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

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

A. **ACORP Status.**

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

2. VA Station Name (City) and 3-Digit Station Number► **VA Connecticut Healthcare System, 689**

3. Protocol Title► **Interventional Therapies after Spinal Cord Injury**

4. Animal Species covered by this ACORP►**Vervet**

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

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

6. Related Documentation for IACUC reference.

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

      (1) Title of project►

      (2) If approved by the R&D Committee, give the date of approval►

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

      (1) Identify the studies described in the previously approved ACORP that have already been completed
      ► We have completed 6 NHPs that received C5 hemisection and anti-NOGO (Nogo decoy molecule)and or control treatment by intrathecal application for 3 months. In addition, we have carried out intracortical injections of BDA to trace descending cortical axons These animals have had full behavioral testing (fine hand movement and open field analysis) and all histology has been carried out on their brains and spinal cords. These data indicate a strong trend for improved functional outcome and an increase in descending corticospinal and 5-HT axons below the level of the lesion. The current group of animals will receive the same treatment protocol and behavioral and histological analysis, but will importantly increase our n to assess statistical significance.

(2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly
► We have completed 6 NHPs that received C5 hemisection and anti-NOGO (Nogo decoy molecule)and or control treatment.

(3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other Items below.
► The study results are generally as expected and no changes have been made to the protocol.

(4) Animals on hand that have had no previous usage are transferred from an expired ACORP to a three year ACORP renewal, and are deducted from the total number of animals approved for use on the new protocol. If there are animals on census in ongoing experiments that have had procedures covered by the expired protocol, but these procedures are not proposed in the renewal, a scientific justification and brief description of the procedures performed on the animals transferred must be provided.
►

(5) If there are animals on census that have had procedures under the old protocol and you anticipate additional procedures under the new protocol, the old and new procedures need to be described. All transferred animals must be justified in the total number requested.
►Jonagold has had spinal cord surgery and catheter/pump placement and behavior training. She will get a craniotomy and BDA injection 6 months after the first surgery.

MacIntosh has had spinal cord surgery and catheter/pump placement and behavior training. She will get a craniotomy and BDA injection 6 months after the first surgery.

Idared had had spinal cord surgery and is scheduled to get an intra-thecal catheter and pump in September. She will needa craniotomy and BDA injection in 6 months' time.

Cortland is being behavior tested.

Gala is being behavior tested.

Honeycrisp is being behavior tested.

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

(1) Title of other protocol ►
(2) IACUC approval number of other protocol ►
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►

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

►(X) Research
►( ) Teaching or Training

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

## Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of people and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.
► The primary cause of disability after SCI is the disconnection of networks by the transection of the nerve fibers (or axons), which are required for electrical signaling between cells. The proposed study is to probe the effects of interventional therapy on the recovery of the injured spinal cord of the non-human primate. If even minimal regrowth of axons could be achieved from surviving neurons, it would provide profound clinical benefit for patients suffering from spinal cord injury. Regeneration of as few as 5% of nerve fibers would allow some arm and leg movement, even if strength were not normal.

C. **Experimental Design.**

1. **Lay Summary**. Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.
► We will study the effects of intrathecal delivery of a specialized protein after spinal cord injury. We will look at behavior testing results before and after surgery and follow the progression with recovery. We will also examine the spinal cord after euthanasia under a microscope for histological analysis.

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

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.
**► NOGO-66 is a powerful inhibitory protein for axon regeneration, which can be counteracted by blockading its receptor with NgR(310)-FC. The proposed study is to probe the effects of NOGO inhibitors on the recovery of the injured spinal cord of the vervet.**
**1) Vervet spinal cord hemisection:**
**a) NgR group - delivering NgR(310)-FC intrathecally (n=3)**
**b) Control group- delivering saline intathecally (n=3)**
**Each animal will undergo an initial spinal cord(SC) surgery, C5/C6 spinal cord hemi-section. Approximately one month after SC surgery a second surgery will take place to insert an intra-thecal catheter at L3 connected to an osmotic pump placed between the scapula. Pumps will be replaced 3 times. Approximately 6 months to a year after the initial surgery Biotinylated dextran amine (BDA) will be injected into the left motor cortex followed by cardiac perfusion one month later.**
**All vervets will also undergo behavior testing. The Kocsis primate behavior training program has three goals: 1) Provide enrichment for singly housed NHP's. 2) Teach animals to participate in behavior tests that will allow evaluation of recovery post-operatively. 3) Teach animals to participate in health evaluations, veterinary care procedures, and animal husbandry chores such as cage changes. Although some behaviors relate directly to evaluation of an experimental medical procedure, these behaviors are also a form of enrichment. The other behaviors are**

**designed to improve care and quality of life for our animals. These behaviors include targeting, drinking from a squeeze bottle, desensitization to squeeze back, presenting for injections, staircase test, precision grip test, well reach and open field testing.**

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.
► **We anticipate from our previous studies that six animals will be needed for each experimental condition in order to achieve statistical significance. While we have been very successful with these experimental procedures, some lesions are incomplete or in others there is excessive focal axon loss. WE ARE REQUESTING 6 ANIMALS TO COMPLETE THIS PART OF THE PROJECT. We suspect that 2 animals per group will not be sufficient for analysis; therefore a minimum of 4 animals is expected to be appropriate for analysis. This number was determined from previous studies in our laboratory where statistical significance is required. Data is analyzed with an unpaired Student's T-test. If we find we can get significant results using fewer animals we will do so.**

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►Animals will undergo a surgical procedure on the spinal cord, a hemisection and intrathecal catheter and pump changes, craniotomy and BDA injections and finally cardiac perfusion. These procedures are detailed in Appendix 5. They will alsc under go behavior training which is described in detail in Appendix 6. These behaviors include targeting, drinking from a squeeze bottle, desensitization to squeeze back, presenting for injections, staircase test, precision grip test, well reach and open field testing.

D. **Species.** Justify the choice of species for this protocol.
► Vervet- WE HAVE USED VERVET MONKEYS IN OUR PREVIOUS STUDIES. THEY ARE CALM, EASY TO HANDLE AND MODERATE IN SIZE. THESE ANIMALS DO NOT NATURALLY CARY THE HERPES B VIRUS. THEY ARE A NON-HUMAN PRIMATE SO WE CAN GET A GOOD INDICATION OF HOW THIS THERAPY WILL REACT IN A PRIMATE MODEL.

## Personnel

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

1. PI

Name► [redacted]
Animal research experience ► [redacted]**has a PhD in anatomy and over 25 years' experience in survival surgery with rodents, cats and primates.** [redacted]**has carried out many studies using the vervet.**

Qualifications to perform specific procedures

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

| | |
|---|---|
| **Oversee and partake in all aspects of this protocol including survival surgery, post-operative care, post-op monitoring and analgesia, cardiac perfusion and tissue harvest.** | **over 25 years experience with the these procedures in vervets** |

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

Name▶[redacted]
Animal research experience ▶ [redacted] **has over 25 years experience working in my lab with cell preparation, culture, tissue harvest and processing for histological analysis.**

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| **Cell preparation, processes tissue, histology** | [redacted] **has over 15 years experience with these procedures** |

Name▶[redacted]
Animal research experience ▶ [redacted] **has a degree in Laboratory Animal Technology and is LATg certified.** [redacted] **has over 20 years experience working with primates and rodents, sterile surgical technique, anesthesia and post-operative care of animals.** [redacted] **has worked in my lab for 15+ years.**

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| **Behavioral studies, anesthesia, post-procedure monitoring and analgesia, cardiac perfusion, euthanasia** | **Over 20 years experience with these procedures in the vervet.** |

Name▶[redacted]
Animal research experience ▶ [redacted] **has a BS degree in Veterinary Technology.** [redacted] **has over 6+ years experience with primates.**

Qualifications to perform specific procedures

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

| | |
|---|---|
| **Behavioral studies, anesthesia, post-procedure monitoring and analgesia, cardiac perfusion, euthanasia** | **Over +6 years experience with these procedures in the vervet.** |

Name►[redacted]
Animal research experience ► **Dr.[redacted] has an MD degree from [redacted] Medical School in [redacted]. [redacted]has been involved in spinal cord research in non-human primates in [redacted]and rodent spinal cord injury at [redacted]since 2004.**

