

**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 of [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
Main Body	
A.1 Full Name of PI(s)	VASDHS page 1
A.2 VA Station Name and Number	VASDHS page 1
A.3 Protocol Title	VASDHS page 1
A.4 Animal Species covered	Section 4.3 VASDHS page 5
A.5 Funding Source(s)	Section 10, VASDHS page
A.6.a Project Title and date of R&D Committee Approval	Section 6 VASDHS page 6 VASDHS Approval letters (pages 1-4)

	[REDACTED] Approval Letters, Pages 7-8
A.7 Type of animal Use	Section 4.5, VASDHS page 6
B. Description of Relevance and Harm/Benefit Analysis	Section 5, VASDHS Pages 9
C.1 Lay Summary	Section 12, VASDHS Pages 10
C.2 Complete description of the proposed use of animals (including experiment plan, number justification, and procedural descriptions)	Section 14, VASDHS Pages 12-16 Modifications pages 25-47
D. Species	Section 4 VASDHS page 7
E. Personnel qualifications and training	Section 18, VASDHS Page 20-23
F. Training to be provided	N/A
G. Occupational Health and Safety	Section 18, Appendix A, VASDHS Page 23-24
H. Animals to be Used	Section 13, VASDHS Page 12
I. Numbers of animals requested	Section 13f, VASDHS Page 12
J. Management of USDA Category D procedures	Section 7f, VASDHS Page 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 24
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 9, VASDHS Page 15
S. Surgery	Section 9, VASDHS Page 13-14
T. Endpoint Criteria	Section 15, VASDHS Page 17-20
U. Termination or removal from the protocol (including euthanasia methods and other disposition)	Section 16, VASDHS Page 20 Section 14, VASDHS Page 16
V. Special Procedures	Section 9, VASDHS Page 10
W. Consideration of Alternatives and Prevention of Unnecessary Duplication	Section VASDHS Pages 11-12
X.1 Controlled drugs	Section 14b, VASDHS Page 16-17
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 48-51
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 16-17
2. Summary of How Materials will be Administered	Section 14b, VASDHS Page 16-17
3. Anesthesia, Sedation, or Tranquilization	Section 14b, VASDHS Page 16-17
4. Toxic Agents	Section 8, VASDHS Page 10
5. Infectious Agents	Section 8, VASDHS Page 10
6. Biological Agents	Section 8, VASDHS Page 10
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	Appendix A, VASDHS page 23
11. Signatures	VASDHS Pages 48-51
Appendix 4 Antemortem Specimen Collection	
1. Summary of Specimens	Section 14a. 6.c VASDHS Pages 15 SOP FF-10 (p. 2)
2. Use of Anesthetics, Tranquilizers, or Analgesics	Section 14b, VASDHS Page 16-17
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, VASDHS Pages 13-14
3. Personnel	Section 14e, VASDHS Page 17
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 14b, VASDHS Page 16-17
5.c Pre-operative preparation of the surgical site	SOP II-02 (p.12)
6.a Intra-operative medications	Section 14b, VASDHS Page 16-17
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 17
7.c Post-operative analgesia	Section 14b, VASDHS Page 16-17
7.d Other Post-operative medications	Section 14b, VASDHS Page 16-17
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 48-51
Appendix 6 Special Husbandry and Procedures	
1. Table of procedures	Sections 7, 9, 14.a.2, VASDHS Pages

	9,10,12-16
1.a Complete description of each procedure	Section 7, VASDHS Pages 12-16
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, DAACLAM	[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

DEPARTMENT OF VETERANS AFFAIRS VA
San Diego Healthcare System - 664
3350 La Jolla Village Dr.
San Diego, CA 92161

Institutional Animal Care and Use Committee

Date: 06/25/2015
From: Institutional Animal Care and Use Committee
To: [REDACTED], MD, PhD
Subject: APPROVED: Renewal of UC Davis Animal Use Protocol # [REDACTED]
Stem Cell Therapy for Treatment of Spinal Cord Injury [REDACTED]
Title: [REDACTED]
Species: Rhesus Macaque
VA R&D Project #: [REDACTED] ([REDACTED])
CC: VA Research & Development Committee
IACUC Initial Approval Date: 07/11/2013
Annual IACUC Approval Date: 06/26/2015
3-year expiration Date: 06/25/2018

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 (07/11/2013), your protocol is renewed until 06/25/2016, 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

DEPARTMENT OF VETERANS AFFAIRS VA
San Diego Healthcare System - 664
3350 La Jolla Village Dr.
San Diego, CA 92161

Institutional Animal Care and Use Committee

Date: 07/09/2014
From: Institutional Animal Care and Use Committee
To: [REDACTED], MD, PhD
Subject: **APPROVED: Renewal of UCSD Animal Use Protocol # [REDACTED] Stem Cell Therapy for Treatment of Spinal Cord Injury ([REDACTED])**
Title: [REDACTED]
Species: Rhesus Macaque
VA R&D Project #: [REDACTED] ([REDACTED])
CC: VA Research & Development Committee
IACUC Initial Approval Date: 07/11/2013
Annual IACUC Approval Date: 06/26/2014
3-year expiration Date: 06/25/2016

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 continuing review approval (06/26/2014), your protocol is renewed until 06/25/2015, 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: UCD Animal Use Protocol # [REDACTED]**
Stem Cell Therapy for Treatment of Spinal Cord Injury ([REDACTED])
Title: [REDACTED]
Species: Rhesus Macaque
Number of Animals: 30
VA R&D Project #: [REDACTED] ([REDACTED])
CC: VA Research & Development Committee
IACUC Approval Date: 07/11/2013
Annual Expiration Date: 07/10/2014
3-year expiration Date: 07/10/2016

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 [REDACTED] Protocol [REDACTED], 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 [REDACTED]

4.2 Protocol Information

Protocol PI / Number:

[REDACTED]

Date of Approval (if known)

07/11/2013

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
☒	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:		30	
Total Number Approved:		30	

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

UNIVERSITY [REDACTED]

[REDACTED]

Institutional Animal Care and Use Committee
One Shields Avenue

[REDACTED]

Fax: [REDACTED]

July 7, 2015

To Whom it May Concern:

The following application was reviewed and approved by the [REDACTED] IACUC on July 02, 2015. Active protocols are reviewed annually.

Title: Stem Cell Therapy for Treatment of Spinal Cord Injury

Principal Investigator: [REDACTED]

Protocol #: [REDACTED]

Institution: [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]

[REDACTED]

Institutional Animal Care and Use Committee
One Shields Avenue

[REDACTED]

Fax: [REDACTED]

February 25, 2014

To Whom it May Concern:

The following application was reviewed and approved by the [REDACTED] IACUC on July 11, 2013. Active protocols are reviewed annually.

Title: **Stem Cell Therapy for Treatment of 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: 11-Jul-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:

Stem Cell Therapy for Treatment of Spinal Cord Injury

3. Protocol Type:

Research

4. Species:

Common Names	Total Number for Study	Name of Source of the Animals
primate - <i>rhesus</i>	30	[REDACTED]

USDA: Yes

Detrimental Species: No

5. Brief Summary of Procedures:

Adult rhesus macaques will be implanted with small sensors to measure activity and motor control. They will then be trained to walk on a treadmill and to perform separate food retrieval tasks with their hands. Monkeys will then have a unilateral hemisection of the spinal cord that will affect motor control of one side of the body. The experimental group of monkeys will receive stem cells to stimulate neuron growth and repair. All animals will receive behavioral rehabilitation to enhance functional recovery.

6. Animal Location(s):**Study Area/Laboratory:**

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

Overnight Housing (vivarium):

Vivarium(s)
[REDACTED] er

Animals will be maintained by:

Vivarium

7. Special Husbandry Requirements:

Monkeys will have a spinal cord injury 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 at approximately 2 weeks post-hemisection and improving gradually 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 observed multiple times per day 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:

Yes - Appendix A in Section 19 will be used to gather details regarding work with hazardous materials when present in a vivarium.

