

**MAPPING TABLE
 (VERSION 1, 5/8/2013)**

When the IACUC of a non-VA affiliate institution serves as the IACUC of Record for a VA facility, the protocol form used by the affiliate institution may be used for work that is to be supported by VA funding, provided that all of the information required on the ACORP (Animal Component of Research Protocol, Version 4) is submitted for Just-in-Time processing.

To document the mapping between the affiliate form and the ACORP, please complete the table below to show the location in the affiliate form for the information required in each of the items in the ACORP. Enter the name of the affiliate institution, the name and number of the VA station served, and the name of the animal protocol form in the header of the table, following the “►” symbols. Please refer to the ACORP (Main Body and Appendices 1-9) and the instructions for completing the ACORP (available at www.research.va.gov/programs/animal_research) for details of the specific information required.

The Mapping Table will be reviewed by the Office of the CVMO, and any items that are required on the ACORP but are not covered in the affiliate’s form will be evaluated to determine whether some sort of VA appendix to the affiliate’s form may be required for JIT submission. If so, the appendix will be entered into the Mapping Table for those items on the ACORP. When the Mapping Table and any required appendix are approved by the Office of the CVMO, the “Date of CVMO Office approval” will be entered below the table.

A scanned copy of the approved Mapping Table, showing all dated signatures, must be submitted along with the protocol approved by the affiliate IACUC serving as the VA IACUC of Record, when documentation for Animal Subjects Research is required for JIT processing. Any information required by the ACORP that does not appear in the affiliate form must be provided as an appendix/attachment, reviewed and approved by the IACUC, along with the affiliate form.

Animal Component of Research Protocol (ACORP) Version 4	Name of Affiliate Institution ► University [REDACTED] Name and Number of the VA Station for which the Affiliate’s IACUC Serves as the IACUC of Record ► Name of Animal Protocol Form (including the date or version number) ► PROTOCOL FOR ANIMAL USE & CARE PI: [REDACTED]
Main Body	
A.1 Full Name of PI(s)	VASDHS Approval Letter, Page 1
A.2 VA Station Name and Number	VASDHS Approval Letter, Page 1
A.3 Protocol Title	VASDHS Approval Letter, Page 1
A.4 Animal Species covered	Section 4 VASDHS page 5
A.5 Funding Source(s)	Section 10, VASDHS page 6
A.6.a Project Title and date of R&D Committee Approval	VASDHS Approval letters pages 1-4 [REDACTED] Approval Letters pages 7-8

A.7 Type of animal Use	Section 4, VASDHS page 6-7
B. Description of Relevance and Harm/Benefit Analysis	Section 5, VASDHS Pages 9
C.1 Lay Summary	Section 12, VASDHS Pages 10-11
C.2 Complete description of the proposed use of animals (including experiment plan, number justification, and procedural descriptions)	Section 14, VASDHS Pages 12-15 , Modifications pages 25-45
D. Species	Section 4 VASDHS page 5
E. Personnel qualifications and training	Section 18, VASDHS Page 20-23
F. Training to be provided	N/A
G. Occupational Health and Safety	N/A
H. Animals to be Used	Sections 4.3, VASDHS Page 5
I. Numbers of animals requested	Section 13f, VASDHS Page 12
J. Management of USDA Category D procedures	Section 7, VASDHS Page 9-10
K. Justification of Category E procedures	N/A
L. Veterinary Support	Section 11, VASDHS Page 10
M.1 Caging needs	Section 6, VASDHS Page 9
M.2 Enrichment	Appendix C , VASDHS Page 23
M.3 Customized routine husbandry	Section 7, VASDHS Page 9
N. Housing Sites	Section 6, VASDHS Page 9
O. Antibody Production	N/A
P. Biosafety	Section 8, VASDHS Page 10
Q. Locations of procedures	Section 6, VASDHS Page 9
R. Body Fluid, Tissue, and Device Collection	Section 14.7, VASDHS Page 15
S. Surgery	Section 9, VASDHS Page 10
T. Endpoint Criteria	Section 15, VASDHS Page 17-20
U. Termination or removal from the protocol (including euthanasia methods and other disposition)	Section 14.8, 16, VASDHS Pages 15 & 20
V. Special Procedures	Section 9, VASDHS Page 10
W. Consideration of Alternatives and Prevention of Unnecessary Duplication	Section 13, VASDHS Pages 11-12
X.1 Controlled drugs	Section 14b, VASDHS Page 15-16
X.2 Human patient care equipment or procedural areas	N/A
X.3 Explosive agents	N/A
Y. Standard Operating Procedures	N/A
Z. Certifications	VASDHS Pages 46-49
Appendix 2 Antibody Production	
1. Immunization	
2. Survival Blood Collection	
3. Terminal Blood Collection	
4. Harvesting Feeder Cells	
5. Expansion of Hybridoma Cell Line(s) <i>in vivo</i>	
Appendix 3 Biosafety	
1. Summary of All Materials Administered	Section 14b, VASDHS Page 15-16

	Section 9, VASDHS page 10
2. Summary of How Materials will be Administered	Section 14b, VASDHS Page 15-16
3. Anesthesia, Sedation, or Tranquilization	Section 14b, VASDHS Page 15-16
4. Toxic Agents	N/A
5. Infectious Agents	N/A
6. Biological Agents	N/A
7. Radioactive Agents	N/A
8. Agents Containing Recombinant Nucleic Acid	N/A
9. Potential for Pain or Distress	Section 15, VASDHS pages 17-20
10. Protection of Animal Facility Staff	N/A
11. Signatures	VASDHS Pages 46-49
Appendix 4 Antemortem Specimen Collection	
1. Summary of Specimens	Section 14a. 7; VASDHS Page 15 SOP FF-10 (p. 2)
2. Use of Anesthetics, Tranquilizers, or Analgesics	Section 7b, VASDHS Page 16
3. Volume Replacement for Fluid Collections	N/A
4. Monitoring the animals	Section 15, VASDHS Page 17-20
Appendix 5 Surgery	
1. Surgery Classification (including justification of multiple survival surgeries)	Section 9, VASDHS Page 10
2. Description of Surgeries	Section 14a.4, VASDHS Page 14
3. Personnel	Section 14e, VASDHS Page 16
4. Location of surgery	Section 14e, VASDHS Page 17
5.a Pre-operative procedures	SOP II-1 (p.18), II-02 (p.12), FF-1 (p.4)
5.b Pre-operative medications	Section 7b, VASDHS Page 16
5.c Pre-operative preparation of the surgical site	SOP II-02 (p.12)
6.a Intra-operative medications	Section 7b, VASDHS Page 16
6.b Intra-operative physical support	SOP II-02 (p.12)
6.c Intra-operative monitoring	SOP II-02 (p.12)
7.a Measures for maintaining sterility for survival surgeries	SOP II-1 (p.18)
7.b Post-operative support	Section 14d, VASDHS Page 16-17
7.c Post-operative analgesia	Sections 14b, VASDHS Pages 17
7.d Other Post-operative medications	Section 14e, VASDHS Pages 17
7.e Post-operative monitoring	Section 14e, VASDHS Page 17
7.f Post-operative consequences and complications	Section 15a, VASDHS Page 17-20
7.g Post-surgical medical records	Section, VASDHS Page
8. Signature	VASDHS Pages 46-49
Appendix 6 Special Husbandry and Procedures	
1. Table of procedures	Sections 7, 9, 14.a.2, VASDHS Page 9-10, 13-

	15
1.a Complete description of each procedure	Section 7, VASDHS Page 7-8 Section 14, VSDHS pp 13-15
1.b Why each procedure is necessary	
2. Personnel	
3. Table of Potential Pain or Distress	
3.a Alleviation of potential pain or distress	
3.b Justification for not alleviating or preventing potential pain or distress	
4. Monitoring	Section 15, VASDHS Page 17-20
Appendix 7 Use of Patient Care Equipment and/or Areas for Animal Studies	
2.a Identify the equipment to be used	
2.b Procedure(s) to be performed with the equipment	
2.c Addressing contamination of the equipment	
3.a Location(s) of human patient care areas to be used	
3.b Animal species to be used in these areas	
3.c Number of animals to be used in these areas	
3.d Date(s) of use	
3.e Time(s) of day of use	
3.f Procedure(s) to be performed on the animals in these areas	
3.g Protection and cleaning of the areas	
3.h Benefits to the patients	
3.i Necessity for the use of these areas	
3.j Animal transport	
3.k Preventing humans from being affected by the presence of the animals	
4. Signatures	
Appendix 8 Use of Explosive Agent(s)	
2.a Identify the explosive agents	
2.b Locations where the explosive agents will be used	
2.c Procedure(s) to be performed	
2.d Precautions for preventing explosions	
2.e Period of use	
2.f Animals that will be administered the explosive agents	
3. Personnel	
4. Signatures	
Appendix 9 Departures	
Description of each IACUC-approved "departure" that is part of this protocol	Section 9, VASDHS Page 10

Signatures

The signatures of the IACUC Chair and the Attending Veterinarian representing the VA, below, certify that the IACUC of the affiliate institution identified above, serving as the IACUC of Record for the VA station identified above, has voted that:

- (1) the affiliate's animal use form provides the information required by the ACORP as shown in the Mapping Table above, and
- (2) the IACUC will review (and approve if compliant with regulatory requirements) any VA appendix that is required by the Office of the CVMO and is therefore identified in the Mapping Table to cover items that are required in the ACORP but are not included in the affiliate's form.

Name of Attending Veterinarian for the VA	Signature	Date
[REDACTED], DVM, DACLAM	[REDACTED]	7/30/2015
Name of IACUC Vice-Chair	Signature	Date
[REDACTED], PhD	[REDACTED]	7/30/2015

The signature of the CVMO below certifies that the affiliate's animal use form and any VA appendix identified in the Mapping Table above are acceptable for animal use protocols to be submitted for JIT processing required for release of VA funding support.

Name of the CVMO	Signature	Date

Institutional Animal Care and Use Committee

Date: 07/30/2015
From: Institutional Animal Care and Use Committee
To: [REDACTED], MD, PhD
Subject APPROVED: Renewal of [REDACTED] Animal Use Protocol # [REDACTED]
Title: Contusion Injury as a Model for Spinal Cord Injury ([REDACTED])
Species Rhesus Macaque
VA R&D Project #: [REDACTED] ([REDACTED])
CC: VA Research & Development Committee
IACUC Initial Approval Date: 08/16/2013
Annual IACUC Approval Date: 08/16/2015
3-year expiration Date: 08/15/2016

The VASDHS Institutional Animal Care and Use Committee (IACUC) has reviewed and **approved** your renewal to [REDACTED] Animal Use Protocol # [REDACTED] used in VA-supported studies. Based on the date of original approval (08/16/2013), your protocol is renewed until 08/15/2016, when the next VA continuing review is required. This is your official approval letter from the VASDHS IACUC; please maintain a copy with your protocol.

It is your responsibility as Principal Investigator to ensure that all members of your laboratory staff have a copy of the protocol and that they understand their individual responsibilities. Your signature on the protocol renewal application indicates that you understand and will comply with all VASDHS policies and procedures related to animal use.

Thank you for your cooperation in complying with federal regulations pertaining to the care and use of laboratory animals.

[REDACTED]

[REDACTED], PhD
Chair, Institutional Animal Care and Use Committee

Institutional Animal Care and Use Committee

Date: 08/12/2014
From: Institutional Animal Care and Use Committee
To: [REDACTED], MD, PhD
Subject: APPROVED: Renewal of [REDACTED] Animal Use Protocol # [REDACTED]
Title: Contusion Injury as a Model for Spinal Cord Injury ([REDACTED] [REDACTED])
Species: Rhesus Macaque
VA R&D Project #: [REDACTED] ([REDACTED])
CC: VA Research & Development Committee
IACUC Initial Approval Date: 08/16/2013
Annual IACUC Approval Date: 08/16/2014
3-year expiration Date: 08/15/2016

The VASDHS Institutional Animal Care and Use Committee (IACUC) has reviewed and **approved** your renewal to [REDACTED] Animal Use Protocol # [REDACTED] used in VA-supported studies. Based on the date of original approval (08/16/2013), your protocol is renewed until 08/15/2015, when the next VA continuing review is required. This is your official approval letter from the VASDHS IACUC; please maintain a copy with your protocol.

It is your responsibility as Principal Investigator to ensure that all members of your laboratory staff have a copy of the protocol and that they understand their individual responsibilities. Your signature on the protocol renewal application indicates that you understand and will comply with all VASDHS policies and procedures related to animal use.

Thank you for your cooperation in complying with federal regulations pertaining to the care and use of laboratory animals.

[REDACTED]

[REDACTED], PhD
Chair, Institutional Animal Care and Use Committee

APPROVAL LETTER

Date: March 27, 2014
From: Institutional Animal Care and Use Committee
To: [REDACTED], MD, PhD
Subject **APPROVED:** [REDACTED] Animal Use Protocol # [REDACTED]
Title: **Contusion Injury as a Model for Spinal Cord Injury** ([REDACTED] [REDACTED])
Species Rhesus Macaque
Number of Animals 12
VA R&D Project #: [REDACTED]
CC: VA Research & Development Committee
IACUC Approval Date: 08/16/2013
Annual Expiration Date: 08/15/2014
3-year expiration Date: 08/15/2016

1. The VASDHS Institutional Animal Care and Use Committee (IACUC) has conducted a review and has **approved** the use of your [REDACTED] Animal Use Protocol # [REDACTED] for VA-supported studies. This is your official approval letter from the VSASDHS IACUC; please maintain a copy with your protocol. You may use this approval letter for funding proposal documentation. This is your official approval letter from the IACUC; please maintain a copy with your protocol. You may use this approval letter for funding proposal documentation.
This protocol is a three-year rewrite and replacement of VA ACORP , which has been made inactive by this approval.
2. Please note that R&D review and approval of the above-referenced projects must be granted by the R&D prior to animal acquisition or commencement of animal use activities described in this protocol. Please contact [REDACTED] , Research Projects Section Director, for clarification of R&D status.
3. It is your responsibility as Principal Investigator to ensure that all members of your laboratory staff have a copy of the protocol and that they understand their individual responsibilities. Your electronic signature on the protocol application indicates that you understand and will comply with all VASDHS policies and procedures related to animal use and the Investigator's Assurance (attached).
4. Thank you for your cooperation in complying with federal regulations pertaining to the care and use of laboratory animals.