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| **Spinal Cord surgery, Perfusion (euthanasia)** | **[redacted]has over 5 years experience performing the spinal cord hemi-section in vervets. [redacted]has many years' experience performing the contusion surgery in rodents.** |

Name►[redacted]
Animal research experience ► **[redacted]has a BS in Health Sciences and 5 years experience in my lab working with rodents and primates.**

Qualifications to perform specific procedures

| Specific procedure(s) that this individual will perform | Experience with each procedure in the species described in this ACORP |
|---|---|
| **Post-procedure monitoring and analgesia, cardiac perfusion and tissue harvest and histology** | **[redacted]has over 5 years' experience in these procedures in the vervet.** |

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

Name►

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

| Specific support procedure(s) assigned to this individual | Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training) |
|---|---|
| | |
| | |

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

| Name of Individual | Working with the VA IACUC | ORD web-based species specific course (NHP) | Any other training required locally (Identify the training) |
|---|---|---|---|
| [redacted] | 07/06/15 (#[redacted]) | 07/06/15 (#[redacted]) | |
| [redacted] | 06/29/15 (#[redacted]) | 06/29/15 (#[redacted]) | |
| [redacted] | 07/28/15 (#[redacted]) | 07/28/15 (#[redacted]) | |
| [redacted] | 09/10/15 (#[redacted]) | 09/10/15 (#[redacted]) | |
| [redacted] | 12/01/15 (#[redacted]) | 11/23/15 (#[redacted]) | |
| [redacted] | 10/27/17 (#[redacted]) | 05/04/15 (#[redacted]) | |

F. **Training to be provided.** List here each procedure in Item E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, enter "N/A"
►NA

G. **Occupational Health and Safety.**

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

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

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

► ( ) Yes. Describe them ►

► (X) No.

## Animals Requested

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

| Description (include the species and any other special features not shown elsewhere in this table) | Gender | Age/Size on Receipt | Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony) | Health Status |
|---|---|---|---|---|
| **Vervet** | **Either** | **Young adult-adult** | **[redacted], [redacted] or other vendor approved by VCS** | **Herpes B negative** |
| | | | | |

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

**USDA Category B**

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

**USDA Category C**

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

**USDA Category D**

| Procedures▶ Spinal cord surgery, Craniotomy | | | | | | |
|---|---|---|---|---|---|---|
| Species / Experimental Group / Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | **Category D TOTAL** |
| **Vervet** | ***6** | **0** | | **0** | | **6** |
| ***animals on protocol: MacIntosh, Jonagold, Idared, Cortland, Gala and Honeycrisp** | | | | | | |

**USDA Category E**

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

**TOTALS over all Categories**

| Species / Experimental Group /Procedure(s) | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | **GRAND TOTAL** |
|---|---|---|---|---|---|---|
| **Vervet** | | | | | | **6** |
| | | | | | | |

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

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

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

| Procedure | Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery) | Person(s) responsible for the monitoring | Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered) |
|---|---|---|---|
| **Spinal cord hemisection** | **See Appendix 5 for details** | | |
| **Craniotomy and BDA injections** | **See Appendix 5 for details** | | |
| **CARDIAC PERFUSION** | **See Appendix 5 for details** | | |
| **OSMOTIC PUMP SX** | **See Appendix 5 for details** | | |

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

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

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

## Veterinary Care and Husbandry

L. **Veterinary Support.**

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

   Name► [redacted], DVM, [redacted]
   Institutional affiliation► Consultant to VACHS
   email contact► [redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted► [redacted], DVM, [redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ►09/14/2017

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

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

| a. Species | b. Type of housing* | c. Number of individuals per housing unit** | d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***) | e. Estimated maximum number of housing units needed at any one time |
|---|---|---|---|---|
| vervet | standard | 1 | yes | 6 |
| | | | | |

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:
► SOP

** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a justification if any animals will be housed singly (if species is not considered "social", then so note)
► From our experience when pair housing vervets, there is a high rate of injury resulting in trauma from fight wounds and extremely few successful pairs/groups. Typically, the injury is located on the head, hand and/or limb and result in deep wounds which require suture and antibiotic therapy. Our research involves behavior testing and fine movements of the limbs. Potential damage to the fingers and limbs could result in the animal being useless to our study. The injuries from fighting also interfere with the training and testing process delaying experimental surgery and testing. Intact hands, limbs and head are critical for the success of our scientific research. Therefore, we are requesting an exemption from the "Nonhuman Primate Housing" policy allowing us to singly house all vervets on this study. Animals

on this study receive additional enrichment and interaction through behavior training. SINCE THE ANIMALS POST-SURGERY CAN HAVE DEFECITS, SOME ANIMALS WOULD BE AT A COMPETETIVE DISADVANTAGE POST OPERATIVELY, FURTHER ADDING TO CONCERNS OF INJURIES.
***Use Appendix 9 to document "departures" from the standards in the *Guide*.

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

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

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

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

► ( ) This ACORP INCLUDES genetically modified animals.

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

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

► (X) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.
**►rubber mats in cage after spinal cord surgery, food in bowls if necessary**

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

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

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

| Building | Room number | Inside of VMU? | |
|---|---|---|---|
| | | Yes | No |
| VA Building [redacted] | [redacted] | (X) | ( ) |
| Or any appropriate room designated by the AF supervisor | | ( ) | ( ) |
| | | ( ) | ( ) |

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

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

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

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

## Special Features

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

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

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

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

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

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

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

| Procedure | Surgical? | | Bldg/Room Number | Requires transport through non-research areas? | |
|---|---|---|---|---|---|
| | Yes | No | | Yes – describe method of discreet transport | No |
| **Cardiac perfusion** | **(X)** | **( )** | [redacted] | **( )** | **(X)** |
| **Spinal cord hemisection, intrathecal catheter and pump change, Craniotomy and BDA injections** | **(X)** | **( )** | [redacted] | **( )** | **(X)** |
| | **( )** | **( )** | | **( )** | **( )** |
| | **( )** | **( )** | | **( )** | **( )** |

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

| Body Fluid, Tissue, or Device to be Collected | Collected AFTER Euthanasia | Collected BEFORE Euthanasia | | |
|---|---|---|---|---|
| | | Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production") | Collected as Part of a Surgical Procedure (Appendix 5, "Surgery") | Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection") |
| Brain, spinal cord | **(X)** | **( )** | **( )** | **( )** |
| | **( )** | **( )** | **( )** | **( )** |
| | **( )** | **( )** | **( )** | **( )** |

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

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

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

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

► **The animal will be removed from the study and euthanatized if it:**

- **Has not recovered motor function to the point where it can sit up for an extended period without assistance within ten days post-operatively and is not showing steady daily improvement in neurologic function and spends most of its time recumbent**

- **Is incurring significant self-injury or pressure sores**
- **Loses 15% percent of its pre-surgical body weight**
- **Is unable to feed itself within ten days post-operatively**
- **PROGRESSIVE, UNRESOLVED INFECTION AT THE SITE OF PUMP IMPLANTATION**

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

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

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

| Check each method that may be used on this protocol | Method of Euthanasia | Species | AVMA Classification | | |
|---|---|---|---|---|---|
| | | | Acceptable | Acceptable w/Conditions | Unacceptable |
| ( ) | $CO_2$ from a compressed gas tank<br>Duration of exposure after apparent clinical death►<br>Method for verifying death►<br>Secondary physical method► | | ( ) | ( ) | ( ) |
| ( ) | Anesthetic overdose<br>Agent►<br>Dose►<br>Route of administration► | | ( ) | ( ) | ( ) |
| ( ) | Decapitation under anesthesia<br>Agent►<br>Dose►<br>Route of administration► | | ( ) | ( ) | ( ) |

| | | | | | |
|---|---|---|---|---|---|
| ( ) | Exsanguination under anesthesia<br>Agent►<br>Dose►<br>Route of administration► | | ( ) | ( ) | ( ) |
| (X) | Other (Describe) ► Cardiac perfusion/exsanguination under anesthesia | | (X) | ( ) | ( ) |
| ( ) | Other (Describe) ► | | ( ) | ( ) | ( ) |

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

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

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.
   ► [redacted], [redacted], [redacted], [redacted], [redacted] all have experience with cardiac perfusion and euthanasia.