Type	Material	Location
Hazardous Chemicals	Bromodeoxyuridine	Lab, Vivarium
Human Tissue	human neural stem cells (hNSCs)	Lab, Vivarium
Infectious Agents	autologous monkey neural stem cells (mNSCs)	Lab, Vivarium

9. Special Procedures and/or Activities:

Anesthetics/Tranquilizers/Sedatives
Survival Surgical Procedures
Multiple Major Survival Surgical Procedures
Prolonged Restraint

Justification for Multiple Major Survival Surgical Procedures:

Animals on this protocol will undergo multiple major survival surgeries. The order of surgeries will be as follows:

1. Motor Telemetry Implant surgery
2. Hemisection of the spinal cord
3. Administration of treatment or control substances
4. Motor Cortex Tracer surgery*

Each of these surgeries adds to the body of knowledge we can gain about recovery from spinal cord injury. The implant surgery will allow us to measure electromyography (EMG) of recovering muscle groups. Treatments administered on a separate day from the lesion surgery make our investigation more clinically relevant. 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.

*Monkeys in Group 1b will undergo motor cortex tracer surgery on the same day as the hemisection surgery.

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 function 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, but no more than 2 hours.

10. Funding Source(s):

Veterans Administration
Department of Defense
California Institute for Regenerative Medicine

11. Veterinary Care:

12. Objectives and Significance:

Objectives:

The hypothesis of this study is that neural stem cell grafts, derived either from human tissue or from the monkey's own cells, may aid in repairing the spinal cord after injury. These grafts serve a two-fold purpose: 1) to create a more growth-friendly environment in the lesion cavity, as axons do not grow through the empty spaces commonly found at sites of spinal cord injury and 2) to extend axons into the host spinal cord, forming neuronal relay circuits that carry information across the spinal cord lesion. To test for anatomical changes in response to the stem cells, we examine different central nervous

system components, most notably the corticospinal, raphespinal, coeruleospinal, and reticulospinal systems, for signs of regeneration or sprouting.

Because the ultimate goal is to restore functional motor control in the cases of spinal cord injury, we also test the monkeys' ability to walk on a treadmill, perform food retrieval tasks, and engage in various locomotor and manipulative behaviors in an open-field environment. Monkeys are first taught the behavioral tasks, then have a wireless EMG recording apparatus implanted to allow recording of muscle activity during task performance. After baseline performance parameters are established, the monkeys undergo spinal surgery to cut the right side of the spinal cord. Treatment-group monkeys receive stem cell grafts, whereas control-group monkeys receive either a lesion only or a fibrin matrix without stem cells. Following spinal surgery, the monkeys' performance on the behavioral tasks is assessed for approximately 32 weeks (except for group 1b, who will be assessed for only 12 weeks). Finally, to allow visualization of the corticospinal and reticulospinal tracts, we inject neural tracers into the monkeys' motor cortices, reticular nucleus, spinal cord, and/or target muscles. Detailed histological analyses are performed after the monkeys are euthanized, and the results are correlated with any observed functional recovery.

Our previous studies (Rosenzweig et al. 2009; Rosenzweig et al. 2010) identified spontaneous CST sprouting as a novel target for therapeutic intervention, which had not been revealed in rodent studies. Our aim is to further investigate these findings and also to extend the promising results obtained in similar experiments performed in rodent models of spinal cord injury to the more clinically relevant rhesus macaque.

Significance:

The intent of this study is to find a potential treatment for patients with spinal cord injury. These potential treatments may lead to recovering partial or complete use of their affected limbs.

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	"spinal cord injury AND "stem cell"; "spinal cord injury" AND "stem cell" AND behavior; "spinal cord injury" AND "stem cell" AND monkey	23-May-2013
PubMed/Medline	1960-present	monkey AND electrode AND spinal	27-Jun-2013
PubMed/Medline	1960-present	monkey AND bromodeoxyuridine	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "immunosuppression therapy"	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "EMG recording"	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "corticospinal tract tracer"	27-Jun-2013
PubMed/Medline	1960-present	monkey AND stereotaxic	27-Jun-2013
PubMed/Medline	1960-present	"stem cell" spinal AND monkey	27-Jun-2013
PubMed/Medline	1960-present	monkey AND craniotomy	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "percutaneous endoscopic gastrostomy"	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "telemetry implant"	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "skin biopsy"	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "bone marrow"	27-Jun-2013
PubMed/Medline	1960-present	monkey AND "restraint chair"	27-Jun-2013
Web of Science	1900-present	"spinal cord injury" AND "monkey" AND "stem cell"	23-May-2013
Primate Portal	1940-present	"hemisection" AND "monkey"; "spinal cord injury"	23-May-2013

b) Refinement:

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, the San Diego lab 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. 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).

There are currently no known *additional* alternatives to the surgical and other procedures we have described. Over the years, we have made the following changes to our procedures to minimize pain, distress, and discomfort. We will continue to look for alternatives and implement them as we are able.

1. Replaced the use of external wires in a jacket that were attached to a cutaneous button in favor of an entirely implanted telemetry system.

2. Working to replace the nasogastric intubation delivery system for immunosuppression drugs with the percutaneous endoscopic gastrostomy
3. Opted for a hemisection injury rather than a full transection injury
4. Conduct BrDU dosing while the animal is awake and in a restraint chair rather than sedating for 5 days in a row
5. Altered the timing and duration of immunosuppression (MMF starts 1 week rather than 2 weeks before, MMF stops 1 month after transplantation rather than until endpoint)
6. To improve subjects' mental and physical well-being, we introduced the exercise cage (where animals can move about in an open-field enclosure for 3 hours per week) and have developed an automated, self-initiated, in-cage rehabilitation system (which gives animals more opportunities to engage in an interesting task).

c) Has this study been previously conducted?

No

d) Replacement (Species Rationale):

There has been extensive research in spinal cord injury utilizing the rat model. While these experiments have identified promising approaches to treatment of spinal cord injury, there is a need to determine if the same treatment approaches work in a species more closely related to humans, the rhesus macaque. Our pilot work in stem cell transplantation in the non-human primate has revealed multiple challenges not observed in rodents that are likely present in humans as well. These include substantially heavier flow and greater volume of cerebral spinal fluid as well as complications resulting from immunosuppression. 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 which would be translatable to humans has occurred.

e) Reduction (Animal Numbers Justification):

We conducted a power analysis (using a standard deviation of 0.2 and an effect size of 0.4) and determined that using 6 animals per treatment group (1a, 1b, 2, and 3) will give us a power of 89%. Group 4a and 4b combined give us a group size of 6 and we do not expect there to be a difference between the two control groups. The subdivision into two groups of 3 is meant to allow comparison with historical lesion-only controls (from our own past studies) while using fewer subjects than two full control groups.

f) Study Groups and Numbers Table:

Group	Species	Number of Animals	Procedures/Treatments
1a	primate	6	Treatment Group: Hemisection + "NeuralStem" stem cells Survival Time = approximately 8 months
1b	primate	6	Treatment Group: Hemisection + "NeuralStem" stem cells Survival Time = approximately 3 months
2	primate	6	Treatment Group: Hemisection + approved UCSF4 stem cells Survival Time = approximately 8 months
3	primate	6	Treatment Group: Hemisection + transdifferentiated autologous stem cells Survival Time = approximately 8 months
4a	primate	3	Control Group: Hemisection only Survival Time = approximately 8 months
4b	primate	3	Control Group: Hemisection + Fibrin Matrix only Survival Time = approximately 8 months

14. Procedure Details:

a) Describe the use of animals in your project.

