Notice of Award

RESEARCH
Department of Health and Human Services
National Institutes of Health

Federal Award Date: 02/28/2020

NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

Grant Number: 1R01NS110605-01A1 REVISED
FAIN: R01NS110605

Principal Investigator(s):
Michel A. Lemay, PHD

Project Title: Intrathecal pump delivery of neurotrophins for locomotor recovery after spinal cord injury

MR. JOHN D. PENNER
SENIOR GRANTS AND CONTRACTS SP
1852 North 10th Street
TASB
Philadelphia, PA 191226023

Award e-mailed to: nih@temple.edu

Period Of Performance:
Budget Period: 04/01/2020 – 03/31/2021
Project Period: 04/01/2020 – 03/31/2024

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to TEMPLE UNIV OF THE COMMONWEALTH in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Neurological Disorders And Stroke of the National Institutes of Health under Award Number R01NS110605. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Elizabeth E Conklin
Grants Management Officer
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

Additional information follows

SECTION I – AWARD DATA – 1R01NS110605-01A1 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$218,750
Federal F&A Costs	$123,580
Approved Budget	$342,330
Total Amount of Federal Funds Obligated (Federal Share)	$342,330
TOTAL FEDERAL AWARD AMOUNT	$342,330

AMOUNT OF THIS ACTION (FEDERAL SHARE) $0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$342,330	$342,330
2	$342,330	$342,330
3	$342,330	$342,330
4	$342,330	$342,330

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name:	Extramural Research Programs in the Neurosciences and Neurological Disorders
CFDA Number:	93.853
EIN:	1231365971A1
Document Number:	RNS110605B
PMS Account Type:	P (Subaccount)
Fiscal Year:	2020

IC	CAN	2020	2021	2022	2023
NS	8472428	$342,330	$342,330	$342,330	$342,330

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: eRA Commons User Name / **OC**: 41021 / **Released**: eRA Commons User Name 02/28/2020
Award Processed: 02/28/2020 07:00:55 PM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R01NS110605-01A1 REVISED

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1R01NS110605-01A1 REVISED

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R01NS110605. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:

Additional Costs

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This revision changes the budget and project period. The new dates are *04/01/2020 and 03/31/2021.*

THE PREVIOUS TERMS AND CONDITIONS STATED BELOW REMAIN IN EFFECT.

In order to meet Institute program objectives within Fiscal Year 2020 budget constraints, the recommended levels for this grant have been reduced. Future year recommended levels of support have also been reduced.

Per the NINDS funding strategy:
http://www.ninds.nih.gov/funding/ninds_funding_strategy.htm

In accordance with the Notice: NOT-OD-19-036, published on November 27, 2018 in the NIH Guide for Grants and Contracts, total direct costs (salary, fringe benefits and tuition remission) for graduate students are provided at a level not to exceed the NIH maximum allowable amount (zero level of the Ruth L. Kirschstein National Research Service Award stipend in effect at the time of the competing award). Support recommended for future years has been adjusted accordingly, if applicable.

The full guide Notice describing the level of compensation allowed for a graduate student can be found at: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-19-036.html

In future years, awards under the Streamlined Non-Competing Award Process (SNAP) must submit a non-competing application via the eRA Commons by the 15th of the month preceding the month in which the budget period ends. The non-competing application can be submitted using the Research Performance Progress Report (RPPR) format via the RPPR link in eRA Commons.

The use of the eRA Research Performance Progress Report (RPPR) Module for submitting Type 5 Progress Reports is required for all awards with start dates on or after October 17, 2014. See Guide Notice: NOT-OD-15-014 http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-014.html

The funds in this award shall not be used to pay the salary of an individual at a rate in excess of Executive Level II ($192,300) per year effective January 6, 2019. See NIH Guide Notice: NOT-OD-19-099 https://grants.nih.gov/grants/guide/notice-files/NOT-OD-19-099.html

To register to use the Commons go to https://commons.era.nih.gov/commons/. Questions regarding the Commons should be addressed to Commons Support at 1-866-504-9552 or commons@od.nih.gov.

Other documents applicable to this grant should be faxed to (301) 451-5635 or mailed to:

Grants Management Branch
National Institutes of Neurological Disorders and Stroke
6001 Executive Boulevard, Suite 3290, MSC 9537
Rockville, MD 20852 (Express Mail)
Bethesda, MD 20892-9537 (Regular Mail)

For additional information, you may access the NIH home page at http://www.nih.gov/ ; and the NINDS Home Page at http://www.ninds.nih.gov

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Denise Chatman
Email: chatmand@ninds.nih.gov **Phone**: 301-496-3993 **Fax**: 301-451-5635

Program Official: Linda Louise Bambrick

SPREADSHEET SUMMARY
GRANT NUMBER: 1R01NS110605-01A1 REVISED

INSTITUTION: TEMPLE UNIV OF THE COMMONWEALTH

Budget	Year 1	Year 2	Year 3	Year 4
TOTAL FEDERAL DC	$218,750	$218,750	$218,750	$218,750
TOTAL FEDERAL F&A	$123,580	$123,580	$123,580	$123,580
TOTAL COST	$342,330	$342,330	$342,330	$342,330

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4
F&A Cost Rate 1	58.5%	58.5%	58.5%	58.5%
F&A Cost Base 1	$211,248	$211,248	$211,248	$211,248
F&A Costs 1	$123,580	$123,580	$123,580	$123,580

PI: **Lemay, Michel A.**	Title: Intrathecal pump delivery of neurotrophins for locomotor recovery after spinal cord injury	
Received: 03/05/2019	FOA: PA19-056 Clinical Trial:Not Allowed	Council: 10/2019
Competition ID: FORMS-E	FOA Title: Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
1 R01 NS110605-01A1	Dual: HD	Accession Number: 4280041
IPF: 8240301	Organization: TEMPLE UNIV OF THE COMMONWEALTH	
Former Number:	Department: ENGINEERING:BIO ENGINEERING (2	
IRG/SRG: CNNT	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 250,000 Year 2: 250,000 Year 3: 250,000 Year 4: 250,000 Year 5: 250,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: Evaluative Info HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
MICHEL LEMAY	Temple University - Of The Commonwealth System of	PD/PI
Redacted by agreement		

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
NS110605

b. Agency Routing Number

2. DATE SUBMITTED
2019-03-05

Application Identifier
264569

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 0571231920000

Legal Name*: Temple University - Of The Commonwealth System of
Department: ENGINEERING:BIO ENGINEERING (2
Division:
Street1*: 1801 North Broad Street,
Street2:
City*: Philadelphia
County: Philadelphia
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19122-6003

Person to be contacted on matters involving this application
Prefix: MR. First Name*: JOHN Middle Name: D. Last Name*: PENNER Suffix:
Position/Title: SENIOR GRANTS AND CONTRACTS SP
Street1*: 1852 North 10th Street
Street2: TASB
City*: Philadelphia
County: Philadelphia
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19122-6023
Phone Number*: (215)-707-3887 Fax Number: (215)-707-8387 Email: tuf81570@temple.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1231365971A1

7. TYPE OF APPLICANT* X: Other (specify)

Other (Specify): Public, Nonprofit, State-related Inst of Higher Ed

Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Intrathecal pump delivery of neurotrophins for locomotor recovery after spinal cord injury

12. PROPOSED PROJECT
Start Date* 09/01/2019
Ending Date* 08/31/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
PA-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: MICHEL Middle Name: Last Name*: LEMAY Suffix:
Position/Title: PROFESSOR
Organization Name*: Temple University - Of The Commonwealth System of
Department: ENGINEERING:BIO ENGINEERING (2
Division:
Street1*: 1947 North 12th Street
Street2:
City*: Philadelphia
County: USA
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19122-6018
Phone Number*: (215)7079407 Fax Number: () Email*: tuf44624@temple.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$1,956,170.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$1,956,170.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: MS. First Name*: KAREN Middle Name: D. Last Name*: MITCHELL Suffix:
Position/Title*: ASSISTANT VICE PRESIDENT
Organization Name*: Temple University - Of The Commonwealth System of
Department: RESEARCH: EXECUTIVE LEADERSHIP
Division:
Street1*: Temple University Services Bldg
Street2: 1852 North 10th street
City*: Philadelphia
County: Philadelphia
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19122-6023
Phone Number*: (215) 707-7547 Fax Number: (215) 707-8387 Email*: karen.mitchell@temple.edu

Signature of Authorized Representative*
MS. KAREN D. MITCHELL

Date Signed*
03/05/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Temple University - Of The Commonwealth System of
Duns Number:	0571231920000
Street1*:	1801 North Broad Street,
Street2:	
City*:	Philadelphia
County:	Philadelphia
State*:	PA: Pennsylvania
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	19122-6003
Project/Performance Site Congressional District*:	PA-002

Additional Location(s) File Name:

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3594-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract A1.pdf
8. Project Narrative*	Narrative only RO1 A1.pdf
9. Bibliography & References Cited	references cited A1.pdf
10.Facilities & Other Resources	facilities and resources A1.pdf
11.Equipment	MAJOR EQUIPMENT A1.pdf

Project Summary/Abstract

Modified fibroblast grafts releasing BDNF or NT-3 neurotrophins into the spinal transection site promote the recovery of plantar weight-bearing treadmill locomotion in adult spinal cats (even without body-weight supported training) by increasing the activity of the locomotor center region. Used in combination with body-weight supported treadmill training, neurotrophins augment the recovery obtained with training and could serve as a rehabilitative supplement to treadmill training and epidural stimulation. The current delivery method requires exposing the spinal cord and inserting the cells into the injury site which carries high risks of further damage to the spinal cord. Our preliminary data show that intrathecal delivery of neurotrophins to the lumbar area via an implanted mini-pump is just as efficacious at promoting locomotor recovery and leads to increased interneuronal firing. While these preliminary results are in acutely spinalized animals, recent results with delivery of neurotrophins via cellular grafts show that delivery of neurotrophins to the spinal cord can re-engage the locomotor circuitry in chronic spinal cord injury models, suggesting that intrathecal delivery might re-activate locomotor centers chronically isolated from supraspinal inputs. The choice of neurotrophin used to re-engage the locomotor circuitry may have implications for the development of neuropathic pain, which is a significant issue to consider prior to clinical applications. BDNF and NT-3 have different actions on sensory afferents and dorsal horn's response to sensory stimuli, with a number of conflicting reports on whether the neurotrophins enhance or reduce pain responses. Confirming the role of either neurotrophin alone in recovery is imperative, as is establishing the effects of both neurotrophins on dorsal horn neurons' responses to innocuous and noxious stimuli.

Based on our preliminary data we hypothesize that intrathecal delivery of either neurotrophin (BDNF or NT-3) will promote treadmill weight-bearing stepping in both acute and chronic models of spinal cord injury by increasing intermediate zone interneuronal activity in a plastic manner. We predict that either neurotrophin will reduce the ratio of laminae I-II neurons to laminae III-V neurons responsive to low level stimuli (mechanical allodynia), but that BDNF will lead to a clear increase in laminae I-II neurons' response to high-level stimuli (noxious stimuli).

Narrative

The proposed experiments aim to develop, validate and explore the effects on lumbar locomotor center activity of a clinically translational method of neurotrophin delivery to the lumbar cord that restores stepping ability in a large mammalian model of spinal cord injury. The proposal will also explore the potential side effects of these neurotrophins on the development of sensory dysfunction such as neuropathic pain.

Facilities and Resources

Scientific Environment: The facilities and other resources available to Dr. Lemay in his own department of Bioengineering and Redacted by agreement include everything needed to undertake and successfully complete the proposed research project. Dr. Lemay's laboratory is located in the Redacted by agreement has a rich collaborative tradition and Dr. Lemay has routine access to the shared facilities. The intellectual environment is rich with other extramurally funded investigators Redacted by agreement doing clinical and basic science spinal cord injury research. These facilities and colleagues collectively provide a scientific environment that is strongly supportive of the project.

Laboratory: Behavioral and neurophysiological recording experiments will be conducted in Dr. Lemay's laboratory which occupies located in Redacted by agreement A motorized treadmill for cat and a 6 cameras Vicon MX system for real-time video acquisition and analysis are available to train and evaluate the locomotor recovery of cats. In addition, a split-belt treadmill with force measuring capabilities is also available to measure ground reaction forces during locomotion. A spinal frame is available that enables rigid fixation of the skull and spinal column and precise cannula or electrode placements into either brain or spinal targets. Two (2) microdrives (ASI Instruments MD-2500, resolution 0.1µm) are available to drive the electrodes/cannula. A surgical microscope is available to facilitate placement. Large animal homeostasis equipment for chronic/acute experiments (ventilator, blood pressure monitor, etc.) is available. Stimulators are available to generate rectangular, regulated current pulse for microstimulation, along with 20 channels of EMG amplifiers to record muscle or nerve activity.
laboratory where most of the histological work will be conducted occupies Square Footage including 6 half benches and 6 desks for technicians, graduate students and fellows in Redacted by agreement

Clinical: N/A

Animal: Animal facilities Redacted by agreement, renovated to conform to AAALAC standards include 2 fully-equipped animal surgery suites. Special services and facilities (surgery, x-ray, laboratory, research, etc.) are also available. The animal facility employs 3 staff veterinarian and 5 staff veterinary technicians available for consultation, teaching, and research support.

Computer: Dr. Lemay is equipped with a personal computer for word processing, graphics, statistical analysis, access to online literature search services and printers. One of the lab computers is dedicated to data acquisition/control and used to collect multiunit data via the RZ2 system. A second computer is dedicated to data acquisition and video analysis via the Vicon system. Additional computers are available for graduate students and postdoctoral fellows. All computers are on an internal university network.
Office: Both Drs. Lemay and have their respective offices. Conference rooms, printers, fax machines and copy machines are readily available for this project.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Lemay Laboratory: Tucker-Davis Technologies RZ2 multi-unit acquisition system including: PZ2-128, 128 channels multi-unit pre-amplifier; 2 RA8GA pre-amplifier for analog signals; 4 channel RX7-2 stimulator and MS4 stimulus isolator. Plexon pre-amplifiers for multi-units electrodes (32 channels) are also available, equipped with cards to measure local-field-potential in addition to single units voltages. Both systems are located in the Lemay laboratory.

6-camera Vicon Motion Analysis system, motorized treadmill, motorized split-belt treadmill with force measuring capabilities for cat.

Redacted by agreement ***Laboratory***: Microplate reader, electroporator, UV transilluminator, Photodocumentation system, several DNA, RNA and protein electrophoresis apparatus, 4 ependorph microfuges (1 refrigerated), 4 operating microscope, static and shaking water baths, bacteriology incubators, 2 refrigerators, 2 freezers, microwave, Lab-Line Bacterial Shaker, liquid N2 cell freezer (holds 10,000 vials), a -80° C freezer, Spectronic 601 spectrophotometer, 2 stereotaxic apparatuses, 4 nanoinjectors, 1 microinjector, pipette puller, and beveler. We have a thermal stimulator (Ugo Basile), a Randall and Selitto analgesymeter (paw pressure, Ugo Basile), Dunnet Style Grip strength instrument (Linton Inst., inc), staircase device (Lafayette Instrument Co), random grid walkway, Von frey hairs, Sony digital video camera.

Redacted by agreement

Redacted by agreement consists of Square Footage of office and lab space on Redacted by agreement

Redacted by agreement includes Square Footage of laboratory space. There are two rooms for centrifuges (2 ultracentrifuges and 2 high speed), shakers and freezers, two tissue culture rooms, a BL2+ viral production facility, histology core facilities, two cold rooms, two bacterial equipment rooms, a dark room, and a dishwashing facility with autoclave.

Amongst the major facilities from that will facilitate this project is the Imaging core that contains:

Imaging Core Facilities. Core imaging and histology facilities include the following rooms housing the listed equipment:

A) Fluorescence and confocal microscopy rooms Redacted by agreement – Square Footage
   a. One Nikon C2-Ti Inverted Confocal Microscope Systems
   b. Two Nikon Eclipse 80i Epifluorescence Microscope with adapter for confocal laser scanner, allowing rapid conversion to an upright confocal
   c. One Olympus BX53 Epifluourescence Microscope
   d. One Leica TCS SP8 Upright Spectral Confocal Microscope System

B) Laser Microdissection Room ( ) – Square Footage
   a. Leica LMD7000 Laser Microdissection System

C) Multiphoton microscopy room ( ) – Square Footage
   a. Leica TCS SP5 Upright Multi-Photon and Spectral Confocal Microscope System

D) Electrophysiology and fluorescence stereomicroscopy room Redacted by agreement) – Square Footage
   a. Olympus SZX-12 fluorescence stereomicroscope
   b. Zeiss Axioskop Fluorescence Microscope

E) Total internal reflection fluorescence microscopy area (Redacted by agreement) – Square Footage
   a. Zeiss TIRF3
   b. Zeiss Axio-Observer Z1 microscope

F) *In vivo* fluorescence microscopy room – Square Footage
   a. Leica MZ16F Fluorescence Stereomicroscope
   b. Olympus BX51 Wide Field Epifluorescence Microscope track-mounted on custom stage for *in vivo* imaging.

