressed each concern; the color coded font to indicate the location of revised text is greatly appreciated. All concerns raised have been resolved; the ACORP is approved and represents no bar to funding the application.</u></p>
Items C.2, Appendix 5, item 16a, and Appendix 6 (0)	<p>The investigator makes reference to the cat SCI being similar to Brown-Sequard Syndrome (BSS) in humans and indicates the daily conditioning approach was selected for that reason. Individuals suffering from BSS have ipsilateral spastic paralysis, ipsilateral loss of vibration, proprioception, and fine touch and contralateral loss of pain and temperature sensation. BSS patients may experience urinary and bowel incontinence, may only walk with a cane or walker, and may experience a burning or tingling sensation. The investigator indicates the low thoracic hemisection model is well tolerated by cats, but a clearer understanding of SCI and training is needed to concur with this assertion. Please respond to each of the following points:</p> <ul style="list-style-type: none"> Describe the degree of expected impairment in the cats post SCI and elaborate on the persistent quantifiable deficits in item C.2. <u>Adequately addressed.</u> The description of post operative complications in item 16a of Appendix 5 is helpful but should be expanded to address concerns about potential bowel incontinence, the ability to stand and/or ambulate during the initial recovery period, self-mutilation due to abnormal sensation, etc. Please address these points in Appendix 5, item 16a. <u>Adequately addressed.</u>

(cont.)

	<ul style="list-style-type: none"> List and describe all of the skilled tasks (ladders and pegboards, as well as other tests related to paw placement, limb trajectory, and balance) in Appendix 6. <u>Adequately addressed.</u> Also indicate how a cat with unilateral spastic paralysis and other deficits will be able to accomplish the skilled locomotor tasks without beginning therapy with basic locomotor tasks. This question is based on the following statement in item C.2: <i>“Thus, some tasks post-injury will be trained, some untrained but familiar, and others novel depending on the training group.”</i> <u>Adequately addressed. The investigator indicated in the memorandum included in the same PDF as the main body of the ACORP that spastic paralysis is not expected and the determination of the cats’ ability to negotiate skilled tasks is a scientific aim of the study.</u> Describe the two novel, untrained tasks requiring adaptations of the stepcycle in Appendix 6. <u>Adequately addressed.</u> Appendix 6 mentions the investigator performing sensory tests to determine the degree of sensation recovery, please elaborate. <u>Adequately addressed.</u> Appendix 6 lists positive support response as a hindlimb reflex test, please describe this test. <u>Adequately addressed. The investigator provided a description of the hindlimb reflex test in the memorandum. In the future, please describe the hindlimb reflex test in the body of the ACORP.</u> Provide a flowchart, which includes the experimental groups, sequence and timetable of events (surgeries, treatments, training, etc.) in item C.2. <u>Adequately addressed.</u>
Item D (0)	<p>The investigator explains why it is necessary to perform ovariohysterectomies in the female cats; however, it is not made clear why male cats or neutered male cats (castration is a minor procedure unlike ovariohysterectomy) could not be used. Neither fluctuating hormone levels nor estrus associated postural changes would be an issue if male cats were used. Please address. <u>Adequately addressed.</u></p>
Item J.1 (0)	<p>Terminal intracardiac perfusion under anesthesia should be listed as a category D procedure in item J.1. Please reconcile. <u>Adequately addressed.</u></p>
Item L.3 (0)	<p>The name(s) of the veterinarians consulted were not listed in L.3. [REDACTED], Grant Support Specialist, kindly provided the following information: <i>The initial review was done by Dr. [REDACTED] on 5/10/10 and the review of the revisions was done on 6/30/10 by Dr. [REDACTED].</i> Please add this information to item L.3. <u>Adequately addressed.</u></p>
Appendix 3 and Appendix 5 (0)	<p>Appendix 3, item 3 indicates the following neurotracers will be administered to the cats: FluoroGold, Biotinylated Dextran Amine (BDA); horseradish peroxidase and fast blue. BDA will be administered by injection only; the other three tracers will be administered by injection or via gel foam application.</p>

(cont.)

	Appendix 5 does not describe the gel foam administration of these agents, please reconcile. <u>Adequately addressed.</u>
Appendix 5 (0)	The surgical descriptions (ovariohysterectomy, laminectomy/hemisectomy with or without port placement, and neurotracers) are not adequate in regard to the approach, the tissue manipulations (dissection, ligations, etc.), or closure (tissue in each layer, suture material, suture pattern, suture removal). Please reconcile. Please also provide more detail about the port (material, size, etc.) and port maintenance/access, since the port will be refilled every other day with ch'abc over the period of one month. <u>Adequately addressed.</u>