1. **ANIMAL SELECTION:** Before animals 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. If animals pass their evaluation, we will consider them for the project.

2. **BEHAVIORAL TESTING:** All animals 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. It is necessary for us to evaluate an animal's competencies before determining the exact array of behavioral testing the animal will undergo. At a minimum, animals will undergo open-field cage testing.

2.A. **Open-Field Cage Training and Exposure.** Monkeys will first be trained 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 approximately 5 times per week for 15-60 minutes per

session both before and after injury

2.B. 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.C. Treadmill Training and Locomotion. Monkeys who will undergo treadmill task testing will first be trained to walk on a treadmill. 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.

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.

2.E. Video and EMG Recording. Video recording of chair tasks, treadmill locomotion, open-field exposures, and home cage testing will be conducted both before and after injury. EMG recording will be limited to those animals who have motor telemetry implants (described in 4.B. below). For animals that have the telemetry implants, the battery pack and transmitter can be turned on using a remote control. Two to four baseline recording sessions are conducted before the hemisection and at approximately 4-, 6-, 9-, 12-, 16-, 20-, and 24-weeks post injury. These will be done for the chair and treadmill tasks, where each session lasts up to 2 hours (but typically 45 minutes). After video and EMG recording, the battery pack and transmitter are turned off. Staff will be present at all times to ensure primate safety. In addition, video recording and behavioral data collection of open-field exposures and home cage testing will also be conducted. Approximately 1-2 sessions per week will be conducted for approximately 1-2 months before hemisection and 1-2 sessions per week will be conducted after hemisection and until the animals end point.

3. BONE MARROW AND SKIN BIOPSY COLLECTION: Animals in Group 3 (who will receive autologous neural stem cells) will undergo bone marrow and/or skin biopsy collection. While the collection of bone marrow for the development of autologous cells is more biologically relevant than skin samples, the process for growth and transfection of bone marrow stromal cells is still being optimized. Therefore, animals may undergo both procedures to ensure appropriate host cells from which to generate the autologous material.

3.A. Skin Biopsy Procedure. Monkeys will be fasted and anesthetized using ketamine and possibly dexmedetomidine and atipamezole according to [REDACTED] SOP FF-1. Monkeys may also receive atropine IM, if recommended by therapeutic staff. Next, an area near the shoulder blades will be shaved and prepped with a depilatory cream (such as Nair®). Then using a dermatome, a thin piece (approximately 1-2mm) of skin will be sliced. If necessary, the biopsy site will be sutured with 3-0 vicryl. If given dexmedetomidine, atipamezole IM will be given as a reversal agent. Following the procedure, animals will receive either ketoprophen IM or acetaminophen orally for pain management. Monkeys will also receive either cefazolin (IM) or cephalixin (oral) to prevent infection. The skin biopsy sample will be shipped to [REDACTED] for culture and transgene insertion.

3.B. Bone Marrow Collection Procedure. Bone marrow will be collected in accordance with SOP GG-5 by a member of the therapeutic staff. Based on a recommendation from our Clinical Labs here at the [REDACTED], we will need bone marrow that contains 75,000 nucleated cells. Under most circumstances, we will be able to accomplish this within a single sedation; however, we may occasionally require more than one sedation to reach this target cell number. Bone marrow volumes will fall within [REDACTED] guidelines. We will do up to 3 sedations for bone marrow collection per monkey and aspirate for bone marrow up to 4 times per sedation. If multiple sedations for bone marrow collection are necessary, we will wait 2 weeks between successive attempts. Following the procedure, animals will receive either ketoprophen or acetaminophen for pain management. The bone marrow sample will be shipped to [REDACTED] for culture and transgene insertion.

4. SURGICAL PROCEDURES: This section describes all survival surgeries that will take place in this protocol. At most, animals will receive 4 survival surgeries: telemetry implants (4.B), hemisection (4.C.), treatment or control surgery (4.D.), and tracer surgery (4.E). There may be other procedures described in other sections of this summary which occur between these described survival surgeries; 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 [REDACTED] SOP FF-1. Animals will then be prepped for surgery according to [REDACTED] SOP II-1 & II-12.

4.B. Motor Telemetry Implant Surgery. Animals may undergo a motor-telemetry implant surgery to enable EMG recording of target muscle groups. There are 8 motor telemetry implants that are shared between the [REDACTED] and the [REDACTED] laboratory. At this time, it is not possible to know when and how many implants will be available. Some animals on this protocol will receive implants and some will not.

EMG will be recorded from muscle targets on the impaired side and possibly non-impaired side of the body (depending on the model of implant that is available to use at the time of surgery). Internal EMG electrodes (Konigsberg Instruments, model T33F-1B OR T33F-4) will be implanted into various muscles of the right and possibly left arm to record muscle activation during behavioral tasks. We will use one telemetry implant and -- if implanting both arms -- will split the leads to implant both the right and left sides of the body. Whether one or both arms are being implanted, only one battery source and wireless transmitter will be implanted. The battery source and transmitter are encased in a silicone sheath with rounded edges (measuring 1½" x 2" x 3/8") and will be implanted 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. Muscle and skin layers of the will be closed according to the closing procedures described in 4.F. below.

4.C. Hemisection Procedures. All monkeys on this protocol will undergo a hemisection injury. A dorsal midline incision will be made over the cervical vertebral area. The spinal cord will be visualized by removing some of the cervical spinous processes in the C4-C8 area. Next, a unilateral hemisection will be made with a stereotaxically guided knife to lesion half of the spinal cord. Last, we will verify that a complete hemisection has been made by visualizing the most ventral portion of the cord. The spinous processes will not need to be stabilized because they will be minimally disrupted.

4.D. Treatment and Control Substances. One to four weeks following the hemisection (which is a clinically relevant time point), animals will be prepped for surgery as described in 4.A. and a dorsal midline incision will be made over the cervical vertebral area and the spinal cord visualized. Then, animals will receive one of the following treatment or control substances into the lesion site. Our current [REDACTED] protocol (# [REDACTED]) has not yet been updated to reflect the range of stem cells we will use. We will not begin any work using the new stem cell lines until we have received approval from both the IACUC and [REDACTED] committees.

4.D.i. Treatment: "NeuralStem" stem cells (n = 12)

4.D.ii. Treatment: [REDACTED] 4 stem cells, NIHhESC-10-0044 (n = 6); Details: http://grants.nih.gov/stem_cells/registry/current.htm?id=200

4.D.iii. Treatment: Transdifferentiated Autologous stem cells (n = 6)

4.D.iv. Control: Fibrin Matrix only (n = 3)

4.D.v. Control: Hemisection Only (n = 3)

4.E. Tracer Procedures. Animals will undergo a corticospinal tract tracer surgery on the same day as the hemisection (Group 1b) or approximately 6 months after the treatment (or control) surgery (described in 4.D) has been conducted (all other groups). Labeling the corticospinal tract enables detailed histological analysis of the altered pathways of the injured spinal cord.

4.E.i. Tracers. The tracers that will be used 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.E.ii Procedure. A midline incision will be made, then a craniotomy approximately 2x4cm will be made using a sterilized surgical drill. Approximately 125µl of tracer will be injected per cortical hemisphere across a maximum of 75 sites (typically 50-60 sites) per hemisphere. Tracing will be done in only one hemisphere (for Group 1b) or both hemispheres (for all other groups).

4.F. Closing Procedures. Due to the length of these surgeries, it is not likely that the medetomidine will need to be reversed; however, if recommended, animals will receive atipamezole IM as a reversal. Monkeys will receive post-operative analgesics and antibiotics as described in Section 14b. 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.F.i. Cervical Area. The dura of the spinal cord will be closed with 6-0 vicryl, and 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.F.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. BROMODEOXYURIDINE (BrDU) DOSING: Following the injury surgery, animals will receive BrDU, which labels dividing neurons. This will enable histological analysis of cell genesis and differentiation in both grafted tissue and host tissue. 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. 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.