[REDACTED]
[REDACTED], PhD
Chair Institutional Animal Care and Use Committee



Investigator's Assurance For the Humane Care and Use of Animals Used in Research, Teaching and Training

1. I agree to abide by PHS Policy, USDA Regulations, VHA policies for the care and use of animals, the provisions of the ILAR Guide to the Care and Use of Laboratory Animals, and all other federal, state, and local laws and regulations governing the use of animals in research.
2. I understand that emergency veterinary care will be administered to animals showing evidence of pain or illness, in addition to routine veterinary care as prescribed for individual species. I understand that it is my responsibility to provide current and updated emergency contact information for personnel who must be contacted in an animal emergency. I understand that any unanticipated pain or distress must be reported to the veterinarian or his/her designee.
3. I assure that I have consulted a veterinarian in the preparation of this proposal, if it includes procedures that could cause pain and distress to a vertebrate animal.
4. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified biomedical scientist listed on this protocol.
5. I certify that all personnel having direct animal contact, including myself, have been trained in humane and scientifically acceptable procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project.
6. I certify that all personnel in this project will attend Orientation to Animal Research and all mandatory classes as determined by each individual's Personnel Qualifications Form.
7. I understand that the use of hazardous agents in animals may only be initiated after approval from SRS and I am responsible for complying with all safety related information stated under Appendix 3 of the protocol and associated Safety Survey.
8. I certify that all personnel working on this protocol will be given the opportunity to participate in the Medical Surveillance Monitoring Program. All personnel on this protocol will be made aware of the hazards involving the use of live animals and tissues.
9. I understand that I must submit a modification for any proposed changes to this protocol and wait for IACUC approval before beginning the work.
10. I understand that should I use the project described in this application as a basis for a proposal for funding (either extramural or intramural), it is my responsibility to ensure that the description of animal use in such funding proposals are identical in principle to that contained in this application.
11. I understand it is the responsibility of the Principal Investigator to ensure the safe and ethical conduct of all research conducted under this protocol, and to assure that all research is carried out following federal, state, local, and VA policies governing animal research.

Offsite Animal Use Protocol

Project Demographics (Includes total number of animals)

4.1 Name of Other Institution:

University of [REDACTED]

4.2 Protocol Information

Protocol PI / Number:

[REDACTED]

Date of Approval (if known)

08/16/2013

4.3 Animal Species covered by the ACORP (only one; include total approved number of animals):

Limited to one species per ACORP. There is a field to enter the total number of animal approved at the other institution

View Details	Species Name	Scientific Name	Common Name
<input type="checkbox"/>	Rhesus Macaque	Macaca mulatta	Rhesus
Species Name:		Rhesus Macaque	
Scientific Name:		Macaca mulatta	
Common Name:		Rhesus	
Sex:		Male/Female	
Category Type:		D	
Total Number Requested:		12	
Total Number Approved:		12	

If "Other" animal species is selected above, please describe this species here:

[REDACTED]

4.4 Indicate the pain category covered by this protocol (check all that apply):

- B
- C
- D
- E

4.5 Protocol Type:

- Survival
- Terminal
- Both Survival and Terminal

4.6 Is this a three-year rewrite of this protocol?

- Yes
- No

5.0 Protocol Information

5.1 Please summarize any VA-specific information regarding this protocol:

Study is performed offsite at [REDACTED].

5.2 Protocol Locations

Vivarium Building

[REDACTED]

Animal Use (Lab) Locations (building and room number(s)):

Veterinary Medicine [REDACTED]

5.3 Is there an existing VA R&D project supporting this protocol?

- Yes (the next screen will allow you link to existing projects)
- No. A new project application is needed

6.0 Protocol to Project Linkage

6.1 Identify the R&D Project(s) that correspond to this protocol:

Project Status	Proposal Number	Project Title	Principal Investigator
Approved	[REDACTED]	ProjectID [REDACTED] Spinal Cord Injury Translational Collaborative Consortia: Combinatorial Primate Therapy	[REDACTED], MD, PhD

[REDACTED]

Institutional Animal Care and Use Committee

[REDACTED] vis

Fax: [REDACTED]

August 8, 2014

To Whom it May Concern:

The following application was reviewed and approved by the [REDACTED] vis IACUC on August 07, 2014. Active protocols are reviewed annually.

Title: **Contusion Injury as a Model for Spinal Cord Injury**

Principal Investigator: [REDACTED]

Protocol #: [REDACTED]

Institution: **University** [REDACTED]

This institution is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW). The Assurance Number is [REDACTED].

The IACUC is constituted in accordance with U.S. Public Health Service (PHS) Animal Welfare Policy and includes a member of the public and a non-scientist.

[REDACTED]

[REDACTED]
IACUC Administrator

IACUC Office

University [REDACTED]

[REDACTED]

Institutional Animal Care and Use Committee

[REDACTED]

[REDACTED]

Fax: [REDACTED]

February 25, 2014

To Whom it May Concern:

The following application was reviewed and approved by the [REDACTED] IACUC on August 16, 2013. Active protocols are reviewed annually.

Title: **Contusion Injury as a Model for Spinal Cord Injury**

Principal Investigator: [REDACTED]

Protocol #: [REDACTED]

Institution: **University** [REDACTED]

This institution is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW). The Assurance Number is [REDACTED].

The IACUC is constituted in accordance with U.S. Public Health Service (PHS) Animal Welfare Policy and includes a member of the public and a non-scientist.

[REDACTED]

[REDACTED]

IACUC Administrator

IACUC Office

University [REDACTED]



PROTOCOL FOR ANIMAL USE & CARE

Protocol #: [Redacted]
Date Expires: 16-Aug-2015

1. Contacts:

Primary Investigator		Alternate Contact	
Name:	[Redacted]	Name:	[Redacted] *
E-mail:	[Redacted]	E-mail:	[Redacted]
Department:	[Redacted]	Department:	[Redacted]
Telephone:	[Redacted]	Telephone:	[Redacted]
After Hours:	[Redacted]	After Hours:	[Redacted]

*Primary contact for sick animals

2. Title:

Contusion Injury as a Model for Spinal Cord Injury

3. Protocol Type:

Research

4. Species:

Common Names	Total Number for Study	Name of Source of the Animals
primate - rhesus	12	[Redacted]

USDA: Yes
Detrimental Species: No

5. Brief Summary of Procedures:

Animals will be trained on a variety of behavioral tasks, including functional hand tasks in the restraint chair, locomotor training in a treadmill, and an open-field task. Following a period of pre-training, animals will undergo a contusion spinal cord injury with or without application of a neuroprotective treatment, and then undergo post-surgical behavioral testing to assess if the treatment improves functional recovery.

6. Animal Location(s):

Study Area/Laboratory:

Location/Building - Room
[Redacted]
Veterinary Medicine [Redacted]
Veterinary Medicine [Redacted]

Overnight Housing (vivarium):

Vivarium(s)
[Redacted]

Animals will be maintained by:
Vivarium

7. Special Husbandry Requirements:

Monkeys will have injuries of the spinal cord and this will affect daily activity immediately post surgery. Based on previous experiments, we have observed monkeys recovering the ability to locomote over time, beginning with rudimentary movement during the first week post-contusion, followed by gradual improvement until their endpoint. During the period of recovery (the length of which varies by animal), the following special husbandry requirements may apply:



1. The animals will be monitored closely for locomotor problems.
2. They will be housed in cages with no perch bars to minimize the potential for injury while they recover ambulatory ability.
3. While the monkey transitions from a period of being primarily recumbent to primarily upright, they will be housed on some combination of rubber mats, towels, and fleece for increasingly shorter durations of each day. This will minimize the development of pressure sores.

8. Hazardous Materials:

No

9. Special Procedures and/or Activities:

Anesthetics/Tranquilizers/Sedatives
 Survival Surgical Procedures
 Multiple Major Survival Surgical Procedures
 Prolonged Restraint
 Use of Non-Pharmaceutical Grade Drugs

Justification for Multiple Major Survival Surgical Procedures:

Some animals on this protocol will undergo multiple major survival surgeries. The sequence will typically involve a motor telemetry implant surgery, a contusion surgery, and a tracer surgery. Each of these surgeries are critical to informing us about recovery from spinal cord injury. The implant surgery will allow us to measure electromyography (EMG) of recovering muscle groups. Last, the tracer surgery enables examination of specific axonal projections and groups of motor neurons, a critical component in evaluating the efficacy of our treatments.

Justification for Prolonged Restraint:

A portion of our behavioral testing occurs while the animal is in a restraint chair (training procedure described in SOP FF-5). We use the restraint chair to assess hand and/or digit functional recovery following spinal cord injury to ensure the animal performs the task with the affected hand. Without the use of chair restraint, the animal would perform these tasks with the non-affected hand, offering minimal rehabilitation and opportunity for assessment. The period of chair restraint typically lasts from 30-60 minutes.

Justification for use of Non-Pharmaceutical Grade Drugs :

Pharmacy grade BrdU is not available. Using sterile gloves and a sterile needle, we will resuspend the compound within a sterile vial using sterile saline. This procedure follows established [redacted] protocols for sterile technique. We will sterile filtering before infusing into the animal. A culture of the BrDU is also conducted. Any bacteria that are identified will be subjected to an antibiotic sensitivity test.

10. Funding Source(s):

Veterans Administration
 Craig H. Nielsen Foundation

11. Veterinary Care:

[redacted]

12. Objectives and Significance:

Objectives:

Our overall aim is to provide a translational platform for the evaluation of therapies for cervical spinal cord injury (SCI) that moves promising preclinical findings in rodents to the non-human primate (NHP), and ultimately to human trials. We have extensive data on treatments and outcomes in a unilateral contusion cervical SCI in rats, and propose to establish this contusion model in NHPs. The contusion injury is the most common injury in humans and has specific unique characteristics that make it different from transection injuries. The injury is anatomically different from transection injuries; contusion affect gray matter where the segmental circuitry is organized, much more significantly and for greater distances than transection. The white matter is differentially affected as opposed to the transection model; mild contusion specifically affect the propriospinal systems and collaterals of the more peripherally placed descending and ascending tracts, whereas the main tracts are less affected. The corticospinal system, which we have shown to be significantly more plastic in the primate than in the rodent, is partially damaged, the degree depending on the severity of the lesion. Understanding the effects of this type of injury will allow us to better predicts injury outcome and better detect treatment effects.

The first goal of the project is to establish a reproducible contusion injury in this species so that we can understand this unique lesion in the primate, and then secondly, to use this more clinically relevant injury to evaluate promising treatments before going to clinical trial in humans. The closer similarities to humans of the primate vs rodent nervous system include

the anatomical organization, the larger size of the spinal cord and CSF spaces, the immunological reaction to injury, and the similarity in forelimb function, making this species an important intermediate translational model for testing treatment applications for spinal cord injury.

Significance:

Spinal cord injury is a devastating neurological event resulting in loss of a variety of essential functions depending on the level of injury, e.g. loss of motor control, loss of sensation, autonomic dysfunction, loss of bladder, bowel, sexual and respiratory function. This study will continue the establishment of a contusion spinal cord injury model in the NHP by evaluating a novel injury protocol, and then a treatment to blunt the secondary injury cascade. The goal is to provide a translational pathway to evaluate treatments that have shown substantial efficacy in rodent models. The contusion injury at the cervical level is the most common injury in humans, and having an injury model in the NHP with outcome measures that are consistent across species, will help provide a more direct pathway for treatments into the clinic. The knowledge gained from these experiments will further knowledge of the effects of injury to the nervous system and potentially improve outcome after spinal cord injury in both humans and animals.

13. The 3 R's - Refinement, Replacement, and Reduction:

a) Database Search for Alternatives:

Database Name	Years Covered	Keywords/Search Strategy	Date
PubMed/Medline	1960-present	"contusion" AND "monkey"	11-Mar-2013
PubMed/Medline	1960-present	monkey AND "von Frey"	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "spinal cord injury" AND neuroprotection	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "EMG recording"	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "corticospinal tract tracer"	18-Jul-2013
PubMed/Medline	1960-present	monkey AND stereotaxic	18-Jul-2013
PubMed/Medline	1960-present	monkey AND craniotomy	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "telemetry implant"	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "restraint chair"	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "magnetic resonance imaging"	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "CSF tap"	18-Jul-2013
PubMed/Medline	1960-present	monkey AND "spinal cord injury" AND "soluble TNF receptor 1"	18-Jul-2013
Web of Science	1900-present	"contusion" AND "monkey"	11-Mar-2013
Primate Portal	1940-present	contusion	11-Mar-2013

b) Refinement:

To date, our group is the only group developing a behavioral and anatomical model of contusion injury in the non-human primate. We continue to pursue answers to these questions in the primate as there are no suitable alternatives in developing a method applicable for human intervention. All possible effort will be made to minimize pain and distress to the animals. Analgesics which will not compromise the goals of our study will be used when recommended by veterinary staff as well as any other potential strategies that are recommended (such as the use of fleece and/or towels to minimize pressure sores).

c) Has this study been previously conducted?

No

d) Replacement (Species Rationale):

In order for new SCI therapies to be evaluated for translational potential, the NHP spinal cord is key. Rodent studies are useful for screening bioactivity, etc. But the NHP cord, organized anatomically like human cord, is the only species that will enable us to yield clinically translatable data. Furthermore, the functional tests used in this study, such as recovering fine motor movement of the hand, are unique to primate species. These tests will thus provide the necessary information to determine if recovery of function that would be translatable to humans has occurred.

We have previously identified important differences/limitations to conducting experiments in rodents, which do not accurately translate to higher vertebrate species. These include:

- a. Greater intrinsic ability of the cortico-spinal tract to regenerate in monkeys compared to rodents;
- b. Greater digit dexterity in monkeys, which is crucial for the development of behavioral and clinical outcome measures in the clinic;
- c. Greater degree of plasticity in the injured monkeys spinal cord, correlated to injury severity and ability to functionally recover to baseline levels;



- d. Differences in spinal cord size/thickness, amount of cortico-spinal fluid, and organization of locomotor tracts vary between small and large animals species;
- e. Up-scaling effects regarding drug delivery, drug concentration/dose, and pharmacokinetics that are difficult to anticipate/translate from small animal models;
- f. Differences in immune system reaction/response to injury and/or drug administration, which are more clinically comparable when investigated in monkeys;
- g. Toxicity and/or side effects resulting from injury and/or drug administration not detected in rodents that could prove critical in the clinic;
- h. There are physical limitations that are more efficiently explored and with greater human relevance in animals with greater dexterity, such as monkeys.

e) Reduction (Animal Numbers Justification):

Based on previous primate experiments, we anticipate that **12 animals** will be sufficient to conduct these tests.

We currently have extensive data on outcomes after C6-7 hemisections in rhesus with well-developed behavioral, physiological and anatomical measures. This data has been used as background to establish procedures for a novel contusion injury paradigm.

In a previous protocol, we tested a cohort of six animals, who collectively experienced a range of contusion severity. Contusions were produced with an electronically controlled impactor device that had precise readouts of the injury dynamics. These data showed that there is a significant correlation between the peak force at impact with functional recovery at 3 weeks after injury ($p < 0.02$). Animals were maintained for up to approximately 5 months.

The next step is to assess the consistency of the injury protocol with respect to the behavioral and anatomical outcome measures. **Four subjects** will be injured using the same parameters. This will enable us to produce an intermediate injury with partial functional recovery (so that either positive or negative effects of the treatment can be detected). These animals will serve as the control group. In addition, data from two of the animals in our previous cohort of six will also be used for the control group.