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
      ► Refrigerate carcass and immediately contact a lab member

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

      Name►[redacted]
      Contact Information► X[redacted], cell [redacted], home [redacted]

      Name►[redacted]

Contact Information► X[redacted], cell [redacted]

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

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

| Name of Procedure | Identify Where the Details of the Procedure are Documented | | |
|---|---|---|---|
| | SOP (title or ID number)* | Other Items in this ACORP -- specify the Item letter(s) | Appendix 6 |
| Behavior training | | Items: | (X)** |
| Rubber mats, stainless steel bowls for food and water, low hung juice bottle | | Items: | (X)** |
| | | Items: | ( )** |
| | | Items: | ( )** |

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

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

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

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

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   ►

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

| Name of the database | Date of search | Period of years covered by the search | Potentially painful or distressing procedures addressed | Key words and/or search strategy used | Indicate which mandate each search addressed | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Replacement of animals (item W.2) | Reduction in numbers of animals used (item W.3) | Refinement to minimize pain or distress (item W.4) | Lack of unnecessary duplication (item W.5) |
| Pubmed | 08/8/17 | unrestricted | lamenectomy, spinal cord hemisection, thoracotomy | remyelination, dorsal hemisection, lamenectomy, thoracotomy, VERVET, AXON TRACING, OSMOTIC PUMP, alternatives | ( ) | (X) | (X) | (X) |
| Pubmed | 8/8/17 | Unrestricted | lamenectomy, spinal cord hemisection thoracotomy, | In vitro, computer, remyelination, , dorsal hemisection, lamenectomy, thoracotomy, VERVET, AXON TRACING, OSMOTIC PUMP | (X) | ( ) | ( ) | ( ) |
| Web of Science | 8/8/17 | Unrestricted | lamenectomy, spinal cord hemisection thoracotomy, | remyelination, dorsal hemisection, lamenectomy, thoracotomy, AXON TRACING, OSMOTIC PUMP alternatives | ( ) | (X) | (X) | (X) |
| Web of Science | 8/8/17 | Unrestricted | lamenectomy, spinal cord hemisection thoracotomy, | In vitro, computer, remyelination, dorsal hemisection, lamenectomy, thoracotomy, VERVET, AXON TRACING, OSMOTIC PUMP | (X) | ( ) | ( ) | ( ) |
| | | | | | ( ) | ( ) | ( ) | ( ) |

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.
   ▶ When possible we use cells in vitro or rodents, but need to use non-human primates to study the spinal cord lesions and repair in a higher animal.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.

► We have based our numbers on the smallest number of animals that can be used to carry out our objectives and gain significant results that can be used for publication.

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.
► We place an intrathecal catheter to deliver drug in this protocol. In previous protocols, we placed the catheter in the ventricle of the brain. Intrathecal placement is less invasive. If we identify any further refinements, they will be incorporated into the protocol.

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.
► A literature search shows there is no unnecessary duplication of work.

## X. Other Regulatory Considerations.

### 1. Controlled drugs.

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

| Controlled substances | Storage | | Personnel Authorized to Access | Location for Use | | Procurement | |
|---|---|---|---|---|---|---|---|
| | Double-locked | Not Double-locked* | | VA Property | Not on VA Property | VA Phar-macy | Non-VA |
| Ketamine | (X) | ( )* | [redacted] | (X) | ( ) | ( X) | ( ) |
| Sodium Pentobarbital | (X) | ( )* | [redacted] | (X) | ( ) | ( X ) | ( ) |
| Buprenorphine | (X) | ( )* | [redacted] | (X) | ( ) | (X ) | ( ) |

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

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

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

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

► ( ) Other. Explain►

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

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

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

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

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

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

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

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

► ( ) Appendix 1, "Additional Local Information"
► ( ) Appendix 2, "Antibody Production"
► (X) Appendix 3, "Biosafety"
► ( ) Appendix 4, "Ante-mortem Specimen Collection"
► (X) Appendix 5, "Surgery"
► (X) Appendix 6, "Special Husbandry and Procedures"
► ( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
► ( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
► (X) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"
► ( ) Appendix 10, "Rodent Breeding Colony"

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

| Item | SOP | | Approval Date |
|---|---|---|---|
| | Title | ID | |
| Appendix 5.2 | Surgery: Non-Rodent Mammals, Survival and Non-survival. | | 08/04/15 |
| Appendix 6b | Thermal Support of Anesthetized Non-Rodent Mammal | | 07/05/17 |
| M1 | VA Housing Policy_ Nonhuman Primate | | 02/07/17 |
| Appendix 5.2 | VA Anethesia Guidelines and Procedures | | 11/04/14 |
| ACORP M | Housing Policy: Nonhuman Primate, NHP Environmental Enhancement Plan | | 08/01/17 |

| | | | |
|---|---|---|---|
| APP 3 | Use of Non-Pharmaceutical-Grade Experimental Agents and Approval for Use of Classes of Drugs | | 07/05/17 |
| | | | |
| | | | |

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

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

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

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

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

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

I further certify that:

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

| Name(s) of Principal Investigator(s) | Signature | Date |
|---|---|---|
| [redacted], **Ph.D.** | [redacted] | 11.30.17 |
| | | |

b. **Certification by IACUC Officials.**

We certify that:

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

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

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

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

| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
|---|---|---|
| [redacted], **DVM,** | [redacted] | 11/29/17 |
| Name of IACUC Chair | | Date |
| [redacted], **Ph.D.** | | 11/29/17 |

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

3. **Appendix 3. Biosafety.**

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

   We certify that:

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

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

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| [redacted], Ph.D. | [redacted] | 11.30.17 |
| | | |
| Name of Institutional Veterinarian | Signature | Date |
| [redacted], DVM, | [redacted] | 11/29/17 |
| Name of IACUC Chair | [redacted] | Date |
| [redacted], Ph.D. | | 11/29/17 |

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

   - Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "toxic", "infectious", "biological", or "contains recombinant nucleic acid";

   - The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;

   - The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

| Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee | Signature | Date |
|---|---|---|
| [redacted], **M.S.** | [redacted] | 11/30/17 |
| | | |

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

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

| Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee | Signature | Date |
|---|---|---|
| [redacted], **M.S.** | [redacted] | 11/30/2017 |
| | | |

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

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

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

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

| Name(s) of Principal Investigator(s) | Signature(s) | Date |
|---|---|---|
| [redacted], **Ph.D.** | [redacted] | 11.30.17 |
| | | |

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

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

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

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

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

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |

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

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

| Name of IACUC Chair | Signature | Date |
|---|---|---|
| | | |
| Name of Attending Veterinarian (VMO or VMC) | Signature | Date |
| | | |
| Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment | Signature | Date |
| | | |
| Name of ACOS for R&D | Signature | Date |
| | | |
| Name of Chief of Staff | Signature | Date |
| | | |
| Name of Director or CEO of the Facility (Hospital or Clinic) | Signature | Date |
| | | |

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

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

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

I further certify that:

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

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

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

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

| Name of VISN Regional Safety Officer | Signature | Date |
|---|---|---|
| | | |

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

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

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

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

| **Material** (Identify the specific agent, device, strain, construct, isotope, etc.) | **Source** (Identify the vendor or colleague, or specify which animals on this protocol will serve as donors) | **Nature of Material** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Toxic Agent (Item 4) | Infectious Agent (Item 5) -- Enter the CDC Biosafety Level (BSL 1, 2, 3, or 4) | Biological Agent (Item 6) | Radioactive Agent (Item 7) | Contains Recombinant Nucleic Acid (Item 8) | Routine Pre- or Post-Procedural Drug | Euthanasia agent |
| ketamine | VA Pharmacy | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| glycopyrrolate | VA Pharmacy or Schein | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| cefazolin | VA Pharmacy or Schein | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| isoflurane | VA pharmacy | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Lactated ringers | McKesson | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Buprenorphine | VA pharmacy | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Motrin | Schein | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Sodium pentobarbital | VA Pharmacy | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | ( ) | (X) |
| NgR(310)-FC | [redacted]lab | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Baytril | Bayer | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Biotinylated dextran amine (BDA) | Life Technologies | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Tisseel fibrin glue | Baxter Healthcare | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| SALINE | HOSPIRA | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |
| Meloxicam | Pharmacy or Schein | ( ) | ( )BSL_ | ( ) | ( ) | ( ) | (X) | ( ) |

