Award Number: W81XWH-17-1-0197

TITLE: Mechanisms of Gastrointestinal Neuropeptide Signaling in an Animal Model of SCI Following Weight Loss Surgery

PRINCIPAL INVESTIGATOR: Andras Hajnal

CONTRACTING ORGANIZATION: Pennsylvania State University
Hershey, PA 17033-0850

REPORT DATE: July 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
July 2018	**Annual**	1 Jul 2017 - 30 Jun 2018

4. TITLE AND SUBTITLE
Mechanisms of Gastrointestinal Neuropeptide Signaling in an Animal Model of SCI following Weight Loss Surgery

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0197

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Andras Hajnal, PhD
Gregory Holmes, PhD

E-Mail: axh40@psu.edu gmh16@psu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)

Pennsylvania State University, Milton S.
Hershey Medical Center
500 University Drive
PO Box 850, MC H109
Hershey, PA 17033-0850

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Approximately two-thirds of spinal cord injured (SCI) individuals become overweight or obese. The greatest increase from normal to overweight or obese has been reported to occur during the first year after acute rehabilitation. In addition to limiting self-help techniques (e.g., wheelchair transfer) these individuals are susceptible to obesity-related conditions including Type II diabetes. As the life-span of SCI individuals continues to increase, these chronic conditions diminish the quality of life while increasing the financial healthcare burden for veterans with SCI.

Weight loss surgery (WLS), including sleeve gastrectomy (SG) and the Roux-en-Y gastric bypass (RYGB) surgery, is regarded as highly effective in the long-term treatment of obesity and remission of type 2 diabetes. The weight-reduction after WLS does not appear to reflect mechanical restriction of food intake or malabsorption of nutrients, but a change in the regulatory signaling within the gut-brain axis. Examples of these changes include the increased release of anorexigenic gut hormones such as glucagon-like peptide-1 (GLP-1) and alterations in gastrointestinal transit. Given the risk factors for developing obesity following SCI, WLS offers an attractive intervention for SCI-induced obesity. SCI individuals, however, should not be regarded as similar to able bodied individuals. In addition to the immediate loss of motor control, SCI results in impairment of autonomic nervous system function, including the regulation and integrity of the gastrointestinal (GI) tract. These co-morbidities combine to challenge metabolic homeostasis of the SCI individual. Due to current knowledge gaps, it is unknown if obese individuals with SCI respond to WLS similarly to the able-bodied. Therefore, efficacy assessments regarding the mechanisms behind successful bariatric surgical intervention in this special population group are warranted before performing surgery on potentially high-risk individuals.

Our own observations in an animal model of SCI identify persistent GI dysmotility, a transient reduced sensitivity to gut hormones, increased adiposity and reduction of lean tissue mass, upregulation of inflammatory markers, intestinal histopathology, and reduced superior mesenteric artery (SMA) blood flow. We have verified that obese rats with an SCI can tolerate the increasingly preferred SG procedure, which is more tolerable by patients and provides similar benefits to those of RYGB surgery at lower costs. Thus, the rodent model is a viable tool for addressing fundamental questions regarding the mechanisms leading to the development of obesity and the efficacy of intervention strategies.

The proposed studies are based upon the central hypothesis that diminished sensitivity of the vagal gut-brain axis to nutrient-associated GI signaling provokes the development of obesity prior to SG in an animal model of SCI. Sleeve gastrectomy (SG) bariatric surgery will reverse this blunted sensitivity even in the radically altered physiology that accompanies SCI.

Research Idea & Strategy: Following SCI, the sensitivity of neural circuits to our target GI peptide, GLP-1, before and after SG intervention will be investigated in SCI rats with the following specific aims:

Specific Aim 1 will test the hypothesis that sensitivity of the gut-brain axis to nutrient-associated signaling is diminished in SCI rats, thus elevating the risk to developing obesity.

Specific Aim 2 will test the hypothesis that SG intervention in obese SCI rats will restore sensitivity of the gut-brain axis to nutrient signaling and reduce adiposity.