Before proceeding with the treatment group, we will use **two subjects** for a dose assessment that will provide feedback on whether the treatment dosing has a biological effect on the target physiological processes. We have used this method in the rat to confirm that the dosing is effective.

Six subjects will serve as the treatment group. We derived this number by extrapolating from the rodent studies, which show that positive treatment effects can be detected with $N=6$ using similar outcome measures. The resulting data will serve two purposes:

1. It will allow us to establish the consistency and reliability of the contusion model for producing cohorts of animals with the same injuries and outcomes;
2. It will provide an evaluation in the NHP of a neuroprotective strategy that has shown efficacy in rodents.

We have tried to optimize the utility of these low n studies by:

1. Running 2 orders of magnitude dose response and timing studies in our rat model prior to scaling up for translation to the primate
2. Matching the injuries and outcomes between rodent and primate as much as possible,
3. Using multivariate composite metrics alongside of univariate analysis to increase power, and
4. Gathering multiple outcome measures including immunological data. This will provide more information on recovery from contusion in the CST-dominant primate that can facilitate enhanced model development even if we do not see a signal/efficacy with our first anti-inflammatory treatment attempt.

f) Study Groups and Numbers Table:

Group	Species	Number of Animals	Procedures/Treatments
1	primate	2	Contusion + Treatment 48-hr survival, no behavioral testing or tracer surgery
2	primate	6	Contusion + Treatment 6 months survival, with behavioral testing and corticospinal tract tracing
3	primate	4	Contusion + Vehicle (control) 6 months survival, with behavioral testing and corticospinal tract tracing

14. Procedure Details:

a) Describe the use of animals in your project.

1. **ANIMAL SELECTION:** Before animals in groups 2 and 3 are purchased for this protocol, they will undergo a physical exam, blood analyses, and/or a radiograph to examine the spinal cord (SOP LL-31). In addition, animals may receive a urinalysis. Under some circumstances, some of these evaluations may have to be repeated; for example, in the case of a

questionable blood test result, we may want some blood analyses repeated. Animals who have a BCS greater than 2.0, have not experienced significant muscle or bone trauma, and do not have ongoing health or behavioral issues will be considered for the project.

Animals in group 1 will be selected from the primate center cull list. Cull animals used for these procedures will be on the cull list for management purposes. In other words, the animals have chronic diarrhea or are lame, and can continue to survive as long as long-term maintenance procedures are put into place. This type of animals will be able to survive for 48 hours following a contusion injury and treatment procedure and will have to be managed for diarrhea or lameness for the short time period post-procedure.

2. BEHAVIORAL TESTING: Animals in groups 2 and 3 will undergo behavioral testing (chair task testing, treadmill locomotion, open-field exposures, and home cage testing); however, not all animals will receive all behavioral testing.

2.A. Chair Training and Testing. Monkeys who will undergo chair task testing will first be trained to enter and exit the restraint chair using a pole-and-collar system (described in SOP FF-5). This training procedure will be conducted by trained [REDACTED] technicians. After monkeys have been trained to enter/exit the chair, we will train them to perform reaching and grasping tasks for food rewards that are designed to aid in recovery of fine motor movement following the injury surgery. Briefly, animals will be trained to reach out and retrieve food items from a platform and from a vertical post. Animals will also be trained to pull a handle attached to springs of varying tensions. This training typically takes 3-18 sessions, depending on the monkey. Following the behavioral training period, animals will be chair tested up to 5 times per week for 30-60 minutes per session both before and after injury.

2.B. Treadmill Training and Locomotion. Monkeys who will undergo treadmill task testing will first be trained to walk on a treadmill by project staff. First, we will train the animals to enter a jump box and exit into a plexiglass contained treadmill. Monkeys will first be acclimated to the treadmill housing while the belt is immobile; however, over a series of sessions, the animals will be trained to walk successively faster up to 4.0 miles per hour for food rewards. This training typically takes 3-24 training sessions, depending on the monkey. Following the behavioral training period, animals will be treadmill testing up to 5 times per week for 15-45 minutes per session both before and after injury by project staff.

2.C. Open-Field Cage Training and Exposure. Monkeys who will undergo open-field cage testing will first be trained by project staff to enter and exit the jump box and the 10'x6'x7' open-field cage. Following the behavioral training period, animals will have the opportunity to engage in tasks designed to aid in recovery of whole body coordinated movements following injury. They will be able to manipulate small objects and climb on large objects, stair-cased perches, and on the cage mesh to obtain food rewards. Animals will be given access to the open-field cage up to 5 times per week for 15-60 minutes per session both before and after injury.

2.D. Home Cage Testing. A variety of in-cage testing devices have been designed to encourage continued use of the impaired hand. These devices are comprised of an array of reach-and-grasping tasks and can be done while the animal is in his home cage. Tasks include grasping a pole and lifting it in order to retrieve a food item out of a well and reaching, grasping, and pulling a variety of different levers in order to receive a treat from a motorized cart.

2.E. Video and EMG Recording. Video and EMG recording of chair tasks, treadmill locomotion, open-field exposures, and home cage testing may be conducted both before and after injury. The battery pack and transmitter (described in 3.B. below) can be turned on using a remote control. Recording sessions may be conducted up to 2x/week for 2 months pre-injury and up to 2x/week following injury. These will be done for the chair and treadmill tasks, where each session lasts up to 2 hours (but typically 45 minutes). In addition, video and EMG recording and behavioral data collection of open-field exposures and home cage testing will be conducted before injury and periodically throughout recovery (typically weekly, but these can take place between monthly and daily throughout recovery). After video and EMG recording, the battery pack and transmitter are turned off. Staff will be present at all times to ensure primate safety.

2.F. Food Rewards. At the request of the IACUC, this section is added to describe the food rewards that are used on this project. We give animals fruits (e.g. apples, grapes, bananas, oranges, plums, strawberries, or other seasonal items), vegetables (e.g. carrots, cucumbers, lettuce, bok choy, tomatoes, or other seasonal items), dried fruit (e.g. raisins, yogurt covered raisins, mango, fig, etc), and nuts (e.g. almonds, shelled peanuts, unshelled peanuts, etc). We also use nutrition bars (e.g. Odwalla bars). We do not give animals any sort of candy.

3. SOMATOSENSORY EVALUATION: Following injury, monkeys may experience a disruption in sensory function resulting in dysesthesia. For some animals in groups 2 and 3, we will quantify alterations in sensibility using the von Frey hair aesthesiometer.

3.A. Description. The von Frey hair method of light touch sensory evaluation uses nylon filaments of varying diameters that require different degrees of pressure before bending. The force required for each filament (or "hair") to bend is constant and enables examination of mechanical sensation and any potential dysesthesia following injury. As an alternative, we may conduct these tests with an electronic von Frey apparatus, which electronically registers the force applied by a particular filament.

3.B. Procedure. While the monkey is awake and in a stationary position - likely in the restraint chair - the smallest diameter filament will be applied to the surface of the monkeys skin. Progressively greater diameter filaments will be used until the monkey produces a response. For example, if the filament is applied to the heel of the foot, the monkey may orient toward the stimulus or pull his foot away, etc. The smallest filament that produces this response in 50-90% of trials will be recorded. Monkeys will undergo this test up to every week pre-injury and post-injury. Alternatively, if using the electronic von Frey device, we can simplify this process by choosing a single filament,

pressing the filament against the animal's skin, and then after withdrawing obtain a measure of our maximum force applied from the digital read-out.

4. **SURGICAL PROCEDURES:** This section describes all survival surgeries that will take place in this protocol. There may be other procedures described in other sections of this summary which occur between these described survival surgeries (e.g. blood draws); however, we opted to keep the descriptions of all surgeries under one heading for the sake of clarity. Surgical preparation for all procedures is described in 4.A. and surgical closing for all procedures is described in 4.F.

4.A. Surgical Preparation. Prior to all surgeries, monkeys will be fasted and anesthetized with ketamine and possibly dexmedetomidine and atipamezole according to CNPRC SOP FF-1. Animals will then be prepped for surgery according to [REDACTED] SOP II-1 & II-12.

4.B. Motor Telemetry Implants. Animals in groups 2 and 3 may undergo motor-telemetry implants to enable recording of target muscle groups. To enable recording of EMG from muscle targets on the impaired side and possibly non-impaired side of the body, a subset of animals will receive wireless motor telemetry implants. For these animals, internal EMG electrodes (Kongsberg Instruments, model T33F-1B OR T33F-4) will be implanted into various muscles of the right and/or left arm to record muscle activation during behavioral tasks. We will use one telemetry implant and may split the leads to implant both the right and left sides of the body. Eitherway, only one battery source and wireless transmitter (which are encased in a silicone sheath with rounded edges measuring 1½" x 2" x 3/8") will be implanted intramuscularly in between the internal and external oblique muscles of one side of the body. The wires will be led from the transmitter to the forelimb subcutaneously and sutured directly to the desired arm/hand muscles.

4.C. Contusion Injury and Treatment Procedure. All monkeys on this protocol will undergo a unilateral contusion spinal cord injury. Monkeys will also undergo intrathecal infusion of soluble tumor necrosis factor (TNF) receptor 1 (Group 1 and 2) or saline (Group 3).

Contusion Procedure. A dorsal midline incision will be made over the cervical vertebra. The spinal cord will be exposed by removing the dorsal lamina in approximately the C5 area. Next, a probe approximately 5mm in diameter will be positioned over one side of the spinal cord and advanced using an electronic device that controls speed and depth of compression, and has force feedback so the features of the injury are immediately available for assessment. We will use an approximately 4mm single compression (compression rate up to 2m/sec) to produce a consistent contusion injury.

Treatment/Control Procedure. In the same surgery as the contusion, an intrathecal cannula will be placed just rostral to the contusion site after the injury has been complete. At approximately 1.5 hours after the contusion, approximately 35 ng/uL of soluble TNF receptor 1 x 1000uL phosphate buffered saline (Group 1, N=2 and Group 2, N=4) or PBS (Group 3, N=4) will be infused into the CSF in the subarachnoid space surrounding the cord over a 5 min period. The TNF is a highly purified recombinant protein that is made in a GMP facility, and it will be reconstituted in sterile saline for injection. The dose was derived from scaling up the rat dose to account for the approximately 100x larger CSF space in the primate. (No adverse effects have been encountered with this agent at the comparable dosing for the rat.) The intrathecal cannula will be withdrawn after an additional 10 minutes, and the hole in the dura will be plugged with a small piece of muscle tissue followed by a drop of tissue adhesive to seal the defect. The wound will then be closed as described in 4.E below.

4.D. Tracer Procedures. Animals in Group 2 and 3 will undergo a corticospinal tract tracer procedure. The tracer procedure will be done after animals have reached a plateau in behavioral recovery, typically up to 10 months following injury. Labeling the corticospinal tract enables detailed histological analysis of the altered pathways of the injured spinal cord.

4.D.i. *Types of Tracers.* Two types of tracers may be used: Anterograde and/or Retrograde tracers. These tracers are inert and non-toxic to cells. They take - *at minimum* - approximately 6 weeks to reach their target destinations in the injured spinal cord. At present there are no tracers available that require less than 6 weeks of transport time.

4.D.ii *Corticospinal Tract Tracing Procedure.* A midline incision will be made, then a craniotomy approximately 2x4cm will be made using a sterilized surgical drill. Approximately 125mcl of tracer will be injected per cortical hemisphere across a maximum of 75 sites (typically 50-60 sites) per hemisphere. Tracing may be done in both hemispheres or just one hemisphere.

4.E. Closing Procedures. Due to the length of these surgeries, it is not likely that the dexmedetomidine will need to be reversed; however, if recommended, animals will receive atipamezole (up to 50mcg/kg) IM as a reversal. Monkeys will receive post-operative analgesics and antibiotics (described below). **Following the injury and until the animal's endpoint,** no anti-inflammatory analgesics (including ketoprofen and meloxicam) will be administered. Administration of anti-inflammatory analgesics influences recovery from spinal cord injury and may confound the influence of our experimental therapies.

4.E.i. *Cervical Area.* The muscle and skin will be closed in 2 layers using 2-0 and 3-0 vicryl (unless

otherwise recommended by the veterinary staff).

4.E.ii. *Cranial Area* . The skull cap that was removed during the craniotomy will be replaced over a layer of gel foam (unless otherwise indicated by veterinary staff). The skull cap will be adhered to the rest of the skull using dental acrylic and/or cyanoacrylate. Muscle and skin will be closed in 2 layers using 2-0 and 3-0 vicryl (unless otherwise recommended by the veterinary staff).

5. **MAGNETIC RESONANCE IMAGING:** Monkeys in groups 2 and 3 may undergo up to 2 MRIs scanning session pre-surgery (to establish a baseline) and MRIs post-surgery as needed to assess post-surgical recovery (to assess acute and chronic changes, typically 1 per month). These will occur at either the [redacted] or at MRI Facility at the [redacted]. Only [redacted] trained personnel will transport the animals. On the morning of the imaging, the animals will be fasted, boxed and loaded into transport cages (identical to the squeeze-back cages in which they are housed) located in an air conditioned, heated, paneled [redacted] van or animal transport truck and will be driven to the imaging center. Hanging water bottles are available for the animals. Once at the imaging facility, the animals will be anesthetized with ketamine IM and possibly dexmedetomidine and atipamezole (according to [redacted] SOP FF-1). Animals will then be prepped for the MRI and undergo the MRI scanning (according to [redacted] SOP II-31 and II-02). Each scanning session will last approximately 1-3 hours, depending on the MRI sequences acquired and quality of the images.

6. **EMG RECORDING OF EXTERNAL ANAL SPHINCTER (EAS) MUSCLE:** During up to 3 pre-injury and up to 10 post-injury recording session, we may record EMG from the EAS muscle of monkeys in Groups 2 and 3. This procedure will enable us to quantify the effect of our injury procedures on autonomic function.

6.A. **Anesthetization:** We will use ketamine and possibly dexmedetomidine and atipamezole to immobilize the animals according to [redacted] SOP FF-1. Once sedated, either ketamine or propofol will be administered via constant rate infusion (CRI) throughout the procedure (approximately one hour).

6.B. **Spontaneous EMG:** Approximately 6 EMG electrodes will be inserted bilaterally (3 on each side) into the EAS muscle to record muscle activity. Spontaneous EMG activity of the EAS muscle will be recorded.

6.C. **Reflex EMG:** We may also record reflex EMG activity of the EAS muscle. If reflex EMG is recorded, we will use rectal probes to produce brief and gentle stretching designed to mimic the size of a fecal bolus. The probes will be up to 25mm in diameter and will be inserted up to 2cm for up to 10 seconds and then withdrawn initiating rectoanal reflex activation of the external anal sphincter muscle. Insertion of the probe may be repeated up to 8 times.