Redacted by agreement

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: MICHEL Middle Name Last Name*: LEMAY Suffix:

Position/Title*: PROFESSOR
Organization Name*: Temple University - Of The Commonwealth System of
Department: ENGINEERING:BIO ENGINEERING (2
Division:
Street1*: 1947 North 12th Street
Street2:
City*: Philadelphia
County: USA
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19122-6018

Phone Number*: (215)7079407 Fax Number: ()

E-Mail*: tuf44624@temple.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Lemay NIH biosketch A1.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-19-056 . Received Date: 2019-03-05T11:13:16.000-05:00

PROFILE - Senior/Key Person

Redacted by agreement

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 10/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Michel A. Lemay, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Professor of Bioengineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Univ of Sherbrooke, Sherbrooke, Canada	B.S.	1987	Electrical Engineering
Case Western Reserve U., Cleveland, OH	M.S.	1990	Biomedical Engineering
Case Western Reserve U., Cleveland, OH	Ph.D.	1994	Biomedical Engineering
M.I.T. Cambridge, MA	Postdoc	1994-1998	Mechanical Engineering

A. Personal Statement

As a graduate student I was trained in Functional Neuromuscular Stimulation and studied the control of wrist flexion/extension and forearm pronation/supination in C5-C6 quadriplegics. As a post-doc I studied the motor output of the spinal circuitry obtained with intraspinal microstimulation in frogs, and later in cats. My own laboratory studies the effects of providing exogenous neurotrophins on locomotor recovery and spinal circuitry in a large animal model of spinal cord injury (feline). The neurotrophin producing transplants promote a locomotor recovery similar to the one obtained with training after spinal cord injury (see Boyce et al 2007) but their mechanisms of action are still relatively poorly understood. Current projects study the changes in interneuronal activity associated with the recovery obtained with training or neurotrophin transplant following injury. In addition, we are developing clinically applicable delivery methods for these transplants.

This application is a continuation of the prior studies conducted in my laboratory and focuses on elucidating similarities in the locomotor recovery capability of the BDNF and NT-3 neurotrophins and differences in the effects of the NT-3 and BDNF neurotrophins on sensory pathways. We are uniquely positioned to conduct the studies proposed based on our expertise on the feline spinal cord model, and in large scale extracellular neuronal recording and analysis.

B. Positions and Honors

Positions and Employment

1988- 1994 Graduate Research Assistant (depts. of Biomedical Engineering and Orthopedics). Rehabilitation Engineering Center, MetroHealth Medical Center, CASE WESTERN RESERVE U., Cleveland, OH.

1994-1995 Postdoctoral Research Associate (advisor: Prof. Neville Hogan). Eric P. and Evelyn E. Newman Laboratory for Biomechanics and Human Rehabilitation, Department of Mechanical Engineering, MASSACHUSETTS INSTITUTE of TECHNOLOGY, Cambridge, MA.

1996-1998 Postdoctoral Research Fellow (advisors: Profs. Emilio Bizzi and Neville Hogan). Departments of Brain & Cognitive Sciences, and Mechanical Engineering, MASSACHUSETTS INSTITUTE of TECHNOLOGY, Cambridge, MA.

1998-2000 Senior Research Associate. Applied Neural Control Laboratory, Dept. Biomedical Engineering, CASE WESTERN RESERVE U., Cleveland, OH.

2001-2008 Assistant Professor. Dept. Neurobiology and Anatomy, DREXEL UNIVERSITY COLLEGE OF MEDICINE, Philadelphia, PA.
2008-2013 Associate Professor. Dept. Neurobiology and Anatomy, DREXEL UNIVERSITY COLLEGE OF MEDICINE, Philadelphia, PA.
2013-2014 Professor. Dept. Neurobiology and Anatomy, DREXEL UNIVERSITY COLLEGE OF MEDICINE, Philadelphia, PA.
2014-present Professor. Dept. Bioengineering, TEMPLE UNIVERSITY, Philadelphia, PA.

Honors

1990-1993 Fonds pour la formation de chercheurs et l'aide à la recherche (FCAR) scholarship for Ph.D. studies,
1994 National Institute on Disability and Rehabilitation Research (NIDRR) Postdoctoral Fellowship
1996-1997 Paralyzed Veterans of America's Spinal Cord Research Foundation Fellowship
2007 Drexel University College of Medicine 2007 Young Scientist Award

C. Contributions to Science

1) *Interneuronal Activity in the Cat Spinal Cord*: Large scale single unit recordings of interneuronal activity in the mammalian spinal cord (up to 45 units recorded simultaneously during locomotor activity). Our recordings disproved the traveling wave hypothesis that suggested that neuronal activity in the cord during locomotion progressed as a wave from the rostral to caudal end of the cord. The methodology has also been used to study activity in neural progenitor cells grafted into the injured spinal cord in collaboration with I Fischer.

N AuYong, K Ollivier-Lanvin and MA Lemay, “Population spatiotemporal dynamics of spinal intermediate zone interneurons during air-stepping in adult spinal cats”, J Neurophysiology, Vol. 106, pp. 1943-1953, 2011. PMID: 21775722 PMCID: PMC3191843

N AuYong, K Ollivier-Lanvin and MA Lemay, “Preferred locomotor phase of activity of lumbar interneurons during air-stepping in sub-chronic spinal cats”, J Neurophysiology, Vol. 105:1011-1022, 2011. PMID: 21084683 PMCID: PMC3074417

JF Bonner, T Connors, WF Silverman, DP Kowalski, MA Lemay and I Fischer, "Grafted neural progenitors integrate and restore synaptic connectivity across the injured spinal cord", J Neuroscience, Vol. 31: 4675-4686, 2011. PMID: 21430166 PMCID: PMC3148661

2) *Neurotrophins Promote Locomotor Recovery in Spinal Animals in the Absence of Locomotor Training*: We established that delivery of neurotrophins to the injury site of spinalized animals could promote recovery of stepping ability similar to the one obtained with sensorimotor training.

M Dimiskovski, R Scheinfield, D Higgin, AJ Krupka, and MA Lemay, “Characterization and validation of a split belt treadmill for measuring hindlimb ground-reaction forces in able-bodied and spinalized felines”, J Neurosci Methods, 2017, PMID: 28069392 PMCID in process

AJ Krupka, I Fischer, and MA Lemay, “Transplants of neurotrophin-producing autologous fibroblasts promote recovery of treadmill stepping in the acute, sub-chronic, and chronic spinal cat”, J Neurotrauma, Dec 2016, PMID: 27829315 PMCID in process

K Ollivier-Lanvin, I Fischer, V Tom, JD Houlé, and MA Lemay, “Either Brain-Derived Neurotrophic Factor of Neurotrophin-3 only neurotrophin-producing grafts promote locomotor recovery in untrained spinalized cats”, Neurorehabilitation and Neural Repair, Vol. 29, pp.90-100, 2015. PMID: 24803493 PMCID in process

VS Boyce, M Tumolo, I Fischer, M Murray and MA Lemay, “Neurotrophic factors promote and enhance locomotor recovery in untrained spinalized cats” J Neurophysiol, Vol. 98, pp. 1988-96, 2007. PMID: 17652412 PMCID: Commentary by RD de Leon “Could neurotrophins replace treadmill training as locomotor therapy following spinal cord injury?” J Neurophysiol, Vol. 98, pp. 1845-46, 2007.

3) *Reflexes Alterations and Axonal Growth Using Exercise and Neurotrophins*: We studied the modulation in spinal reflexes and the axonal growth obtained with peripheral nerve gowth when combined with exercise and neurotrophin delivery in spinal rats.

Côté, M-P, GA Azzam, MA Lemay, V Zhukareva, and JD Houlé, “Activity-dependent increase in neurotrophic factors is associated with an enhanced modulation of spinal reflexes after SCI”, J Neurotrauma, Vol. 28:299-309, 2011. PMID: 21083432 PMCID: PMC3037803

J Tom, HR Sandrow-Feinberg, K Miller, C Domitrovich, J Bouyer, V Zhukareva, MC Klaw, MA Lemay, and JH Houlé, “Exogenous BDNF enhances the integration of chronically injured axons that regenerate through a peripheral nerve grafted into a chondroitinase-treated spinal cord injury site”, Exp Neurol, Vol. 239C, pp. 91-100, 2013. PMID: 23022460 PMCID in process

4) *Modularity in Spinal Motor Output*: This work is an extension of my training in Functional Neuromuscular Stimulation. I have studied the modularity in spinal motor output in various animal models and using biomechanical modeling. Initial work was with Emilio Bizzi at MIT where I studied the linear summation properties of the end-point force responses evoked by simultaneous activation of intraspinal sites. Since then I have explored the modularity in spinal motor output in intact (collaboration with W Grill) and spinal (in my own laboratory) cats. Our work shows that, as in the frog, the end-point forces produced by intraspinal stimulation in the cat have a limited number of patterns, and these force patterns may be used to produce movements.

VS Boyce and MA Lemay, “Modularity of endpoint force patterns evoked using intraspinal microstimulation in treadmill trained and/or neurotrophin treated chronic spinal cats” J Neurophysiol, Vol. 101, pp. 1309-20, 2009. PMID: 19118106 PMCID: PMC2666421

MA Lemay, D Grasse, and WM Grill, "Hindlimb endpoint forces predict movement direction evoked by intraspinal microstimulation in cats", IEEE Transactions on Neural Systems and Rehabilitation Engineering, Vol. 17, pp. 379-389, 2009. PMID: 19497827 PMCID: PMC3062993

MA Lemay, M Bhowmik-Stoker, GC McConnell and WM Grill, “Role of biomechanics and muscle activation strategy in the production of endpoint force patterns in the cat hindlimb” The Journal of Biomechanics, Vol. 40, pp. 3679-87, 2007. PMID: 17692854 PMCID in process

MA Lemay and WM Grill, “Modularity of motor ouptut evoked by intraspinal microstimulation in cats” J Neurophysiol, Vol. 91, pp. 502-514, 2004.

5) *Role of Afferent Feedback in the Control of Locomotion*: This collaborative work with Drs. Rybak (Drexel University College of Medicine) and Prilutsky (Georgia Institute of Technology) explores the role of afferent feedback in the control of locomotion through modeling of the locomotor circuitry and experimental evaluation of the gait changes obtainable with stimulation of sensory feedback.

SN Markin, MA Lemay, BI Prilutsky, and IA Rybak, “Motoneuronal and muscle synergies involved in cat hindlimb control during fictive and real locomotion: a comparison study, J Neurophysiology, Vol. 107, pp. 2057-2071, 2012. PMID: 22190626 PMCID: PMC3331602

K Ollivier-Lanvin, A Krupka, N AuYong, K Miller, BI Prilutsky and MA Lemay, “Electrical stimulation of the sural cutaneous afferent nerve controls the amplitude and onset of the swing phase of locomotion in the spinal cat”, J Neurophysiology, Vol. 105, pp. 2297-2308, 2011. PMID: 21389308 PMCID: PMC3094182

SN Markin, AN Klishko, NA Shevtsova, MA Lemay, BI Prilutsky and IA Rybak, “Afferent control of locomotor CPG: Insights from a simple neuro-mechanical model,” Annals of NY Academy of Science, Vol. 1198, pp. 21-34, 2010. PMID: 20536917 PMCID in process

H Barbeau, DA McCrea, MJ O’Donovan, S Rossignol, WM Grill, and MA Lemay, "Tapping into spinal circuits to restore motor function," Brain Research Reviews, Vol. 30, pp. 27-51, 1999.

Lemay Bibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/michel.lemay.1/bibliography/44112513/public/?sort=date&direction=ascending.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

P01 NS055976 Houlé (PI) 04/1/13-03/31/18
NIH/NINDS

Spinal Cord Injury, Plasticity and Transplant Mediated Repair
Project #4 - Mechanisms of Locomotor Recovery after SCI in Cats
The long-term objective of this program project is to develop treatments for acute and chronic spinal cord injury. Dr. Lemay's project is concerned with looking at the mechanisms responsible for the locomotor recovery observed with neurotrophins and training in spinal cats.
Role: PI on Project #4.

Private Source Spence (PI) 01/01/18-12/31/20
Private Source
Private Source
The goal of this grant is to determine whether chemogenetic tools can enhance recovery from spinal cord injury, and to investigate the mechanisms by which epidural stimulation improves movement.
Role: Dr. Lemay is collaborator on this project.

Private Source Spence (PI) 01/07/18-12/31/20
Private Source
The goal of this grant is to determine whether chemogenetic tools can enhance recovery from spinal cord injury when delivered into sensory neurons in a contusion injury model.
Role: Dr. Lemay is collaborator on this project.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 10/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

Redacted by agreement

Page 103 of 143 to Page 106 of 143

Withheld pursuant to exemption

Redacted by agreement

of the Freedom of Information and Privacy Act

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Modular Budget

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Budget Period: 1

Start Date: 09/01/2019 End Date: 08/31/2020

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	58.50	241,426.00	141,234.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Ernest Kinneer, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 06/20/2017

Total Indirect (F&A) Costs: 141,234.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 391,234.00

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

PHS 398 Modular Budget

Budget Period: 2	
Start Date: 09/01/2020 End Date: 08/31/2021	

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	58.50	241,426.00	141,234.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Ernest Kinneer, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 06/20/2017

Total Indirect (F&A) Costs: 141,234.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 391,234.00

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

PHS 398 Modular Budget

Budget Period: 3	
Start Date: 09/01/2021 End Date: 08/31/2022	

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	58.50	241,426.00	141,234.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Ernest Kinneer, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 06/20/2017

Total Indirect (F&A) Costs: 141,234.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 391,234.00

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

PHS 398 Modular Budget

Budget Period: 4	
Start Date: 09/01/2022 End Date: 08/31/2023	

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	58.50	241,426.00	141,234.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Ernest Kinneer, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 06/20/2017

Total Indirect (F&A) Costs: 141,234.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 391,234.00

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

PHS 398 Modular Budget

Budget Period: 5

Start Date: 09/01/2023 End Date: 08/31/2024

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	250,000.00
Consortium Indirect (F&A)	0.00
Total Direct Costs*	250,000.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	58.50	241,426.00	141,234.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): DHHS, Ernest Kinneer, (214) 767-3261

Indirect (F&A) Rate Agreement Date: 06/20/2017

Total Indirect (F&A) Costs: 141,234.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 391,234.00

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	1,250,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	
Section A, Total Direct Costs for Entire Project Period ($)	1,250,000.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	706,170.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	1,956,170.00

2. Budget Justifications

Personnel Justification	P264569_LemayM_PersonnelJustificationv2.pdf
Consortium Justification	
Additional Narrative Justification	P264569_LemayM_AdditionalNarrativeJust.pdf

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

Personnel Justification

Personnel

Michel Lemay, PhD (Principal Investigator) (Years 1-5: % FTE)
Dr. Lemay is a Professor in the Department Of Engineering at Temple University. His laboratory specializes in 1) recovery of locomotion following spinal cord injury, 2) recording spinal interneurons in the in vivo cat model and 3) the reorganization of spinal neurons following spinal cord injury and therapeutic interventions. Dr. Lemay will supervise the surgical and experimental approaches outlined in this proposal, and act as the direct supervisor for the post-doctoral fellow and graduate assistant. Dr. Lemay will oversee all activities of this project including obtaining and maintaining appropriate research approvals.

Redacted by agreement

TBA, PhD (Post-Doctoral) (Years 1-5: 100% FTE [12 Calendar Months])
The post-doctoral fellow will assist with all aspects of the project. The fellow will be trained in animal behavioral training, as well as in the surgical procedures proposed. Throughout the duration of the project, the fellow will assist with administrative approvals, scheduling, experimental preparation and data collection, perform treadmill training, and perform needed data management, analysis and manuscript preparation. The fellow will be selected for his/her ability and experience with neural data analysis.

TBA, BS (Graduate Assistant) (Years 1-5: 100% FTE [12 Calendar Months])
The graduate assistant will assist with all aspects of the project. He/She will be trained in animal behavioral training, the surgical procedures proposed, and neurophysiological data analysis. Throughout the duration of the project, the graduate assistant will behaviorally train the animals, assist during data collection locomotor sessions, and during the terminal physiological experiments. The graduate assistant will also be involve with histological analyses, and neural data analyses

Redacted by agreement

Additional Narrative Justification

Modular Budget

The following items are excluded from the Indirect Cost Base:

Tuition

Tuition is requested for a graduate student for 6 credits per semester at $1,429/credit.

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	response to reviewers.pdf
Research Plan Section	
2. Specific Aims	SPECIFIC AIMS A1.pdf
3. Research Strategy*	Narrative_Final_A1.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	vertebrate animal section_1.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	
10. Resource Sharing Plan(s)	Data and Resource Sharing Plan A1_1.pdf
11. Authentication of Key Biological and/or Chemical Resources	Authentication of key resources A1_1.pdf
Appendix	
12. Appendix	

Funding Opportunity Number: PA-19-056. Received Date: 2019-03-05T11:13:16.000-05:00

reviewers' comments

SPECIFIC AIMS

We *hypothesize that the delivery of BDNF or NT-3 to the lumbar spinal cord via an implanted programmable mini-pump promotes the recovery of plantar weight-bearing stepping in both acute and chronic models of SCI, by causing an increase in spinal excitability that leads to sustained plastic changes in neuronal activity patterns*. Our underlying hypothesis is that, as with epidural stimulation, the neurotrophins (NTFs) increase lumbar circuitry excitability which in turn increases the circuitry's responsiveness to hindlimb afferent feedback.