5.B. Timing and Duration of administration. BrDU will be administered daily from 1 day to 6 months post-injury for a maximum of 7 consecutive days (typically only for 3-5 consecutive days). 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.

6. IMMUNOSUPPRESSION PROTOCOL: Animals in groups 1a, 1b and 2 will undergo immunosuppression therapy beginning approximately 1 weeks prior to stem cell transplantation and until their endpoint (See Table 13f for endpoints). This therapy helps to ensure that the stem cells will survive and not be rejected by the body.

6.A. Drugs.

6.A.i. *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).

6.A.ii. *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.

6.A.iii. *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.

6.B. Options for Dosing.

6.B.i. 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 [REDACTED] SOP II-12).

6.B.ii. As an alternative to nasogastric tube dosing, we may place a **percutaneous endoscopic gastrostomy (PEG) tube**. The PEG tube will be placed using the pull method (an outpatient procedure) approximately 2 weeks prior to hemisection:

- 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.

Because this is a newer technique, we will go through a series of practice trials before implanting this into a project animal. The first trial run has already been done (following an approved amendment under a previous protocol) -- the procedure was conducted on a cull animal that had just been euthanized. The second trial will be conducted on a cull animal that will be euthanized immediately *after* the procedure has been completed. The third trial will be conducted on a cull animal that will survive approximately 2 weeks following the procedure. If all of these practice trials are successful, we will perform the PEG tube placement in a project animal.

6.B.iii. 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.

6.C. 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.

7. TERMINAL PROCEDURES UNDER ANESTHESIA: Following the transplantation surgery, monkeys will remain on study for approximately 3 months (**Group 1b**) or 8 months (**Groups 1a, 2, 3, and 4b**) and then be euthanized. Monkeys in **Groups 4a** will stay on study for approximately 8 months following the injury surgery. Monkeys showing unanticipated severe sequelae will be euthanized prior to the planned endpoint. Brains and spinal cords, and possibly limbs will be collected.

12.A. **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.

12.B. **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	Atropine	0.04mg/kg	Intramuscular (IM)	SID For skin biopsy, bone marrow, and/or surgery preparation or as otherwise needed
primate	botulinum toxin	~60 units	Subcutaneous (SC)	Amendments approved 1/23/14 and 2/5/15
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
primate	Oxymorphone	0.15mg/kg	Intramuscular (IM)	TID for 3 days following surgery and additionally when recommended by vet staff
primate	Ketamine	up to 30mg/kg	Intramuscular (IM)	Prior to immobilization. May also be given IV or CRI
primate	Fentanyl	up to 10 mcg/kg/hour	Intravenous (IV)	For the first 24 hours following hemisection, if the animal will tolerate a catheter
primate	Midazolam	0.1 mg/kg	Intramuscular (IM)	As an alternative to haloperidol In response to self-injurious behavior, as needed
primate	Diphenhydramine	2-4 mg/kg	Intravenous (IV)	see amendment approved 4/18/14
primate	Haloperidol	up to 0.05mg/kg	Intramuscular (IM)	In response to self-injurious behavior, as needed
primate	Neurontin	up to 60mg/kg	Oral	In response to self-injurious behavior, as needed
primate	BrDU	50mg/kg	Intravenous (IV)	Daily for a maximum of 7 days from 1 to 6 months post-injury
primate	Dexmedetomidine	up to 0.015 mg/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	Magnevist/gadolinium	0.2 ml/kg	Intravenous (IV)	see amendment approved 4/18/14
primate	Reglan (metoclopramide)	0.20-.5mg/kg	Intramuscular (IM)	see amendment approved 4/18/14
primate	Cerenia (maropitant)	1 mg/kg	Subcutaneous (SC)	see amendment approved 4/18/14
primate	Ondansestron	2 mg/kg	Intramuscular (IM)	see amendment approved 4/18/14
primate	Ondansestron	2mg/kg	Intravenous (IV)	see amendment approved 4/18/14
primate	Diphenhydramine	1-4 mg/kg	Intramuscular (IM)	see amendment approved 4/18/14
primate	Ketoprofen	up to 2mg/kg	Intramuscular (IM)	Following skin biopsy and additionally as recommended by vet staff <u>up to 1 week before injury surgery</u>
primate	Acetaminophen	up to 16 mg/kg	Oral	TID As recommended by vet staff for pain management

primate	Buprenex	up to 0.03mg/kg	Intramuscular (IM)	As recommended by vet staff for pain management TID as needed.
primate	CellCept	up to 100mg/kg	Oral	up to 2x daily beginning up to 14 days before stem cell transplantation and until endpoint
primate	Prednisone	up to 2mg/kg	Oral	daily, beginning up to 1 day before stem cell transplantation and until endpoint
primate	Tacrolimus	up to ~3 mg/kg	Oral	up to 2x daily beginning up to 2 days before stem cell transplantation and continuing until the end date

c) Anesthesia Monitoring:

General Anesthesia. 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.

e) Surgery:

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

Building	Room	Surgeon(s)
[REDACTED] Building	[REDACTED]	[REDACTED]

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.

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 may experience discomfort following the injury surgery. Complications could include head stupor, head pressing, and altered motor function; however, these complications are not anticipated.

Post Tracer Surgery: There is a small possibility that the skull cap removed at the start of the procedure may become detached. 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, some monkeys experience perforation of the tympanic membrane. Animals with tympanic membrane ruptures are treated with antibiotics and are evaluated periodically to ensure the membranes are healing. Furthermore, to minimize the incidence of ruptures,

personnel placing animals in the stereotaxic apparatus receive training prior to attempting placement.

POST PROCEDURE

BrDU Administration: Monkeys may show poor appetite.

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.

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.

LONG TERM EFFECTS

Weight Loss: Weight loss is a specific criterion 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 criterion: 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:

1) Brown Séquard 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). Wound classification "1" or "2" are an expected consequence of the hemisection injury.

2) 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 suggest 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 level of depression will be monitored by therapeutic staff to determine the well-being of the monkeys.

POST PROCEDURE

BrDU Administration: 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.

Immunosuppression: 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.

PEG Tube Placement: 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.

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?

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.

POST PROCEDURE

BrDU Administration: 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.

Immunosuppression: 1. We will utilize special PPE requirements to minimize contamination from other areas of the primate center whenever animals undergo immunosuppression.

2. 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. We will also treat side effects of immunosuppression drugs based on veterinary recommendation. Some examples 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

PEG Tube Placement: • In an amendment approved on our previous protocol (#15851), we described performing this procedure on a cull animal that had been euthanized. This enabled optimization of the procedure itself. • Next, we will perform this procedure on a cull animal that was under anesthesia, but who was euthanized when the procedure was completed. This gave us an idea of the body's response (i.e. blood flow, etc) to the procedure. • Last, we will perform this procedure on a cull animal that will survive up to 2 weeks following the procedure. This will allow us to learn whether there is leakage at the port entry and will reveal any other issues that might come up that we haven't anticipated. NOTE: The animal that will be used for this procedure will be on the cull list for management purposes. In other words, the cull animal has chronic diarrhea or is lame, and can continue to survive as long as long-term maintenance procedures are put into place. This type of animal will be able to survive a short, mildly invasive procedure, such as a PEG tube placement, and will have to be managed for diarrhea or lameness, etc for the short time period post-procedure. • If we determine that there is leakage at the port entry, we will either use a different internal bumper OR switch to the alternate method described in section 1 (which utilizes a non-human primate jacket). • Many of the adverse effects listed in section 15.A. are substantially minimized through the use of endoscopy.

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.