7. **BODY FLUID SAMPLE COLLECTIONS:** Cerebrospinal fluid (CSF) and blood will be collected by trained [redacted] staff. CSF will be collected from all animals on the day of the contusion surgery and at the time of perfusion. Blood samples will be collected from all monkeys on the day of contusion injury and at the time of perfusion. Blood samples will also be collected 48 hours after contusion injury in groups 2 and 3. Samples will be collected according to the following guidelines:

7.A. **Sedation.** Animals may be sedated with ketamine and possibly dexmedetomidine and atipamezole according to [redacted] SOP FF-1. For blood, samples may be collected while the animal is awake (i.e. via arm pull collection).

7.B. **CSF Samples.** Up to 2mL will be collected according to [redacted] SOP II-40. CSF will be collected on the day of the contusion surgery as a baseline measurement and at the time of euthanasia for all to measure chronic inflammatory status as reflected by the presence of proinflammatory cytokine expression.

8.C. **Blood Samples.** Up to 10mL of blood will be collected from any peripheral vessel according to [redacted] SOP GG-5. Animals may be awake for this procedure (i.e. via arm pull collection) or may be sedated as described above. Peripheral blood will be analyzed for TNF-alpha levels and peripheral monocytes will be FACS sorted and assayed for TNF-alpha production.

8. **TERMINAL PROCEDURES UNDER ANESTHESIA:** Following the injury, monkeys in groups 2 and 3 may remain on study for up to 12 months and then be euthanized. Monkeys showing unanticipated severe sequelae will be euthanized prior to the planned endpoint. Brains and spinal cords will be harvested from all subjects after intracardiac perfusion.

8.A. **Pre-Perfusion X-Ray.** Prior to perfusion, animals may undergo an anatomical x-ray. The x-ray will allow visualization of changes that have occurred due to rehabilitation and treatment.

8.B. **Pre-Perfusion Samples.** At the time of perfusion, CSF and blood will be collected. The CSF and blood will be collected according to [redacted] SOPs II-40 & GG-5, respectively.

8.C. **Euthanasia.** Animals will be euthanized according to [redacted] SOP LL-14 and as described in section 16 below.

b) All Drugs and Compounds to be Administered to the Animals (except for euthanasia) - anesthetics, analgesics, neuromuscular blocking agents, antibiotics and/or experimental compounds:

Will drugs be used in this study? Yes

Species	Drug	Dose	Route	When and how often will it be given?
primate	botulinum toxin in local anesthetic (ie lidocaine)	~60 units	Subcutaneous (SC)	amendments approved 5/29/14 AND 2/05/15
primate	gadolinium	0.2ml/kg	Intravenous (IV)	amendment approved 4/18/14

primate	Reglan (metoclopramide)	0.2-0.5 mg/kg	Intramuscular (IM)	amendment approved 4/18/14
primate	cerenia (maropitant)	1 mg/kg	Subcutaneous (SC)	amendment approved 4/18/14
primate	Ondansetron	2 mg/kg	Intramuscular (IM)	amendment approved 4/18/14
primate	Ondansetron	2 mg/kg	Intravenous (IV)	amendment approved 4/18/14
primate	Midazolam	0.1mg/kg	Intramuscular (IM)	In response to self-injurious behavior, as needed
primate	Fentanyl	7-10 mcg/kg/hour	Intravenous (IV)	For the first 24 hours following contusion, if the animal will tolerate a catheter
primate	Neurontin	up to 60mg/kg	Oral	In response to self-injurious behavior, as needed
primate	Ketoprofen	up to 2mg/kg	Intramuscular (IM)	As recommended by vet staff for pain management <u>up to 1 week before injury surgery</u>
primate	Acetaminophen	up to 16mg/kg	Oral	TID As recommended by vet staff for pain management
primate	Buprenex	0.05mg/kg	Intramuscular (IM)	As recommended by vet staff for pain management TID as needed.
primate	Propofol	up to 20mg/kg/hour	Intravenous (IV)	During EAS recordings or as recommended by a veterinarian via CRI
primate	Diazepam	0.05-1.0 mg/kg	Intravenous (IV)	As needed by request of the IACUC
primate	Oxymorphone	0.15mg/kg	Intramuscular (IM)	TID for 3 days following surgery and additionally when recommended by vet staff
primate	Dexmedetomidine	up to 0.015mg/kg	Intramuscular (IM)	Prior to immobilization, if recommended
primate	Atipamezole	see volume note	Intramuscular (IM)	Equal volume as dexmedetomidine. Given following the use of dexmedetomidine, when necessary
primate	Ketamine	up to 30mg/kg	Intramuscular (IM)	Prior to immobilization. May also be given IV or CRI
primate	soluble TNFR1	35ug in 1mL PBS	Intrathecal	1x Treatment to reduce the initial inflammatory response in groups 2 and 3
primate	Haloperidol	up to 0.05mg/kg	Intramuscular (IM)	In response to self-injurious behavior, as needed
primate	Diphenhydramine	2-4 mg/kg	Intramuscular (IM)	amendment approved 4/18/14
primate	Diphenhydramine	2-4 mg/kg	Intravenous (IV)	amendment approved 4/18/14
primate	Atropine	0.04mg/kg	Intramuscular (IM)	SID for surgery preparation or as otherwise needed
primate	Isoflurane	To effect	Inhalation	Continuous during procedures which require inhalation anesthesia
primate	Cephazolin	25mg/kg	Intramuscular (IM)	Every 2 hrs during surgery and BID or TID for up to 7 days following surgery or any other procedure requiring antibiotics
primate	Cephalexin	30 mg/kg	Oral	BID, as an alternative to Cephazolin, when necessary

c) Anesthesia Monitoring:

During surgery, monkeys will be monitored as per standard [REDACTED] neurosurgery procedures. Any or all of the following may be monitored continually: heart rate, respiratory rate, oxygen saturation, blood pressure, core body temperature, CO2 level, MAC iso level.

d) Post-Anesthetic Monitoring:

Observations will be done according to standard [REDACTED] post-operative procedures as well as at veterinary discretion. Standard procedure includes assessment every 2-4 hours from 6am to 10pm for the first 7 days post-surgery. During each assessment, the following items are evaluated:

- Appetite (whether the animal has eaten food that was offered or that was present in the cage)
- Hydration (level of water bottle, whether the animal drinks when offered)



- Presence of urine or feces
- Position of the animal (L/R lateral or sternal)
- Mobility of the animal
- Condition of any bandage (if applicable)
- Appearance of the incision site (clean/dry/intact)
- Pain Score

e) Surgery:

i) Surgery Location(s) and Surgeon(s):

Building	Room	Surgeon(s)
		(contusion, treatment, tracer)
		(contusion, treatment, tracer)
		(contusion, tracer)
		(tracer)
		(contusion, treatment, tracer)
		(telemetry implant)
		(telemetry implant)

ii) Post-Surgical Monitoring:

a) Please identify the parameters monitored, and interval(s) and for what duration of monitoring.

Following surgery, observations will be conducted according to standard [redacted] post-operative procedures as well as at veterinary discretion. Standard procedure includes assessment every 2-4 hours from 6am to 10pm for the first 7 days post-surgery. During each assessment, the following items are evaluated:

- Appetite (whether the animal has eaten food that was offered or that was present in the cage)
- Hydration (level of water bottle, whether the animal drinks when offered)
- Presence of urine or feces
- Position of the animal (L/R lateral or sternal)
- Mobility of the animal
- Condition of any bandage (if applicable)
- Appearance of the incision site (clean/dry/intact)
- Pain Score

b) When will analgesics be administered and at what interval(s)?

>Analgesics will first be given near the end of the surgery and will continue TID for approximately 3 days or until the vet staff deem appropriate.

c) If post-operative analgesics cannot be given, please provide scientific justification.

Not applicable.

15. Adverse Effects:

a. Describe all significant adverse effects that may be encountered during the study.

POST-SURGERY

Post-Telemetry Implant Surgery: Animals will be visually checked at least 3 days/week for infection and erosion. If either is noted, the veterinary staff will be notified immediately and treatment initiated according to veterinary recommendation. This may include surgical repair of skin, analgesics, antibiotics, and/or cleaning the implant or incision site(s). If continual problems are noted, then the implant will be removed per veterinary discretion.

Post Injury Surgery: Monkeys will be paralyzed on one side of the body initially, but will recover substantial movement over a few weeks. If the lesion extends into the gray matter on the opposite side of the injury site, there may be an initial bilateral paresis, but this should recover within a day or two. There will be damage to both gray and white matter on the side where the injury occurred and so paresis will take a longer time to recover. Complications could include bladder or bowel dysfunction, though this is highly unlikely. The spinal cord systems controlling these functions are bilateral and peripherally positioned, making it unlikely that both sides of this system would be damaged by the lesion procedure. Another possible complication is the development of pressure sores. To avoid this, after surgery the animals will be placed on a triple layer of soft bedding that includes fleece, a towel, and porous rubber mats. Animals are turned every 2-4 hours after surgery between 6am and 10pm by primate medicine and project staff who have been trained to work closely with spinal cord injured animals by the Training Coordinator. Animals are also encouraged to sit upright and move around the cage by offering preferred food as soon as possible. If sores would develop, they would be treated immediately as recommended by the vet staff.

Post Tracer Surgery: There is a small possibility that the skull cap removed at the start of the procedure and then replaced at the end of the procedure may become detached. If this occurs, the animal will be reanesthetized and the flap will be re-attached. There is also a small chance that animals may experience mild brain swelling. Personnel performing this procedure undergo training with the lead surgeon prior to attempting the reattachment and we used

dental acrylic and/or cyanoacrylate to adhere the bone securely to the rest of the skull.

Stereotaxic Placement: During placement into the stereotaxic apparatus in preparation for neurosurgery, animals may experience perforation of the tympanic membrane. We have no history of this problem; however, it is a possibility.

LONG TERM EFFECTS

Weight Loss: Weight loss is a specific criteria for euthanasia at the [REDACTED] which animals enrolled in this protocol may experience. However, there are two important factors to consider when deciding whether an animal on this protocol is a candidate for euthanasia under this criteria:

1. Animals are trained to conduct functional tasks through the use of food rewards (described in 14.2). As such, the pre-injury weight of the animal is artificially inflated from the pre-project weight, which is likely a more accurate normal weight for a given animal.
2. Animals typically lose approximately 10-15% of their body weight following the injury surgery due to muscle atrophy NOT a reduction in appetite or a failure to thrive. This conclusion is based on animals observed over the course of the previous several years, some of whom have lost close to 20% of their body weight following the injury surgery (calculated from the pre-injury weight) and still maintain an acceptable body condition score (BCS) of 2.0 or higher.

Based on these two factors, we propose an alternative in section 15.b below for calculating weight loss that more accurately reflects excessive weight loss for animals on this protocol.

Persistent Self Picking: Animals on this project have been known to persistently pick at the leg opposite the lesion (referred to as "contralateral limb") as well as at their cranial and cervical incision sites. In most cases, monkeys who exhibit self-picking behaviors do not appear to be in any obvious distress. In other words, monkeys will engage in these behaviors and yet *fully participate in other study activities, maintain a healthy appetite, and interact in a socially normal manner.* There are two main classifications of self-picking observed: **Brown-Sequard Syndrome** and **Peri-Incisional Pruritis.**

Brown Sequard Syndrome refers to the reduction of pain and temperature sensation on the contralateral side (typically the left side, in our case). Humans with similar injuries describe permanent numbness, tingling sensations, and itching on the opposite side of the body from the injury. We therefore expect non-human primates to experience similar side effects on a *permanent* basis following their injuries.

We have observed animals plucking hair or picking/scratching at the skin on the contralateral limb. We believe this is a manifestation of some or all of the aforementioned symptoms and this behavior often results in small, non-severe wounds forming on the contralateral limb. The severity of these picking wounds can be described using the "Stirling Pressure Ulcer Severity Scale" (<http://www.scireproject.com/outcome-measures/stirlings-pressure-ulcer-severity-scale>):

- "0" = no clinical evidence of a sore
- "1" = discoloration of the intact skin
- "2" = partial-thickness skin loss or damage involving epidermis and/or dermis
- "3" = full-thickness skin loss involving damage or necrosis of subcutaneous tissue
- "4" = full-thickness skin loss with extensive destruction and tissue necrosis extending to bone, tendon or capsule

In many cases, wounds that develop from contralateral limb picking/scratching persist but remain small and non-severe (i.e. a "1" or a "2" on the Stirling scale). Wounds of classification "1" or "2" are an expected consequence of the hemisection injury. These type of wounds have not been observed in our first cohort of contusion injured subjects, likely due to less damage to the ascending spinal tracts relaying pain and temperature. .

Peri-Incisional Pruritis. Following surgery, animals occasionally pick and/or scratch at their suture lines. In most animals to date, the focus of attention is typically at the cervical suture line (following injury, treatment, or tracer surgeries); however, we have also observed animals focusing these behaviors at the suture lines on the cranium (following the tracer surgery) and hand (following the implant surgery). Monkeys exhibiting these behaviors occasionally open full thickness incisions requiring medical attention and additional healing time. Our collaborators have observed similar behaviors following injuries in rats, which suggests that these behaviors may be a result of the injury. However, it is not yet clear whether other sources are implicated as well. Possibilities include reaction to treatments, a particular suture material, the use of cautery during surgery, or inappropriate sterile technique (all of which have been examined).

b. Describe criteria for monitoring the well-being of animals on the study and criteria for terminating/modifying the procedure(s) if adverse effects are observed.

FOLLOWING ALL SURGERIES

Food and water intake, and fecal and urinary output will be monitored through standard health monitoring by the [REDACTED]'s central services ([REDACTED] SOP FF-1). Pain score, neurological status, and signs of depression will be monitored by

therapeutic staff to determine the well-being of the monkeys.

Stereotaxic Placement: To continue to keep the risk low, only primate medicine or project personnel trained to place animals in the stereotaxic apparatus will perform this procedure.

LONG TERM EFFECTS

Weight Loss: The weight of the animal upon assignment to the project (i.e. "pre-project") will be used to calculate weight loss (as approved by the IACUC at the July 1, 2010 meeting) instead of the weight at the time of the injury surgery (i.e. "pre-injury"). Animals will be weighed monthly pre-injury and between weekly and bimonthly post-injury. Animals will also be assigned a pre-project BCS from which additional evaluations of weight loss will be made.

Persistent Self Picking: Monkeys will be observed daily by project SRAs, therapeutic staff, and/or [REDACTED] central services staff for the development of behaviors listed in section 15.A.

c. How will the signs listed above be ameliorated or alleviated?

FOLLOWING ALL SURGERIES

Analgesics, SQ fluids, and orogastric feeding will be done if necessary as per veterinary discretion. If animals show pain or discomfort that can't be alleviated with analgesics and routine veterinary medical care, animals will be euthanized.

Stereotaxic Placement: Animals with tympanic membrane ruptures are treated with antibiotics and analgesics as recommended by the vets, and are evaluated periodically to ensure the membranes are healing.