Responsiveness to afferent feedback changes due to either BDNF or NT-3 will be established by measuring the excitability of dorsal horn interneurons in response to proprioceptive and other large sensory afferent inputs versus nociceptive inputs. The long-term objective is to evaluate the potential sensitization attached to the use of either neurotrophin in the development of dorsal horn excitability that is common in central pain associated with incomplete spinal injury models, as typically occurs in humans. We *hypothesize that NT-3 promotes a similar recovery of locomotor ability but with reduced risks of chronic pain development*, as nociceptive sensory neurons' responses are not enhanced with NT-3 as they may be with BDNF.

Specific Aims

SA1: a) Intrathecal delivery of BDNF or NT-3 into the lumbar cord cerebrospinal fluid promotes recovery of plantar weight-bearing stepping in an acute model of spinal cord injury and b) this recovery is due to increased spinal excitability c) that leads to sustained plastic changes in neuronal firing

Our work showing that NTF (BDNF or NT-3) producing transplants restore plantar weight-bearing stepping in untrained spinal cat[74] indicates that either neurotrophin may be used as an alternative or complement to body-weight supported treadmill training. Our prior studies were based on implantation of xenografts or autologous NTFs producing grafts into the thoracic transection cavity site. These grafts produce additional damage to the cord and control of dosing is poor. Preliminary results show that locomotor recovery in spinal cats using intrathecal delivery of BDNF or NT-3 only in the lumbar cord cerebrospinal fluid is associated with increased intermediate zone interneuronal firing. We hypothesize that direct infusion of human neurotrophin BDNF or NT-3 into the lumbar cord cerebrospinal fluid will promote the recovery of plantar weight-bearing stepping in spinal animals and sustained changes in interneuronal firing activity.

SA2: Locomotor networks are maintained in a chronic injury model and can be re-activated following delivery of neurotrophins that promotes an increase in spinal neuronal firing similar to the one obtained with acute delivery of neurotrophins

We have recently shown that transplants that produce BDNF for a limited period of time restore weight-bearing stepping without exercise even when transplants are delivered 6 weeks post-injury.[60] Since the majority of spinal cord injury individuals are chronic cases, there is a need to demonstrate the efficacy of our intrathecal BDNF or NT-3 delivery therapy in a chronic animal model. We hypothesize that the locomotor networks' responsiveness is maintained in chronic SCI such that intrathecal delivery of BDNF or NT-3 via pump for a 5 week period will restore plantar weight-bearing stepping in spinal cats injured 6 weeks prior to NTF delivery. We hypothesize that the recovery is associated with sustained changes in interneuronal firing which explain why locomotor recovery is maintained for an extended period following cessation of neurotrophin delivery (as in our prior studies with cell transplants).[18, 60]

SA3: Intrathecal delivery of NT-3 promotes increases in low threshold short latency responses of dorsal horn interneurons, while BDNF promotes increases in high threshold long latency responses.

NT-3 may be clinically preferable to BDNF as an agent to re-engage the locomotor circuitry since it is associated with sprouting of large diameter afferents rather than small nociceptive fibers. To test this hypothesis, we will measure mechanical allodynia (withdrawal response to an innocuous stimuli) and thermal hyperalgesia (withdrawal response to a noxious heat stimuli) for the animals of aims 1 & 2. In addition to these behavioral tests of the spinal reflexes hypersensitivity, we will also measure the responses of dorsal horn interneurons to low threshold stimulation of sensory nerves, and to noxious level stimulation of the same sensory nerves in the animals of aims 1 and 2. Enhanced responses to noxious and innocuous stimuli in superficial dorsal horn interneurons (especially those of laminae I-II) are typically associated with allodynia and "pain-like" perception enhancement. Enhancement in the responses of more ventral dorsal horn interneurons (laminae III-V) to low threshold stimuli is expected with NT-3 based on the sprouting of large diameter afferents induced by this neurotrophin. These changes in synaptic connectivity likely contribute to the recovery associated with neurotrophins and are expected to be differentially modulated by BDNF and NT-3.

A) SIGNIFICANCE

Motor functions are significantly disrupted after spinal cord injury (SCI) due to the disconnection between the neural circuits below and above the lesion. Studies in the cat animal model, starting with Sir Charles Sherrington[86], have established that the locomotor circuitry below the lesion remains functional, and can be re-engaged with exercise and other treatments. Studies on this model of SCI and others have contributed to the development of therapies, currently utilized in individuals with SCI, such as body-weight supported training[14, 35, 36, 41, 63] and epidural stimulation.[47, 52]

We have shown that fibroblast grafts releasing neurotrophins (NTF) transplanted into the spinal transection site promote the recovery of plantar weight-bearing treadmill locomotion in adult spinal cats even without body-weight supported training.[18] Delivery of neurotrophins could thus maintain and augment the lumbar locomotor circuitry's output and be used in conjunction with body-weight-supported-training (BWST) or epidural stimulation. Since locomotor recovery with BWST, especially weight support, is poor in complete ASIA A cases, NTFs may become part of the arsenal to increase spinal locomotor output in humans, since we have seen greater recovery in animals receiving both BWST and neurotrophins.[18]

This locomotor recovery does not involve axonal growth through the transplant[18] but rather enhanced excitability of the lumbar spinal circuitry as shown by our preliminary recordings of intermediate zone interneuronal activity following intrathecal BDNF delivery (Figure A1).[61] The locomotor recovery obtained in those animals also suggest that direct delivery of neurotrophins to the lumbar area is as efficacious at promoting locomotor recovery as our previous delivery method, which involved grafting cells into the thoracic transection cavity site. Intrathecal pump delivery offers numerous advantages over cellular or viral vectors methods, most notably safety and precise control of dosing and period of delivery. The proposed intrathecal NTF delivery poses minimal risk to the cord and is already widely used to deliver Baclofen. As with Baclofen[32, 62], we have found in our preliminary studies that the intrathecal doses necessary to promote locomotor recovery are reduced to a level that avoids side effects such as the spasticity noted in other studies.[17, 99] Human BDNF protein, which has been delivered intrathecally in some clinical trials for the treatment of amyotrophic lateral sclerosis (ALS) has limited reversible side effects.[11, 73] Demonstration of the feasibility and rehabilitative efficacy of pump delivery of BDNF in a large mammalian model would represent a significant step towards the development of a clinically applicable BDNF delivery method. This would permit testing of the effects of neurotrophins on locomotor recovery with/without body-weight support treadmill rehabilitation or other interventions, including epidural stimulation,[35, 36] in spinal cord injured individuals and could greatly impact the field of locomotor rehabilitation. The clinical potential of the approach is high, but a greater understanding of the mechanisms of action of the NTFs (BDNF or NT-3) and associated effects on sensory dysfunction, including neuropathic pain, must first be evaluated in animal models.

Robustness and reproducibility

Experimental reproducibility and replication are significant problems in spinal cord injury research[89] and other disciplines.[12, 79] Experiments testing the same interventions often fail to produce an effect as strong as reported in the original experiments, or fail to reproduce the original results. Our proposal recognizes these issues by using objective measures to evaluate the recovery and changes in lumbar circuitry while blinding the evaluator as much as possible to the treatment applied. In addition, our record with reproducibility has been outstanding to date as our locomotor recovery results following neurotrophins delivery have been repeated over multiple experiments in our laboratory,[18, 60, 74] and in other species and laboratories.[17, 99] Some differences in locomotor recovery have been noted for NT-3 between rats and cats, although dosing is hard to control with the viral delivery method used in rats[17] which may have contributed to the differences observed.

B) INNOVATION

Our proposal is innovative in several respects. While the roles of neurotrophins in axonal elongation, sprouting, and regeneration of central or peripheral axons have been extensively studied, we are one of the few groups using NTFs to re-engage the locomotor circuitry via their effects on the function of the lumbar circuitry, delivering it directly at the lumbar circuitry (as opposed to the injury site), in a minimally invasive manner with controlled dosing capabilities (as opposed to cellular or viral delivery methods). In addition, we are the only group (to our knowledge) capable of measuring large scale (up to 128 channels, 4 times what has been achieved in the rat[88]) changes in the activity of intermediate or dorsal horn sensory neurons associated with the neurotrophin delivery during locomotor activity or in response to muscle and cutaneous nerve stimulation. With these recordings we are in a unique position to evaluate the potential effects of either neurotrophin in the development

of sensory dysfunction, and the extend of the chronic neurophysiological plastic changes in interneuronal firing following time- and dose-controlled delivery of BDNF or NT-3.

Overall, the proposal will further develop a potential method of augmenting the re-engagement of the locomotor circuitry to the point where clinical translation would be possible with minimal risks of further damage to the individual's cord. In addition, we will answer important questions about the plastic changes in spinal excitability and potentiation of nociceptive responses associated with two neurotrophins that in our model appear equivalent in the locomotor recovery they provide.

C) APPROACH

1) Preliminary Results and Rationale

This project builds on three lines of expertise within the laboratory: 1) intrathecal neurotrophin delivery in the acute spinal model, 2) neurotrophin delivery in the chronic spinal animal model, 3) interneuronal recordings and afferent nerve stimulation.

Preliminary results and rationale/premise for Aim 1: *BDNF or NT-3 intrathecal delivery in the acute spinal cord injured cat*: We have preliminary results in our cat model that intrathecal delivery of BDNF to the lumbar cord is as effective as cellular delivery to the injury site or BWST at restoring stepping in the untrained acute spinal cat. We have obtained good plantar weight-bearing stepping as early as 3 weeks post-transection/pump implant in four animals where BDNF delivery was initiated immediately following the spinal cord injury and the animals did not receive any body-weight supported training. As shown in Figure 1, those animals recover the ability to plantar weight-bearing step, defined as the ability to execute 10 consecutive plantar steps at the speed tested and all the lower speeds. Animals treated with saline execute stepping on the dorsal aspect of their paw, and only at slow speeds, while BDNF treated animals can reach speeds of 0.8 m/s (maximal speed of our treadmill). We have also established in one animal that NT-3 intrathecal delivery similarly restores plantar weight bearing stepping (Figure 1), confirming our results showing equivalent stepping ability in animals treated with either neurotrophin via cellular delivery at the injury site.[74] For BDNF treated animals, we have preliminary data showing that the number of active interneurons during air-stepping trials [5] is higher than in animals treated with saline, and that the average firing frequency of those units is higher (Figure A1). The rationale for aim 1 is to confirm those findings in a larger population and demonstrate that a similar increase in neuronal excitability occurs with NT-3. We suspect that this increased excitability is responsible for the recovery and we predict that it will be maintained for an extended period following the end of NTFs delivery, as is the locomotor capability.

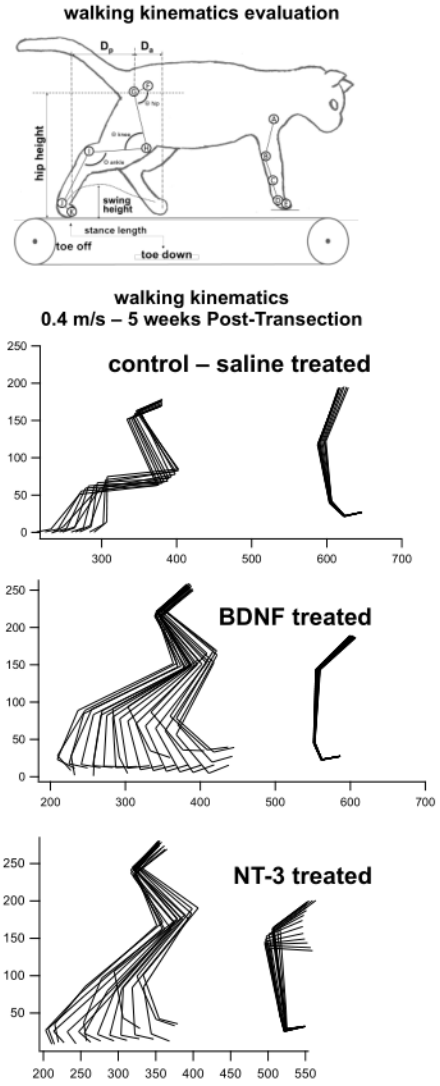

Figure 1. Locomotor recovery with intrathecal delivery of BDNF or NT-3. Stick figures of one exemplar hindlimb step post-transection for animals that received intrathecal delivery of saline, BDNF or NT-3 at the lumbar cord level for 5 weeks immediately starting post-transection. Stance phase is shown from toe-down to toe-up, and the swing from toe-off to toe down. The animals that received neurotrophins stepped at up to 0.8 m/s (10 consecutive plantar weight-bearing steps at that speed and all lower speeds) at 5 weeks post-transection, steps shown are for a speed of 0.4 m/s. The animal that received saline stepped on the dorsal aspect of the footpad, and only at the lower speeds. X-Y axes for the stick figures are in mm.

Preliminary results and rationale/premise for Aim 2: *BDNF or NT-3 intrathecal delivery in the chronic spinal cord injured cat*: Treatments that can re-engage the acutely injured locomotor networks would benefits roughly 17,500/year new SCI cases (in the USA alone), but a treatment must be efficacious in chronic injury in order to benefit the more than 285,000 individuals already living with SCI.[24] A number of prior studies suggest that the locomotor networks are preserved in a chronic injury and this evidence form the rationale for this aim. Animals can be trained to locomote even after an extended delay post-injury[64], and we have shown recovery of stepping in animals where BDNF delivery via transplanted genetically modified autologous fibroblasts was initiated 6 weeks post-transection (Figure 2, and [60]). Those animals recovered plantar weight-bearing stepping ability comparable in performance to the one in animals receiving the cellular graft at the time of transection or 2 weeks following injury. We also found in those animals that BDNF production from the transplanted fibroblasts was downregulated by 5 weeks post-grafting, even

though the animals maintained their locomotor recovery up to 12 weeks post-grafting, the last time point tested.[60] These results suggest that a limited period of neurotrophin delivery may be sufficient to re-engage the locomotor circuitry for an extended period. This suggests that a similar increase in interneuronal excitability can be obtained with NTFs delivery in a chronic injury model, and that increased excitability is maintained for extended period after the end of NTF delivery. We hypothesize that intrathecal delivery of BDNF or NT-3 via pump for a 5 weeks period will restore plantar weight-bearing stepping in untrained spinal cats injured 6 weeks prior to the start of the neurotrophin delivery and lead to a sustained increase in neuronal excitability. Importantly, the limited time of neurotrophin delivery (5 weeks) will avoid the onset of spasticity reported with continuous delivery of neurotrophins[17, 65], yet locomotor recovery and locomotor network excitability will be maintained for an extended period following cessation of BDNF or NT-3 delivery due to plastic changes in neuronal excitability.

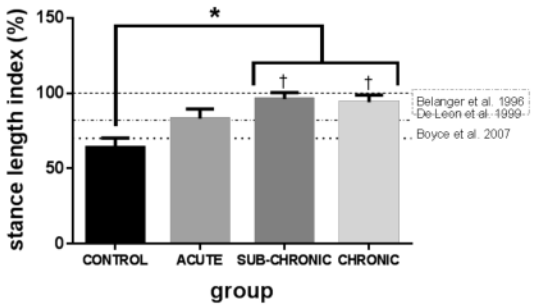

Figure 2. **Recovery of stance length** for a treadmill speed of 0.4m/s 5 weeks after graft of BDNF producing transplants at the time of transection (ACUTE), 2 weeks post-transection (SUB-CHRONIC) or 6 weeks post-transection (CHRONIC) (index is relative to pre-transection values). Recovery was similar in those three groups and on-par with results for body-weight supported locomotor training (Belanger et al., 1996/De Leon et al., 1999), or BDNF-producing xenografts (Boyce et al, 2007). CONTROL animals did not plantar steps on a consistent basis and the recovery shown was calculated from the limited number of plantar steps they took. * - $p < 0.05$; † - pre-transection group mean (100%) falls within the group's 95% CI post-injury

Preliminary results and rationale/premise for Aim 3: *sensory neurons' responses following BDNF, NT-3 or saline treatment*: The decision to study both BDNF and NT-3 arises from two lines of observation. First, while we have established that BDNF or NT-3 are sufficient for locomotor recovery[74], results in the rat using viral vector delivery methods indicate that only BDNF is efficient at promoting recovery, while NT-3 showed none to modest effects on recovery of stepping.[17] This would suggest that BDNF might be the preferable NTF to consider for applications in humans, although dosing may be responsible for the negative results in rodents since even BDNF leads to locomotor impairment when continuously administered in rodents.[99] The current study will provide a repeat of our original experiment under better timing and dosing conditions.