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

| **Material*** (Identify the specific agent, device, strain, construct, isotope, etc.) | **Dose** (e.g., mg/kg, CFU, PFU, number of cells, mCi) and **Volume** (ml) | **Diluent* or Vehicle*** | **Route** of admin | **Frequency or duration** of admin | **Reason** for Administration and **Expected Effects** | Location of **Further Details** in this ACORP (specify "Main Body" or "App #", and identify the Item) | Administration Under **Anesthesia, sedation, or tranquilization** (Y/N) |
|---|---|---|---|---|---|---|---|
| ketamine | 10-15mg/kg;0.5-1.5ml | NA | IM | Once | Anesthesia | App. 5 | N |
| glycopyrrolate | 0.015 mg/kg; 0.3-0.75ml | NA | IM | Once | Pre-anesthetic | App. 5 | N |
| cefazolin | 20mg/kg;.03-0.7ml | Sterile water | IM | 5 days | antibiotic | App. 5 | N |
| isoflurane | 1-3.5% | NA | inhalation | Duration of surgery | anesthesia | App. 5 | Y |
| Lactated ringers | 5-10ml/kg/hr;25-60ML/hr | NA | IV/SQ | Duration of surgery | hydration | App. 5 | Y |
| Buprenorphine | 0.005-0.01mg/kg (0.3mg/ml; .8-0.33ml) | NA | IM | 48 hrs | analgesia | App. 5 | N |
| Ibuprofen | 10 mg/kg; (100mg/tab; ½ tab) | NA | PO | 48 hrs | analgesia | App. 5 | N |
| Sodium pentobarbital | 100mg/kg; 5-10ml | NA | IV | once | anesthesia | App. 5 | Y |
| NgR(310)-FC * | 0.9mg/kg; 2ml | | Intra-thecal | 4 months | To counteract the effects of NOGO an inhibitory protein for axon regeneration | Main body | Y |
| Baytril | 5mg/kg; 1ML | NA | IM | 5 days | antibiotic | App. 5 | N |
| Biotinylated dextran amine (BDA) * | 150nm/site; 5UL total | Saline | direct | once | Axon tracer | App. 5 | Y |
| Tisseel fibrin glue | <1ml | NA | direct | once | Seal dura | App. 5 | Y |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Meloxicam | 0.15mg/kg; 0.1-0.2ML | NA | SQ | 48 hrs | analgesic | App. 5 | N |
| SALINE | 150nm/site; 450nm total | | direct | once | Axon tracer | App. 5 | |

*Each material, diluent, or vehicle that is listed as FDA approved or is labeled "USP" is pharmaceutical grade. Check on-line for formulations that are FDA approved for administration to humans (http://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm) or animals (http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042847). Designate with a * each material and each diluent or vehicle to be used that is not pharmaceutical grade. For each of these, explain here why the use of a non-pharmaceutical grade formulation is necessary, and describe how it will be ensured that the material is suitable for use. (See ACORP App. 3 Instructions, for specifics about the level of detail required.)
► NgR(310)-FC* is made in the lab of Dr. [redacted] under sterile conditions. This protein is not available as pharmaceutical grade.

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

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

   ►Lactacted Ringers will be given IV during surgery to maintain good hydration

   ►Sodium Pentobarbital will be given IV under ketamine anesthesia to deeply anesthetize the animal prior to cardiac perfusion

   ► NgR(310)-FC will be infused by a subcutaneous osmotic pump intrathecally. Initially when placed the animal will be under anesthesia, but the administration will continue after the animal is awake.

   ►Tisseel fibrin glue will be used during surgery while the animal is anesthetized with isoflurane.

   ►BDA will be injected during surgery while the animal is anesthetized with isoflurane.

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

   ► The administration of ketamine, glycopyrrolate, xylazine, cefazolin, baytril, buprenorphine, meloxicam and ibuprofen should at most cause momentary pain that does not require the use of anesthesia.

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

| Name of Toxic Agent | Mutagenic | Teratogenic | d. Select Agent? | e. Other specify toxic properties |
|---|---|---|---|---|

| | | | | Not a Select Agent | Select Agent Used in Sub-threshold Quantities | Select Agent that Requires Registration/Approval | |
|---|---|---|---|---|---|---|---|
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |
| | ( ) | ( ) | ( ) | ( ) | ( ) | ( )* | ( )▶ |

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

Name of agent ▶

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

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

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

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

| | | | | | |
|---|---|---|---|---|---|
| | | (Yes/No) | ( ) | ( ) | ( )** |
| | | (Yes/No) | ( ) | ( ) | ( )** |
| | | (Yes/No) | ( ) | ( ) | ( )** |

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

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

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

Name of agent ▶

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

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

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

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

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

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

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

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

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

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

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

    a. Complete the table below.

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

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

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

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

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

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

| Surgery | | Terminal | Survival | | |
|---|---|---|---|---|---|
| # | Description (specify the species, if ACORP covers more than one) | | Minor | Major | One of Multiple* |
| 1 | Spinal cord transection | ( ) | ( ) | (X) | (X)* |
| 2 | Intrathecal catheter and pump placement | ( ) | (X) | ( ) | ( )* |
| 3 | Pump change | ( ) | (X) | ( ) | ( )* |
| 4 | Craniotomy and injection of Biotinylated dextran amine (BDA) | ( ) | ( ) | (X) | (X)* |
| 5 | Cardiac perfusion | (X) | ( ) | ( ) | ( )* |

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

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:
▶ The first major surgery is to make the spinal cord lesion. The craniotomy and BDA injection surgery is to inject tracer to trace corticospinal axons to and beyond the cervical lesion site to determine if there is increased axonal sprouting/regeneration to determine if our interventional approach (Nogo decoy molecule treatment) elicits axonal regeneration across the spinal cord injury site.

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
▶ The intrathecal catheter and pump placement will be done approximately one month after the initial spinal cord surgery to allow the animal sufficient time to recover from the spinal cord surgery which is a major surgery. If we were to place the pump at the time of the first surgery and the animal had complications, we would be unable to determine if this is a complication from the surgery itself or potentially a reaction to the solution in the pump. Each pump will be changed on a monthly basis which is the amount of time the solution will last in each pump. The craniotomy and BDA injection will be done one month prior to cardiac perfusion (6-12 MONTHS AFTER THE INITIAL SURGERY).

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

3.

Surgery 1 ▶ Spinal Cord Transection and catheter placement: Surgery will be performed under strict sterile conditions in the surgical suite at the West Haven VAMC animal surgical suite. The site will be clipped and prepped using 3M™ Duraprep (patient skin prepping solution meets AORN and CDC guidelines for the reduction of surgical site infections, designed to be applied in a single, painted coat). In this procedure we will remove the bone at C5/C6 vertebral border to expose the dura. Using a

microscapel we will transect the lateral funiculus including the cortical spinal tract. The wound will be closed in layers using a single strand absorbable suture. The subcutaneous tissue will be closed using monofilament absorbable suture. The skin will then be closed using interrupted sutures with monofilament absorbable suture.

Surgery 2 ► We will anesthetize the monkey with ketamine and glycopyrrolate (10mg/kg; 0.015mg/kg). The animal will then be intubated and placed on isoflurane inhalation anesthesia (1.5-3%). Surgery will be performed under strict sterile conditions in the surgical suite at the West Haven VAMC animal surgical suite according to the VA Policy "Surgery: Non-Rodent Mammals, Survival and Non- Survival". The surgical site will be prepped using 3M™ Duraprep. An incision is made over spinal cord L3 and a small catheter is threaded intrathecally and sutured in place. Connecting tubing is tunneled under the skin to the neck and a pocket formed between the scapulas to hold the minipump. The wound will be closed in layers using absorbable suture and the animal allowed to recover. After surgery while still anesthetized a primate jacket (Lomir Biomedical Primate jacket) will be placed on the monkey to prevent the animal from removing the pump and or scratching a the incison site (which did occur on one animal). The jacket will stay on for approximately 5 days. From our experience this is enough time for the animal to become accustomed to having the pump in place and the incision to heal. There have been no complications from having the jacket on and the surgery site has healed uneventfully.