LONG TERM EFFECTS:

Weight Loss: Monkeys will receive items from a range of supplemental foods provided by therapeutic and project staff until the animal's weight stabilizes. When necessary, orogastric tubation feeding will also be used.

Persistent Self Picking: If the behaviors described in section 15.A. are observed and the animals exhibit no signs of distress and do not produce open lesions, then no treatment will be provided. Instead, we will continue to monitor their behavior daily. Based on types of injuries, we do expect that animals will show these behavioral symptoms in at least a minor way until their endpoints.

We will administer treatment if an animal:

- a) Brown Sequard Syndrome: develops more than ten open lesions of any Stirling classification, OR
- b) Brown Sequard Syndrome: has a lesion that is 1cm or bigger regardless of Stirling classification, OR
- c) Peri-Incisional Pruritis: a full thickness wound develops around the incision site that is not showing progress toward healing on its own (such as shrinking or drying up).

When treatment is needed, animals will undergo any combination of bandaging (to prevent additional damage), pharmacological intervention (to reduce their sensory symptoms), antibiotics, and/or topical treatments.

The exact regimen of pharmacological intervention will vary based on the severity of each case and will be decided upon through consultation with veterinary staff and the PI. Once a pharmacological regimen has begun, animals may remain on the regimen until their endpoints. Animals will be periodically evaluated to determine whether they may be weaned off the pharmacological treatment. This evaluation will be based on the severity and/or persistence of the symptoms and in consultation with veterinary staff and the PI. Possibilities for intervention include such drug classifications as:

- antipsychotics (such as haloperidol)
- sedatives (such as haloperidol)
- benzodiazepines (such as diazepam)
- neuropathic analgesics (such as gabapentin)

d. Study endpoints:

FOLLOWING ALL SURGERIES:

Animals will be humanely euthanized if they show pain and distress at any time that is not improving and/or can't be resolved using standard veterinary medical care.

LONG TERM EFFECTS:

Weight Loss: Weight Loss: Weight loss will be calculated using the pre-project weight (weight obtained during the pre-project physical exam), not the pre-injury weight (as approved by the IACUC at the July 1, 2010 meeting) and the following criteria will be used:

- If an animal experiences 20% weight loss (but remains above a BCS of 1.0) and is part of an experimental group that requires a tracer surgery (as described in 14.4.E), then a tracer surgery will be performed as soon as possible, with euthanasia approximately 6 weeks later.



- If an animal experiences 25% weight loss (but remains above a BCS of 1.0), euthanasia will be scheduled - without a tracer surgery – within 48 hours.
- Regardless of weight loss, if an animal's BCS drops to 1.0 or less at any time, the animal will be scheduled for euthanasia - without a tracer surgery - within 48 hours. The BCS must be determined independently by 2 veterinarians.

Persistent Self Picking:

As a result of Brown Sequard Syndrome, we expect that there may be some wounds that persist until the animal's endpoint. As long as these wounds are of no greater severity than classification "2" on the Stirling Scale, we will continue to treat the animal (as described in 15c), but will not euthanize the animal.

Animals will be scheduled for euthanasia - without a tracer surgery - within 48 hours if any of the following criteria are met:

- Brown Sequard Syndrome: Any wound is classified as "3" or greater on the Stirling Scale for more than 14 days, despite treatment (as described in 15.c.). Because wounds of classification less than "3" will likely not require a bandage, this time line of 14 days also puts a time limit on the duration of bandaging/bandage changes.
- Peri-Incisional Pruritis: A wound does not show signs of improvement after more than 30 days, despite treatment (as described in 15.c.).
- An animal suffers a severe wounding incident - involving deep muscle or major physical impairment - that can't be surgically repaired or with other routine veterinary medical care.

16. Euthanasia:

Species	Method	Drug	Dose	Route	Justification for Physical Methods
primate	Exsanguination via paraformaldehyde perfusion	Pentobarbital	~60mg/kg	Intravenous (IV)	N/A

17. Disposition:

Animals will be euthanized at the end of the study, or if there are prolonged post-operative complications.

18. Roster:

Name	E-mail	Occupational Health Participation	ACU 101 Training	Qualifications/Experience	Status
[Redacted]	[Redacted]	24-Jul-2009	13-Mar-2013	Dr. [Redacted] has over 25 years working with nonhuman primates and is an expert in the field of functional recovery following spinal cord injury. Dr. [Redacted] is included in our personnel roster to participate and consult in behavioral testing paradigms.	Approved
[Redacted]	[Redacted]	2-Jul-2007	9-Mar-2013	Dr. [Redacted] has over 25 years experience working with nonhuman primates. [Redacted] is an expert in the field of functional recovery following spinal cord injury and has been collaborating with Dr. [Redacted] on this study for the last 6 years. Dr. [Redacted] has performed spinal cord surgeries in tadpoles, frogs, mice, rats, opossums, cats, and non-human primates. [Redacted] also served as the PI on an NIH-sponsored training course for spinal cord injury methods, which	Approved



				included instruction on how to do contusion injuries, perform intrathecal application of drugs, and cortical injections. These training qualifies [redacted] to do contusion injuries, perform spinal treatment procedures, and conduct cortex tracer surgeries.	
[redacted]	[redacted]	8-Aug-2013	14-Aug-2013	[redacted] has worked with nonhuman primates for over 15 years, and has just started working in neuroscience and behavior. [redacted] is in the process of training and becoming familiar with all aspects of the study in order to provide complete project support.	Approved
[redacted]	[redacted]	12-Feb-2005	26-Jan-2013	Dr. [redacted] is a neurologist with over 25 years of experience from experimental work using animal models for studies of spinal cord, nerve root, and peripheral nerve injuries. [redacted] is an expert in EMG recording of urodynamics and will be conducting the EMG recordings of the external anal sphincter muscle. Dr. [redacted] is a collaborator listed on the grants which describe the procedures in this protocol. [redacted] experience includes over 8 years of experience performing spinal procedures using non-human primate models.	Approved
[redacted]	[redacted]	8-Mar-2011	13-Feb-2013	[redacted] has worked with nonhuman primates for 6 years, the last 5 years in neuroscience and behavior. [redacted] is familiar with and has been trained in all aspects of the study and provides project support, including treadmill, open-field cage testing, and stereotaxic placement (the specific indication for which was requested by the IACUC).	Approved
[redacted]	[redacted]	24-Mar-2011	1-Dec-2014	[redacted] has 10 years of experience working with nonhuman primates, 7 of those years in the area of neuroscience and behavior. [redacted] has been trained in and has mastered all aspects of the study, including treadmill, open-field cage testing, and stereotaxic placement (the specific indication for which was requested by the IACUC), and provides project support.	Approved
[redacted]	[redacted]	13-Nov-2008	2-May-2014	Dr. [redacted] received [redacted] DVM in 1999, DCVIM in 2004,	Approved



				<p>and DACVECC in 2006. [REDACTED] has performed neurosurgeries, including dorsal laminectomies and spinal cord injuries in rats since 2003. [REDACTED] has performed neurosurgeries, including craniotomies, dorsal laminectomies, spinal cord injuries, and cortex tracers in primates under the guidance of Dr. [REDACTED] since 2008. [REDACTED] has also been trained to perform contusion injuries by the protocol PI. [REDACTED] is included on our personnel roster to serve as a back-up surgeon for all surgeries listed on this protocol.</p>	
[REDACTED]	[REDACTED]	2-Jul-2007	1-Nov-2012	<p>Dr. [REDACTED] has over 8 years experience working with nonhuman primates. [REDACTED] has been collaborating with Dr. [REDACTED] on this study for the past 5 years. [REDACTED] has performed neurosurgeries, including craniotomies, spinal laminectomies, cortical injections, and intra-hippocampal electrode implantations in rodents since 2003. [REDACTED] has also performed neurosurgeries, including craniotomies, spinal laminectomies, and cortex and spinal injections in non-human primates under the guidance of Dr. [REDACTED] since 2005. [REDACTED] is included on our personnel roster to conduct surgeries and has been trained by Dr. [REDACTED] on all listed procedures.</p>	Approved
[REDACTED]	[REDACTED]	2-Jul-2007	11-Feb-2013	<p>Dr. [REDACTED] has over 30 years experience working with nonhuman primates. [REDACTED] is an expert in the field of motor telemetry implantation and has been included on our personnel roster to conduct motor telemetry implant surgeries.</p>	Approved
[REDACTED]	[REDACTED]	9-Mar-2012	5-Jan-2015	<p>Dr. [REDACTED] has over 4 years of surgical experience in performing craniotomies (Cynomolgus & Rhesus) and stereotactic drug delivery to the brain. [REDACTED] has also conducted injections in cortico-spinal fluid and lateral ventricles. [REDACTED] is included on our personnel roster to conduct neurosurgery and has been trained to perform contusions, spinal treatment procedures, and cortex tracer injections by the protocol PI.</p>	Approved
[REDACTED]	[REDACTED]	5-May-2011	20-Feb-2015	<p>Dr. [REDACTED] has 12 years of</p>	Approved



				experience working with nonhuman primates and in the area of behavior and neuroscience. [REDACTED] is the SRA managing all study activity on a daily basis and has been trained in all aspects of the study, including treadmill, open-field cage testing, and stereotaxic placement (the specific indication for which was requested by the IACUC). [REDACTED] is enrolled in the Occupational Health Program and has taken the ACU 101 class.	
[REDACTED]	[REDACTED]	20-Sep-2004	7-Jan-2014	Dr. [REDACTED] (MD, PhD) has received surgical training as a physician and has over 20 years of primate neurosurgery experience. [REDACTED] is trained to perform contusions, and conduct spinal and cortex injections. [REDACTED] is on this protocol to serve as a backup for contusion, treatment, and tracer surgeries.	Approved
[REDACTED]	[REDACTED]	25-Apr-2008	2-Oct-2013	[REDACTED] has been workign with nonhuman primates for over 5 years. [REDACTED] has been collaborating with Dr. [REDACTED] on this study for the past 7 years. [REDACTED] is included on our protocol to conduct EMG and video recording sessions, as well as to assist with EMG and/or cortical stimulation during surgery. [REDACTED] has been trained on all of these procedures by [REDACTED] collaborator, [REDACTED]	Approved
[REDACTED]	[REDACTED]	2-Jul-2007	25-Feb-2013	Dr. [REDACTED] has been working with nonhuman primates for the last 6 years. [REDACTED] is an expert in the field of motor telemetry implantation and has been included on our personnel roster to conduct motor telemetry implant surgeries.	Approved

Protocol # [REDACTED] - Appendix C - Non-human Primate Enrichment Program Form

Section 1. Social Enrichment

Pairing conditions from which subjects are to be exemp.

- Continuous pairing
- Intermittent pairing

Duration of exemption: 36 Month(s)

Scientifically based justification for exempting subjects from the pairing conditions.

The adult monkeys on this protocol will receive telemetry implants, which are costly to replace both with respect to the time invested as well as to the overall health of the monkey. Furthermore, any digit trauma - to an implanted OR non-implanted animal - would render the monkey unable to perform the food retrieval and locomotion tasks that are required as part of this protocol. The risk to the monkey as well as to the project as a whole is too great to allow for continuous or intermittent pairing; however, grate pairing would be acceptable for those monkeys who are compatible.



Category/group of animals and the type of social pairing these animals can receive.

Category/Group
N/A

Section 2. Food Enrichment

Food enrichment from which subjects are to be exempt.

Fruit Vegetables Other foods/mixtures
Duration of exemption: 36 Month(s)

Scientifically based justification for exempting subjects from the food enrichment.

We would like animals on this protocol to be eliminated from the administration of biweekly food enrichment (i.e. fruit, vegetables, and other foods/mixtures) by the colony management staff. We would like this exemption to last for the entire duration that the animal is on this project. Animals on this protocol receive ample food reinforcements during daily behavioral testing sessions and through in-cage object manipulation opportunities. An assortment of fruits and vegetables (such as apples, oranges, bananas, peaches, tomatoes, green beans, carrots, and celery) as well as dried fruits, peanuts, and seeds are commonly used for both in-cage and behavioral testing enrichment. Consequently, excessive weight gain is a concern for some animals on this project. When weight loss is a concern, supplemental foods are administered through the primate medicine core. Therefore, the additional food enrichment that is administered biweekly from colony management staff is 1) not necessary and 2) may contribute to obesity. As requested by the IACUC, the investigator confirms that animals will receive enrichment on a minimum schedule as the rest of the animal colony. Animals in the colony typically receive enrichment 2x/week; however, animals on this protocol will typically receive enrichment up to 5x per week. When behavioral testing is not occurring and animals will not receive supplements 5 days per week as result, animals will receive enrichment AT MINIMUM on the same schedule as the rest of the colony.

Category/group of animals and the type of food enrichment these animals cannot receive.

Category/Group	Type of Food Enrichment
N/A	N/A

Section 3. Cage Enrichment

Cage enrichment items from which subjects are to be exempt.

Perch
Duration of exemption: 36 Month(s)

Scientifically based justification for exempting subjects from the cage enrichment.

Monkeys will receive spinal cord lesions which will impair mobility. The presence of a perch introduces the potential of unnecessary hazards, such as the monkey's foot getting caught and/or the monkey falling off the perch.

Category/group of animals and the type of cage enrichment these animals cannot receive.

Category/Group	Type of Cage Enrichment
N/A	N/A

Assurances for the Humane Care and Use of Vertebrate Animals:

I have read and agree to abide by the [redacted] **Policy and Procedure Manual section 290-30** ([redacted]). This project will be conducted in accordance with the **ILAR Guide for the Care and Use of Laboratory Animals**, and the [redacted] **Animal Welfare Assurance** on file with the US Public Health Service. I will abide by all Federal, State, and local laws and regulations dealing with the use of animals in research.

The activities proposed in this application do not unnecessarily duplicate previous experiments [AWA 2.31(d)(1)(iii)].

I will advise the IACUC in writing of any proposed significant changes in the procedures and wait for IACUC approval prior to implementing the change. I will also advise the IACUC of any changes in personnel involved in this project.

I have read and agree with the above statements.



Institutional Animal Care and Use Committee (IACUC)

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[redacted] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact

12 Approved Amendment(s):

Approved: 6-Feb-2014

Add Stem Cell Treatment

1. Proposed Changes:

We would like to add the use of stem cells as a treatment following contusion injury.

Stem Cell Treatment

One to four weeks following the contusion (which is a clinically relevant time point), animals will be prepped for surgery as described in 14.4.4.A. and a dorsal midline incision will be made over the cervical vertebral area and the spinal cord visualized. Then, animals will receive treatment with "NeuralStem" stem cells or [redacted] 4 stem cells (NIHhESC-10-0044 (n = 6); Details: http://grants.nih.gov/stem_cells/registry/current.htm?id=200). Stem cells will be introduced into the spinal cord via spinal injections into the contusion cavity. [redacted] will be the lead surgeon for this transplant procedure.