The potential role of BDNF in pain, and the implications this would have if used in humans with incomplete injuries also raises concerns about its effects on pain processing. Boyce et al. [17] found a significant increase in c-fos expression in the dorsal horn nociception processing region (laminae I-II) in animals treated with BDNF, as well as increased sensitivity to noxious heat in those animals. While intrathecal delivery of BDNF protects against the mechanical hyper-reactivity (allodynia) induced by peripheral nerve stimulation at levels eliciting flexion withdrawal in spinal animals[53], others have shown increases in mechanical reactivity and dorsal horn neurons' responses to peripheral stimulation with BDNF delivery in intact animals (neonatal slice preparation or adult intact).[25, 46] Our histological analysis shows that intrathecal delivery of BDNF leads to sequestration of the protein within the DRGs and cells of the grey matter (Figure 3), likely modulating the response of first order interneurons (and deeper interneurons). The rationale for aim 3 is that since BDNF is likely involved in pain processing[23, 45, 46, 70] and delivered to DRG neurons, it becomes imperative to explore the dorsal horn interneurons' responses to afferent stimulation in our animals. While chronic pain cannot be studied in the transection spinal cord injury model (no sensory information ascending past the injury site in a full transection), the information gained will provide valuable information for investigators working

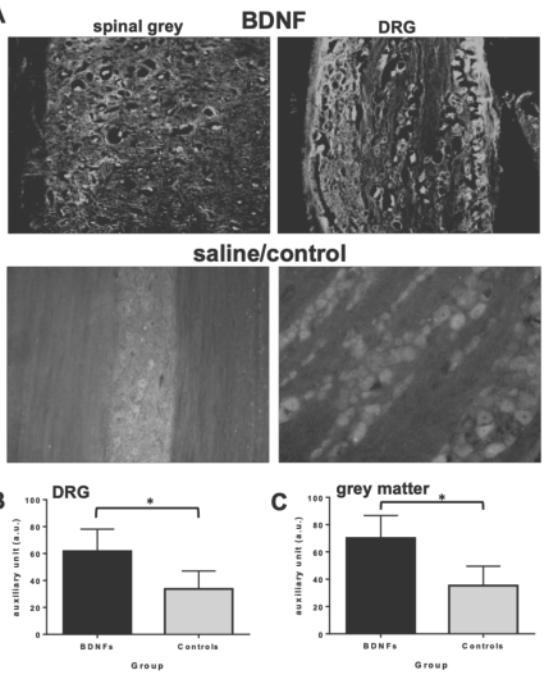

Figure 3. **Immunohistochemical BDNF staining** (BDNF purified MAXPab mouse polyclonal antibody (Abnova B02P)). (A) shows BDNF max fluorescence density in the ***cells*** of the DRGs and of the grey matter at the catheter tip/delivery site for a spinal cat that received intrathecal BDNF for 5 weeks and one that received saline for the same amount of time. No significant accumulation above background levels was observed at the delivery site of any of the animals that received saline. (B) shows the average (mean±SD) maximum fluorescence in the cells of the DRGs at the delivery site for BDNF treated (n=4) and saline (Controls) treated (n=4) cats. Fluorescence was significantly higher in the BDNF treated animals (Independent Samples *t*-test, p<.05). (C) shows the average (mean±SD) maximum fluorescence in the cells of the spinal grey matter at the level of the delivery site for BDNF treated (n=4) and saline (Controls) treated (n=4) cats. Fluorescence was again significantly higher in the BDNF treated animals (Independent Samples *t*-test, p<.05). Fluorescence in the DRG cells extended throughout the low lumbar cord while fluorescence in the cells of the grey matter was mostly at the delivery site (not shown). The results also confirm earlier reports demonstrating the stability of the protein in solution.[56, 66]

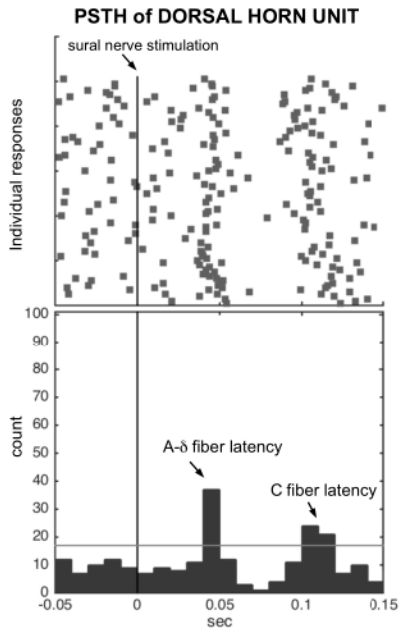

Figure 4. Effects of sural nerve stimulation on the firing of an L7 dorsal horn neuron. The raster plot on top demonstrates the firing responses (each line is a response) of a single unit following delivery of single pulse stimulus to the sural nerve (100μs duration, 10mA amplitude, i.e. ~20T). The bottom peri-stimulus-time histogram (PSTH) shows the histogram of the unit's response to stimulation. The latency of the responses peaks (about 40 msec and 100 msec) indicates that the effects are likely mediated by slow conducting A-δ (~5-20 m/s) or C-fibers (~1-2 m/s)[19] (measured distance of about 25 cm from the foot pad to the L7 cord). The orange horizontal line indicates the 95% confidence limit for the unit spontaneous firing, indicating that the two peaks above the line are statistically significant.[1] The figure demonstrates our ability to detect interneuronal firing in response to stimulation of afferent pathways. This particular unit was at a depth of 1100μm (lamina V) and may likely be a second order neuron as cells of origin of the spinothalamic tract are located within that lamina while C-fibers are not known to terminate that deeply in the dorsal horn. We plan to target dorsal units involved in nociception as well as mechanoreceptor processing units and compared responses between animals treated with BDNF, NT-3 or saline (CONTROL).

on partial SCI models. We believe that the potential negative impact of BDNF on nociception (reviewed in [72]) may depend on dosage, time of delivery and injury model. While exploring the full effects of those parameters in the development of nociception is beyond the scope of this project, we can establish the direct effect of BDNF delivery under our proposed acute and delayed treatment plans. In our model, we have developed tools to assess the dorsal horn interneuronal responses to nerve afferent stimulation at levels recruiting the nociceptive fibers. Our approach is based on stimulating peripheral nerves at varying stimulation levels and measuring the response of interneurons in the dorsal horn in spinals animals treated with saline versus those treated with NTF. Figure 4 shows the post-stimulus histogram for an interneuron recorded in lamina V (depth 1100 μm) of the L7 segment dorsal horn in one spinalized cat in response to stimulation of the sural nerve at a level (10mA) recruiting large sensory as well as small nociceptive fibers. Stimulation levels at which a unit is recruited and the latency of the response can then be used to confirm the unit types. Measurements over multiples sites are then used to establish the overall effects of neurotrophins on the interneuronal responses.

2) Responsiveness to the NIH Mandate for Reproducibility

The investigators are aware of potential problems, limitations, and difficulties in conducting unbiased science and those have been addressed as much as possible in the experimental design. The surgeon and experimenter (Lemay and staff) will be blinded to the nature of the solution used to fill the pump. The NTF or saline solution will be prepared by the Redacted by agreement and delivered to the Lemay laboratory at the time of surgery and pump refills. Cats will be randomly assigned to groups and the nature of the solution delivered to the spinal cord will not be revealed to the Lemay laboratory until all analyses (behavioral, kinematics, neurophysiological, histological, etc.) have been completed. Since the locomotor recovery obtained with NTFs is significant, we acknowledge that the Lemay laboratory staff is likely to have a good guess as to the nature of the solution in the pump following the locomotor treadmill sessions. The urinary system of the cat prevents the use of male spinal cats, but other biological variables such as age, weight, and underlying health conditions are fully considered. Biostatistical Computing Services available at the Lewis Katz School of Medicine will assist in statistical analyses. We will validate all commercial antibodies in the lab using western blots of normal cat spinal cord tissue and, when possible, tissue from spinal animals to determine the specificity of the antibodies.

3) Experimental Design

A) Study Design:

SA1. Intrathecal delivery of BDNF or NT-3 in an acute model of spinal cord injury

Animals: Adult female domestic short-hair cats will be used for this study. Females are used because they are easier to maintain post-spinalization (bladder expression is significantly more difficult in the male spinal cat and often involves daily catheterization which can lead to infections and bladder/sphincter/urethra damage).

General Experimental Protocol: A total of 30 animals will be used for this aim. All animals will receive an implant of a commercially available mini-pump connected to a cannula implanted through the L7/S1 inter-vertebral space. The pump will deliver saline (n=5, 5wks & n=5 12wks ACUTE CONTROL), BDNF (n=5, 5wks & n=5 12wks ACUTE BDNF) or NT-3 (n=5, 5wks & n=5 12wks ACUTE NT-3) over the course of 5 weeks following pump implant and simultaneous T11/T12 spinal transection. BDNF delivery rate was chosen to match the average daily production of BNDF by the cells in our previous results (50ug/day) and sufficient to restore treadmill stepping in our preliminary results without leading to any overt spasticity signs.[17, 56, 65] NT-3 dosage was similarly established. The period of delivery was limited to 5 weeks since our evidence suggests that neurotrophin production is down regulated by 5 weeks post-transplant when using genetically modified cells to deliver

BDNF/NT-3.[60] Recovery of plantar weight-bearing stepping will be evaluated at 3 and 5 weeks post-transection/implant using our standard kinematic measures[18, 60, 74] (see Detailed Methods below), n=5 of the animals in each treatment condition will be kept for 6 weeks following the end of neurotrophin delivery and their kinematic performance will be evaluated at the end (12 wks post-spinal transection).

The 12 weeks end-point matches most of our prior work with cellular delivery[18, 60, 74] where locomotor capabilities are maintained for a least 6 weeks after cellular neurotrophin delivery is down-regulated.[60] Similar results are expected for intrathecal delivery of NTFs. The animals receiving saline (CONTROL) are expected to show only minimal stepping on the dorsal aspect of the paw with no weight-support. We expect animals receiving neurotrophin intrathecal infusion (ACUTE BDNF or ACUTE NT-3) to recover plantar-weight bearing stepping by 5 weeks post-transection and to maintain this capability to 6 weeks post-end of neurotrophin delivery (as seen with cellular delivery). Mechanical allodynia and thermal sensitivity will be evaluated in all the animals at 3 and 5 weeks post-transection (and at 12 weeks in animals maintained to that point); animals receiving saline will serve as control for the animals receiving neurotrophins.

Thermal sensitivity will be evaluated using a tail/paw-flick method where the latency of the tail or paw's response to a heat stimuli is measured.[49] Mechanical allodynia will be measured using a repeated sub-threshold mechanical stimulus applied using a purpose-made device developed by Topcat Metrology (Topcat Metrology Ltd, Cambs, UK).[51]

Soleus H-reflex, dorsal horn interneurons' response to electrical stimulation of sensory afferents and intermediate zone interneurons' activity during a locomotor task (air-stepping) will be measured for our groups in a terminal experiment at the 6 or 13 weeks time points. Animals will be sacrificed following the neurophysiological recordings and their spinal cords analyzed to establish 1) the potential damage to the cord from the cannula implant (n=30 animals), 2) the extent of the spread of neurotrophin within the spinal tissue (all NTF groups, see Figure 3). All aim 1 animals will have the right sciatic nerve exposed 1 week prior to the terminal experiments, and the nerve will be injected with Cholera Toxin B (CTB) to trace large diameter fibers' potential sprouting in NTF treated animals. Calcitonin gene related peptide (CGRP) staining will be used to similarly evaluate the extend of the spread of small diameter fibers in the dorsal horns (see Figure 5).

SA2. Intrathecal delivery of BDNF in a chronic model of spinal cord injury

Animals: Adult female domestic short-hair cats will be used for this aim.

General Experimental Protocol: A total of 15 animals (n=5 CHRONIC CONTROL with saline delivery; n=5 CHRONIC BDNF; and n=5 CHRONIC NT-3) will be used for this aim. Animals will receive an implant of the same mini-pump connected to a cannula implanted at the L7/S1 inter-vertebral space. The pump will deliver saline, BDNF or NT-3 over the course of 5 weeks starting 6 weeks post-spinal transection. BDNF and NT-3 dosage of delivery will be the same as in aim 1, but a higher dose may be used in later animals if we observe a drop in performance over time or no recovery at the lower dose with chronic spinal animals. Recovery of plantar weight-bearing stepping will be evaluated using our standard kinematic measures and is expected to be similar to the one obtained for the NTF treated animals of SA1. Animals will be maintained up to twelve weeks post the onset of neurotrophins delivery and their locomotor characteristics and sensory responses (allodynia and thermal sensitivity) will be evaluated at 3, 5, and 12 weeks post-onset of neurotrophin infusion (matching with the animals of aim 1) to establish the long-term benefits and plasticity associated with the transient delivery of neurotrophins. Neurophysiological recordings as in SA1 will be conducted at 19 weeks post-spinalization (one week post CTB injection into the right sciatic nerve) and the animals will be sacrificed afterwards. Their spinal cords will be analyzed via histological methods as in SA1.

SA3. Dorsal horn interneuron responses to afferent stimulation and intermediate zone interneuron activity during air-stepping in BDNF, NT-3 or saline (CONTROL) treated spinal cats

Animals: Adult female domestic short-hair cats of Aims 1 & 2 will be used for this aim.

General Experimental Protocol: A total of 45 animals (all animals from Aims 1 & 2) will be used. The potential increase in spasticity in the neurotrophin treated animals will be evaluated by measuring the soleus h-reflex and its frequency adaptation during the terminal recording experiments. In addition, we will measure the responses of dorsal horn interneurons to noxious and non-noxious afferent stimuli to establish if BDNF and NT-3 have differential effects on the responses of interneurons in the complete spinal transection model, and the correlations between those changes and the behavioral responses.

During the final portion of the terminal experiments, we will measure the activity of intermediate zone interneurons during a locomotor activity (air-stepping). Details about the measurements and analyses to be performed are described below in the *Detailed Methodology* section.

	TIME RELATIVE TO TRANSECTION AND PUMP IMPLANT (in weeks)								
AIM	WK [-4,0]	WK 0	WKS 3 & 5	WK 6	WKS 9 & 11	WK 12	WK 13	WK 18	WK 19
AIM 1: Intrathecal delivery of BDNF or NT-3 in acute SCI	animals acclimated to treadmill, n=30	spinal transection + intrathecal pump implant n=10 animals, saline infusion n=10 animals, BDNF infusion n=10 animals, NT-3 infusion	locomotor kinematics, mechanical allodynia and thermal sensitivity assessments, n=30 sciatic nerve CTB injection, n=15	terminal recordings + perfusion, n=15		locomotor kinematics, mechanical allodynia and thermal sensitivity assessments, n=15 sciatic nerve CTB injection, n=15	terminal recordings + perfusion, n=15		
AIM 2: Intrathecal delivery of BDNF or NT-3 in chronic SCI	animals acclimated to treadmill, n=15	spinal transection + intrathecal pump implant n=15 animals, saline infusion	WK5: locomotor kinematics, mechanical allodynia and thermal sensitivity assessments	n=5 animals, saline infusion n=5 animals, BDNF infusion n=5 animals, NT-3 infusion	locomotor kinematics, mechanical allodynia and thermal sensitivity assessments at WK 11: infusion stopped, n=15			locomotor kinematics, mechanical allodynia and thermal sensitivity assessments sciatic nerve CTB injection, n=15	terminal recordings + perfusion, n=15
AIM 3: Measure responses of dorsal horn interneurons to noxious and innocuous stimuli				terminal recordings + perfusion, n=15 AIM 1 animals				terminal recordings + perfusion, n=15 AIM 1 animals	terminal recordings + perfusion, n=15 AIM 2 animals

Table 1. Experimental timeline for the animals of aims 1-3. Table shows the time at which measurements and procedures will occur relative to the time of transection and pump implant that are set as week 0 (WK 0).

SA1, SA2 and SA3: *Randomization and/or blinding*

[redacted] personnel will be preparing the solution to be injected into the pump in a randomized fashion. Due to the large effects observed with neurotrophins, Dr. Lemay's group is likely to become aware of an animal's group (NTF or saline) following the 1st or 2nd kinematic evaluation session. This may lead to a potential bias in the histological analyses that we will prevent by having Dr. Lemay removed the animal's identity from the spinal tissue before giving it to the individual responsible for histological analyses. Other variables (gait parameters, reflexes, neuronal responses) are strictly quantified and less susceptible to bias even if the Lemay Lab guesses the animal's group based on its locomotor recovery.

B) Detailed Methodology:

i) Animal Training and Kinematic Evaluation

Cats will be trained to walk on a motorized treadmill and the kinematics of the hindlimbs during locomotion will be collected using a high-speed video motion analysis system (Vicon Motion Systems Inc., USA).[18]

Testing of locomotor capability sessions will involve measuring the animal's hindlimb stepping on the treadmill with the evaluator providing balance support by holding the tail. Walking will commence at the lowest speed (0.2 m/s) and be increased in 0.1 m/s increments up to 0.8 m/s; 40-60 steps at each speed will be collected in each session. Perineal stimulation is used to engage locomotion in all animals as previously reported.[60, 74] In our experience, minimal stimulation is needed in the non-locomotor trained animals receiving neurotrophins (BDNF or NT-3), while untrained animals not receiving neurotrophins are incapable of more than a few plantar steps at the lower speeds even with significantly more stimulation.[18, 60, 74]

We will assess the extent of locomotor recovery using quantitative measures extracted from the kinematics measurements, and the animal's ability to perform weight-bearing plantar steps. Maximum speed of stepping is a good general indicator of the locomotor recovery following spinal transection[21] (10 consecutive steps must also be performed at all the lower speeds). With the cellular transplant producing neurotrophins, the locomotor recovery follows a binomial distribution after adult spinal transection and no treadmill body-weight supported training: animals receiving neurotrophin producing transplants recover the ability to plantar steps at speeds up to 0.8 m/s, while the animals that do not receive neurotrophin producing transplants cannot perform 10 consecutive plantar weight-bearing steps at any of the speeds tested[18, 74] or only at the lower speeds.[60] Other

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measures of hindlimb motor recovery include stride length, step height and a number of other kinematic parameters.[13, 60] Linear mixed models with the measure of interest as the dependent variable and groups (CONTROL, ACUTE BDNF, ACUTE NT-3, CHRONIC BDNF, CHRONIC NT-3) or conditions (pre/post-transection) as factors, with steps and time points as repeated measures, are used to establish the significance of the differences observed.[18, 60, 74]

ii) Heat Sensitivity and Mechanical Allodynia Measurements

Heat sensitivity will be measured using two tests: tail flick response and limb withdrawal response (to compare heat sensitivity with mechanical allodynia for the hindlimb). The tail flick response measures the latency of tail withdrawal from a noxious heat stimuli applied to the ventral distal end (3.5 cm distal segment) of the tail.[49] The shaved ventral distal end of the tail is placed over a notch and a noxious heat stimulus is applied on the ventral surface, and the response to withdrawal is recorded by a photosensor when the tail flicks away from the heat source. The test is terminated if the animal does not flick the tail after 10 seconds to avoid tissue damage. A series of four readings will be taken at 10 min intervals at each of the experimental time points (**Table 1**). The latency of the hindlimb withdrawal to a noxious heat stimulus applied to the footpad will be similarly measured. A linear-mixed model with treatment (CONTROL, ACUTE BDNF, etc) as factors and time points (3 weeks post-infusion, 5 weeks, etc) as repeated measures will be used to determine the effects of treatment or time on the heat sensitivity at both the tail and foot pad, with post-hoc tests used to compare average withdrawal time between animal groups.