Impact: Complications of SCI include reduced gastrointestinal integrity and motility (ileus), poor energy homeostasis and an increased risk for obesity. It is unknown how bariatric surgical intervention for the able-bodied population translates similarly to the SCI population. Our proposal for a neurophysiological study of the beneficial mechanisms of SG in an animal model of SCI is singular, novel, and likely to provide significant evidence-based data for applying this intervention to persons with SCI.

Innovation & Military Relevance: We are only just beginning to understand the multi-organ responses following SCI. This proposal will combine GI physiology and animal models of WLS to explore the efficacy of SG to reverse the effects of obesity in an experimental model of SCI. Success of this proposal will develop evidence based strategies for the treatment of obesity following SCI.

15. SUBJECT TERMS

None provided

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	13	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

Table of Contents

Page

1. **INTRODUCTION:** Approximately two-thirds of spinal cord injured (SCI) individuals become overweight or obese. Weight loss surgery (WLS), including sleeve gastrectomy (SG) is regarded as highly effective in the long-term treatment of obesity and remission of type 2 diabetes. Given the risk factors for developing obesity following SCI, WLS offers an attractive intervention for SCI-induced obesity. SCI individuals, however, should not be regarded as similar to able bodied individuals. In addition to the immediate loss of motor control, SCI results in impairment of autonomic nervous system function, including the regulation and integrity of the gastrointestinal (GI) tract. These co-morbidities combine to challenge metabolic homeostasis of the SCI individual. It is unknown how bariatric surgical intervention for the able-bodied population translates similarly to the SCI population. This project combines GI physiology and animal models of WLS to explore the efficacy of SG to reverse the effects of obesity in an experimental model of SCI. The proposed studies are based upon the following two main hypothesis, tested in two Specific Aims: 1) diminished sensitivity of the vagal gut-brain axis to nutrient-associated GI signaling provokes the development of obesity prior to SG in an animal model of SCI, 2) SG will reverse this blunted sensitivity even in the radically altered physiology that accompanies SCI. Success of this project will develop evidence based strategies for the treatment of obesity following SCI.

2. **KEYWORDS:** Spinal Cord Injury, Obesity, Metabolic Disorder, Type-2 Diabetes, Weight Loss Surgery, Sleeve Gastrectomy, Anima Models, Gut-Brain Axis, Vagus Nerve, Nucleus Tractus Solitarius, Gastrointestinal Hormones, Glucagon-Like Peptide-1, Sugar, Fat, Food Preferences, Taste, Behavioral Tests, Nerve Recording, Neurophysiology

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

  This project mechanistically investigates the effect of SCI and SCI coupled with weight loss surgery (WLS) on the gut-brain axis. The principal goals are to 1) identify the post-SCI alterations in the neural signals emanating from the gut that control food intake; 2) identify the post-SCI alterations in the neural circuit within the brain where gut signaling is integrated (the nucleus tractus solitarius, NTS); 3) the resulting behavior that is modulated by these neural circuits; and 4) the efficacy of WLS on restoring normal function in SCI.

  Research-specific tasks:

Task	Months	Project Oversight	Overarching Deliverable Data Set
IACUC approval of proposed animal techniques has been secured (#46425, effective date 4/27/2016). Modifications for full JIT congruency will only require 1 month	JIT for award	Holmes	Approval
Standard of care measures: All experiments record daily BW and food intake	Project duration	Holmes/Hajnal	Mean Energy Intake
Metabolic indices of obesity: Animals are monitored for plasma glucose, body composition, oral glucose tolerance test	Project duration	Hajnal	Obesity profile