Immunosuppression Protocol and Drugs

Animals receiving stem cells will undergo immunosuppression therapy beginning approximately 1 weeks prior to stem cell transplantation and until their endpoint (approximately 3-6 months). This therapy helps to ensure that the stem cells will survive and not be rejected by the body.

Tacrolimus (Prograf) will be administered orally 1-2x/day beginning approximately 2 days before stem cell transplantation. Dosages will be adjusted based on blood trough levels. Tacrolimus doses will begin at up to 1mg/kg and not exceed 3mg/kg (target therapeutic range = 4-10ng/mL).

Mycophenolate Mofetil (CellCept) will be administered orally 1-2x/day beginning approximately 7 days before stem cell transplantation. Dosages will be adjusted based on blood trough levels, but will not exceed 100mg/kg. The target therapeutic range for CellCept is 3-6ug/mL. One month following stem cell transplantation, dosages for CellCept will be tapered over the course of one week down to 0.

Prednisone will be administered orally 1x/day beginning approximately 1 day before stem cell transplantation. Doses will begin at 2mg/kg and then be reduced to 1mg/kg within 3 day following transplantation and continuing until the animal's endpoint.

Options for Immunosuppression Dosing It may be necessary to chair train animals who will be receiving immunosuppression therapy. If animals stop volunteering to take the drug doses orally, we will use this option to administer drugs through a nasogastric tube (according to CNPRC SOP II-12).

As an alternative to nasogastric tube dosing, we may place a percutaneous endoscopic gastrotomy (PEG) tube. The PEG tube will be placed using the pull method (an outpatient procedure) approximately 2-4 weeks prior to contusion:

- The animal will be sedated with ketamine and atropine, intubated and maintained on Isoflurane.
- An endoscopy will be performed to identify the fundus and the great curvature of the stomach, the stomach will be gently moved toward the body wall.
- A local anesthetic with lidocaine will be performed on the skin and the body wall (where the needle and the tube will be inserted). The endoscopic light will guide the placement of a percutaneous needle/catheter onto the fundus of the stomach.

- A wire will be thread through the needle and will be captured by the endoscope. The wire and the endoscope will be gently removed through the mouth.
- The wire will be attached to a PEG tube and gently tread through the esophagus to the stomach and will exit to the outside of the body.
- A bumper (inherent part of the tube) will secure the placement of the tube inside the stomach and another bumper or mesh will secure the tube outside the body wall
- The percutaneous portion of the tube will then be tunneled subcutaneously on the back and an implantable port will be attached and secured subcutaneously.

As an alternative to the above PEG tube placement - where all components remain inside the body - we may need to use a method where the port is located outside the body. For this alternative procedure, all steps remain the same as in 10.B.ii. except that - in the last step - the PEG tube will be tunneled subcutaneously to the back and the tube will be secured to the skin on the outside of the body. In this case, we would fit the animal with a non-human primate jacket to protect the port and prevent the animal from accessing it.

Please Note: If either PEG tube placement procedures are used, immunosuppression dosing will not begin until the animal has fully healed, as per veterinary recommendation.

Blood Sampling.

To ensure appropriate monitoring of blood trough levels and clinical chemistry values while animals are undergoing immunosuppression, we will collect blood samples at least once per week in accordance with the frequency and volume restrictions outlined in [REDACTED] SOP GG-5.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

Previous research has examined the effect of genetically altered cells in both rodents and non-human primates on anatomy and histology of the brain and injured spinal cord. However, to date, our group is the only group investigating these effects on both anatomy/histology AND functional recovery. Moreover, our collaborator [REDACTED] is pursuing a line of investigation that, in rodents, has yielded results an order of magnitude greater than anything we have seen in the spinal cord injury field. We continue to pursue answers to these questions in the primate as there are no suitable alternatives in developing a method applicable for human intervention. Rodents and primates differ greatly in immune function, as well as CNS anatomy, function, and size. The corticospinal tract, for example, is critical for fine hand motor function in primates, but not in rodents. Heretofore, this investigation has taken place in the hemisection injury model. Given the clinical relevance of the contusion injury, stem cell therapy in a contusion injury model is the next step in the investigation of this treatment paradigm.

Requested Updated Literature Search (conducted on 2014 Jan 31)

1. PubMed/Medline (1960 - present): monkey AND "immunosuppression therapy"; "stem cell" spinal AND monkey; monkey AND "percutaneous endoscopic gastrostomy"
2. Primate Portal (1940 - present): "stem cell"; immunosuppression; "percutaneous endoscopic gastrostomy"
3. Web of Science (1900 - present): "stem cell" AND monkey; immunosuppression AND monkey; "percutaneous endoscopic gastrostomy" AND monkey

3. Potential Adverse Effects:

Immunosuppression

The following list is based on side effects experienced by humans in clinical trials.

Tacrolimus may produce diabetes mellitus/hyperglycemia, hyperkalemia, allergic reaction, nephrotoxicity, neurological changes, hypertension, and cardiac damage. Note: Subject should avoid grapefruit or grapefruit juice due to a possible interaction with tacrolimus.

Mycophenolate Mofetil may produce diarrhea, leukopenia, sepsis, vomiting, a higher incidence of opportunistic infections, and reactivation of Herpes B virus.

Prednisone and Prednisolone may produce adrenal suppression, myopathy, and delayed wound healing.

Animals will be observed multiple times per day for behavioral changes (at morning health, AM and PM feeding, and during daily behavioral testing). Changes in behavior will be recorded in the animal's health record. Biochemical screen will take place up to multiple times per week as needed. Blood trough levels and clinical chemistry values will be measured as well.

To alleviate adverse effects, we will utilize special PPE requirements to minimize contamination from other areas of the primate center whenever animals undergo immunosuppression. In addition, treatment of the side effects of



immunosuppression drugs will be based on veterinary recommendation. Some examples may include:

- Administration of insulin for diabetes mellitus/hyperglycemia
- Diet manipulation or medication for hyperkalemia or nephrotoxicity
- Anticholinergic or other medication for neurological changes
- Pharmacological treatment of hypertension

Based on our calculations of the dose of MMF, we would classify our dose as "low." Therefore, the risk of reactivation of Herpes B virus in animals undergoing MMF immunosuppression is also low. At a minimum, we will ensure personnel who work with these animals are aware of this possibility by posting appropriate signage in the ante-room of the project room, which will indicate that the animals undergoing MMF immunosuppression may be at a higher risk for Herpes B virus. 3.

Percutaneous Endoscopic Gastrostomy (PEG) Tube Placement

PEG tube placement may result in visceral puncture or visceral entrapment between the stomach and body wall. If the PEG tube is placed too close to the upper or lower esophageal sphincter, it may cause protracted vomiting. Even when the PEG tubes is properly placed, approximately 10-20% become displaced. This may result in peritonitis. For the procedure that will leave all components inside the body, there is a possibility of leakage at the port entry near the skin and development of cellulitis.

Daily food and water intake and fecal and urinary output will be monitored through standard health monitoring by the [redacted]'s central services ([redacted] SOP FF-1). Pain score, neurological status, and level of depression will be monitored by therapeutic staff to determine the well-being of the monkeys

Many of the adverse effects listed above are substantially minimized through the use of endoscopy.

The criteria that will be used to determine whether euthanasia is to be performed:

Animals undergoing stem cell treatment will be humanely euthanized if they show pain and distress at any time that is not improving and/or can't be resolved using standard veterinary medical care. We will use the same endpoints for euthanasia that are outlined the Section 15.D. of the currently approved protocol.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

Institutional Animal Care and Use Committee (IACUC)

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██████████ - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact ▼

12 Approved Amendment(s):

Approved: 18-Apr-2014

Add Gadolinium to MRI procedures ▼

1. Proposed Changes:

We would like to add the option to use the Magnevist/gadolinium contrast agent during the MRI procedures. If administered, Magnevist would be giving IV at a dose of 0.2ml/kg.

Uploaded File(s):

[DocUpload-02-██████████_AMENDMENT.pdf](#)

2. Justification for Proposed Changes:

Contrast agents highlight areas of vasculature and enable clear visualization of normal tissue from abnormal tissue (if there is any) and would be a useful tool in identifying the extent of lesion and surgical grafting prior to the animal's end point.

3. Potential Adverse Effects:

Hunt et. al. (2009) conducted a study examining adverse effect of gadolinium by conducting a retrospective review of 158,439 human patients who received gadolinium. 0.04% (64) of patients showed either hives or nausea. Over 75% of those patients (i.e. 49) were characterized as having "mild" adverse effects and required no treatment other than observation. 15% of those patients (i.e. 7) were characterized as having "moderate" adverse effects and were given diphenhydramine. The remaining 10% (or 0.005% of the entire population) were characterized as having "severe" adverse effects and were given epinephrine or other drugs. There were no patient deaths among those that received gadolinium.

Animals will be observed for nausea or allergic reactions. Based on veterinary recommendation, the following may be administered:

Nausea:

- Reglan (metoclopramide) at 0.2-0.5mg/kg IM
- Cerenia (maropitant) at 1mg/kg SC
- Ondansetron at 2mg/kg IM/IV

Allergic Reactions: Diphenhydramine at 2-4mg/kg either IM or IV

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[REDACTED] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact ▼

12 Approved Amendment(s):

Approved: 29-May-2014

Botulinum Toxin ▼

1. Proposed Changes:

We would like to add the option to use of botulinum toxin as a method to inhibit peri-incisional pruritis following contusion. Based on veterinary recommendation, we would also like the option to use it as a clinical application in other peri-incisional sites, such as the PEG tube, as needed. There are two chemically and antigenically distinct forms of botulinum toxin. At this time we propose to use type A (BOTOX).

If used, botulinum toxin in 1mL of local anesthetic (such as lidocaine) will be injected into the subcutaneous space around the cervical incision site up to 7 days prior to hemisection (Group 4A) or on the day of hemisection (all other groups). Approximately 100 units will be dissolved in 1mL of local anesthetic. Approximately 60 units would be used per animal, as further explained below. Injections will be done in a grid-like fashion around the cervical incision site, injecting approximately 0.05mL at each site.

Uploaded File(s):

[DocUpload-03-\[REDACTED\].AMENDMENT.pdf](#)

2. Justification for Proposed Changes:

Injections of botox will turn off peripheral nociceptors in the area of the injections for up to 3 months. If the peri-incisional pruritis that we have observed in several of our hemisected subjects is due to neuropathic pain, then this single set of botulinum toxin injections may alleviate much of this adverse effect. In the two hemisected subjects that have undergone this treatment, no peri-incisional pruritis has been observed. This would lead to far less use of bandaging and haldol, and substantially reduce the number of sedations required to monitor the peri-incisional pruritis. Peri-incisional pruritis has not yet been observed in contusion animals; however, the greater number of occurrences has followed the second spinal procedure. So far only one contusion animal has undergone more than one procedure.

Updated Literature Search for alternatives to *botulinum toxin*

Date Databases Searched: 2014 May 16th

Database: Pubmed/Medline

Years Covered: 1960-present

Keywords Used: botox AND "neuropathic pain"; myobloc AND "neuropathic pain"

Database: Web of Science

Years Covered: 1900-present

Keywords Used: botox AND "neuropathic pain"; myobloc AND "neuropathic pain"

Additional Justification for Proposed Dose

The concentration we have proposed is within the range of human use and can also be received at most medical spas, with the effects wearing off after approximately 3-4 months. This dose doesn't change according to weight, pre-existing conditions, age, etc. In addition, Scott & Suzuki (1988) showed that the lethal dose of botulinum toxin in macaque



monkeys is 38-42 units/kg (a total of 304-336 units for the lightest monkey on our study), with systemic toxicity occurring at approximately 33 units/kg (264 units for the lightest monkey on our study). If the average cervical opening is approximately 4 inches, and we inject approximately 3 sites per inch in the grid-like fashion described in section #1), then we would be injecting at approximately 12 sites. At 5 units per site, this is a total of 60 units. This is far below the system toxicity dose cited in Scott & Suzuki (1988).

3. Potential Adverse Effects:

In 2005, a paper was published listing the adverse effects that had been reported to the FDA following use of BOTOX in both therapeutic and cosmetic cases. Adverse effects included respiratory compromise, flu-like syndrome, cardiovascular system issues (unspecified), nervous system issues (unspecified), muscle weakness, seizure, gastrointestinal system issues (unspecified), dysphagia, and allergic reactions. The authors go on to state that the majority of serious adverse events were the result of high doses (i.e. over 500 units) given to patients that also had complicated underlying diseases.

Animals undergoing this treatment will be monitored closely for at least 14 days following injection. Adverse effects will be ameliorated based on consultation with vet staff and the PI.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A



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Approved Amendment(s) for Active Protocol:

[redacted] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact ▼

12 Approved Amendment(s):

Approved: 20-Jun-2014

Transfer Of Animal To Another IACUC Approved Protocol ▼

1. Proposed Changes:

As cited in IACUC Protocol # [redacted] /Contusion Injury as a Model for Spinal Cord Injury, section #17, Disposition, we are requesting to allow the transfer of an animal to another IACUC approved protocol depending on surgical history.

Specifically we are requesting the transfer of a completely surgically naïve animal ([redacted]) from IACUC Protocol # [redacted] /Contusion Injury as a Model for Spinal Cord Injury to IACUC Protocol # [redacted] /Stem Cell Therapy for Treatment of Spinal Cord Injury. This subject has been trained and tested on behavioral tasks that are exactly parallel between both protocols.

Uploaded File(s):
No Files Found.

2. Justification for Proposed Changes:

By utilizing an animal already trained and behaviorally tested for both protocol behavioral programs, this will eliminate the need to procure and replace the animal with another CNPRC colony animal.

3. Potential Adverse Effects:

Adverse effects will remain the same as stated in the original protocol.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A





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Approved Amendment(s) for Active Protocol:

[redacted] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact

12 Approved Amendment(s):

Approved: 14-Oct-2014

Add BrDU Dosing

1. Proposed Changes:

BROMODEOXYURIDINE (BrDU) DOSING: Following the injury surgery, animals in Group 2 who receive stem cells will receive BrDU. For BrDU administrations while the animal is sedated, animals will receive ketamine and possibly dexmedetomidine and atipamezole according to [redacted] SOP FF-1. For BrDU administrations while the animal is awake, see 6.C. below.

5.A. Dose and Route of administration. 50mg/kg of BrDU will be dissolved in saline and administered IV in a volume of up to 20mL of saline. BrDU will be infused into a peripheral vessel. Pharmacy grade BrDU is not available. Using sterile gloves and a sterile needle, we will resuspend the compound within a sterile vial using sterile saline. This procedure follows established [redacted] protocols for sterile technique. We will sterile filtering before infusing into the animal. A culture of the BrDU is also conducted. Any bacteria that are identified will be subjected to an antibiotic sensitivity test.