Mechanical Allodynia is measured using a device (Topcat Metrology Ltd, Cambs UK) that applies a repeated innocuous mechanical stimulus to the lateral aspect of the metatarsus of the hindlimb. The stimuli are produced by a rounded metal pin (2.5 mm in diameter tip) mounted on an actuator. The actuator is attached by a band to the hindleg, and a stimulus with an intensity of 2N is repeatedly applied at intervals of 2.5 seconds up to 30 stimuli. The number of stimuli needed to elicit a withdrawal response is then noted, and the test repeated in a second session 2-4 hours later. The average number of stimuli between the two sessions is the value for that experimental time point. Parameters chosen were based on values used in arthritic cats,[51] but may be adjusted in initial sessions with spinal animals. A similar linear-mixed model analysis will be used to determine the effects of treatment (BDNF, NT-3, saline) and time post-injury on the mechanical allodynia and the estimated marginal means will be used to evaluate differences between animal groups.

iii) Terminal Neurophysiological Recordings

Under anesthesia, the sciatic, sural and medial plantar (MPL) nerves of the left and right hindlimbs will be implanted with nerve cuffs for stimulation/recording; bifilar EMGs will be implanted bilaterally in major hindlimb muscles (including *soleus*, *tibialis anterior*, *medial gastrocnemius*, *vastus lateralis*, *biceps femoris anterior*/*posterior*, *sartorius anterior*) (surgical details in [6, 7, 76]). Following an L3-L7 laminectomy and electrodes implantation, the animals will be transferred to a stereotaxic frame and decerebrated. H-reflex and interneuronal responses will be taken in the decerebrate state, avoiding the confounding effects of anesthetic agents.

iii-a) H-reflex measurements

Soleus H-reflex response to sciatic nerve stimulation will be measured to examine potential spasticity in the neurotrophin treated animals. Although we have not observed clinical manifestations of spasticity in our treated animals (heightened cutaneous reflexes, hopping rather than walking during treadmill walking, etc) the reflex measurements will provide a much more accurate measure of the effects of neurotrophins on the h-reflex which is typically heightened in spasticity.[3, 16, 67] We will obtain recruitment curves for the M-wave (muscle response) and H-wave (reflex response), as well as the frequency-dependent depression (FDD) of the H-reflex to repeated stimulation of the sciatic nerve (H-reflex adaptation to train of pulses at increasing frequency) (see [75] for methods). Variables analyzed include: motor threshold (MT), H-reflex threshold, maximal response amplitude for both M and H-wave (M_{max} and H_{max}), H_{max}/M_{max} ratio (which is believed to give an estimate of motoneuronal excitability), and FDD percentages at 3 stimulation frequencies (0.3, 5 and 10Hz). One-way ANOVA is then used to compare differences between groups of animals. By using both legs we should obtain a number of observations sufficient to obtain significant differences as we observed for similar reflex measurements in rats.[75]

iii-b) Dorsal horn interneuronal response to sural and MPL nerve stimulation

Interneuronal activity of lumbar dorsal horn interneurons in response to nerve stimulation[2, 30, 83] will be measured using multiunit electrode recording techniques developed in our laboratory, see [6, 7, 15] and Figure 4 above. Multiunit electrodes (A8x8-5mm-200-200-177, Neuronexus, Ann Arbor MI) are inserted into the dorsal horns of the L5-L7 cord covering the depths ranging from 500-1900 (Laminae I-VI in the cat). Single units are identified, and the responses of the units to trains of pulses (2 Hz, biphasic 100µs pulses) applied to the sural

and MPL nerves are measured. The cuff around the sciatic nerve is used to measure the stimulation threshold T (1st volley of evoked activity in the sciatic nerve in response to stimulation of the distal nerve) for the 2 stimulated nerves, allowing us to choose stimulus levels known to activate large diameter afferents such as Aβ (mechanoreceptors) (up to 2.3T), all the way to levels activating small nociception-carrying C-fiber afferents (10-50T).[19] We plan to stimulate at 1.5T (which recruit mechanoreceptors and proprioceptors, but Ia and Ib fibers terminate in deeper laminae than the ones targeted) and 20-30T (C-fibers responses, as well as lower threshold fibers). The animals are paralyzed with pancuronium bromide (1 mg/kg) to prevent extraneous sensory feedback from the movements induced by the nerve stimulation. The stimulated nerves (*sural*, MPL) were chosen based on our previous experience with them[76] and published evidence that synaptic transmission through those pathways is modulated by locomotor training.[27, 28] In addition, both nerves innervate the footpad and produce flexion withdrawal reflexes in response to noxious stimuli,[86] but not when activated at lower levels (<2T) where their effects is often dependent on the phase of gait at which stimulation is delivered.[31, 33, 34, 38, 39, 42, 43, 76, 84, 85]

Peri-stimulus-time histogram (PSTH) will be used to evaluate the changes in single units' firing in response to the afferent nerve stimulation.[1, 48] The responsiveness of single units to afferent stimulation will be evaluated with statistical testing of the significance of the peaks in PSTH.[1, 93] The PSTHs will provide information about the coupling of the sensory afferent to the dorsal horn interneurons sampled, e.g. latencies of the responses can determine if units are receiving monosynaptic or di/poly-synaptic inputs (we have observed significant peaks at latencies up to 25-30ms in some units, indicating that sufficient integration occurs at the synapses involved to produce spiking in the recorded units). We expect to measure responses in 200-300 units/animal based on previous experience with this preparation, which should be sufficient to obtain statistically significant differences in responsiveness between groups.[6, 7] Changes in responsiveness to nerve stimulation (noxious and non-noxious stimuli) between conditions (CONTROL (acute and chronic), ACUTE BDNF, ACUTE NT-3, CHRONIC BDNF, CHRONIC NT-3) will be evaluated by comparing the proportions of units responsive to stimulation (normalized to # sites sampled) using MANOVAs (with groups and stimulus levels as factors) or non-parametric χ^2 tests, and by comparing the response latencies of the units responding.

In addition to general increases in responsiveness due to BDNF or NT-3 infusion, some of the specific changes to explore include: **measurement 1**) ratio of dorsal (600-800µm depth, laminae I-II (some III)) to more ventral (1000-1600µm) dorsal horn units responsive to low level stimuli in BDNF, NT-3 and saline treated animals, **measurement 2**) ratio of dorsal (600-800µm depth, laminae I-II (some III)) to ventral (1000-1600µm) units responsive to high level stimuli in BDNF, NT-3 and saline treated animals.

Increases in **measurement 1**) with BDNF infusion would indicate an increase in nociception processing units (lamina I-II neurons[9, 57]) responsive to mechanoreceptor level of stimulation with BDNF, which could be the physiological substrate for the increased allodynia reported by some groups with BDNF administration.[25, 40] We do recognize that not all cells of origin of the spinothalamic tract are from lamina I (lamina V is also a minor contributor[9]) and that increases in conductivity to these deeper laminae neurons from laminae I-II could be responsible for the allodynic-like behavior observed. We might be able to observe this phenomenon as long latency responses in laminae III-V neurons in response to non-noxious stimuli. We do not expect an activity increase in nociception processing units for mechanoreceptor level stimuli for NT-3 treated animals, but we do expect in increased response in the laminae III-V neurons where larger diameter afferents responsive to NT-3 terminate.

Measurement 2) will provide information regarding the relative increase in responsiveness of nociception processing units (laminae I-II) and mechanoreceptor processing units (laminae III-V) to stimulation level recruiting both large (mechanoreceptors) and small (C-fibers) fibers. Increase in the ratio with BDNF infusion would suggest a greater increase in connectivity of noxious afferents to laminae I-II with no concurrent increase in connectivity of those same afferents to the termination zone of mechanoreceptor afferents with BDNF. Results will be analyzed for short latency responses (fast conduction fibers) and long latency responses (slow conduction fibers) to separate the contribution of the different receptor types (varying number of synapses in the transmission pathway produce latency differences in the order of 1-2 ms (for 2-4 additional synapses) while conduction velocities (1-2 m/s for C-fibers versus 30 m/s for mechanoreceptors' afferents) produce latency differences on the order of 100 ms for a 25 cm distance between stimulation and recording sites). The prediction with NT-3 infusion are that this ratio will decrease with NT-3 infusion, suggesting a greater increase in connectivity to the termination zone of mechanoreceptor afferents with no concomitant increase in connectivity to the nociception processing units of laminae I-II.

Our hypotheses are that transmission via both pathways will be enhanced (greater number of responsive units per sites sampled) in NTF treated animals (compared to saline infused animals) but that the proportions of

laminae I/II neurons to laminae III-V responsive to mechanoreceptor levels of stimulation will be lower in BDNF treated animals compared with saline treated animals, but not as low as for NT-3 treated animals. The increased responsiveness in mechanoreceptor's 2nd order neurons (laminae III-V) with BDNF may thus contribute to reducing allodynic pain-like behaviors (painful response to innocuous stimuli) by "gating"[69] the responses of the nociceptive 2nd order neurons (laminae I-II) to non-noxious stimuli. These neurophysiological measurements should then correlate with lower mechanical allodynia in BDNF treated animals compared to saline, but higher mechanical allodynia than in NT-3 treated animals. Our hypothesis about increased responsiveness in laminae I/II neurons to noxious stimuli in BDNF treated animals predicts a decreased latency to the heat stimuli in BDNF treated animals, but similar latencies for the NT-3 and saline treated animals.

iii-c) Intermediate zone interneuronal activity during locomotor task

In addition to the responses of dorsal horn interneurons to afferent stimulation, we are planning to record the activity of intermediate zone interneurons (laminae VII) during episodes of air-stepping (see [7] and Figure A1). To obtain longer and more robust bouts of locomotion, clonidine (500μg/kg) will be administered intravenously to prime locomotor behavior once pancuronium bromide's neuromuscular block washes out (~1/2 hour). The same multiunit electrodes will be used but the depth of penetration is increased and more segments are sampled (L3-L7). Our goal is to establish the changes in interneuronal activity in NTFs treated animals. As in our preliminary data, we hypothesize an increase in the number of active interneurons (normalized to the number of sites sampled) and firing frequency of these interneurons. In addition, we hypothesize that this increased excitability is maintained, in parallel with the locomotor recovery, up to 12 weeks following the end of NTFs delivery. Changes in activity between groups (CONTROL/ACUTE NTF (5 & 12 wks)/CHRONIC NTF) will be evaluated by comparing the number of active units/locomotor trials, average peak firing frequencies, average ratios of peak firing frequency/lowest firing frequency (modulation depth) for the units obtained (using ANOVA with post-hoc tests or non-parametric tests to compare the means between the different groups). Our preliminary results between animals that received saline of BNDF intrathecally at the lumbar cisterna for 5 weeks following transection have shown an increase in activity with those simple measures. Additional measures that we plan to explore will assess the modulation patterns of single unit activity during a step cycle.[37] For many units obtained in sub-chronic spinal cats, we found that the activity was most concentrated around a particular phase of gait, or preferred phase.[7] The proportion of units tuned to a phase of gait may increase with NTF, or the percentage of the phase of gait (flexion or extension) during which a tuned unit is active may also increase with NTF. The interneuronal activity data set will be particularly rich.

iv) Surgical Procedures

Transection: Animals are transected at the T11/T12 vertebral level following our published procedure.[18]

Mini-pump implantation: The pump (iPrecio SMP-200) offers a refillable 900 μL reservoir and can be programmed to deliver fluid at rates between 1.0 μL/hr to 30.0 μL/hr. The pump is actually a peristaltic infusion pump and allows for in-vivo refilling or emptying of its reservoir, giving us the ability to deliver neurotrophins at varying concentration for as long or as little as necessary. The pump is implanted on the back just rostral of the pelvis tip and is well tolerated by the cat. Entry into the dura mater is at the L7/S1 junction through a minute hole made in the dura matter with a 30G needle, the cannula (24G, ReCathCo LLC, Allison Park, PA) is inserted subdurally for approximately 1 cm rostral to the entry site. The cannula is then bonded to the dura mater with cyanoacrylate glue and further secured to the S1 dorsal process with a small silicon sheet. The cannula is then tunneled subcutaneously to the pump.

BDNF (Abnova H00000627-P02) or NT-3 (Abnova H00004908-P01) is prepared just before implant as in [66] (1 μg BDNF in 500 μL sterile 0.9 saline, delivery rate of 1.0 μL/hr). With a full reservoir, delivery is possible for 37 days (900 μL/24 μL). Saline infusion will be at the same rate (1.0 μL/hr) in the CONTROL groups.

Sciatic nerve injection: The right sciatic nerve will be exposed under anesthesia, after completion of the last kinematic and thermal and mechanical sensitivity evaluation sessions, and injected with 10-20μl of 1% cholera toxin B (CTB) subunit (Alexa Fluor labeled (594), Invitrogen) using a Hamilton syringe with a 30 gauge needle. This will label large caliber myelinated axons[58], without affecting neuronal firing obtained during the terminal neurophysiological recordings that will be conducted the week following nerve injection.[98]

v) Histology and Immunohistochemistry

Animals will be euthanized and perfused with 4% paraformaldehyde solution following the terminal neurophysiology recordings. The perfused spinal cords will be prepared following standard cryopreservation techniques,[18] sectioned at 30 μm intervals and mounted in a serial order. The injury site will be evaluated for

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lesion completeness using Nissl-myelin staining. The absence of trauma from the cannula insertion will be verified by GFAP and hematoxylin and eosin (H&E) staining of the lumbosacral cord from the insertion site (L7/S1) to the location of the tip of the cannula visualized at explantation.

The area of diffusion of BDNF or NT-3 within the spinal cord parenchyma will be visualized using a monoclonal anti-BDNF antibody western blot (Abnova)[66] or monoclonal anti-BDNF antibody western blot (Abnova). The antibody is specific to either the human BDNF or NT-3 infused with the pump and will allow us to specifically determine the diffusion of the exogenous BDNF/NT-3 applied with the pump. BDNF/NT-3 staining will be compared between neurotrophin treated animals and the ones receiving saline infusion (CONTROL).

Following a suggestion from our primary reviewer, we will label the extend of dorsal horn penetrations for small (calcitonin gene related peptide positive(CGRP+)) and large (cholera toxin B (CTB)) fibers using histology and quantification techniques in routine use in the [redacted] Briefly, outlines of laminas I-VI are used as templates[82] and applied to the appropriate locations in spinal cord images by an individual blinded to the experimental conditions. The CGRP+ or CTB stained axons occupying areas within the outline of each lamina are quantified using a macro that applies a standardized optical density threshold to each image and measures the area of staining equal or greater than the threshold using Nikon Elements Software.[92] Data across groups are then compared using ANOVA followed by *post-hoc* tests to determine significant differences between animals treated with saline, BDNF or NT-3.

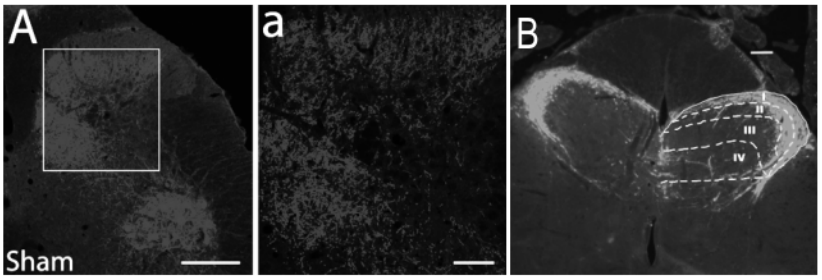

Figure 5. Labeling of dorsal horn large and small diameter fibers. Panel A shows labeling of large diameter axons in laminae III-VI as well as motor neurons following cholera toxin B (CTB) injection into the sciatic nerve prior to sacrifice in rats.[58] Panel a shows a higher magnification image or the boxed area in A. (Scale bar=300µm in A, 100µm in a and B). Panel B shows calcitonin gene related peptide positive (CGRP+) axons staining of the S3 spinal cord in a spinal cat that received intrathecal delivery of saline for 5 weeks following spinalization (CONTROL). The laminae demarcations are overlayed over the dorsal horns. CGRP+ and CTB stained axons penetrations into the laminar levels will be quantified and compared between experimental groups as described in the proposal.[58]

Redacted by agreement assisted in the preparation of the preliminary histological results presented in Figures 3 & 5 and is primarily responsible for both histology analyses and behavioral/spinal tests of heat sensitivity and mechanical allodynia.