Surgery 3 ► Osmotic pump exchange: Surgery will be performed under strict sterile conditions in the West Haven VAMC animal surgical suite under gas anesthesia (isoflurane 1.0 -3%) after induction with ketamine and glycopyrrolate IM (10-15mg/kg;0.015mg/kg). A small incision will be made in the skin above the pump, the pump will be removed and a new one will be implanted. The wound will be closed in layers using a single strand absorbable suture.

Surgery 4 ► Surgery will be performed under strict sterile conditions in the surgical suite at the West Haven VAMC animal surgical suite. The surgical site will be clipped and prepped using 3M™ Duraprep. The animal will be anesthetized with isoflurane and positioned into a stereotaxic frame. A midline incision of the scalp will be made and the temporalis muscle will be reflected laterally. Frontoparietal craniotomy will be performed to expose the dura over the central sulcus overlying the hand and arm region. The craniotomy will be approximately 2cm in size. A 2cm circle will be made using a drill and diamond cut bit. The bone is cooled with saline flush during the drilling process. The bone is gently lifted using a Dumont forceps and placed in a bowl of sterile saline. We have used this method in a previous approved stroke model protocol with a 5cm craniotomy. Biotinylated dextran amine (BDA) will be injected into the motor cortex spanning the arm and hand regions of the motor cortex targeting to cortical layer V at via a glass micropipette. The injections will be started 1 mm rostral to the central sulcus and continue at 1-mm intervals parallel to the central sulcus. The dura will be sealed with fibrin glue and cranioplasty with bone flap and skin closure are performed. The temporalis muscle will be replaced over the craniotomy site and sutured to gala aponeurtica. This muscle flap will hold the bone in place as it heals. The subcutaneous tissue will be closed using monofilament absorbable suture. The skin will then be closed using interrupted sutures with monofilament absorbable suture.

Surgery 5 ► Cardiac Perfusion- Animals will be anesthetized with ketamine (10-15mg/kg), and then deeply anesthetized with sodium pentobarbital i.v. (100 mg/kg) just prior to transcardial perfusion with saline and buffered paraformaldehyde solution.

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

| Name | Surgery #(s) (see Item 1) | Role in Surgery | | | |
|---|---|---|---|---|---|
| | | Surgeon | Assistant | Manage Anesthesia | Other (describe) |
| [redacted] | 1-5 | ( ) | (X) | ( ) | ( ) |
| [redacted] | 1-5 | (X) | ( ) | ( ) | ( ) |
| [redacted] | 1-5 | ( ) | ( ) | (X) | ( ) |
| [redacted] | 1-5 | ( ) | ( ) | (X) | ( ) |
| [redacted] | 5 | ( ) | (X) | ( ) | ( ) |

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

| Building | Room Number | Surgery #(s) (see Item 1) | Type of Space | | |
|---|---|---|---|---|---|
| | | | Dedicated Surgical Facility | Other Dedicated Surgical Space | Other Space not Dedicated to Surgery |
| Building [redacted] | [redacted] | 1-4 | (X) | ( )* | ( )* |
| Building [redacted] | [redacted] | 5 | ( ) | ( *) | (X)* |
| | | | ( ) | ( )* | ( )* |
| | | | ( ) | ( )* | ( )* |

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

**▶ Room [redacted] is a procedure room in the NHP area that will be used for terminal surgery, cardiac perfusion. This room is equipped with a perfusion table and running water necessary to carry out this procedure**

6. **Pre-operative protocol.**

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

| Surgery #(s) (see Item 1) | Fast (Specify Duration) | Withhold Water (Specify Duration) | Place Intravenous Catheter(s) (Specify Site(s)) | Other – Describe |
|---|---|---|---|---|
| 1 | (X) -- overnight | ( ) -- | (X) --Cephalic or saphenous vein | ( ) -- |

| | | | | |
|---|---|---|---|---|
| 2 | (X) -- overnight | ( ) -- | (X) -- Cephalic or saphenous vein | ( ) -- |
| 3 | (X) -- overnight | ( ) -- | ( ) -- | ( ) -- |
| 4 | (X) -- overnight | ( ) -- | (X) -- Cephalic or saphenous vein | ( ) -- |
| 5 | ( ) -- | ( ) -- | (X) -- Cephalic or saphenous vein | ( ) -- |

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

| Agent | Surgery #(s) (see Item 1) | Dose (mg/kg) & volume (ml) | Route of administration | Frequency of administration (e.g., times/day) | Pre-operative period of treatment (e.g., immediate, or # of days) |
|---|---|---|---|---|---|
| **Ketamine** | **1-5** | **10-15mg/kg** | **IM** | **once** | **immediate** |
| **Glycopyrrolate** | **1-5** | **0.015 mg/kg** | **IM** | **once** | **immediate** |
| **Cefazolin** | **1** | **20mg/kg** | **IM** | **twice** | **immediate** |
| **Baytril** | **2** | **5mg/kg** | **IM** | **once** | **immediate** |
| **Meloxicam** | **4** | **0.15mg/kg** | **SQ** | **once** | **immediate** |

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

Surgery 1-4 ▶ The surgical site will be clipped using a #20 surgical blade, the animal is transported to the OR and placed on warm water recirculating heating pad and positioned for surgery. The skin is then prepped and scrubbed with a commercial product 3M™ DuraPrep™ Surgical Solution, a patient skin prepping solution in a self-contained applicator.

Surgery 5 ▶ The site will be clipped using a #20 surgical blade

7. **Intra-operative management.**

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

| Agent | Paralytic* | Surgery #(s) (see Item 1) | Dose (mg/kg) & volume (ml) | Route of administration | Frequency of dosing |
|---|---|---|---|---|---|
| **Isoflurane** | | **1-4** | **1-3.5% to effect** | **Inhalation** | **Continuous** |
| **Lactated ringers** | | **1,2,4** | **5-10ml/kg/hr (30-60ml/hr)** | **IV** | **Continuous drip** |
| **Sodium Pentobarbital** | | **5** | **100mg/kg (** | **IV** | **To effect** |

| | | | | | |
|---|---|---|---|---|---|
| **Buprenorphine** | | **1,2** | **0.005-0.01mg/kg(0.3mg/ml;.08-0.33ml;** | **IM** | **Once completion of surgery** |

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

b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).
► The animal is placed on a padded surface with a warm water recirculating heat pad covered with a blanket and a second warm water heat pad. Intravenous fluids are heated using the iWarm™ IV fluid warmer according to VA Guidelines "Thermal Support of Anesthetized Non-Rodent Mammals"

c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.
► Oxygen saturation, heart rate, blood pressure, body temperature, respirations and end tidal CO2 are monitored throughout surgery and recorded every 15 minutes.

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

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

| Surgery # (see Item 1) | Survival Period | Measures for Maintaining Sterility | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sterile Instruments | Surgical Cap | Sterile Gloves | Surgical Scrub | Sterile Drapes | Sterile Gown | Face Mask | Other* |
| 1 | 6-12 months | (X) | (X) | (X) | (X) | (X) | (X) | (X) | ( )* |
| 2 | 6-12 months | (X) | (X) | (X) | (X) | (X) | (X) | (X) | ( )* |
| 3 | 6-12 months | (X) | (X) | (X) | (X) | (X) | (X) | (X) | ( )* |
| 4 | One month | (X) | (X) | (X) | (X) | (X) | (X) | (X) | ( )* |

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

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

Surgery 1 ► Heat pad and SQ fluids

Surgery 2 ► Heat pad and SQ fluids

Surgery 3 ►heat pad

Surgery 4 ► Heat pad and SQ fluids

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

| Surgery # (see Item 1) | Agent* | Dose (mg/kg) & Volume (ml) | Route of Administration | Frequency of Dosing (e.g., times/day) | Period of treatment (e.g. days) |
|---|---|---|---|---|---|
| 1 | **Buprenorphine** | **0.005-0.01mg/kg(0.3 mg/ml;.08-0.33ml;** | **IM** | **Every 6-12 hours: Twice a day** | **48 hrs** |
| 2 | **Buprenorphine** | **0.005-0.01mg/kg(0.3 mg/ml;.08-0.33ml;** | **IM** | **Every 6-12 hours: Twice a day** | **48 hrs** |
| 3 | **Ibuprofen** | **10mg/kg( ½-¾ tablet)** | **PO** | **twice** | **48 hrs** |
| 4 | **Buprenorphine**<br><br>**Meloxicam** | **0.005-0.01mg/kg(0.3 mg/ml;.08-0.33ml**<br><br>**0.1mg/kg; 0.1-0.2ml** | **IM**<br><br>**SQ** | **Every 6-12 hours: Twice a day**<br><br>**Once/day** | **48 hrs**<br><br>**48 hrs** |