Aim 1: Sensitivity of the gut-brain axis to nutrient-associated signaling is diminished in SCI rats, thus elevating the risk to developing obesity.			
Sub-Aim 1a: Gastrointestinal vagal afferents after SCI are non-responsive to GLP-1 analogs (Nerve recording)	1-6	Holmes	Vagal afferent function
Sub-Aim 1b: Central gastrointestinal vago-vagal neurocircuitry is non-responsive to GLP-1 analogs after SCI (c-Fos)	7-9	Holmes	Central NTS sensitivity
Sub-Aim 1c: Central gastrointestinal vago-vagal neurocircuitry is non-responsive to GLP-1 analogs after SCI (single unit NTS recording)	8-16	Hajnal	Central NTS sensitivity
Sub-Aim 1d: Peripheral gastrointestinal vagal afferents down regulate GLP-1 receptor expression following SCI	9-12	Holmes	Central NTS sensitivity
Sub-Aim 1e: Taste Functions – brief-access sucrose and lipid intake studies	1-4	Hajnal	Behavioral relevance
Sub-Aim 1f: Food preferences – 24-hour sucrose and lipid intake studies	5-8	Hajnal	Behavioral relevance
Aim 2: SG intervention in obese SCI rats will provoke elevated sensitivity of the gut-brain axis to nutrient signaling and reduced adiposity.			
Sub-Aim 2a: Gastrointestinal vagal afferents of T3-SCI SG rats are more responsive to GLP-1 analogs than T3-SCI rats serving as SG surgical controls (Nerve recording)	13-16	Holmes	Vagal afferent function
Sub-Aim 2b: Central gastrointestinal vago-vagal neurocircuitry of SCI rats regains sensitivity to GLP-1 analogs after SG surgery vs SG controls (c-Fos)	17-22	Holmes	Central NTS sensitivity
Sub-Aim 2c: Central gastrointestinal vago-vagal neurocircuitry of SCI rats regains sensitivity to GLP-1 analogs after SG surgery vs SG controls (single unit NTS recording)	17-24	Hajnal	Central NTS sensitivity
Sub-Aim 2d: Peripheral gastrointestinal vagal afferents down regulate GLP-1 receptor expression following SG surgery vs SG controls (nodose, NTS?)	24-28	Holmes	Central NTS sensitivity
Sub-Aim 2e: Taste Functions – brief-access sucrose and lipid intake studies	24-29	Hajnal	Behavioral relevance
Sub-Aim 2f: Food preferences – 24-hour sucrose and lipid intake studies	30-33	Hajnal	Behavioral relevance
Conclude project: Submit final reports, remaining manuscripts	34-36	Hajnal/Holmes	Publications
Manuscript preparation is anticipated to aggregate along Aims 1a & 1b; 1c & 1d; 1e & 1f; 2a & 2b; 2c & 2d; 2e & 2f and will begin after the completion of each grouping.			

- **What was accomplished under these goals?**
  - 1) Major Activities: All activities during the reporting period was related to Specific Aim1.
  - 2) Specific Objectives: The milestones/target dates for specific objectives and the actual completion dates or the percentage of completion were as follows:

Major Task 1	Target Dates	Actual Completion Dates	Percentage of Completion
Subtask 1: IACUC approval	Month 1	06/20/2017	100%
Subtask 2: ACURO approval	Month 2-3	(it was delayed) 12/05/2017	100%
Subtask 3: Hire and train new Technician - for Immunohistochemistry and neurophysiological recordings	Month 2-6	Hire was made on 09/01/2017 Training is completed	100%
Subtask 4: Order and receive immunohistochemical supplies	Month 1-8	Completed	100%
Subtask 5: Nerve recording Aim 1a: Gastrointestinal vagal afferents after SCI are non-responsive to GLP-1 analogs	Month 4-18	Vagal nerve recording is ongoing	35%
Subtask 6: c-Fos Histology Aim 1b: Central gastrointestinal vago-vagal neurocircuitry is non-responsive to GLP-1 analogs after SCI (c-Fos)	Month 7-9	c-Fos histology is ongoing	25%
Subtask 7: Behavioral studies Aim 1e: Taste Functions – brief-access sucrose and lipid intake studies	Month 1-8	Initiated, ongoing	15%

- 3) Key Outcomes:

Subtask 1: IACUC Approval. Outcome: Approval of proposed animal techniques has been secured (#47686, effective date 06/20/2017).