C) Expected Results:

Our working hypotheses (supported by our preliminary data) are that neurotrophins transiently delivered into the cerebrospinal fluid at the level of the lumbar cord will re-engage the locomotor circuitry and allow the spinal cat to plantar-weight step on a treadmill without prior training. We expect that animals whose lumbar cord are infused with BDNF or NT-3 (acute or chronic spinal) will recover plantar weight-bearing stepping and at speeds up to 0.8 m/s, while the animals receiving saline will be unable to step (10 consecutive plantar weight-bearing steps) at any of the speeds tested. We expect recovery of other kinematic measures to be similar to our results with cell delivery of neurotrophins into the transection site.

Our preliminary data (Figure A1) suggest that the recovery correlates with increased activity of the intermediate zone interneurons of the lumbar cord. We hypothesize that this increased activity is plastic and is maintained after delivery of NTF has ceased. While we predict differential actions of BDNF and NT-3 on sensory response, we hypothesize that the increase in activity for intermediate zone interneurons will be similar with either neurotrophins because of the widespread distribution of afferent inputs onto interneurons. Nevertheless, the two neurotrophins are expected to differentially modulate sensory modalities. Anatomically, we expect to see increased CGRP staining in laminae I-II in BDNF treated animals, with a potential increase in deeper laminae staining. NT-3 is expected to preferentially increase large afferent sprouting (CTB stain) into the deeper dorsal horn laminae (III-V).

We propose that a transient 5-weeks delivery of BDNF into the lumbar cord cerebrospinal fluid will not lead to the development of spasticity symptoms in the animals. Spasticity measures will not be statistically different between groups infused with neurotrophins and the group infused with saline. As in the rats, we expect BDNF infusion to normalize the frequency-dependent depression observed in spinal animals.[26] H_{max}/M_{max} ratio (measure of motor pool excitability) is expected to be slightly increased in the BDNF treated cats as it was in exercised rats with elevated spinal BDNF levels[26]). Interneuronal responses to non-noxious (mechanoreceptor) and noxious stimuli are difficult to predict and will require careful interpretation. While intrathecal delivery of BDNF protects against the mechanical hyper-reactivity (allodynia) induced by peripheral nerve stimulation at levels eliciting flexion withdrawal in spinal animals,[53] others have shown increases in mechanical reactivity and

dorsal horn neurons' responses to peripheral stimulation with BDNF delivery in intact animals (neonatal slice preparation or adult intact).[25, 46] Our experiments should provide useful information about the changes in mechanoreceptor responses that may be beneficial in functional tasks such as locomotion (e.g. footpad load feedback promotes extensor activation in the stance phase[68]) but also on increases in both low (mechanoreceptor) and high-threshold (Aδ and C-fiber) afferents' responsiveness which may contribute to hyperalgesia to mechanical stimuli and chronic pain after spinal cord injury.

Similar results are expected in terms of h-reflex responses for infusion of NT-3. Our results on the modification of the h-reflex in trained rats correlated with an increase in BDNF and NT-3 spinal levels,[26] so it is difficult to pin the increase in H_{max}/M_{max} ratio and the normalization of the frequency dependent response on specifically one neurotrophin. NT-3 is more specifically linked to increase in the monosynaptic afferent pathway connection strength,[4, 44, 71] which would predict larger h-reflex responses, although we found no study reporting on h-reflex modifications following increases in NT-3 only. The role of NT-3 in neuropathic pain (reviewed in [59, 87]), is as mixed as it is for BDNF, but the current consensus seems to indicate that NT-3 may Increase mechanical allodynia, as observed following spinal intrathecal delivery in normal rats,[95, 96] but reduce thermal hyperalgesia.[97] The sprouting of Aβ fibers into lamina II observed in intact rats[95] would predict an increase in upper laminae (II specifically) interneuronal firing for low level nerve stimulation, but a potential decrease in the interneurons' response to high level stimuli. Behavioral effects to mechanical and thermal stimuli may be dependent on the ratio of mechanoreceptors to noxious interneurons activated and the potential gating of nociceptive responses as described above.

While chronic pain cannot be studied in the transection spinal cord injury model (no sensory information ascending past the injury site in a full transection), the information gained will provide valuable information for investigators working on partial spinal cord injury models. We predict that changes in dorsal horn interneuronal responses will correlate with our thermal and mechanical sensitivity measurements. Studies in nerve or root injuries typically find strong correlations between the appearance of sensory dysfunction and changes in the dorsal horn neurons' responses to nerve stimuli[90, 91, 94], although the time course of the changes may vary. These strong correlations between dorsal horn neurons' responses to peripheral nerve stimuli and sensory dysfunction behavioral assessment measures in spinal intact animals are evidence that our experiments in a full transect model are a valid approach to elucidating the potential role of NTFs in the development of sensory dysfunction in incomplete spinal cord injuries.

A full answer to the role of neurotrophins in chronic pain is definitely outside the scope of this project, but we believe that the potential negative impact of BDNF and NT-3 on pain[59, 72, 87] may depend on dosage, time of delivery and injury model.[50, 54] The source of BDNF may also affect the development of pain with BDNF from glia being particularly involved in the development of neuropathic pain.[29] In our cat SCI model, neurotrophins delivery promotes load-bearing rather than foot withdrawal, suggestive of the expected greater increase in mechanoreceptor afferent responses (compared to nociceptive responses increase) with neurotrophin intrathecal delivery. BDNF and NT-3 may offer different balances between the responses to low mechanical threshold afferents, and nociceptive inputs. One potential outcome is that both neurotrophins have similar effects to mechanical/large afferent stimuli, but that NT-3 shows significantly less sensitization to noxious stimuli.

We expect minimal to no damage from the cannula system, and limited diffusion of the neurotrophins due to the sticky nature of the molecules, the propensity of TrkB/TrkC receptors in the spinal cord, and previous results with delivery into the spinal cord's cerebrospinal fluid in rats.[66]

D) Anticipated Problems and Alternative Strategies:

Our experience (n=8 animals) with the proposed pump and cannula, and with the spinal cat model, give us confidence that technical problems related to the delivery methods will be minimal, and that aims 1 & 2 can be achieved without major modifications or adjustments in the proposed methods. Some adjustments to the infusion protocol made be necessary for the animals of aim 2 where NTF infusion is to start 6 weeks post-injury and only last for 5 weeks. We have obtained good recovery that was sustained for at least 12 weeks post-NTF delivery in chronic SCI animals where the NTF producing cells were implanted 6 weeks following spinalization.[60] In that study, BDNF only was delivered via autologous cells whose neurotrophin production is down-regulated by 5 weeks post-grafting. If we observe no recovery by 5 weeks after the onset of NTF infusion, we may extend the period of NTF delivery or the dosage used. Based on the results with autologous cells delivered in a similar chronic injury model and the wide variability in the cell NTF production, we expect that our selected dose will be sufficient to re-engage the locomotor circuitry in the animals of aim 2.

The new groups of aim 1 animals serve two purposes: 1) evaluate the "longer"-term plasticity of the increase in neuronal firing and locomotor recovery (primary reviewer experimental design concern), and 2) compare

recovery and neuronal responses (dorsal horn and intermediate zone neurons) at the same time point post-NTF delivery onset for acute or delayed delivery of neurotrophins (secondary reviewer experimental design concern). We recognize that the animals of aims 1 and 2 will be evaluated at different time points post-spinal transection (13 weeks *versus* 19 weeks) and this may potentially influence reflex and interneuronal activity, although the time difference (6 weeks) is not particularly long compared with the time post-injury (minimum 13 weeks). Nevertheless, we will carefully examine our data for systematic changes related to time in the reflex and neuronal responses at the 6 weeks, 13 weeks and 19 weeks terminal experiments. If we suspect an effect of time post-spinal transection on the terminal recordings, we will take some of the animals of aim 1 out to 19 weeks post-spinalization.

Intermediate zone interneuron recordings during air-stepping are routine in the laboratory, but the added dorsal interneurons recordings will add time to the terminal sessions. We may need to prepare additional animals if a number of preparations do not survive long enough to allow all the measurements to be completed in every animal. Clonidine administration (given after the dorsal horn interneuron responses to nerve stimuli are collected) is necessary to obtain bouts of locomotion sufficiently long to establish statistically significant differences in activity. It may produce an over-estimation of the number of neurons active during air-stepping as it has been shown to increase polysynaptic excitation in trained and untrained spinal cats, with the excitation being more prevalent in trained cat and likely responsible for the increased locomotor capability of trained animals.[28] The increased neuronal activity with NTF delivery is likely a similar result of the effects of clonidine and NTF. We may attempt epidural stimulation in some early experiments and see if it produces sufficiently long (>45s) bouts of locomotion following interruption of the stimulation. Otherwise, the stimulus artifacts produced by electrical stimulation prevent the use of those methods to elicit locomotion.

Thermal hyperalgesia and mechanical allodynia are new behavioral measurements for the laboratory but [redacted], our collaborator, has years of experience with these tests in the rat, and we have adapted our proposed tests from published methods used successfully in the cat. Our terminal electrophysiological results will also allow us to investigate the mechanisms associated with the results of the behavioral tests. We are quite familiar with H-reflex measurements[26] and do not anticipate issues with this test. The interneuronal response to noxious and non-noxious stimuli will provide information about dorsal horn interneuronal responsiveness to mechanoreceptors and C-fibers afferents with/without BDNF/NT-3 delivery.

E) Overall Summary:

Although major progress in locomotor rehabilitation strategies for spinal cord injuries have been made, BWST in complete SCI individuals (ASIA A), even with epidural stimulation, still results in limited stepping and weight-bearing capability. We have developed an approach that can be used in combination with both BWST and epidural stimulation that may further the recovery in humans, based on the combinatorial possibility shown in animals. This proposal will advance our knowledge of the ability of neurotrophins at re-engaging the locomotor when directly delivered to the spinal circuits in acute and chronic models of spinal injury. Further we will explore the differential effects of the two neurotrophins shown to produce stepping on the sensory responses of the dorsal horn interneurons to innocuous and noxious stimuli and on the activity of the lumbar locomotor centers. Those results will be important in guiding future clinical applications of the neurotrophins to spinal cord injured individuals.

F) Timeline:

The timeline allows ample time for analyses of the multiunit activity, which is labor intensive and time consuming. We have budgeted ample time over the five years of the study to the analyses of both neural and kinematic data. This will ensure timely publication of our results.

AIM	TASK	Y 1	Y 2	Y 3	Y 4	Y 5
AIM 1: Intrathecal delivery of BDNF or NT-3 in acute SCI	• Conduct sensory behavioral assays preliminary testing on spinal animals	■				
	• Spinalize/Implant pump in n=30 animals with BDNF or saline or NT-3 delivery	■	■	■		
	• Prepare and submit manuscripts		■	■		
AIM 2: Intrathecal delivery of BDNF or NT-3 in chronic SCI	• Spinalize/Implant pump in n=15 animals with BDNF or saline or NT-3 delivery		■	■	■	■
	• Prepare and submit manuscripts				■	■
AIM 3: Measure responses of dorsal horn interneurons to noxious and innocuous stimuli	• Measure interneuronal responses to innocuous and noxious stimuli for the animals of Aim 1	■	■	■		
	• Measure interneuronal responses to innocuous and noxious stimuli for the animals of Aim 2		■	■	■	■

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No
Other Requested information	

Vertebrate Animals

All surgical interventions, pre- and post-surgical care will be provided in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council), and approval from the Institutional Animal Care and Use Committee of Temple University.

1. Description of Procedures

Veterinary care of animals Animals will be housed in Redacted by agreement of Temple University Redacted by agreement Animal facilities Redacted by agreement were renovated to conform to AAALAC standards and include 2 fully-equipped animal surgery suites. Special services and facilities (surgery, x-ray, laboratory, research, etc.) are also available. The animal facility employs 3 staff veterinarians and 5 staff veterinary technicians available for consultation, teaching, and research support. In addition to the veterinary round, a member of the research team will check twice daily on the animals.

Behavioral Procedures Animals are acclimated to walk on an enclosed treadmill using food reward and other positive reinforcements. The other behavioral tests (mechanical allodynia and thermal sensitivity) are conducted on the limbs rendered insensate by the spinalization, and measures such as skin inspection and time limits are used to ensure that the animals's limbs are unharmed during the procedure.

Anesthesia and Surgical Procedure The spinalization and sciatic nerve injection are survival surgeries to be conducted under aseptic conditions. These surgeries are to be performed in the surgical suite of the Animal Facility. Details of the spinalization procedure used in our laboratory and the post-spinalization training procedures can be found in (Boyce et al. 2007). The sciatic nerve injection involves exposing the sciatic nerve while the animal is under isoflurane anesthesia and injecting 10-20µl of 1% cholera toxin B (CTB) subunit (Alexa Fluor labeled (594), Invitrogen) using a Hamilton syringe with a 30 gauge needle. Muscles and skin are then closed in layers.

During the acute terminal experiments, animals will be fully anesthetized until decerebration to alleviate pain and avoid suffering. Food will be withheld for 12 hours before anesthesia. The animal is anesthetized using ketamine HCl (Ketaset, 15 mg/kg, IM) given in combination with atropine sulfate (.05mg/kg, IM), masked with isoflurane (1-5% in O2), intubated and maintained at a surgical level of anesthesia with isoflurane (1-3% in O2). The cephalic vein will be catheterized to administer fluid/drugs during the procedure. During surgery, anesthesia will be maintained with gaseous isoflurane (1-3% in oxygen). Anesthesia level will be determined by monitoring heart rate, blood pressure through an arterial line, and withdrawal and blink reflexes. Animal will be ventilated at a controlled rate, body temperature will be maintained between 37° and 39° C using thermal pads, warm 0.9% saline with 8.4 mg/cc sodium bicarbonate and 5% dextrose added will be administered IV (~20 cc/hr), and blood pressure and expired CO2 will be monitored throughout the experiment. The decerebration procedure involves the following: The bone over the occipital and parietal lobes will be removed. The carotid arteries will be occluded, and the brainstem transected just rostral to the superior colliculi and continuing rostroventrally to a point caudal to the mammillary bodies, producing a mesencephalic or postmammilary preparation. The brain rostral to the transection will be removed (including cortex and thalamus) and the skull packed with Surgicel, Avitene, and Agar to control bleeding. Visual verification of the complete removal of the cortex and thalamus is sufficient to assure proper decerebration as concluded by the decerebration subcommittee of the MCP Hahnemann University Institutional Animal Care and Use Committee (White paper on Decerebrate Mammalian Preparations). Dextran will be administered if needed to maintain blood pressure. Once the decerebration is completed, anesthesia is discontinued prior to undertaking recordings in the cord. Discontinuing anesthesia in favor of decerebration will increase the neural responsiveness to the perineal stimulation used to initiate locomotion.

Following completion of the recording experiments (typically 16-20 hours after anesthesia induction), the animals will be euthanized with an overdose of Euthasol (0.4 mg/kg, IV), a method consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

An animal will be removed from the study if it is unable to eat or drink. This will be determined by monitoring the animal's weight to be certain that loss does not exceed 20% of the pre-spinal transection value (animals lose a fair amount of muscle mass in the hindlimbs which often leads to >15% weight loss in the initial month following transection). If an animal loses more than 20% of its pre-transection weight, its dietary food will

be enriched, and the animal will be removed from the study if it can't gain weight back. If it is determined that the animal has an urinary or skin infection, the ULAR Clinical Veterinarian will be consulted and a 10 day course of antibiotics will be initiated and the animal will be evaluated after this treatment. If the condition(s) described above worsens (urinary blockade, which we have never observed in over 20 years), the animal will be removed from the study. In rare instances of self-mutilation (chewing of the hindlegs or tail) the animal will be euthanized.

Post-procedural care of spinalized animals Following the spinalization procedure described in the application, animals will be housed in individual cages lined with a foam mattress to provide cushioning, warmth, and moisture absorption. Cats will be checked and treated twice per day by members of the investigative team. The bladder will be expressed manually or reflex micturition will be initiated by light tactile stimulation of the perineum at least twice per day. The bowel will be evacuated manually or by enema, as required. Subcutaneous lactated Ringer's solution (25cc) will be administered daily to maintain normal hydration in the immediate post-operative period. Antibiotics (ampicillin, 15 mg/day) will be administered as needed. Analgesics (buprenorphine, 0.005-0.01 mg/kg, SQ, every 12 hours or transdermal fentanyl patches, 25µg/hr for 48 hrs) will be administered if pain is indicated by guarding or vocalization. Analgesia will be indicated by normal appetite and absence of signs of pain. The area around the perineum will be shaved and cleaned as needed to prevent dermatitis. The skin of the hindlimb will be massaged to prevent pressure sores, and the hindlimbs will be moved throughout their range of motion to prevent orthopedic problems. Animals will be fed dry chow and distilled water ad libidum. Dry chow will be supplemented or replaced by canned food (CD) as needed. In addition, 1 vitamin tablet will be administered daily. Restriction to distilled water and use of urine acidifer food greatly reduce the incidence of bladder infections (Eldridge 1984).

2. Justifications

Animal models of SCI are used for this research as no reduced preparations or computer modeling can capture and reproduce the effects of spinal cord injuries and interventions on the locomotor behavior of the animals and its effects on the motor and sensory systems.