5.B. Timing and Duration of administration. BrDU will be administered once per day for 3 - 7 consecutive days beginning from 1 day to 6 months post-injury. BrDU dosing done at later time points will help determine the duration of grafted-cell proliferation.

5.C. Injection in Restraint Chair. If the animal will tolerate BrDU administration while awake, we will administer the dose while the animal is in a restraint chair. This will be done with a flexible intravenous catheter. The injections will typically follow a chair training session; however, occasionally, we will need to chair the animal solely for the purposes of BrDU administration.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

BrDU labels dividing neurons. This will enable histological analysis of cell genesis and differentiation in both grafted tissue and host tissue.

Updated Literature Search, conducted 2014 Sept 11

Pubmed (1960 - present): "monkey" AND "bromodeoxyuridine"

Pubmed (1960 - present): "monkey" AND "restraint chair"

3. Potential Adverse Effects:

For Section 8: BrDU is a hazardous chemical that will be stored in both the lab area as well as in the vivarium following infusions.

For Section 15:

A.: Monkeys may show poor appetite.

B: Daily food and fecal output will be monitored through standard health monitoring by the [redacted] s central



services ([redacted] SOP FF-1).

C: Veterinary staff may recommend feeding via orogastric tube (OGT) to supply additional nutrients until the monkey's appetite returns. If animals show pain or discomfort that can't be alleviated with analgesics and routine veterinary medical care, animals will be euthanized.

D: Animals will be humanely euthanized if they show pain and distress at any time that is not improving and/or can't be resolved using standard veterinary medical care.

For Appendix A

2: BrdU is a white crystalline water-soluble compound commonly used in the detection of proliferating cells in living tissues. It has shown to be "taken up" by actively dividing, newly formed cells, thus allowing for histological analysis of neurogenesis.

3: It is hazardous to both humans and animals.

4: This agent will not spread because it does not leave the animal after infusion.

5: Research has indicated that BrDU has cytotoxic, strongly teratogenic and mutagenic properties. Primary routes of occupational exposure to BrDU include aerosol exposure, ingestion, accidental injection, and tissue/transplacental absorption.

6.d and e: Animal carcasses will be labeled and disposed of via incineration and standard facility disposal method

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[REDACTED] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact

12 Approved Amendment(s):

Approved: 2-Oct-2014

Modifications to Diet, Immunosuppression, and PEG Procedure

1. Proposed Changes:

We request the following modifications to two separate aspects of this protocol.

Added Section: 14.A.2.F. Food Rewards, Feeding, and Diet Manipulation

Food Rewards. This protocol uses food rewards for behavioral testing. We give animals fruits (e.g. apples, grapes, bananas, oranges, plums, strawberries, or other seasonal items), vegetables (e.g. carrots, cucumbers, lettuce, bok choy, tomatoes, or other seasonal items), dried fruit (e.g. raisins, yogurt covered raisins, mango, fig, etc), and nuts (e.g. almonds, shelled peanuts, unshelled peanuts, etc). We also use nutrition bars (e.g. Odwalla bars). We do not give animals any sort of candy.

Feeding and Diet Manipulation. Some monkeys may require decreased chow for behavioral testing purposes. Feeding will be done in coordination with [REDACTED] staff depending on the requirements of the experiments that are being conducted at any given time for any given monkey. Animals will always have their daily caloric needs met. If an altered diet is needed, changes will be conducted with veterinary consultation and oversight.

Modified Section: 14.A.6.A.ii. Mycophenolate Mofetil (CellCept)

This section currently reads: "(CellCept) will be administered orally 1-2x/day beginning approximately 7 days before stem cell transplantation. Dosages will be adjusted based on blood trough levels, but will not exceed 100mg/kg. The target therapeutic range for CellCept is 3-6ug/mL. One month following stem cell transplantation, dosages for CellCept will be tapered over the course of one week down to 0."

At the end of this paragraph, we would like to add: "If an animal does not appear to tolerate the dosage of CellCept (shows signs of nausea, drooling, listlessness), we may decide to taper the animal before the one month time point."

Added Section 14.A.6.A.iv. Stool Softener

To prevent possible intestinal impaction, we may use a stool softener (such as Colace) or fiber supplements (such as inulin or fiber bites). Dosing for Colace will be approximately 1.5-2mg/kg, administered 2x/day. The need for continued use would be determined via an abdominal xray (conducted according to CNPRC SOP II-06) to assess impaction collected. If -- based on veterinary consultation -- impaction is not observed, Colace would be discontinued and used again, only if clinically indicated.

Modified Section: 14.A.6.B.ii. As an alternative to nasogastric tube dosing, we may place a percutaneous endoscopic gastrostomy (PEG) tube.

To this procedural summary, we would like to add -- per veterinary request -- the option of pre-passing two non-absorbable sutures to the bumper of the PEG tube and anchoring these sutures to the abdominal muscle to prevent any movement of the PEG tube. This extra step won't require any additional opening or surgery.



Uploaded File(s):
No Files Found.

2. Justification for Proposed Changes:

Added Section: 14.A.2.F. Food Rewards, Feeding, and Diet Manipulation

Some animals enrolled on this protocol receive more calories than they need and gain weight to an unhealthy degree. We modify the food rewards that are used where possible (substituting vegetables for fruit); however, animals sometimes need a diet modification such that their total daily caloric needs are not exceeded.

Modified Section: 14.A.5.A.ii Mycophenolate Mofetil (CellCept)

Some animals experience nausea while on CellCept, and we would like the option of tapering them off the medication earlier than one month post-grafting.

Added Section 14.A.5.A.iv. Stool Softener

A recent necropsy report indicated that the cecum of an animal was moderately impacted. As a preventative measure, we would like to regularly use a stool softener. This may also help with any potential nausea.

Modified Section: 14.A.6.B.ii. As an alternative to nasogastric tube dosing, we may place a percutaneous endoscopic gastrostomy (PEG) tube.

This additional step will help keep the bumper in place so that it doesn't move into the main compartment in the stomach.

3. Potential Adverse Effects:

Added Section: 14.A.2.F. Food Rewards, Feeding, and Diet Manipulation

There are no potential adverse effects of animals eating no more than their daily caloric needs. The animals are weighed monthly pre-injury and from weekly to monthly post-injury. Their weight will be monitored and veterinary staff will be consulted to ensure the animal continues to have his daily caloric needs met.

Modified Section: 14.A.5.A.ii Mycophenolate Mofetil (CellCept)

There are no potential adverse effects of tapering an animal off CellCept at an earlier time point.

Added Section 14.A.5.A.iv. Stool Softener

Diarrhea is a possible side-effect of Colace. Animals will be monitored daily and Colace will be stopped if diarrhea is observed.

Modified Section: 14.A.6.B.ii. As an alternative to nasogastric tube dosing, we may place a percutaneous endoscopic gastrostomy (PEG) tube.

There are no additional adverse effects outside those listed for the PEG procedure in the main protocol.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A



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Approved Amendment(s) for Active Protocol:

[redacted] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact ▼

12 Approved Amendment(s):

Approved: 13-Nov-2014

Modify Disposition ▼

1. Proposed Changes:

For animal disposition, we currently state "Animals will be euthanized at the end of the study, or if there are prolonged post-operative complications."

We request to be able to transfer animals from protocol # [redacted] that have undergone behavioral testing, imaging, blood draws, and EMG, (but no other procedures without individual animal IACUC approval) to other IACUC approved protocols.

Therefore we request the disposition of animals be amended to read: "Animals will be euthanized at the end of the study, or if there are prolonged post-operative complications. Alternatively, animals that have undergone behavioral testing, imaging, blood draws, and EMG, (but no other procedures without individual animal IACUC approval) may be transferred to another IACUC-approved protocol."

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No Files Found.

2. Justification for Proposed Changes:

After animals move into the project room and take part in behavioral testing and occasionally some early, non-invasive, procedures such as imaging, blood collection, and EMG procedures, we often see behavioral or physiological characteristics exhibited that lead us to believe an animal will thrive best on a particular spinal project protocol, and on occasion it may not be the protocol they were originally assigned to.

The [redacted] veterinary staff discussed potential animal transfers from this protocol and would approve transfer of animals who have undergone behavioral testing, imaging, blood draws, and EMG, but no other procedures without individual IACUC approval.

3. Potential Adverse Effects:

None anticipated.

4. Additional Animals:

Additional animals are not required at this time. It is possible that animal numbers may require adjustment in the future to accommodate research goals based on transfer "balance" history. This amendment is not intended to change the number of animals currently approved and justified to meet the project objectives.

5. Justification for Additional Animals:

N/A

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Approved Amendment(s) for Active Protocol:

[redacted] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact

12 Approved Amendment(s):

Approved: 8-Jan-2015

Extend Interval Between Contusion and Grafting

1. Proposed Changes:

We would like to extend the interval between contusion and grafting. In the amendment "Add Stem Cell Treatment" approved February 6, 2014, we indicate that animals will undergo stem cell grafting into the spinal cord approximately 1-4 weeks following contusion injury. One animal underwent contusion on November 4th and was scheduled to undergo stem cell grafting on December 12th; however, there were problems with the stem cells and the procedure had to be cancelled. We anticipate that this animal will be able to undergo stem cell grafting on January 29th, which is approximately 12 weeks post-contusion. We would like to extend the interval from contusion to grafting for all subsequent animals on this protocol to allow grafting anywhere from approximately 1 week to 12 weeks post-contusion.

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No Files Found.

2. Justification for Proposed Changes:

In the approved amendment "Add Stem Cell Treatment" approved February 6, 2014, we indicate that grafting 1 to 4 weeks post-injury is a clinically relevant time point. Our lab has shown that stem cells are effective in promoting axonal growth at greater time points post-injury. As such, we want to expand the interval of contusion to grafting in Group 2, which is our control + treatment group.

3. Potential Adverse Effects:

Animals will have a longer period of time to recovery post contusion prior to undergoing immunosuppression and stem cell grafting, which helps to prevent adverse effects. There will be no increase in the total survival time due to increasing the interval between contusion and grafting. The amount of time the PEG tube has to heal before immunosuppression begins will also increase. As a result, no additional adverse effects are expected as a result of increasing this interval.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A



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Approved Amendment(s) for Active Protocol:

[redacted] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact ▼

12 Approved Amendment(s):

Approved: 3-Feb-2015

Add Pre-Contusion X-Ray ▼

1. Proposed Changes:

We would like to add the option of performing an x-ray prior to the contusion procedure. The steps would be as follows:

- Animal will be sedated, delivered to the surgery prep area, and prepped for surgery as is already described.
- A non-sterile marker or patch will be placed on the animal's back (such as "Target Tape," <http://target-tape.com/>)
- The x-ray is performed by trained [redacted] personnel in accordance to [redacted] SOP II-6.
- The animal is placed in the stereotaxic frame with the non-sterile marker/patch still attached. A surgical pen or small bit of suture will be used to mark the skin in the appropriate location for the contusion using the information from the radiograph.
- The non-sterile marker/patch is removed and the sterile surgery prep is completed.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

This patch will enable us to use more accurate landmarks to identify our target site for contusion. This will minimize variation due to atypical anatomy or variation in anatomy, leading to more accurate and reliable experimental results.

3. Potential Adverse Effects:

Potential adverse effects of the x-ray and use of non-sterile marker or patch are minimal and will not exceed those already described in the existing protocol.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[REDACTED] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact

12 Approved Amendment(s):

Approved: 5-Feb-2015

Add Option to Administer 2nd dose of Botox

1. Proposed Changes:

We would like to add the option of administering botulinum toxin A (hereafter called "botox") on the day of the grafting procedure.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

The proposed duration between contusion and grafting is 1 to 4 weeks. The "Botulinum Toxin" amendment that was approved on May 29th, 2014 indicates we will administer botox on the day of contusion because botox turns off peripheral nociceptors in the area injected for a period of 3 months. As a result, botox administered at the time of contusion keeps nociceptors turned off following the contusion surgery, treatment (i.e. grafting) surgery, and for several weeks beyond the treatment surgery. This technique has significantly reduced the onset of peri-incisional pruritis ("PIP," described in the "Adverse Effects" section of our main protocol). Only 1 in 10 animals who were administered botox treatment showed signs of PIP compared to approximately 3 in 5 that showed PIP with no botox.

We are asking for approval to administer botox on the day of the grafting procedure because there have been a few incidents where animals were unable to undergo the grafting procedure in the time frame described in our protocol (based on clinical reasons such as weight loss and PEG tube problems). Delaying grafting allowed the animal to recover more fully following contusion and enable a more successful grafting surgery and survival. However, delaying the grafting procedure also reduced the time period in which nociceptors are turned off AFTER the grafting procedure. In the case where the grafting procedure takes place 2 months or longer following contusion, we would like the option to administering a second dose of botox.

3. Potential Adverse Effects:

In our "Botulinum Toxin" amendment approved on May 29th, 2014, we review data from Scott and Suzuki (1988) that shows our typical dosing of approximately 60 units is far below the toxic dose for a rhesus monkey (toxic = ~264 units). Further, the prescribing information included with the botox lists 360 units in a 3-month period as the maximum safe dose. The administration of a 2nd dose of botox 2 or more months following the first dose would increase the total dose to approximately 120 units in a 60-day (or longer) period, which does not exceed either these maximums.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[REDACTED] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact

12 Approved Amendment(s):

Approved: 9-Apr-2015

Collar Use Without Restraint Chair Testing

1. Proposed Changes:

We request the option to use a collar for rehabilitative purposes in animals who are not undergoing behavioral testing in the restraint chair.

Following spinal cord injury, it is typical for individuals to show a range of mobility. Our research group has developed a variety of techniques to promote movement in animals following spinal cord injury. One technique has been to utilize the pole-and-collar system to gently guide and assist the animal in performing activities, which promote movement. Animals who have undergone contusion injuries and who require collaring for rehabilitative purposes (see subgroup information in Section 2) will be acclimated to the collar as is described in [REDACTED] SOP FF-5, Section 5.1.1 to 5.1.3, albeit in a slightly modified way to account for the fact that the animal will have a spinal cord injury. Specifically:

- Consistent with section 5.1, animals will be given approximately 24 hours to acclimate to the collar before it is used for rehabilitative purposes. Collar fitting and underlying skin condition will be checked daily and any time an animal is sedated for any purpose.
- Collar use for rehabilitation will only be done with animals post-contusion. As a result, arm presentation and pole attachment training as is described in section 5.2 will not be used.
- Beginning at section 5.3.4 and 5.3.5, the cage door will be partially opened and secured with a padlock. The pole is then attached to the collar of the impaired animal. We will maintain control of the animal; however, we do not anticipate springing out of the cage, or any crawling and climbing. We will maintain position until the animal stops struggling, and then release pressure and allow the animal to relax at the door of the cage.
- Section 5.3.6 describes placement of the chair, which is not relevant to this amendment. Instead, a second animal handler places the jump box (with both doors removed) on the cage and helps the first animal handler place the pole through the front of the jump box and out the back. The second handler then threads the back door back on the jump box (but doesn't latch it), and then unlocks the padlock from the cage door.
- Consistent with section 5.3.7, the first handler gently guides the animal, who is unable to get into the jump box completely on his own, into the jump box. Resistance is dealt with as described in the section consistent with 5.3.5 above. Once the animal is inside the jump box, the second animal handler closes the front door of the jump box. The pole is then removed from the animal's collar and the back door of the jump box is fully closed and latched.
- Consistent with section 5.3.8, when the animal responds correctly, positive reinforcement with food rewards is offered.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

This is a safer and less stressful alternative to hand catching or other forms of physical manipulation. For much of the history of this project, animals have worn collars as a standard component of our behavioral testing routine to rehabilitate hand movements in a restraint chair. However in the recent past, our behavioral testing paradigm has expanded to include the use of in-cage tasks, which has led to less reliance on the restraint chair for rehabilitation of hand movements. As a



result, a portion of animals enrolled on this protocol have not undergone chair task testing and therefore have not needed to wear a collar. These animals may be in either Group 2 or Group 3.