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: 1. antipsychotics (such as haloperidol) 2. sedatives (such as haloperidol) 3. benzodiazepines (such as midazolam or diazepam) 4. neuropathic analgesics (such as gabapentin)

d. Study endpoints:

FOLLOWING ALL SURGERIES and ALL PROCEDURES: 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 will be calculated using the pre-project weight, 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 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, 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 Séquard 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 a "3" or greater on the Stirling Scale for more than 14 days, despite treatment (as described in 15.c.). • Peri-Incisional Pruritis: A wound does not show signs of improvement after more than 30 days, despite treatment. • An animal suffers a severe wounding incident - involving deep muscle or major physical impairment - that can't be surgically repaired or treated using other routine veterinary medical care.

16. Euthanasia:

Species	Method	Drug	Dose	Route	Justification for Physical Methods
primate	Exsanguination via paraformaldehyde perfusion	Pentobarbital	about 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. Amendment approved 11/13/14 for transfer options; see amendment.

18. Roster:

Name	E-mail	Occupational Health Participation	ACU 101 Training	Qualifications/Experience	Status
[REDACTED]	[REDACTED]	2-Jul-2007	9-Mar-2013	Dr. [REDACTED] (Ph.D.) 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 included instruction on how to do contusion injuries, section the spinal cord, and perform intrathecal application of drugs. [REDACTED] is	Approved

				included in our personnel roster to consult on and perform behavioral testing procedures and to be a back-up surgeon.	
[REDACTED]	[REDACTED]	31-Oct-2006	3-Jan-2013	Dr. [REDACTED] (PhD) has 7 years experience working with nonhuman primate tissues. [REDACTED] has been collaborating with Dr. [REDACTED] on this study for the last 7 years. Dr. [REDACTED] has been included on our protocol personnel roster to be a non-sterile assistant during surgical procedures and has received training on these tasks from the protocol PI.	Approved
[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] 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 all aspects of the study and provides project support.	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] is familiar with and mastered all aspects of the study and provides project support.	Approved
[REDACTED]	[REDACTED]	13-Nov-2008	2-May-2014	Dr. [REDACTED] (DVM, PhD) has performed neurosurgeries, including dorsal laminectomies and spinal cord injuries in rats	Approved

				<p>since 2003. [REDACTED] has performed neurosurgeries, including craniotomies, dorsal laminectomies, spinal cord injuries in primates under the guidance of Dr. [REDACTED] since 2008. [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] (PhD) has over 8 years experience working with nonhuman primates. He has been collaborating with Dr. [REDACTED] on this study for the past 5 years. He 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 motor 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] (Ph.D.) has over 30 years experience working with nonhuman primates. [REDACTED] is an expert in the field of motor telemetry implantation and has been collaborating with Dr. [REDACTED] on this study for the past 15 years. Dr. [REDACTED] is included on our personnel roster to conduct motor telemetry implant surgeries.</p>	Approved
[REDACTED]	[REDACTED]	9-Mar-2012	5-Jan-2015	<p>Dr. [REDACTED] (PhD) 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 on all procedures by the protocol PI.</p>	Approved
[REDACTED]	[REDACTED]	5-May-2011	20-Feb-2015	<p>Dr. [REDACTED] (PhD) has 12 years of experience working with nonhuman primates and in the area of behavior and neuroscience.</p>	Approved

				[REDACTED] is the SRA managing all study activity on a daily basis.	
[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 the principle PI on the grants funding this work and is the primary surgeon for the cortical injections and stem cell transplantations.	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.	Approved
[REDACTED]	[REDACTED]	2-Jul-2007	25-Feb-2013	Dr. [REDACTED] (PhD) 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 collaborating with Dr. [REDACTED] on this study for the past 6 years. Dr. [REDACTED] is included on our personnel roster to conduct motor telemetry implant surgeries. [REDACTED] has been trained in all procedures by Dr. [REDACTED].	Approved

Protocol # [REDACTED] - Appendix A - Room/Lab Safety Information
1. Material(s):
autologous monkey neural stem cells (mNSCs) Bromodeoxyuridine human neural stem cells (hNSCs)
2. Provide a short description of the agent:
Bromodeoxyuridine (BrDU) is a white crystalline water-soluble compound that is frequently used in cancer treatments/research for inducing increased susceptibility to radiation therapy, and as a topical anti-viral treatment. It has also shown to be "taken up" by actively dividing, newly formed cells, thus allowing for histological analysis of neurogenesis.
Human Neural Stem Cells are human neural progenitor cells derived either from fetal tissue or from an NIH-approved embryonic stem cell line (WiCell H9, NIH code WA09).
3. This agent/material is hazardous for:
Humans and Animals <i>For which animal species?</i> primate
4. The agent can be spread by:
Does not leave animal

5. Describe any human health risk associated with this agent:

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.

Human embryonic stem cells can never be shown to be free of all human pathogens, once injected into the animal they should not pose a risk to animal care personnel.

6. The precautions below apply to this experiment:

- a. The following items must be assumed to be contaminated with hazardous material:
- b. Cages must be autoclaved before cleaning - No
- c. Label cages and remove label after decontamination - No
- d. Animal carcasses must be labeled and disposed of as follows:
Incineration
- e. All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:
Use Standard Facility Disposal Method
- f. The researcher or their technicians are responsible for the feeding and husbandry of these animals - No

7. Personal Protective Equipment Required:

- a. The following personal protective equipment must be worn/used in the room:
Lab Coat/Coveralls
Disposable Gloves
Eye Protection/Face Shield
Shoe Covers/Booties
Other - Gown (and shoe covers) when any animals are undergoing immunosuppression
- b. Personal protective equipment must be removed before leaving the room/ante room - Yes
- c. Personal protective equipment must be discarded or decontaminated at the end of the project - Yes
- d. Hands and arms must be thoroughly washed upon leaving the room - Yes
- e. Full shower, including washing of hair, must be taken upon leaving the room - No
- f. Decontaminate Room (Inform vivarium area supervisor when cage and/or room can be returned to general use) - No

8. Provide any other information needed to safely work in this room:

Our approved [REDACTED] protocol number is [REDACTED] Our approved [REDACTED] protocol number is # [REDACTED]

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: 12 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. On occasions when project testing is not occurring or during periods of SRA vacation, 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: 12 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)

Protocol Amendment Information

This page will only display amendments that were submitted using the online system. Amendments approved prior to June 26, 2008 can be viewed by opening the approved protocol. They will be located at the end of the approved protocol form.

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[IACUC Menu](#) | [Add New Request](#) **Approved Amendment(s) for Active Protocol:**

[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved: 22-Aug-2013 ▼

1. Proposed Changes:

We would like to add the option of somatosensory evaluation for animals on this protocol.

Description. The von Frey hair aesthesiometer is a light touch sensory evaluation method that uses nylon filaments of varying diameters and 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.

Procedure. While the monkey is awake and in a stationary position in a 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. The filament will be applied to approximately half a dozen different locations on both the left and right side of the body (in succession, not simultaneously), including the shoulder, thorax, hand, hip, knee, and foot. Testing sessions typically last approximately 30 minutes, but no more than one hour.

Uploaded File(s):
No Files Found.

2. Justification for Proposed Changes:

Following injury, monkeys may experience a disruption in sensory function resulting in dysesthesia. This procedure will enable us to establish baseline sensory function and quantify the change that results following injury as well as recovery over time. Animals need to be in a restraint chair to ensure the animal stays in a fixed position where each of the body areas can be touched in the same spot across all testing sessions. Without the use of the restraint chair, animals would likely not cooperate and any data collected would likely not be usable.

3. Potential Adverse Effects:

No adverse effects are expected as a result of this procedure. No special changes are needed in animal care and treatment based on this procedure. If we learn that animals are experiencing increased sensitivity that may indicate chronic pain, we will inform veterinary staff so that appropriate therapy can be initiated.