The animal model proposed for this study is the cat (spinal females since they are easier to care for and remain healthier after spinalization (Roy et al. 1992)), which has been the model of choice for the large majority of systems level neural studies because of its similarities to the human system. The somatic motor system, and particularly the hindlimb motor system and its reflexes (Jankowska 1992), bears a strong resemblance to what is known of the human motor system (Holstege 1996). The skeletal motor system of the cat has been studied extensively, and use of the existing data will avoid unnecessary duplication of research. They are the smallest cursorial mammals and walk with extended legs as do humans. Cats are easy to handle, and the size and structure of their spinal cord is comparable to humans. The length and diameter of the human and feline spinal cords differ, but available data suggest that there is a scaling with body size between the two species and that a similar laminar relationship exists in the two species. The length of the human spinal cord is approximately 26% of body height with the mean length ranging from 40-45 cm in adults (Perese and Fracasso 1959), while the mean length of the feline spinal cord is 34 cm (Blinkov and Glezer 1968).

Furthermore, in humans the lumbar segments occupy approximately 5.4 cm (13%) and the sacral segments occupy approximately 3 cm (7.3%) of the total cord length (Lassek and Rasmussen 1938). In the feline, the lumbar segments are 8.2 cm (24%) in length and the sacral segments are 2.0 cm in length (5.9%). The cross sectional area of the adult human spinal cord varies from 0.36-0.97 cm^2. In the lumbar cord the grey matter and white matter occupy 0.19 cm^2 and 0.46 cm^2 respectively, and in the sacral segments occupy 0.16 cm^2 and 0.19 cm^2 respectively (Lassek and Rasmussen 1938). A similar reduction in cross sectional area and ratio of white matter to gray matter occurs in the cat (Blinkov and Glezer 1968). Thus, although the lumbar segments are greater in number (7 in cat, 5 in man), and occupy a greater percentage of the cord length in feline than in man, there is a similar relationship between the feline and human lumbosacral spinal cord geometry.

Number of Animals Used A total of 45 cats are proposed and this number is sufficient to obtain statistically significant differences in the gait parameters and neuronal responses. Our previous results have established that groups of 4 animals (Boyce et al. 2007) are sufficient to establish significance for the parameters of gait between conditions (trained/untrained/neurotrophin treated), and we assume similar size effects and disruption in gait in this population of spinal animals, with a similar treatment effect for the neurotrophins. Power analysis

for the mechanical allodynia, thermal hyperalgesia and h-reflex responses shows that a groups size of 5 should allow us to detect differences of about 20% with a type I error rate (α) of .05 and a power of .80 if the underlying variance is about 11%. Based on the available literature on mechanical allodynia and thermal hyperalgesia in cats, our estimates of the variances in those tests are reasonable (Goldstein and Malseed 1979; Guillot et al. 2014) so we should be able to detect changes as small as 20% with our sample size. Studies on correlating neuronal activity from cortex/brainstem with motor activity during gait were successfully completed with n less than 4 per group if sufficient units were recorded per animal (Drew et al. 1986; Drew et al. 1996; Kably and Drew 1998; Lavoie and Drew 2002; Matsuyama and Drew 2000a; b). We expect a similar outcome for our terminal interneuronal recordings since the yield in neurons recorded is high with multiunit electrodes. Group sizes of 5 will allow us to compensate for the potential lost of animals at decerebration.

Randomization and/or blinding

Redacted by agreement personnel will be preparing the solution to be injected into the pump in a randomized fashion. Dr. Lemay's group will be blinded to the nature of the solution until all kinematics, histological and electrophysiological analyses are completed. Due to the large effects observed with neurotrophins, Dr. Lemay's group is likely to become aware of an animal's group (NTF or saline) following the 1st or 2nd kinematic evaluation session. Animal identity will be blinded by Dr. Lemay before the spinal tissue is passed to the individual (post-doc or graduate assistant) responsible for histological analyses.

Data collected include the kinematics of gait which are stored within the Vicon (motion analysis system) database system which is automatically backed up weekly. The thermal hyperalgesia and mechanical allodynia are noted within each animal's laboratory notebook and remain with the investigator. Finally the neural data of the terminal experiments (h-reflex, interneuronal recordings) are collected and stored both as raw data on the RS-4 (streaming RAID array attached to the TDT acquisition system) of the TDT system and on a local server which is backed up following each experiment. Statistical analyses are performed with the SPPS environment (SPSS Statistics 24, IBM Corp, Armonk, NY). The individual statistics to be run on each variables collected are detailed in the application.

3. Minimization of Pain and Distress

The anesthetics and analgesia regimen used to alleviate pain and discomfort as well as the post-surgical provisions and care are described above in the *Description of Procedures* section. An animal will be removed from the study if it is unable to eat or drink. This will be determined by monitoring the animal's weight to be certain that loss does not exceed 20% of the pre-spinal transection value (animals lose a fair amount of muscle mass in the hindlimbs which often leads to >15% weight loss in the initial month following transection). In rare instances of self-mutilation (chewing of the hindlegs or tail) the animal will be euthanized. Animals removed from the study prematurely will be euthanized with euthasol while under anesthesia and death will be confirmed by bilateral thoracotomy.

4. Method of Euthanasia

As stated above, the animals will be euthanized with an overdose of Euthasol (0.4 mg/kg, IV), a method consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

References cited in Vertebrate Animals Section

Blinkov SM, and Glezer II. *The human brain in figures and tables; a quantitative handbook [by] Samuil M. Blinkov, and Ilya I. Glezer*. New York: Basic Book, 1968, p. 482.

Boyce VS, Tumolo M, Fischer I, Murray M, and Lemay MA. Neurotrophic factors promote and enhance locomotor recovery in untrained spinalized cats. *J Neurophysiol* 98: 1988-1996, 2007.

Drew T, Dubuc R, and Rossignol S. Discharge patterns of reticulospinal and other reticular neurons in chronic, unrestrained cats walking on a treadmill. *J Neurophysiol* 55: 375-401, 1986.

Drew T, Jiang W, Kably B, and Lavoie S. Role of the motor cortex in the control of visually triggered gait modifications. *Can J Physiol Pharmacol* 74: 426-442, 1996.

Eldridge L. Lumbosacral spinal isolation in cat: surgical preparation and health maintenance. *Exp Neurol* 83: 318-327, 1984.

Goldstein FJ, and Malseed RT. Evaluation of narcotic analgetic activity using a cat tail-flick procedure. *Journal of Pharmacological Methods* 2: 333-338, 1979.

Guillot M, Taylor PM, Rialland P, Klinck MP, Moreau MM, Martel-Pelletier J, Pelletier JP, and Troncy E. Evoked temporal summation in cats to highlight central sensitization related to osteoarthritis-associated chronic pain: a preliminary study. *PLoS One* 9: e97347, 2014.

Holstege G. The somatic motor system. *Prog Brain Res* 107: 9-26, 1996.

Jankowska E. Interneuronal relay in spinal pathways from proprioceptors. *Prog Neurobiol* 38: 335-378, 1992.

Kably B, and Drew T. Corticoreticular pathways in the cat. II. Discharge activity of neurons in area 4 during voluntary gait modifications. *J Neurophysiol* 80: 406-424, 1998.

Lassek AM, and Rasmussen GL. A quantitative study of newborn and adult spinal cords of man. *Journal of Comparative Neurology* 69: 371-379, 1938.

Lavoie S, and Drew T. Discharge characteristics of neurons in the red nucleus during voluntary gait modifications: a comparison with the motor cortex. *J Neurophysiol* 88: 1791-1814, 2002.

Matsuyama K, and Drew T. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. I. Walking on a level surface. *J Neurophysiol* 84: 2237-2256, 2000a.

Matsuyama K, and Drew T. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. II. Walking on an inclined plane. *Journal of neurophysiology* 84: 2257-2276, 2000b.

Perese DM, and Fracasso JE. Anatomical considerations in surgery of the spinal cord. A study of vessels and measurements of the cord. *Journal of Neurosurgery* 16: 314-325, 1959.

Roy RR, Hodgson JA, Lauretz SD, Pierotti DJ, Gayek RJ, and Edgerton VR. Chronic spinal cord-injured cats: surgical procedures and management. *Lab Anim Sci* 42: 335-343, 1992.

References Cited

[1] M. Abeles, "Quantification, smoothing, and confidence limits for single-units' histograms," Journal of neuroscience methods, vol. 5, pp. 317-25, May 1982.

[2] D. Andrew and A. D. Craig, "Quantitative responses of spinothalamic lamina I neurones to graded mechanical stimulation in the cat," J Physiol, vol. 545, pp. 913-31, Dec 15 2002.

[3] R. W. Angel and W. W. Hofmann, "The H Reflex in Normal, Spastic, and Rigid Subjects," Arch Neurol, vol. 9, pp. 591-6, Jun 1963.

[4] V. L. Arvanian, P. J. Horner, F. H. Gage, and L. M. Mendell, "Chronic neurotrophin-3 strengthens synaptic connections to motoneurons in the neonatal rat," J Neurosci, vol. 23, pp. 8706-12, Sep 24 2003.

[5] N. AuYong, "Characterizing spinal interneuronal activity during air-stepping in sub-chronic spinal cats," in Neurobiology and Anatomy Philadelphia: Drexel University College of Medicine, 2009, p. 371.

[6] N. Auyong, K. Ollivier-Lanvin, and M. A. Lemay, "Population spatiotemporal dynamics of spinal intermediate zone interneurons during air-stepping in adult spinal cats," Journal of Neurophysiology, vol. 106, pp. 1943-53, Oct 2011.

[7] N. Auyong, K. Ollivier-Lanvin, and M. A. Lemay, "Preferred locomotor phase of activity of lumbar interneurons during air-stepping in subchronic spinal cats," Journal of Neurophysiology, vol. 105, pp. 1011-22, Mar 2011.

[8] H. Barbeau and S. Rossignol, "Recovery of locomotion after chronic spinalization in the adult cat," Brain Res, vol. 412, pp. 84-95, May 26 1987.

[9] G. Battaglia and A. Rustioni, "Substance P innervation of the rat and cat thalamus. II. Cells of origin in the spinal cord," J Comp Neurol, vol. 315, pp. 473-86, Jan 22 1992.

[10] J. M. Bauman and Y. H. Chang, "High-speed X-ray video demonstrates significant skin movement errors with standard optical kinematics during rat locomotion," J Neurosci Methods, vol. 186, pp. 18-24, Jan 30 2010.

[11] BDNF Study Group, "A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III)," Neurology, vol. 52, pp. 1427-33, Apr 22 1999.

[12] C. G. Begley and L. M. Ellis, "Drug development: Raise standards for preclinical cancer research," Nature, vol. 483, pp. 531-3, Mar 28 2012.

[13] M. Belanger, T. Drew, J. Provencher, and S. Rossignol, "A comparison of treadmill locomotion in adult cats before and after spinal transection," J Neurophysiol, vol. 76, pp. 471-91, 1996.

[14] M. Belanger, T. Drew, and S. Rossignol, "Spinal locomotion: a comparison of the kinematics and the electromyographic activity in the same animal before and after spinalization," Acta Biol Hung, vol. 39, pp. 151-4., 1988.

[15] J. F. Bonner, T. M. Connors, W. F. Silverman, D. P. Kowalski, M. A. Lemay, and I. Fischer, "Grafted Neural Progenitors Integrate and Restore Synaptic Connectivity across the Injured Spinal Cord," The Journal of neuroscience : the official journal of the Society for Neuroscience, vol. 31, pp. 4675-86, Mar 23 2011.

[16] L. J. Bour, B. W. Ongerboer de Visser, J. H. Koelman, G. J. van Bruggen, and J. D. Speelman, "Soleus H-reflex tests in spasticity and dystonia: A computerized analysis," J Electromyogr Kinesiol, vol. 1, pp. 9-19, 1991.

[17] V. S. Boyce, J. Park, F. H. Gage, and L. M. Mendell, "Differential effects of brain-derived neurotrophic factor and neurotrophin-3 on hindlimb function in paraplegic rats," Eur J Neurosci, vol. 35, pp. 221-32, Jan 2012.

[18] V. S. Boyce, M. Tumolo, I. Fischer, M. Murray, and M. A. Lemay, "Neurotrophic factors promote and enhance locomotor recovery in untrained spinalized cats," J Neurophysiol, vol. 98, pp. 1988-96, Oct 2007.

[19] I. A. Boyd and K. U. Kalu, "Scaling factor relating conduction velocity and diameter for myelinated afferent nerve fibres in the cat hind limb," J Physiol, vol. 289, pp. 277-97, Apr 1979.

[20] E. J. Bradbury, S. Khemani, R. Von, King, J. V. Priestley, and S. B. McMahon, "NT-3 promotes growth of lesioned adult rat sensory axons ascending in the dorsal columns of the spinal cord," The European journal of neuroscience, vol. 11, pp. 3873-83, Nov 1999.

[21] E. Brustein and S. Rossignol, "Recovery of locomotion after ventral and ventrolateral spinal lesions in the cat. I. Deficits and adaptive mechanisms," J Neurophysiol, vol. 80, pp. 1245-67, 1998.

[22] W. B. J. Cafferty and M. S. Ramer, "Promoting sensory axon regeneration across the PNS-CNS interface," in Glial Interfaces in the Nervous System, H. Aldskogius and J. Fraher, Eds.: IOS Press, 2002.

[23] P. Carroll, G. R. Lewin, M. Koltzenburg, K. V. Toyka, and H. Thoenen, "A role for BDNF in mechanosensation," Nat Neurosci, vol. 1, pp. 42-6, May 1998.
[24] N. S. C. I. S. Center, "Spinal Cord Injury facts and figures at a glance," Birmingham, AL: University of Alabama, 2017, p. 2.
[25] L. Constandil, R. Aguilera, M. Goich, A. Hernandez, P. Alvarez, C. Infante, and T. Pelissier, "Involvement of spinal cord BDNF in the generation and maintenance of chronic neuropathic pain in rats," Brain Res Bull, vol. 86, pp. 454-9, Nov 25 2011.
[26] M. P. Cote, G. A. Azzam, M. A. Lemay, V. Zhukareva, and J. D. Houle, "Activity-dependent increase in neurotrophic factors is associated with an enhanced modulation of spinal reflexes after spinal cord injury," Journal of neurotrauma, vol. 28, pp. 299-309, Feb 2011.
[27] M. P. Cote and J. P. Gossard, "Step training-dependent plasticity in spinal cutaneous pathways," The Journal of neuroscience, vol. 24, pp. 11317-27, Dec 15 2004.
[28] M. P. Cote, A. Menard, and J. P. Gossard, "Spinal cats on the treadmill: changes in load pathways," J Neurosci, vol. 23, pp. 2789-96, Apr 1 2003.
[29] J. A. Coull, S. Beggs, D. Boudreau, D. Boivin, M. Tsuda, K. Inoue, C. Gravel, M. W. Salter, and Y. De Koninck, "BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain," Nature, vol. 438, pp. 1017-21, Dec 15 2005.
[30] A. D. Craig and K. D. Kniffki, "Spinothalamic lumbosacral lamina I cells responsive to skin and muscle stimulation in the cat," J Physiol, vol. 365, pp. 197-221, Aug 1985.
[31] C. Crone, H. Hultborn, L. Mazieres, C. Morin, J. Nielsen, and E. Pierrot-Deseilligny, "Sensitivity of monosynaptic test reflexes to facilitation and inhibition as a function of the test reflex size: a study in man and the cat," Exp Brain Res, vol. 81, pp. 35-45, 1990.
[32] A. Dario and G. Tomei, "A benefit-risk assessment of baclofen in severe spinal spasticity," Drug Saf, vol. 27, pp. 799-818, 2004.
[33] A. M. Degtyarenko, E. S. Simon, and R. E. Burke, "Differential modulation of disynaptic cutaneous inhibition and excitation in ankle flexor motoneurons during fictive locomotion," J Neurophysiol, vol. 76, pp. 2972-85, Nov 1996.
[34] A. M. Degtyarenko, E. S. Simon, T. Norden-Krichmar, and R. E. Burke, "Modulation of oligosynaptic cutaneous and muscle afferent reflex pathways during fictive locomotion and scratching in the cat," J Neurophysiol, vol. 79, pp. 447-63, Jan 1998.
[35] V. Dietz, "Body weight supported gait training: From laboratory to clinical setting," Brain Research Bulletin, vol. 78, pp. I-VI, 2009.
[36] B. Dobkin, D. Apple, H. Barbeau, M. Basso, A. Behrman, D. Deforge, J. Ditunno, G. Dudley, R. Elashoff, L. Fugate, S. Harkema, M. Saulino, and M. Scott, "Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI," Neurology, vol. 66, pp. 484-93, Feb 28 2006.
[37] T. Drew and S. Doucet, "Application of circular statistics to the study of neuronal discharge during locomotion," J Neurosci Methods, vol. 38, pp. 171-81, Jul 1991.
[38] J. Duysens and G. E. Loeb, "Modulation of ipsi- and contralateral reflex responses in unrestrained walking cats," J Neurophysiol, vol. 44, pp. 1024-37., 1980.
[39] J. Duysens and R. B. Stein, "Reflexes induced by nerve stimulation in walking cats with implanted cuff electrodes," Exp Brain Res, vol. 32, pp. 213-24., 1978.
[40] D. P. Feliciano, P. Sahbaie, X. Shi, M. Klukinov, J. D. Clark, and D. C. Yeomans, "Nociceptive sensitization and BDNF up-regulation in a rat model of traumatic brain injury," Neurosci Lett, vol. 583, pp. 55-9, Nov 7 2014.
[41] E. J. Fox, N. J. Tester, C. P. Phadke, P. M. Nair, C. R. Senesac, D. R. Howland, and A. L. Behrman, "Ongoing walking recovery 2 years after locomotor training in a child with severe incomplete spinal cord injury," Phys Ther, vol. 90, pp. 793-802, May 2010.
[42] A. Frigon and S. Rossignol, "Adaptive changes of the locomotor pattern and cutaneous reflexes during locomotion studied in the same cats before and after spinalization," The Journal of physiology, vol. 586, pp. 2927-45, Jun 15 2008.
[43] A. Frigon and S. Rossignol, "Plasticity of reflexes from the foot during locomotion after denervating ankle extensors in intact cats," Journal of Neurophysiology, vol. 98, pp. 2122-32, Oct 2007.
[44] O. Gajewska-Wozniak, M. Skup, S. Kasicki, E. Ziemlinska, and J. Czarkowska-Bauch, "Enhancing proprioceptive input to motoneurons differentially affects expression of neurotrophin 3 and brain-derived neurotrophic factor in rat hoffmann-reflex circuitry," PLoS One, vol. 8, p. e65937, 2013.