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

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

| Surgery # (see Item 1) | Medication | Dose (mg/kg) & Volume (ml) | Route of Administration | Frequency of dosing (e.g. times/day) | Period of treatment (e.g. days) |
|---|---|---|---|---|---|
| 1,2,4 | **Lactated ringers** | **30 ml/lb/day (Flecknell) minus the amount given Intravenously during surgery** | **SQ** | **once** | **Immediate post-op** |
| 1, 4 | **Cefazolin** | **20mg/kg** | **IM** | **Twice a day** | **5 days** |
| 2 | **Baytril** | **5mg/kg** | **IM** | **Once/day** | **5 days** |
| | | | | | |

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

(1) Immediate post-operative monitoring

| Surgery # (see Item 1) | Frequency of Monitoring | Duration at this Frequency | Name(s) of Responsible Individual(s) |
|---|---|---|---|
| 1,2, 4 | 2X a day | 5 days or more if needed | [redacted] or [redacted] |
| 3 | once a day | 2 days or more if needed | [redacted] or [redacted] |

- All the above include weekends and holidays

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

| Surgery # (see Item 1) | Frequency of Monitoring | Duration at this Frequency | Name(s) of Responsible Individual(s) |
|---|---|---|---|
| 1 | At least once a day | Until eating normally and sitting up for extended periods | [redacted] or [redacted] |
| 4 | At least once a day | Until eating normally and urinating normally | [redacted] or [redacted] |

- All the above include weekends and holidays

f. Post-operative consequences and complications.

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

Surgery 1 ▶ Initially we expect to see hemiplegia on one side and hemiparesis on the contralateral side. This may make it difficult for the animal to sit up initially. Rarely may they not be able to self-feed and/ or can sit up unassisted. Rubber mats will be placed in the cage for comfort when lying down and to prevent pressure sores. Animals that cannot self-feed will be given food with a long forceps directly to the mouth. Bowls will be placed in the cage and water bottles will be hung low on the cage. We expect to see gradual daily improvement. It is expected that the animal should be able to sit up for short periods of time and self-feed the day after surgery. Another rare complication could be self-mutilation of the paretic hand. If this happens the hand will be bandaged to prevent further injury and antibiotics and/or analgesic will be given as directed by the veterinarian antibiotics as listed or recommended by the veterinarian and NSAIDS [meloxicam (0.1 mg/kg q24hrs)]. If an animal is unable to sit up unassisted and self-feed it will be reported to the IACUC as an adverse event and the animal will be placed in category E.

Surgery 2 ▶ A possible complication would be self-removal of the osmotic pump and or catheter. If possible we would surgically repair or replace the catheter or pump making a total of 4 pump surgeries if it became necessary to remove a pump to allow infection to clear.

Surgery 3 ▶ A possible complication would be self-removal of the osmotic pump or possibly infection. One animal did develop an infection isolated to the pocket surrounding the pump which was treated with antibiotics and replacing the pump.

Surgery 4 ► We do not expect any major consequences or complications however with brain injections there is always a risk of a brain bleed resulting in stroke like symptoms. If we were to see that the animal was unable to feed itself or paralysis of one side, we would give supportive care under the guidance of the veterinarian. If an animal develops post-operative neurologic signs (e.g. ataxia, seizures, depressed mentation, etc.) the veterinarian on call will be contacted to recommend appropriate treatment or euthanasia if deemed necessary.

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

Surgery 1 ► If the animal

- Has not recovered motor function to the point where it can sit up for an extended period without assistance within ten days post-operatively.
- Is not showing steady daily improvement in neurologic function and spends most of its time recumbent.
- Is incurring significant self-injury or pressure sores
- Loses 15% percent of its pre-surgical body weight

Surgery 2 ► If this could be repaired surgically we would do so, if not the animal may be euthanized.

Surgery 3 ► If this could be repaired surgically we would do so, if not the animal may be euthanized. If the animal developed an infection that spread to the spinal cord it would be euthanized.

Surgery 4 ► If the animal exhibited stroke like symptoms that do not show improvement after supportive care was given and after consultation with the veterinarian and Dr. [redacted] it is determined that it is becoming a humane issue then the animal would be euthanized or scheduled for cardiac perfusion.

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

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

| Surgery # (see Item 1) | Location of Records | Name(s) of Individual(s) Responsible for Maintaining Written Records | Research Personnel | Veterinary Staff |
|---|---|---|---|---|
| 1-4 | Outside of designated animal room | [redacted], [redacted] | (X) | ( ) |

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

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

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

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

| Special Procedure | | Features | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Brief Description | Husbandry | Restraint | Noxious Stimuli | Exercise | Behavioral Conditioning | Irradiation | Imaging | Other** |
| 1 | Behavior training | ( ) | ( ) | ( ) | ( ) | (X) | ( ) | ( ) | ( ) |
| 2 | Rubber mats, stainless steel bowls for food and water, low hung juice bottle | (X) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) |
| 3 | | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) |
| 4 | | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | ( ) |

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

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

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

Special Procedure 1 ► **Behavior training:**

**Targeting– Animal is taught to touch a ½ "delron (plastic) rod. This behavior allows the trainer to then move the animal about the cage, move an animal from cage to cage, observe the use of all limbs, and visualize all sides of the animal. Once this behavior has been trained the animal husbandry staff and veterinary staff can be taught the cues for this behavior and use it when moving or examining an animal.**
**Stage 1 – present small rewards at one fixed place at center of cage – if none are taken, leave them in the food box after working with all animals; goal is to have animal take treats from trainer**

**Stage 2 –give bridging cue and present small rewards; goal is to acquaint animal with bridge to establish it as a secondary reinforcer.**
**Stage 3 – using a food lure (treat is visible in hand), present the treat at different quadrants of cage, always choosing a quadrant the animal is not in. Bridge as soon as animal enters the target quadrant –**

**not necessary for him to come and sit in front of trainers hand; goal is to reward for moving into proximity of the trainer's hand and strengthen bridging cue.**
**Stage 4 – conduct as stage 3, only fade the lure by having the trainer's hand closed, but with food in it. Bridge as in stage 3, as soon as animal moves into the quadrant; goal is to reward for moving into proximity of the trainer's hand but without having to see food lure.**
**Stage 5 – exactly as stage 4, only have target stick in the other hand; goal is to desensitize the animal to the target stick.**
**Stage 6 – present food treats with the target stick 6-8" behind food treat. No bridging cue; goal is to desensitize the animal to the target stick.**
**Stage 7 - present food treats with the target stick 2" behind food treat. No bridging cue; goal is to desensitize the animal to the target stick.**
**Stage 8 – present target stick with food lure tucked behind the tip of the target, bridge and reward when the target is touched even if the animal is focused on retrieving the reward. Just work at one location; goal is to have animal touch the target stick.**
**Stage 9 - present target stick with food 2" behind the tip of the target (so that target is easier to touch than food), bridge and reward when the target is touched. Just work at one location; goal is to have animal touch the target stick.**
**Stage 10 – remove food lure, and use target stick to move animal around the cage as in stage 3. Bridge as soon as animal enters quadrant; goal is to use the target stick to move animals around.**
**Stage 11 – present target stick at front of cage in different quadrants. Bridge and reward when animal touches target stick; goal is to have animal touching the end of the target stick.**

**Drinking from a Squeeze Bottle – This allows us to give measured fluids post-operatively. Most animals show little fear of the juice bottle. Steps may include**
**1) Squirting some juice on the side of cage and moving the bottle away so animal feels comfortable investigating.**
**2) Holding the bottle to the front of the cage with a drop of juice at the end of the spout.**
**3) Actively squeezing the bottle when the sipper is in the animals mouth**
**4) Removing the bottle any time the animal attempts to touch or hold the sipper with a hand – this is a bad habit that needs to be discouraged from the start.**