An unforeseen consequence of having a subset of animals without collars is the possibility that animals may simply refuse to locomote and/or do not have the opportunity to strengthen their core/abdominal muscles. As such, having the option to place a collar and use the pole-and-collar system in the absence of the restraint chair will substantially improve the rehabilitative progress of this subset of animals. We don't anticipate the number of animals in this subset to be large (N ~ 6). Further, animals in this "N ~ 6" subset may be amenable to locomotion and activity without the use of a collar. However, in the circumstance whereby an animal **doesn't** have a collar and **is not willing to move**, we request the option to place a collar on the animal for rehabilitative purposes.

Updated Literature Search for alternatives to pole-and-collar training:

Date Databases Searched: 2015 March 18
Database: Pubmed/Medline and Primate Portal
Years Covered: 1960-present
Keywords Used: monkey AND "pole-and-collar"

Date Databases Searched: 2015 March 30
Database: Pubmed/Medline and Primate Portal
Years Covered: 1960-present
Keywords Used: monkey AND "physical therapy"

3. Potential Adverse Effects:

We anticipate no adverse effects as a result of pole-and-collar use outside those that have already been reported. In fact, we anticipate only positive side effects from the use of the pole and collar for rehabilitative purposes.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A



Institutional Animal Care and Use Committee (IACUC)

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Amendment Request(s) for Active Protocol:

[redacted] - Contusion Injury as a Model for Spinal Cord Injury - Alt. Contact

1 Request(s) Submitted: # [redacted] - 17-Apr-2015

1. Proposed Changes:

Add 4th Group

We would like to add a 4th group of animals (N = 2) that will survive for an intermediate period of time between the currently proposed groups (approximately 5-10 weeks post contusion). Animals in group 4 will also undergo only one surgery (no PEG tube, no treatment).

In Section 13.f. "Study Groups and Numbers Table," please add the following:

- Group: 4
- Species: Primate
- Number of Animals: 2
- Procedures/Treatments: Contusion only

In 14.A.4.C "Contusion Procedure," we state: *A dorsal midline incision will be made over the cervical vertebra. The spinal cord will be exposed by removing the dorsal lamina in approximately the C5 area. Next, a probe approximately 5mm in diameter will be positioned over one side of the spinal cord and advanced using an electronic device that controls speed and depth of compression, and has force feedback so the features of the injury are immediately available for assessment. We will use an approximately 4mm single compression (compression rate up to 2m/sec) to produce a consistent contusion injury.*

Beginning with the two animals proposed in Group 4, we would like to open the dura *prior* to positioning the probe as described in the above paragraph. We have not previously opened dura, so these first two animals will be pilot animals who will survive for approximately 5-10 weeks post-contusion. Following the contusion injury, the dura will be closed and the remaining closing will be done as described in 14.A.4.E of the parent protocol.

Expand Blood Collection

Currently, we are approved to collect blood at the time of the contusion injury and at the time of perfusion. For all animals enrolled in this protocol, we would like the option to collect blood at the additional time points listed below. All blood will be collected by trained [redacted] staff according to SOP F-10 and in accordance with blood collection guidelines described in [redacted] SOP GG-5.

- 1-day post contusion
- 1 week post-contusion
- 2 weeks post-contusion
- 6 weeks post-contusion

Uploaded File(s):

2. Justification for Proposed Changes:

Add 4th Group

Opening the dura enables greater visualization of the midline of the spinal cord, which will provide greater consistency of injury across animals. The group of two pilot animals with an intermediate survival period is proposed in order to determine how opening the dura will affect:

1. the biomechanical readout from the electronic contusion device at the time of injury,
2. the behavioral recovery of the animal (which is currently well predicted by the biomechanical readouts), and



3. the histological readouts of the lesion

We need the pilot animals to survive longer than 48 hours in order to collect sufficient behavioral data; however, survival past 10 weeks will likely not be substantially more informative. As such, we request the intermediate survival time of 5-10 weeks. If this procedure produces more consistent injuries, we would like to remove the dura on future contusion procedures performed on animals enrolled in other groups on this protocol.

Expand Blood Collection

The collection of blood at these additional time points will enable us to better characterize the immunological response to spinal cord contusion injury.

3. Potential Adverse Effects:

Add 4th Group

Our research group routinely opens the dura for other procedures described on this and other protocols (e.g. hemisection, delivery of stem cells, injection of treatments into the cord) without incident and do not anticipate problems with this procedure. If a blood vessel on the surface of the cord is damaged during the impact, application of gelfoam with light pressure will be used to stop bleeding, and then dural closure will commence.

Expand Blood Collection

No additional adverse effects are expected beyond those described in the parent protocol.

4. Additional Animals:

We are adding a 4th group of animals, n = 2. We request 2 additional animals be added to this protocol.

5. Justification for Additional Animals:

This is a pilot study. Two subjects will be sufficient for us to determine the effect of opening the dura on our biomechanical, behavioral, and histological outcomes.

Amendment Request Status: Assigned and Being Reviewed

IACUC Staff Reviewer:	[Redacted]
Telephone:	[Redacted]
Email:	[Redacted]

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[REDACTED] - Contusion Injury as a Model for Spinal Cord Injury ▼

15 Approved Amendment(s):

Approved: 7-May-2015

Add 4th Group of Animals PLUS Expand Blood Collection ▼

1. Proposed Changes:

Add 4th Group

We would like to add a 4th group of animals (N = 2) that will survive for an intermediate period of time between the currently proposed groups (approximately 5-10 weeks post contusion). Animals in group 4 will also undergo only one surgery (no PEG tube, no treatment).

In Section 13.f. "Study Groups and Numbers Table," please add the following:

Group: 4

Species: Primate

Number of Animals: 2

Procedures/Treatments: Contusion only

In 14.A.4.C "Contusion Procedure," we state: *A dorsal midline incision will be made over the cervical vertebra. The spinal cord will be exposed by removing the dorsal lamina in approximately the C5 area. Next, a probe approximately 5mm in diameter will be positioned over one side of the spinal cord and advanced using an electronic device that controls speed and depth of compression, and has force feedback so the features of the injury are immediately available for assessment. We will use an approximately 4mm single compression (compression rate up to 2m/sec) to produce a consistent contusion injury.*

Beginning with the two animals proposed in Group 4, we would like to open the dura *prior* to positioning the probe as described in the above paragraph. We have not previously opened dura, so these first two animals will be pilot animals who will survive for approximately 5-10 weeks post-contusion. Following the contusion injury, the dura will be closed and the remaining closing will be done as described in 14.A.4.E of the parent protocol.

Animals in this group will be excluded from motor telemetry implants, treatment procedures (as described in 14.A.4.C and the stem cell amendment), and tracer procedures. Animals in this group may undergo behavioral testing, a single pre-contusion and post-contusion MRI, botox injections, BrDU infusions, and/or be subject to diet manipulations and collar use without restraint chair testing.

Expand Blood Collection

Currently, we are approved to collect blood at the time of the contusion injury and at the time of perfusion. For all animals enrolled in this protocol, we would like the option to collect blood at the additional time points listed below. All blood will be collected by trained [REDACTED] staff according to SOP F-10 and in accordance with blood collection guidelines described in [REDACTED] SOP GG-5.

- 1-day post contusion
- 1 week post-contusion
- 2 weeks post-contusion
- 6 weeks post-contusion

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

Add 4th Group

Opening the dura enables greater visualization of the midline of the spinal cord, which will provide greater consistency of injury across animals. The group of two pilot animals with an intermediate survival period is proposed in order to determine how opening the dura will affect:

1. the biomechanical readout from the electronic contusion device at the time of injury,
2. the behavioral recovery of the animal (which is currently well predicted by the biomechanical readouts), and
3. the histological readouts of the lesion

We need the pilot animals to survive longer than 48 hours in order to collect sufficient behavioral data; however, survival past 10 weeks will likely not be substantially more informative. As such, we request the intermediate survival time of 5-10 weeks. If this procedure produces more consistent injuries, we would like to remove the dura on future contusion procedures performed on animals enrolled in other groups on this protocol.

Expand Blood Collection

The collection of blood at these additional time points will enable us to better characterize the immunological response to spinal cord contusion injury.

3. Potential Adverse Effects:

Add 4th Group

Our research group routinely opens the dura for other procedures described on this and other protocols (e.g. hemisection, delivery of stem cells, injection of treatments into the cord) without incident and do not anticipate problems with this procedure. If a blood vessel on the surface of the cord is damaged during the impact, application of gelfoam with light pressure will be used to stop bleeding, and then dural closure will commence.

Expand Blood Collection

No additional adverse effects are expected beyond those described in the parent protocol.

4. Additional Animals:

We are adding a 4th group of animals, n = 2. We request 2 additional animals be added to this protocol.

5. Justification for Additional Animals:

This is a pilot study. Two subjects will be sufficient for us to determine the effect of opening the dura on our biomechanical, behavioral, and histological outcomes.



A. **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], MD, PhD	[Redacted Signature]	7/30/15

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, DACLAM		7/30/2015
Name of IACUC Chair	Signature	Date
[Redacted], PhD	[Redacted]	7/30/2015

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], MD, PhD	[REDACTED]	7/30/15
Name of Institutional Veterinarian	Signature	Date
[REDACTED], DVM, DACLAM		7/30/2015
Name of IACUC Vice-Chair	Signature	Date
[REDACTED], PhD	[REDACTED]	7/30/2015

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]		7/30/2015

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
N/A		

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], MD, PhD	[REDACTED]	7/30/15

Departures from “Must” and “Should” Standards in the *Guide*. No signatures required.

Secondary Just-In-Time ACORP Review

PI	STATION	CYCLE	APPLICATION TITLE
██████████	San Diego, CA-664	MERIT/Summer 2014	The VA Gordon Mansfield SCI Consortium NHP ██████████ protocol ██████████

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

(cont.)

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

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

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

REVIEWER FEEDBACK

ACORP Item number(s) (score)	Comments/Concerns
ACORP (NHP)	This protocol uses rhesus monkeys to develop a reproducible contusion model of spinal cord injury and understand the effects of this type of injury. The investigator and his team are commended for his efforts to reduce the number of animals used in the proposed study. Some concerns were identified.
Items 7 and 15 (1)	<p>The investigator states that the spinal cord injury will affect the monkey's daily activity immediately post surgery. During the first week, they may be able to make rudimentary movements and may recover the ability to locomote in time. The nursing care for these monkey is challenging and the investigator has provided detailed information about many aspects of the care plan; however, some aspects need to be clarified:</p> <ul style="list-style-type: none"> • During the first week after surgery, please elaborate on the rudimentary movements the monkey can likely make. • The investigator indicates that after surgery the monkey is turned every 2-4 hours between 6am and 10pm by trained staff. By what means is this accomplished? • How does the monkey access food and water during the first week after hemi-section surgery? • How is skin care managed to prevent fecal soiling and urine scalding? • How is it determined that monkey has recovered enough function to advance to the open-field cage training? • Is the open-field cage training continuously supervised by trained staff members who could intervene if necessary?
Items 13, 14 and Appendix C (1)	Item 13- table f) study groups and numbers table indicates three experimental groups, with Group 1 surviving 48 hours and Group 2 and 3 surviving 6 months. Item 14.8 Terminal Procedures Under Anesthesia

(cont.)

	<p>states that survival time is 12 months but Appendix C indicates that monkeys are exempted from social, food, and cage enrichment for 36 months. It would be helpful to the understanding of this protocol to include a flowchart with a timeline (including the survival time for each group) that indicates all the procedures and manipulations that an individual monkey of a given experimental group may undergo. Please reconcile.</p>
<p>Item 14 (1)</p>	<p>In regard to treadmill training and locomotion, the length of treadmill walking varies from 15-45 minutes per session. Will the treadmill walking be stopped if the monkey appears to be tired? Will the treadmill stop automatically if the monkey falls? Are the animal's tarsal and plantar pads checked for injury before and after each treadmill session?</p> <p>When are the skin sutures removed from healed incisions?</p>
<p>Amendment (add stem cell treatment) (1)</p>	<p>The stem cell treatment description lacks sufficient detail. The amendment should stand alone, please fully describe "NeuralStem" stem cells or UCSF4 stem cells procedure. Appendices 3 and 5 of the VA ACORP form may be used as a template to ensure the procedure is adequately described.</p> <p>Monkeys that undergo immunosuppression will receive three drugs (i.e. tacrolimus, mycophenolate mofetil, and prednisone) simultaneously; is that correct?</p> <p>In regard to percutaneous endoscopic gastrostomy (PEG) tube placement, the investigator notes that even when properly placed, approximately 10-20% become displaced. The investigator notes that if a PEG tube leaks, a different internal bumper will be used or an alternate method using a NHP jacket will be used. On average how many PEG tube surgeries or alternative procedures does an individual monkey undergo?</p>
<p>Amendment (add gadolinium to MRI procedures) (1)</p>	<p>Would Magnevist/gadolinium contrast agent be administered every time an MRI procedure is performed? Presumably, an IV catheter will be placed; please address and elaborate further on the contrast agent administration.</p> <p>Are animal pretreated with any of the medications listed (metoclopramide, maropitant, ondansetron, or diphenhydramine) to possibly offset the risk of adverse reactions?</p>
<p>Amendment (modifications to diet, immunosuppression, and PEG Procedure) (1)</p>	<p>The investigator indicates that some monkeys may require reduced chow for behavioral testing purposes; when utilized, restriction will apparently be specific to each monkey. The brief description suggests the monkey is being food restricted to encourage the animal to work harder to receive food rewards during testing. If this is the case, the investigator needs to provide more detailed information, such as the percentage that an individual animal's food will be reduced by (e.g. 10%?), how long the food restriction will be in place, and the monitoring plan to ensure adequate nutrition and body weight. Note: the <i>Guide</i> (pg. 31) states "Body weights should be recorded at least weekly and more often for animals requiring greater restrictions (NRC 2003b)." Please address.</p>

(cont.)