[45] S. M. Garraway, A. J. Anderson, and L. M. Mendell, "BDNF-induced facilitation of afferent-evoked responses in lamina II neurons is reduced after neonatal spinal cord contusion injury," J Neurophysiol, vol. 94, pp. 1798-804, Sep 2005.
[46] S. M. Garraway, J. C. Petruska, and L. M. Mendell, "BDNF sensitizes the response of lamina II neurons to high threshold primary afferent inputs," Eur J Neurosci, vol. 18, pp. 2467-76, Nov 2003.
[47] Y. P. Gerasimenko, V. D. Avelev, O. A. Nikitin, and I. A. Lavrov, "Initiation of locomotor activity in spinal cats by epidural stimulation of the spinal cord," Neurosci Behav Physiol, vol. 33, pp. 247-54, Mar 2003.
[48] G. L. Gerstein and N. Y. Kiang, "An approach to the quantitative analysis of electrophysiological data from single neurons," Biophysical journal, vol. 1, pp. 15-28, Sep 1960.
[49] F. J. Goldstein and R. T. Malseed, "Evaluation of narcotic analgetic activity using a cat tail-flick procedure," Journal of Pharmacological Methods, vol. 2, pp. 333-338, 1979.
[50] J. W. Grau, J. R. Huie, K. H. Lee, K. C. Hoy, Y. J. Huang, J. D. Turtle, M. M. Strain, K. M. Baumbauer, R. M. Miranda, M. A. Hook, A. R. Ferguson, and S. M. Garraway, "Metaplasticity and behavior: how training and inflammation affect plastic potential within the spinal cord and recovery after injury," Front Neural Circuits, vol. 8, p. 100, 2014.
[51] M. Guillot, P. M. Taylor, P. Rialland, M. P. Klinck, M. M. Moreau, J. Martel-Pelletier, J. P. Pelletier, and E. Troncy, "Evoked temporal summation in cats to highlight central sensitization related to osteoarthritis-associated chronic pain: a preliminary study," PLoS One, vol. 9, p. e97347, 2014.
[52] S. Harkema, Y. Gerasimenko, J. Hodes, J. Burdick, C. Angeli, Y. Chen, C. Ferreira, A. Willhite, E. Rejc, R. G. Grossman, and V. R. Edgerton, "Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study," Lancet, vol. 377, pp. 1938-47, Jun 4 2011.
[53] J. R. Huie, S. M. Garraway, K. M. Baumbauer, K. C. Hoy, Jr., B. S. Beas, K. S. Montgomery, J. L. Bizon, and J. W. Grau, "Brain-derived neurotrophic factor promotes adaptive plasticity within the spinal cord and mediates the beneficial effects of controllable stimulation," Neuroscience, vol. 200, pp. 74-90, Jan 3 2012.
[54] K. J. Hutchinson, F. Gomez-Pinilla, M. J. Crowe, Z. Ying, and D. M. Basso, "Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats," Brain, vol. 127, pp. 1403-14, Jun 2004.
[55] T. Iwahara, Y. Atsuta, E. Garcia-Rill, and R. D. Skinner, "Spinal cord stimulation-induced locomotion in the adult cat," Brain Res Bull, vol. 28, pp. 99-105, Jan 1992.
[56] L. B. Jakeman, P. Wei, Z. Guan, and B. T. Stokes, "Brain-derived neurotrophic factor stimulates hindlimb stepping and sprouting of cholinergic fibers after spinal cord injury," Exp Neurol, vol. 154, pp. 170-84, Nov 1998.
[57] M. W. Jones, A. V. Apkarian, R. T. Stevens, and C. J. Hodge, Jr., "The spinothalamic tract: an examination of the cells of origin of the dorsolateral and ventral spinothalamic pathways in cats," J Comp Neurol, vol. 260, pp. 349-61, Jun 15 1987.
[58] L. Kelamangalath, X. Tang, K. Bezik, N. Sterling, Y. J. Son, and G. M. Smith, "Neurotrophin selectivity in organizing topographic regeneration of nociceptive afferents," Exp Neurol, vol. 271, pp. 262-78, Sep 2015.
[59] N. Khan and M. T. Smith, "Neurotrophins and Neuropathic Pain: Role in Pathobiology," Molecules, vol. 20, pp. 10657-88, Jun 09 2015.
[60] A. J. Krupka, I. Fischer, and M. A. Lemay, "Transplants of Neurotrophin-Producing Autologous Fibroblasts Promote Recovery of Treadmill Stepping in the Acute, Sub-Chronic, and Chronic Spinal Cat," J Neurotrauma, Dec 20 2016.
[61] M. A. Lemay, F. Marchionne, and A. Krupka, "Interneuronal activity in spinal felines treated with BDNF delivered intrathecally to the lumbar spinal cord," in Proceedings of the 47th Annual Meeting of the Society for Neuroscience, Washington, DC, 2017.
[62] K. S. Lewis and W. M. Mueller, "Intrathecal baclofen for severe spasticity secondary to spinal cord injury," Ann Pharmacother, vol. 27, pp. 767-74, Jun 1993.
[63] R. G. Lovely, R. J. Gregor, R. R. Roy, and V. R. Edgerton, "Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat," Exp Neurol, vol. 92, pp. 421-35., 1986.
[64] R. G. Lovely, R. J. Gregor, R. R. Roy, and V. R. Edgerton, "Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cats," Brain Res, vol. 514, pp. 206-18., 1990.
[65] P. Lu, A. Blesch, L. Graham, Y. Wang, R. Samara, K. Banos, V. Haringer, L. Havton, N. Weishaupt, D. Bennett, K. Fouad, and M. H. Tuszynski, "Motor axonal regeneration after partial and complete spinal cord transection," J Neurosci, vol. 32, pp. 8208-18, Jun 13 2012.

[66] W. Martin Bauknight, S. Chakrabarty, B. Y. Hwang, H. R. Malone, S. Joshi, J. N. Bruce, E. Sander Connolly, C. J. Winfree, M. G. Cunningham, J. H. Martin, and R. Haque, "Convection enhanced drug delivery of BDNF through a microcannula in a rodent model to strengthen connectivity of a peripheral motor nerve bridge model to bypass spinal cord injury," J Clin Neurosci, vol. 19, pp. 563-9, Apr 2012.
[67] W. B. Matthews, "Ratio of maximum H reflex to maximum M response as a measure of spasticity," J Neurol Neurosurg Psychiatry, vol. 29, pp. 201-4, Jun 1966.
[68] D. A. McCrea, "Spinal circuitry of sensorimotor control of locomotion," J Physiol, vol. 533, pp. 41-50., 2001.
[69] R. Melzack and P. D. Wall, "Pain mechanisms: a new theory," Science, vol. 150, pp. 971-979, 1965.
[70] L. M. Mendell, "Neurotrophin action on sensory neurons in adults: an extension of the neurotrophic hypothesis," Pain, vol. Suppl 6, pp. S127-32, Aug 1999.
[71] J. B. Munson, R. D. Johnson, and L. M. Mendell, "NT-3 increases amplitude of EPSPs produced by axotomized group Ia afferents," J Neurophysiol, vol. 77, pp. 2209-12, Apr 1997.
[72] J. Nijs, M. Meeus, J. Versijpt, M. Moens, I. Bos, K. Knaepen, and R. Meeusen, "Brain-derived neurotrophic factor as a driving force behind neuroplasticity in neuropathic and central sensitization pain: a new therapeutic target?," Expert Opin Ther Targets, pp. 1-12, Dec 18 2014.
[73] G. Ochs, R. D. Penn, M. York, R. Giess, M. Beck, J. Tonn, J. Haigh, E. Malta, M. Traub, M. Sendtner, and K. V. Toyka, "A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis," Amyotroph Lateral Scler Other Motor Neuron Disord, vol. 1, pp. 201-6, Jun 2000.
[74] K. Ollivier-Lanvin, I. Fischer, V. Tom, J. D. Houle, and M. A. Lemay, "Either brain-derived neurotrophic factor or neurotrophin-3 only neurotrophin-producing grafts promote locomotor recovery in untrained spinalized cats," Neurorehabil Neural Repair, vol. 29, pp. 90-100, Jan 2015.
[75] K. Ollivier-Lanvin, B. E. Keeler, R. Siegfried, J. D. Houle, and M. A. Lemay, "Proprioceptive neuropathy affects normalization of the H-reflex by exercise after spinal cord injury," Exp Neurol, vol. 221, pp. 198-205, Jan 2010.
[76] K. Ollivier-Lanvin, A. J. Krupka, N. AuYong, K. Miller, B. I. Prilutsky, and M. A. Lemay, "Electrical stimulation of the sural cutaneous afferent nerve controls the amplitude and onset of the swing phase of locomotion in the spinal cat," Journal of Neurophysiology, vol. 105, pp. 2297-308, May 2011.
[77] K. G. Pearson, "Proprioceptive regulation of locomotion," Curr Opin Neurobiol, vol. 5, pp. 786-91., 1995.
[78] J. V. Priestley, M. S. Ramer, V. R. King, S. B. McMahon, and R. A. Brown, "Stimulating regeneration in the damaged spinal cord," Journal of physiology, Paris, vol. 96, pp. 123-33, Jan-Mar 2002.
[79] F. Prinz, T. Schlange, and K. Asadullah, "Believe it or not: how much can we rely on published data on potential drug targets?," Nat Rev Drug Discov, vol. 10, p. 712, Aug 31 2011.
[80] M. S. Ramer, T. Bishop, P. Dockery, M. S. Mobarak, D. O'Leary, J. P. Fraher, J. V. Priestley, and S. B. McMahon, "Neurotrophin-3-mediated regeneration and recovery of proprioception following dorsal rhizotomy," Molecular and cellular neurosciences, vol. 19, pp. 239-49, Feb 2002.
[81] P. J. Reier, M. A. Lane, E. D. Hall, Y. D. Teng, and D. R. Howland, "Translational spinal cord injury research: preclinical guidelines and challenges," Handb Clin Neurol, vol. 109, pp. 411-33, 2012.
[82] B. Rexed, "A cytoarchitectonic atlas of the spinal cord in the cat," J Comp Neurol, vol. 100, pp. 297-380, 1954.
[83] H. G. Schaible, R. F. Schmidt, and W. D. Willis, "Responses of spinal cord neurones to stimulation of articular afferent fibres in the cat," J Physiol, vol. 372, pp. 575-93, Mar 1986.
[84] B. J. Schmidt, D. E. Meyers, M. Tokuriki, and R. E. Burke, "Modulation of short latency cutaneous excitation in flexor and extensor motoneurons during fictive locomotion in the cat," Exp Brain Res, vol. 77, pp. 57-68, 1989.
[85] E. D. Schomburg, N. Petersen, I. Barajon, and H. Hultborn, "Flexor reflex afferents reset the step cycle during fictive locomotion in the cat," Exp Brain Res, vol. 122, pp. 339-50., 1998.
[86] C. S. Sherrington, "Flexion-reflex of the limb, crossed extension reflex and reflex stepping and standing," J Physiol, vol. 40, pp. 28-121, 1910.
[87] D. Siniscalco, C. Giordano, F. Rossi, S. Maione, and V. de Novellis, "Role of neurotrophins in neuropathic pain," Curr Neuropharmacol, vol. 9, pp. 523-9, Dec 2011.
[88] W. Song and J. H. Martin, "Spinal cord direct current stimulation differentially modulates neuronal activity in the dorsal and ventral spinal cord," J Neurophysiol, vol. 117, pp. 1143-1155, Mar 1 2017.

[89] O. Steward, P. G. Popovich, W. D. Dietrich, and N. Kleitman, "Replication and reproducibility in spinal cord injury research," Exp Neurol, vol. 233, pp. 597-605, Feb 2012.
[90] E. Tabo, S. L. Jinks, J. H. Eisele, Jr., and E. Carstens, "Behavioral manifestations of neuropathic pain and mechanical allodynia, and changes in spinal dorsal horn neurons, following L4-L6 dorsal root constriction in rats," Pain, vol. 80, pp. 503-20, Apr 1999.
[91] Y. Takemura, S. Kobayashi, E. Kato, S. Yamaguchi, and Y. Hori, "Peripheral nerve injury-induced rearrangement of neural circuit in the spinal dorsal horn revealed by cross-correlation analysis," Neurosci Lett, vol. 662, pp. 259-263, Jan 1 2018.
[92] X. Q. Tang, D. L. Tanelian, and G. M. Smith, "Semaphorin3A inhibits nerve growth factor-induced sprouting of nociceptive afferents in adult rat spinal cord," J Neurosci, vol. 24, pp. 819-27, Jan 28 2004.
[93] J. Ushiba, Y. Tomita, Y. Masakado, Y. Komune, and Y. Muraoka, "Statistical test for peri-stimulus time histograms in assessing motor neuron activity," Medical & Biological Engineering & Computing, vol. 40, pp. 462-8, Jul 2002.
[94] H. R. Weng, J. V. Cordella, and P. M. Dougherty, "Changes in sensory processing in the spinal dorsal horn accompany vincristine-induced hyperalgesia and allodynia," Pain, vol. 103, pp. 131-8, May 2003.
[95] D. M. White, "Contribution of neurotrophin-3 to the neuropeptide Y-induced increase in neurite outgrowth of rat dorsal root ganglion cells," Neuroscience, vol. 86, pp. 257-63, Sep 1998.
[96] D. M. White, "Neurotrophin-3 antisense oligonucleotide attenuates nerve injury-induced Abeta-fibre sprouting," Brain Res, vol. 885, pp. 79-86, Dec 01 2000.
[97] T. D. Wilson-Gerwing, M. V. Dmyterko, D. W. Zochodne, J. M. Johnston, and V. M. Verge, "Neurotrophin-3 suppresses thermal hyperalgesia associated with neuropathic pain and attenuates transient receptor potential vanilloid receptor-1 expression in adult sensory neurons," J Neurosci, vol. 25, pp. 758-67, Jan 19 2005.
[98] T. Yasaka, S. Y. Tiong, E. Polgar, M. Watanabe, E. Kumamoto, J. S. Riddell, and A. J. Todd, "A putative relay circuit providing low-threshold mechanoreceptive input to lamina I projection neurons via vertical cells in lamina II of the rat dorsal horn," Mol Pain, vol. 10, p. 3, Jan 17 2014.
[99] E. Ziemlinska, S. Kugler, M. Schachner, I. Wewior, J. Czarkowska-Bauch, and M. Skup, "Overexpression of BDNF increases excitability of the lumbar spinal network and leads to robust early locomotor recovery in completely spinalized rats," PLoS One, vol. 9, p. e88833, 2014.

Data and Resource Sharing Plan:
In addition to publishing papers on our results we will make the data available upon request to fellow investigators. We have had few such requests over the years, but we have shared EMGs and neural data with a number of investigators.

Authentication of Key Biological and/or Chemical Resources:
Key biological materials that will be used in this research and fall under the NIH criteria as 1) likely to differ from laboratory to laboratory, or over time, 2) vary in qualities or qualifications that could influence the research data, were divided in two categories: Antibodies and Neurotrophins.

Antibodies:
Antibodies used for immunohistochemistry labeling are tested for specificity and sensitivity. A number of tests are used to test for immunolabeling specificity and sensitivity:
1) verify the lack of immunostaining in tissues with no antigen, and positive immunostaining in cells and locations well-know to express the antigen (e.g. GFAP staining at the SCI injury site).
2) compare the patterns of immunoreactivity to the expected locations of immunoreactivity, both within the cell and in the cell populations. Immunolabeling localization should correspond to cellular regions where the target antigen exerts its function: membrane (i.e. channels, receptors, membrane cell markers), nucleus (i.e. transcription factors) or cytoplasm (i.e. enzymes, cytoskeleton or organelle proteins). In addition, the cell populations expressing the antigen should agree with prior reports and with locations of cells expressing the mRNA.
3) use western blots of normal cat spinal cord tissue and, when possible, tissue from spinal animals to determine the specificity of the antibodies.

Neurotrophins:

In addition to the SDS-PAGE stained with Coomassie Blue methodology used by Abnova (our BDNF and NT-3 neurotrophin supplier) to separate the BDNF or NT-3 protein, we will conduct chick dorsal root ganglia growth assays to evaluate the potency of our neurotrophins at the time of preparation and at the time of explant.