**Desensitization to Squeeze-back– This is a simple desensitization/habituation plan where the animal is squeezed, released and rewarded. After several sessions, the animal is then squeezed, rewarded, and then released. Taking food while being squeezed is indicative of an animal that is not stressed by the squeezing procedure. The animal can then be squeezed and asked to perform other behaviors such as targeting while squeezed. It is important to randomly squeeze and release animals when an injection is not going to be given to maintain the animal's comfort level with the squeezing process. Squeezing only for injections will quickly lead to animal becoming fearful of the squeezing process.**

**Presenting for an IM injection– This involves teaching an animal which is not "squeezed" to press his thigh against the front bars of the cage and allow a needle to be inserted and the contents to be injected without moving. Actual injections (saline) would only take place during the final stages of training, and would be repeated less than once a week during training. After the behavior is trained and reliable, it can be maintained with sessions only once/month or even longer.**

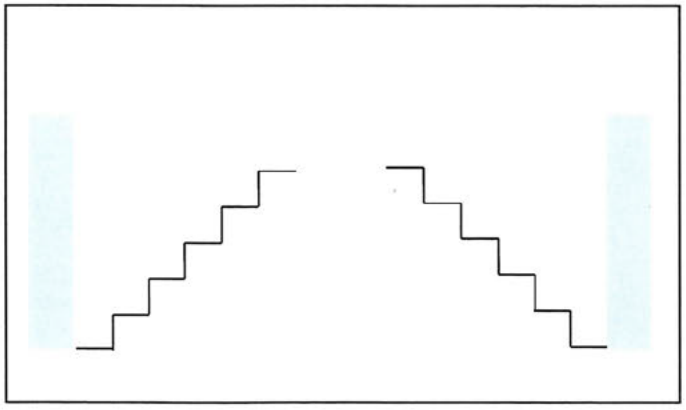
- **Stage 1 – "Capture" the animal when he is lateral to the front of the cage. (Capturing a behavior mean waiting for it to occur or luring the behavior and bridging and reinforcing when it happens) This may require using a food lure or slightly pulling forward the squeeze back.**
- **Stage 2 – Desensitize to syringe. Ask for the side presentation while holding a capped, blunt needle syringe. Gradually move the syringe closer the cage front while asking for the side presentation.**

- **Stage 3 – touch animal on the thigh with the capped, blunt needle syringe, through the bars of the cage.**
- **Stage 4 – touch the animal multiple times with the capped, blunt needle syringe. Do not reinforce if the animal moves away.**
- **Stage 5 – touch animal on the thigh with the un-capped, blunt needle syringe, through the bars of the cage.**
- **Stage 6 - touch the animal multiple times with the un-capped, blunt needle syringe. Do not reinforce if the animal moves away.**
- **Stage 7 – While the animal is holding and being touched with the blunt needle, pull on hairs of thigh. Reinforce animal for maintaining lateral position. Eventually you are able to actually pull out a hair or two while the animal maintains his position.**
- **Stage 8 – Quickly insert and remove a 1cc VanishPoint syringe with a 25g needle into the animal's upper thigh. Reinforce with a large piece of fruit. Animal <u>will</u> move away from the front of the cage, but should still be reinforced. Follow this with a request to present at front of cage. Touch with blunt needle and reinforce.**
- **Stage 9 – Go back to blunt needle work. Increase duration of time animal is required to hold at front of cage.**
- **Stage 10 – Insert a 1cc VanishPoint syringe with a 25g needle filled with sterile 0.5 mls saline. Inject contents of the syringe. Vary training with stages 5, 7, and 10 once/ week or less to maintain the behavior.**

**Staircase Test**

**A ¼" thick Lexan panel is attached to the front of the animal's home cage. The panel fits just inside openings (2.5"x 4.5") on the far left and far right side of their home cage. There is a set of 5 steps outside each opening. The steps are 4 inches long with a 1" tread and a 1" rise. The animals are required to reach through the opening to remove a food item from each of the steps. The positioning of the stairs respective to the opening forces the animal to use the left hand to remove food from the stairs on the left and the right hand to remove food from the stairs on the right.**

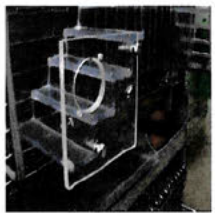
**A stainless steel covers is placed over the openings, one raisin, fruit gem (BioServ) or other treat is placed on the far end of one step. The cover is removed and animals are given a maximum of 30 seconds to initiate each trial, 10 seconds to complete each trial. The elapsed time from the animal's hand passing through the opening in the cage to the retrieval of the morsel of food and bringing hand back through the opening is recorded for each trial. Each step will be timed individually. All trials are videotaped. The series of trials is then repeated on the opposite side.**



**Well/Reach Test**

**This test examines the animal's fine motor dexterity by requiring it to use just two fingers to pick up a piece of food from inside a small well. The well block is made from ½" thick Lexan. Wells are 1 ¼" long, ½' wide, and 3/8" deep. The well block is secured on the first step of the staircase. The stainless steel panel is put in place, raisins or Fruit Gems (BioServ) are placed in the center of one of four wells. The cover is removed and animals are given a maximum of 30 seconds to initiate each trial, 10 seconds to complete each trial. The elapsed time from the animal's hand passing through the opening in the cage to the retrieval of the morsel of food and bringing hand back through the opening is recorded for each trial. Each well will be timed individually. All trials are videotaped. The series of trials is then repeated on the opposite side.**

**For the Staircase and Well/Reach tests, the animals participate in 3 trials on each task. Tasks are alternated so that the animal completed one round on each task before moving on to the second round of trials.**



**Precision Grip Test**

**Monkeys are required to reach out an opening (2.5"x 4.5") on the far left or far right side of their cage to remove a morsel of food using only their index finger and thumb from a pin fixed in the center of a modified, open ended 60ml syringe . The syringe is placed in the opening of a Lexan panel affixed to the front of the stairs approximately 25cm from the floor of the cage and 5cm back from the opening in the cage. Each experiment consists of five consecutive trials on the left or right side. The hand tested first is alternated for each experiment so that all experiments do not begin with the same hand. Animals are given a maximum of 30 seconds to initiate each trial, 10 seconds to complete a trial. The elapsed time from the animal's hand passing through the opening in the cage to the retrieval of the morsel of food and bringing hand back through the opening is recorded for each trial. All trials are videotaped.**

**Open Field Test**

**A clear lexan panel is placed on the end of the cage. The animal is given the top half of the cage to move about. Food is placed on the top of the cage and a challenger ball with food is hung inside the cage. The animal's movements are video recorded for approximately 10-15 minutes. Open field testing allows us to evaluate the animal's movements within the cage, including ambulation, use of hands and arms to retrieve food from the top of the cage and the challenger ball.**

**During the initial training period testing may be done as often as 5 times a week. During training the maximum number of trials on the test being taught at that time will vary. If good progress is being made they may have up to 25 repetitions. Once the task is learned, frequency may vary depending on the animal. No animal is ever forced to work, and most animals appear to enjoy the interaction once trained. Post-operatively animals will be tested at day 7, 14, 21, 28, 42 and monthly thereafter. During post-operative testing the staircase, well reach, precision grip and bar grip testing will consist of 3**

**trials on each side. If an animal loses interest, the test may be repeated in the afternoon with 3 more trials.**

Special Procedure 2 ▶**Rubber mats, stainless steel bowls for food and water, low hung juice bottle will be used post-operatively.**

Special Procedure 3 ▶

Special Procedure 4 ▶

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

Special Procedure 1 ▶ **Perhaps one of the most important aspects of animal training is its ability to provide for the animals' overall physical and mental welfare. The [redacted] primate behavior training program has three goals: 1) Provide enrichment for NHP's. 2) Teach animals to participate in behavior tests that will allow evaluation of recovery post-operatively. 3) Teach animals to participate in health evaluations, veterinary care procedures, and animal husbandry chores such as cage changes. Although some behaviors relate directly to evaluation of an experimental medical procedure, these behaviors are also a form of enrichment. The other behaviors are designed to improve care and quality of life for our animals.**

Special Procedure 2 ▶ **Rubber mats are placed in the cage for the comfort of the animal and to prevent pressure sores to animals that spend more time sitting on the bottom of the cage or laying down for interrupted periods of time post- surgery. Food and/ or water will be placed in stainless steel bowls inside the cage to allow easy access for the animal until it can maneuver food out of the hopper and reach the water bottle. Juice bottles will be hung low on the cage after surgery to entice the animal to drink to maintain proper hydration.**