4. Additional Animals:

N/A

Institutional Animal Care and Use Committee (IACUC)

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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved: 5-Dec-2013

Add External Anal Sphincter Recording ▼

1. Proposed Changes:

During up to 3 pre-injury and up to 10 post-injury recording sessions, we would like the option of recording EMG from the EAS muscle of monkeys in Groups 2 and 3. Pre-injury recording sessions will be at least 2 weeks apart. Post-injury recording sessions will be at least one month apart. Further, post-injury recording sessions will be dependent on overall health of the animal as recommended by a veterinarian. For example, the animal should have a stable weight (without the use of repeated orogastric tube feedings) in order for this procedure to be performed.

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). Anesthetic monitoring procedures will follow the procedures outlined in the approved protocol.

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.

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.

To carry out this procedure, we would like to add [REDACTED] to our protocol as well.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

This procedure will enable us to quantify the effect of our injury procedures on autonomic function. It will also serve as a clinical measure of recovery.

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.

Updated literature search: conducted on November 19, 2013

PubMed/Medline: keywords = monkey AND "external anal sphincter muscle" AND spinal

Web of Science: keywords = monkey AND "external anal sphincter muscle"

Summary: Neither PubMed or Web of Science include publications on the use of EAS recordings to quantify the

effect of cervical spinal cord injury. One article (Schwarz et. al. 1997) indicated that this technique was effective at differentiating patients with Parkinson's from those with multiple system atrophy, based on abnormal spontaneous activity.

3. Potential Adverse Effects:

Animals may feel painful following the procedure.

Based on veterinary recommendation, following past procedures on **pre-injury** animals, we have given one dose of ketoprofen IM or IV (at up to 2mg/kg, according the [REDACTED] formulary).

Based on veterinary recommendation, following past procedures on **post-injury** animals, we have given one dose of buprenex IM (at up to 0.03 mg/kg, according the [REDACTED] formulary). For any animals who undergo this procedure within one week of injury, we would also like to use buprenex as the post-procedure analgesic in accordance with our description of the use of this drug in our 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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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact

17 Approved Amendment(s):

Approved: 23-Jan-2014

Botulinum Toxin

1. Proposed Changes:

We would like to add the option to use of botulinum toxin as a method to reduce peri-incisional pruritis following hemisection. 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 subjects is due to neuropathic pain, then this single set of botulinum toxin injections may alleviate much of this adverse effect. 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.

Updated Literature Search for alternatives to *botulinum toxin*

Date Databases Searched: 2013 December 18th

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:

NA

5. Justification for Additional Animals:

NA

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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved: 20-Mar-2014

Add Magnetic Resonance Imaging ▼

1. Proposed Changes:

All monkeys in this protocol may undergo up to 2 MRI scanning sessions pre-surgery (to establish a baseline) and up to 3 post-surgery as needed. Animals in groups 1a, 1b, and 2 will have percutaneous endoscopic gastrostomies (unless one of the alternate methods of immunosuppression drug administration is used, see section 14.A.6.B.i and 6.B.iii). The metal components of the PEG tube are titanium and thus MRI-compatible. One animal on a different protocol who has a PEG tube had an MRI in early-March with no adverse effects.

MRIs will occur at either the [REDACTED] or at MRI Facility at the [REDACTED] Annex. 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 then reversed with atipamezole (according to [REDACTED] SOP FF-1). Animals will then be prepped for the MRI, which will include placement into an MRI-compatible stereotaxic apparatus. MRI scanning will be done as described in [REDACTED] SOP II-31 using general inhalant anesthesia as described II-02. Each scanning session will last approximately 1-3 hours, depending on the MRI sequences acquired and quality of the images. If MRI images are taken, scanning will take place within approximately 6 weeks following hemisection and up to approximately every month thereafter.

Please Note: We would like to conduct the first post-surgical MRIs on our recently implanted animals (one from Group 2 and one from Group 3) during the week of March 24th. *We request that this amendment be added to the IACUC committee meeting on March 20th.*

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

MRIs will be used to assess acute and chronic changes following hemisection and/or implantation with autologous stem cells. By using a stereotaxic apparatus, we ensure quality imaging of the spinal cord area of interest (i.e. C5-C7).

3. Potential Adverse Effects:

No additional adverse effects are expected.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 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-04-\[REDACTED\].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] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact

17 Approved Amendment(s):

Approved: 29-May-2014

Extend end date for one stem cell animal

1. Proposed Changes:

We request an extension on the end date of one of our current stem cell animals. The animal is in Group 1A, and our protocol currently indicates that animals will survive approximately 8 months post-transplant surgery.

This animal underwent hemisection on September 12th, Stem Cell Transplant on October 1st, and Motor Cortex Tracer on March 31st. He is in good health with stable levels of the immunosuppression drugs, and we wish to extend his end date until approximately June 23rd (~ 9 months post-transplant).

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

Previous subjects have survived for 2.25 - 4.75 months after grafting. Anatomical analyses of those subjects suggest that the grafted cells are maturing at a fairly slow rate (possibly corresponding to the rate of maturation of human neural progenitors in vivo). This means that newly-formed neuronal relay circuits are also immature, and may not yet be fully functional. Allowing this animal to survive for approximately 9 months post-graft will provide an improved opportunity to: 1) confirm the maturation rate of the grafted cells; and 2) observe functional consequences of newly-formed circuitry. June 23rd, specifically, will be 12 weeks post-tracer surgery and is a time point at which we can still get quality labeling of the corticospinal tract.

3. Potential Adverse Effects:

As long as this animal continues to be in good health, there will be no adverse effects of extending his end date. If, however, this animal's health becomes compromised (poor appetite, diarrhea, difficulty maintaining immunosuppression levels), we will elect to perfuse at that time, pending veterinary recommendation.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact

17 Approved Amendment(s):

Approved: 12-Jun-2014

Weight Loss

1. Proposed Changes:

One animal that has undergone the PEG tube procedure (April 28 2014) and the hemisection (May 13 2014) was scheduled to undergo a stem cell transplant (June 3 2014). This surgery was cancelled due to weight loss and was immediately started on a supplemental food regimen. Our plan is to reschedule the stem cell transplant at a later date after the animal has gained some weight. This animal has also developed a series of Brown-Sequard related self-picking spots on his contralateral foot and ipsilateral hand. As of Monday (June 9), some of the spots had achieved a classification (based on the Stirling Pressure Ulcer Severity Scale described in our approved protocol) of "2," with others of a lower classification. As is described in our protocol, bandages were applied to these areas and pharmaceutical intervention has been added.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

Regarding weight loss, our current protocol states:

- The starting weight of an animal will be the weight closest to the date of assignment to the project.
- An animal that loses 20% of their body weight will have a tracer scheduled as soon as possible (if the experiment calls for a tracer) with perfusion 6 weeks later.
- An animal that loses 25% body weight will be euthanized within 48 hours.

The animal in question had a starting weight of 13.34kg (April 9, 2013).

- On the day of the PEG procedure, he weighed 12.44kg (April 28, 2014, 7% loss). This is one of the exceptions on our project where an animal loses weight after assignment. However, this animal was solid muscle with a robust appetite and had excellent behavioral skills in all of our tasks. He was the picture of health.
- On the day of his hemisection surgery, he weighed 11.35kg (May 13, 2014, 15% loss).
- On the Friday before his scheduled stem cell transplant surgery, he weighed 10.42kg (May 30 2014, 22% loss) and has a BCS of 1.5.
- As of Friday, June 6th, he weighed 10.45 kg.

Our protocol indicates a tracer surgery be conducted; however, this animal has not yet undergone treatment with a stem cell graft. As a result, tracing this animal would put him through a neurosurgery that would give us no useful information. Based on veterinary consultation, this animal was immediately enrolled in a supplemental food regimen (including cage-side supplements, project food reward items, and administration of boost via orogastric tube). Within two days, the animal appeared more bright, alert, and reactive than he had earlier in the week.

Our plan is to delay stem cell transplantation until the animal gains weight and has a BCS of 2.0. We will weigh the animal twice per week. If there continues to be an upward trend in his weight, we will continue weighing twice per week until he weighs 11.34 kg (or 15% loss). If he loses weight, the campus veterinarian will be notified and he will be assessed for BCS by two separate veterinarians. Based on veterinary recommendation, the weight gain can't be simply from increasing

the subcutaneous fat pad. We will continue to offer supplemental foods as well as provide exercise opportunities (exercise cage and treadmill, depending on tolerance and health) to help build muscle mass.

If the animal loses 25% of his body weight or has a BCS of 1.0, he will be euthanized within 48 hours.

Regarding Brown Sequard related self-picking, our protocol indicates that a series of intervention steps be undertaken when the spots are at a classification level of "2" or less. We are consistent with what our protocol has described and no spots are of a greater classification at this time.

3. Potential Adverse Effects:

The monkey will experience no adverse side effects from these interventions; however, the animal may experience additional weight loss despite these efforts.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

Institutional Animal Care and Use Committee (IACUC)

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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved with Condition: 26-Jun-2014

Spinal Cord Injury & Stem Cell Treatment Study Surgical Proposition ▼

The IACUC approved your amendment with the following condition(s):

For [REDACTED]: 1) During the two week time frame discussed in the amendment, the overall weight loss previously capped at 25% may not exceed 28%. 2) A BCS of less than 1.5 requires euthanasia. 3) Animal activity levels must be monitored daily with decreased activity reported to the [REDACTED] veterinary staff. 4) An independent veterinary review, intended to assess this animal's status and suitability to continue with the proposed plan as described in this amendment, is required. A senior veterinarian from Campus Veterinary Services with extensive non-human primate experience will conduct this assessment on 6/27/14 and report back to the campus Attending Veterinarian.

1. Proposed Changes:

[REDACTED] received a hemisection surgery on 05/13/14. Following hemisection, he lost considerable weight and is currently at 22% weight loss. Per protocol, this animal must be euthanized if he reaches 25% weight loss. All experimental manipulations have been postponed until weight gain occurs (as per amendment approved by IACUC on 06/12/14). The animal's attitude and demeanor remain excellent, and he eats well although he is somewhat selective about the foods he consumes, tending to prefer liquids and softer items over chow.

On 6/22/14, radiographs confirmed displacement of his PEG tube such that the internal bumper is adjacent to the pylorus. It is the opinion of the veterinary staff that this displacement may be contributing to his low body weight and selective appetite.

Correction of the PEG tube may require major surgery. All animals tend to lose weight in the acute post-operative period due to the effects of general anesthesia and poor appetite in the 2-4 days following surgery. For this animal, that acute post-operative weight loss will likely cause him to reach 25% weight loss.

We are requesting that the IACUC consider a one-time, short-term exception for the weight loss stipulation to allow the performance of this clinically-necessary procedure. We would like to allow >25% weight loss for up two weeks post-surgical repair of this condition. If the animal does not reach pre-surgical body weight in this two-week period, he will be scheduled for humane euthanasia.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

By utilizing an animal already trained, behaviorally tested for protocol behavioral programs and implanted with a PEG Tube, this will eliminate the need to procure and replace the animal with another [REDACTED] colony animal.

3. Potential Adverse Effects:

The placement of PEG tubes has been extremely beneficial for ease of treatment with chronic immunosuppressive and other drug therapies in these animals. After placement of PEG tubes in 5 animals over the past 9 months, we have identified the following adverse events related to the PEG tube.

As with any chronic implants, there is a risk of abscessation around the device. In some cases, the skin over the implant may become thin and/or ulcerated due to pressure necrosis. The PEG tube may separate at the junction between tube types causing the inadvertent SQ administration of oral medications. The presence of the PEG tube may cause gastric mucosal

irritation/ulceration. The PEG tube may become impacted with gastric contents (food particles or hair). The PEG tube may migrate further into the stomach allowing the internal portion of the tube to obstruct the pylorus. Although it has not been seen to date, additional future complications could include gastric ileus following placement of the PEG tube or leakage of gastric contents through the stoma into the SQ space or into the peritoneal cavity.

All adverse effects will be assessed by the veterinarian and treated at the discretion of the veterinary staff. Abscesses may be treated by cleaning, lancing, and/or flushing as well as provision of topical and/or systemic antibiotic therapies. Skin wounds may be managed with topical treatments, debridement and primary closure, healing by second intention, bandaging, or systemic antibiotics and/or analgesics as deemed appropriate by the veterinarian. Impacted or separated tubing may need to be removed and replaced with minor surgery. Gastric ulceration may be treated with gastric protectants and acid reducers such as omeprazole, famotidine, sucralfate, or other similar compounds.

Tubes that migrate into the stomach may need to be surgically removed and repaired or replaced. Clinical signs related to this type of migration may include inappetance, vomiting, and/or bloat. Symptoms may be alleviated by provision of alternate diets, and anti-emetic therapies, but repair of the migrated tubing would require major surgery to perform a gastrotomy for removal of the existing tube, debridement of the stoma, and placement of a new PEG tube via the same gastrotomy site. This type of repair surgery is already listed in the approved protocol to be implemented following PEG tube failure.

On 6/17/14, two animals were identified with migrated PEG tubes at the time of necropsy (one 9 months post-PEG tube placement and one 4 months post-PEG tube placement). One animal ([REDACTED]) was euthanized due to scheduled project end date but did have PEG tube failure in the last few days of his project which prompted advancing his euthanasia date slightly. The second animal ([REDACTED]) was euthanized due to acute clinical complications that were attributed to PEG tube migration and subsequent pyloric/duodenal obstruction. Both of these animals also had large intestinal fecal accumulations, presumably secondary to ileus. It is unclear what the cause of the colonic ileus was but it cannot easily be attributed to the presence of the PEG tube given that the small intestines were normal in both animals. The intestinal mucosa and serosa was grossly normal and the intestines were not dilated or distended. Both animals were still passing stool.

Assessment of the remaining 3 animals with PEG tubes has revealed at least one more animal with a similar presentation that remains subclinical ([REDACTED]). A second animal ([REDACTED]) may have a lesser degree of tube migration. Radiographic assessment will need to be repeated in this animal to confirm the degree of migration and serial radiography may be used in the future to assess whether or not additional migration has occurred that may ultimately require intervention as well.

The underlying problem has been identified as an inability of the external bumper of the PEG tube to properly stabilize the tube after placement. The most distal portion of the tubing appears to stretch over time so despite properly securing the injection port, the more proximal tubing is allowed to slide back into the stomach rather than remain in the SQ space as designed. Several members of the veterinary staff have been exploring alternate placement methods to prevent recurrence of this in future placements. A new type of external bumper has been identified that appears to more tightly hold the PEG tubing in place and should prevent additional migration of the tubing into the stomach. The [REDACTED] vets also feel that it may be advantageous to shorten the total length of tubing to minimize any migration that may be possible. In addition, we have considered more thorough tacking of the stomach wall to the body wall during placement to limit movement of the stomach, suturing the internal bumper to the gastric mucosa during placement, or suturing or using an adhesive to affix the tubing to the external bumper. At this time, it is agreed that using an alternate bumper and shortening the overall length of tubing will offer the most successful solution.