Special Procedure 3 ▶

Special Procedure 4 ▶

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

| Procedure Number (see Item 1) | Responsible Individual(s) | |
|---|---|---|
| | Carrying Out Procedure | Monitoring the Animals |
| 1 | [redacted], [redacted] | [redacted], [redacted] |
| 2 | | |
| 3 | | |
| 4 | | |

3. **Potential Pain or Distress.** Complete the table below for each special procedure identified in Item 1, above, indicating for each procedure, whether potential pain and/or distress is expected, and, if so,

describing the potential pain and/or distress and indicating whether any measures are to be taken to prevent or alleviate it.

| Procedure Number (see Item 1) | Expected Potential Pain and/or Distress | | | |
|---|---|---|---|---|
| | No | Yes | | |
| | | Description | To Be Relieved | Not to Be Relieved |
| 1 | (X) | Behavior testing | ( )[a] | ( )[b] |
| 2 | (X) | Rubber mats, bowls for food and water | ( )[a] | ( )[b] |
| 3 | ( ) | | ( )[a] | ( )[b] |
| 4 | ( ) | | ( )[a] | ( )[b] |

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

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

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

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

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

Special Procedure 1 ►

Special Procedure 2 ►

Special Procedure 3 ►

Special Procedure 4 ►

4. **Monitoring.** Describe how the condition of the animals will be monitored during and after each of the

special procedures, and list the criteria that will be used to determine when individual animals will be removed from groups undergoing these procedures, because of pain or distress (see ACORP App. 6 Instructions, for details):

| Procedure Number (see Item 1) | Monitoring Methods | Endpoint Criteria |
|---|---|---|
| 1 | Visual observation during test | If an animal is unwilling to participate in testing after approximately 10 minutes, testing will be stopped for the day. |
| 2 | Observation, written records | When animal is eating well from hopper food bowls will be discontinued. When the animal moves around and the threat of pressure sores is gone, rubber mat may be removed. |
| 3 | | |
| 4 | | |

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

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

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

Copy the lines below for each departure.

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

►Single housing of social species should be the exception and justified based on experimental requirements

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

►Vervets on this protocol will be singly housed. They will have visual, auditory, and olfactory contact with other vervets in the room. They also receive positive interaction from the laboratory staff during behavior training sessions. Animals are observed daily by laboratory staff and/or animal care staff and any aberrant behavior is immediately reported to Veterinary Clinical Services.

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

► From our experience when pair housing vervets, there is a high rate of injury resulting in trauma from fight wounds and extremely few successful pairs/groups. Typically, the injury is located on the head, hand and/or limb and result in deep wounds which require suture and antibiotic therapy. Our research involves behavior testing and fine movements of the limbs. Potential damage to the fingers and limbs could result in the animal being useless to our study. The injuries from fighting also interfere with the training and testing process delaying experimental surgery and testing. Intact hands, limbs and head are critical for the success of our scientific research. Therefore, we are requesting an exemption from the "Nonhuman Primate Housing" policy allowing us to singly house all vervets on this study.

**REQUEST FOR VA ACORP AMENDMENT**
**VA Connecticut Healthcare System/689**
**Institutional Animal Care and Use Committee**

Protocol # **JK0025** Title: **Interventional Therapies after Spinal Cord Injury**

Principal Investigator [redacted] Office Phone: [redacted] Email: [redacted]

Please check all applicable changes to be made to the approved protocol. Attach a narrative (see instructions) that describes the modification. **Changes must not be implemented until IACUC approval is granted.**

| **A) Personnel, Procedure/Housing, Funding, Departures** | **List the actual change being requested** |
|---|---|
| ____ Adding new personnel: Attach a Q of P form | |
| ____ Deleting personnel/change of PI | |
| ____ Additional procedure/surgery/housing room | |
| ____ Title change/additional funding: Attach funding form | |
| ____ Requesting an IACUC Exemption or Departure | |
| ____ Other | |

**B) Adding Species/Breeding Colony, Increasing # of animals**

| | |
|---|---|
| ____ Addition of new species-include USDA category & # | |
| ____ Addition of new strain/transgenics-include USDA cat | |
| Increase in the # of an already approved species | |
| ____ Include USDA category & # of animals | |
| ____ Adding a Breeding Colony: Attach Appendix 10 | |

**C) Changing Euthanasia, Hazardous, Therapeutic, Anesthetic Agents**

| | |
|---|---|
| X Add/ change euthanasia method | **Add Euthasol** |
| ____ Add/change radiation, infectious, or biohazard agent | |
| ____ Add/ change therapeutic or anesthetic agent | |

**D) Procedures:**

| | |
|---|---|
| ____ Addition of an invasive procedure* | |
| ____ Addition of a non-invasive procedure* | |

**NOTE**: An amendment to your **Research Protocol Safety Survey** may be required if you propose changes in biological, chemical, or radiological agent use. (Submit to Subcommittee on Research Safety, SRS).

*If this amendment includes new procedures, I certify that I have consulted with the following sources and, to the best of my knowledge, the new procedures requested in this protocol do not unnecessarily duplicate previous experiments or unnecessarily use animals. (Check all applicable sources):

___ Medline ___ Nat Agricultural Library ___ Library of Congress __ Other (please specify) _____

***The table below must be completed for new NHP procedures that are USDA Cat D or E**

**INVESTIGATOR ASSURANCE:** I assure that these activities do not unnecessarily duplicate previous experiments conducted [redacted] ee to conduct this project in accordance with applicable provisions of the Anima[redacted]c Health Service Policy and the Guide for the Care and Use of Laboratory Animals.

**PI Signature** [redacted] **Date** **08/28/2018**

Revised & IACUC Approved: 12/05/17

("Per" signature not acceptable

**Consideration of Alternatives and Prevention of Unnecessary Duplication**

**This table must be completed when requesting new USDA Category D or E procedures for USDA covered species, (e.g. nonhuman primates, rabbits, etc.). It is the responsibility of the investigator to search for ways to reduce, refine or replace animal procedures. Complete a search for each potentially painful and/or distressing procedure proposed in this amendment. A minimum of 2 databases must be searched.**

| Name of the database | Date of search | Period of years covered by the search | Potentially painful or distressing procedures addressed | Key words and/or search strategy used | Indicate which mandate each search addressed | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Replacement of animals | Reduction in numbers of animals used | Refinement to minimize pain or distress | Lack of unnecessary duplication |
| | | | | | ( ) | ( ) | ( ) | ( ) |
| | | | | | ( ) | ( ) | ( ) | ( ) |
| | | | | | ( ) | ( ) | ( ) | ( ) |
| | | | | | ( ) | ( ) | ( ) | ( ) |

**For each procedure listed, please describe your consideration of alternatives and how it was determined that alternatives were not available.**

We will not have enough of pharmaceutical grade Sodium Pentobarbitol used for cardiac perfusion/ euthanasia for the last 2 monkeys scheduled for perfusion on Tuesday 9/4/2018. We are requesting to use Euthasol for one animal at a dose of 100mg/kg intravenous. The Euthasol will be provided by VCS at the time of the procedure.

[redacted]

**From:** [redacted]
**Sent:** Tuesday, September 04, 2018 7:31 AM
**To:** [redacted]
**Cc:** [redacted]
**Subject:** Corrected - Protocol Amendment Approval - Designated Member Review - #JK0025 - Add Euthasol
**Attachments:** VA Protocol Amendment Euthasol 8 28 18.doc

[redacted], **Ph.D.**
Protocol **#JK0025**
**"Interventional Therapies after Spinal Cord Injury"**
Protocol Amendment:
Add change euthanasia method: **Add Euthasol**
Safety Approval Date: **N/A**
IACUC Approval Date: **09/03/2018**

Good Morning Dr. [redacted]

The Subcommittee for Animal Studies reviewed the above noted animal studies protocol amendment via the IACUC approved Designated Member Review process.

This is to serve as notice that the amendment has been approved.

Once the documentation is complete, I will send a copy of the approval in the interoffice mail. If you have any questions or require additional information, please don't hesitate to contact me.

[redacted]

[redacted]
VA Coordinator for Animal Studies and Research Safety
[redacted]
Fax # [redacted]