6/26/14 update: Ideally, we would try this device in a short term animal prior to its use in a long term experimental animal. However, the migrated tubing noted in [REDACTED] posed a risk of acute decompensation due to the potential for obstruction. Given the clinical implications, we conducted the clinically necessary surgical repair of this animal during the week on 6/25/14 at the recommendation of the [REDACTED] veterinarian. In reviewing the radiographs of [REDACTED], we determined that surgical correction of the tubing for that animal is warranted as well.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact

17 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 [REDACTED] 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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[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved: 15-Oct-2014

Request One-Time Additional Skin Biopsies ▼

1. Proposed Changes:

Two animals enrolled in this protocol had skin biopsies collected several months ago (March 11, 2014 and May 29, 2014). We request approval to collect one additional skin biopsy on each of these animals. The procedure will be conducted exactly the same as is described in the main protocol.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

Our collaborators have had trouble culturing stem cells from these particular skin biopsies from these two animals. Instead of waiting additional time for the cells to grow, they wish to start again with fresh samples from the same animals. The "trouble" is not related to a problem in the technique but rather to three main sources of difficulty in culturing stem cells from skin:

1. If the biopsy is too thick or too thin, it can be difficult to prepare sections of skin for in vitro growth;
2. Foamy virus that may be present in the tissue requires use of antiviral agents, which can sometimes slow growth;
3. skin cultures are occasionally subject to fungal growth, and the antifungal agents can also have deleterious effects on growth.

We take precautions to alleviate these effects in every stage of the process; however, due to these reasons, there are sometimes difficulties in growing stem cells from skin.

3. Potential Adverse Effects:

The site of the previous biopsy has completely healed. We expect no additional adverse effects outside of those listed in the main protocol.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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#17523 - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved: 10-Oct-2014

Modify disposition ▼

1. Proposed Changes:

For animal disposition, protocol # [REDACTED] currently states "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 surgically naive animals from protocol # [REDACTED] to other IACUC approved protocols. We request the disposition of animals to be amended to read: "Animals will be euthanized at the end of the study, or if there are prolonged post-operative complications. Alternatively, surgically naïve animals may be transferred to another IACUC-approved protocol."

Uploaded File(s):
No Files Found.

2. Justification for Proposed Changes:

After animals move into the project room and take part in behavioral testing, we often see behavioral 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.

3. Potential Adverse Effects:

N/A

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

Institutional Animal Care and Use Committee (IACUC)

Protocol Amendment Information

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If you have more than one active protocol, select the active protocol below to find a specific approved amendment.

[IACUC Menu](#) | [Add New Request](#)

Approved Amendment(s) for Active Protocol:

[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact

17 Approved Amendment(s):

Approved: 16-Oct-2014

Transfer Two Animals To A Different Protocol

1. Proposed Changes:

We request the transfer of two surgically naïve animals ([REDACTED] and [REDACTED]) from IACUC Protocol # [REDACTED]/Stem Cell Therapy for Treatment of Spinal Cord Injury to IACUC Protocol # [REDACTED]/Chondroitinase Delivery as Treatment for Spinal Cord Injury. These subjects have been trained and tested on behavioral tasks that are exactly parallel between both protocols. In addition to behavioral testing, one subject ([REDACTED]) has undergone a single skin biopsy. The skin biopsy was a minor procedure requiring a single injection of ketamine and dexmedetomidine, followed by a single injection of ketoprophen per [REDACTED] routine procedures. No sutures were required.

We request an expedited review of this amendment in order to stay on schedule with the 2 very precisely timed events already scheduled for this animal. The first of these timed procedures is scheduled for October 22nd (hemisection, requiring out of town surgeons), followed by spinal injections on November 19th (requiring out of town surgeons).

Uploaded File(s):
No Files Found.

2. Justification for Proposed Changes:

After animals move into the project room and take part in behavioral testing, we often see behavioral 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. 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 [REDACTED] colony animal.

3. Potential Adverse Effects:

Not applicable.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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Approved Amendment(s) for Active Protocol:

[Redacted] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved: 5-Nov-2014

Add Funding Source (CIRM) ▼

1. Proposed Changes:

We request to add our grant from the California Institute for Regenerative Medicine (CIRM) as a funding source for work approved on this protocol.

Uploaded File(s):
No Files Found.

2. Justification for Proposed Changes:

This grant has been awarded to fund work approved on this protocol.

3. Potential Adverse Effects:

Not applicable.

4. Additional Animals:

N/A

5. Justification for Additional Animals:

N/A

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[IACUC Menu](#) | [Add New Request](#) **Approved Amendment(s) for Active Protocol:**

[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact

17 Approved Amendment(s):

Approved: 13-Nov-2014

1. Proposed Changes:

For animal disposition, we are currently able to transfer surgically naive animals from protocol # [REDACTED] to other IACUC approved protocols.

We would like to modify the disposition to read (changes in **bold**):

"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.**"

Uploaded File(s):
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:

N/A

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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[IACUC Menu](#) | [Add New Request](#) **Approved Amendment(s) for Active Protocol:**

[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact

17 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:

For all groups described in this protocol, the proposed duration between hemisection and grafting is 1 to 4 weeks. The "Botulinum Toxin" amendment that was approved on January 23rd, 2014 indicates we will administer botox on the day of hemisection (or up to 7 days before) 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 hemisection keeps nociceptors turned off following the hemisection 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 hemisection 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 hemisection, we would like the option to administering a second dose of botox.

3. Potential Adverse Effects:

In our "Botulinum Toxin" amendment approved on January 23rd, 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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[IACUC Menu](#) | [Add New Request](#) **Approved Amendment(s) for Active Protocol:**

[REDACTED] - Stem Cell Therapy for Treatment of Spinal Cord Injury - Alt. Contact ▼

17 Approved Amendment(s):

Approved: 12-Mar-2015

Collar Use Without Restraint Chair Testing ▼

1. Proposed Changes:

At the request of the IACUC committee, this amendment is being submitted to approve the use of a collar for rehabilitative purposes in animals who are not undergoing behavioral testing in the restraint chair.

Uploaded File(s):

No Files Found.

2. Justification for Proposed Changes:

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. Specifically, the pole is attached to the collar on an impaired animal. Then the pole is placed through the front of our jump box and out the back. The animal, who is unable to get into the jump box completely on his own, is *gently* guided into the jump box. Once the animal is inside the jump box, the pole is removed from the collar. 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 will most likely be in group 1b (N = 6).

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, which is Group 1B plus the potential for an animal or two from other groups, though this is very unlikely). 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 Feb 26

Database: Pubmed/Medline

Years Covered: 1960-present

Keywords Used: monkey AND "pole-and-collar"

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

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]	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 a NHP model of spinal cord injury to evaluate the potential of neural stem cell grafts to facilitate spinal cord repair. The investigator is commended for the measures taken to refine the study procedures (see item13.b). 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?
Item 13 and Appendix C (1)	In item 13, table f) Study Groups and Numbers Table indicates the survival time for all groups except 1b (3 months) is 8 months. Appendix C -Section1. Social Enrichment and Section 3. Cage Enrichment indicates the length of exemption as 12 months but Section 2. Food Enrichment lists the duration of the exemption as 36 months. Please explain.

(cont.)

	<p>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 a 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?</p> <p>The stem cell treatment description lacks sufficient detail; please fully describe "Neuralstem" stem cells, [REDACTED]4, NIHhESC-10-0044 stem cells, transdifferentiated autologous stem cells treatment/procedure. Appendices 3 and 5 of the VA ACORP form may be used as a guide to ensure the appropriate information is provided.</p> <p>Sub-item 6.A. – Monkeys that undergo immunosuppression receive all three drugs (i.e. tacrolimus, mycophenolate mofetil, and prednisone); is that correct?</p> <p>Under the heading 6.B <u>Options for Dosing</u>, the investigator indicates that series of practice trials on cull monkeys for implanting PEG tubes was conducted under an approved amendment to a previous protocol. Please list the protocol number and the amendment number (if applicable).</p>
<p>Item 15 (1)</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 a monkey on a study 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 animals 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</p>

	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.