Subtask 2: ACURO Protocol was approved on 12/05/2017.

Subtask 3: Training: Ms. Lisa Willing was hired as a research technician in Dr. Holmes lab on 09/01/17 at 50% effort on the project. Her training has been completed. She is currently running the c-Fos histological studies (Subtask 6).
Existing technician Ms Horvath in the Hajnal Lab (at 25% effort, starting on 09/01/17) has been trained for SG. She is currently running the behavioral studies (Subtask 7).

Subtask 4: Laboratory supplies (required for the training) have been ordered.

Subtask 5: Electrophysiology: Vagal nerve recording. Electrophysiology, we have made progress with vagal neural recordings and established technique to screen for effects of GI hormones, e.g. CCK, GLP-1 (see Fig 1.)

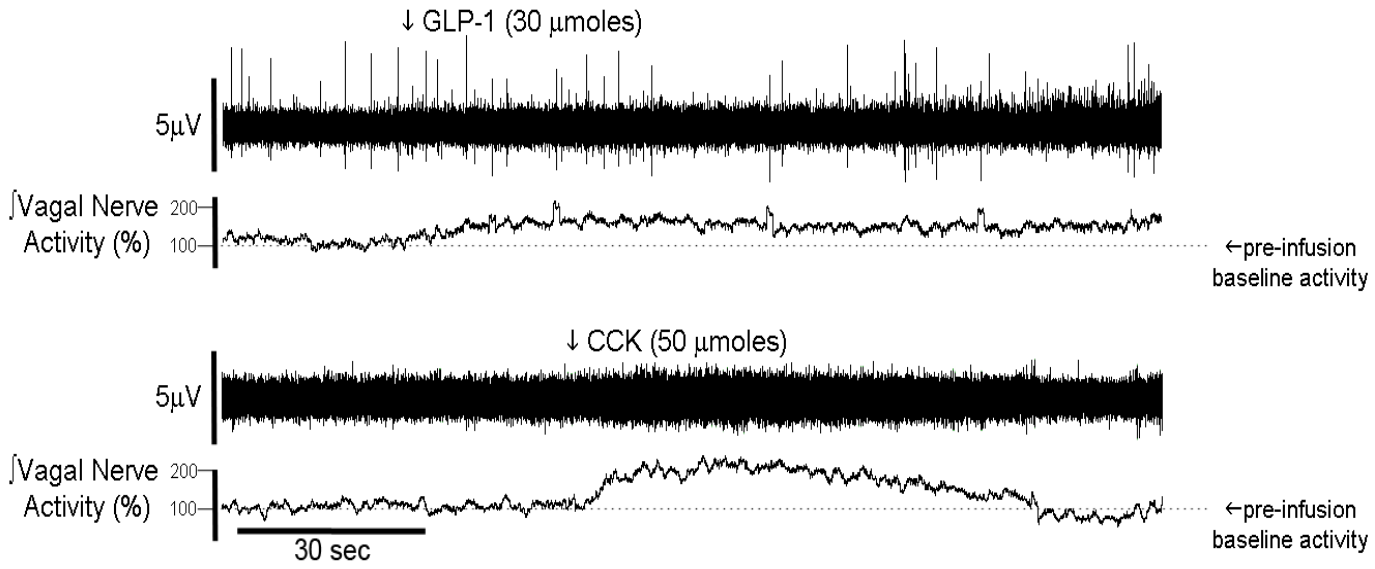


Fig. 1. Vagal nerve recording from a single, non-injured, animal exposed to superior mesenteric artery infusion of GLP-1 (30 mmoles) or CCK-8s (50 mmoles). GLP-1 firing reflects a more subtle level of vagal nerve firing that is longer duration. This is consistent with previous reports demonstrating a prolonged elevation of vagal firing rate beyond the circulating availability of exogenous GLP-1 (Nishizawa et. al., 1996). In contrast, the response of the vagus to CCK-8s is more pronounced and of shorter duration. This demonstrates the feasibility of our refined vagal afferent recording technique.

Subtask 6: C-Fos histology.

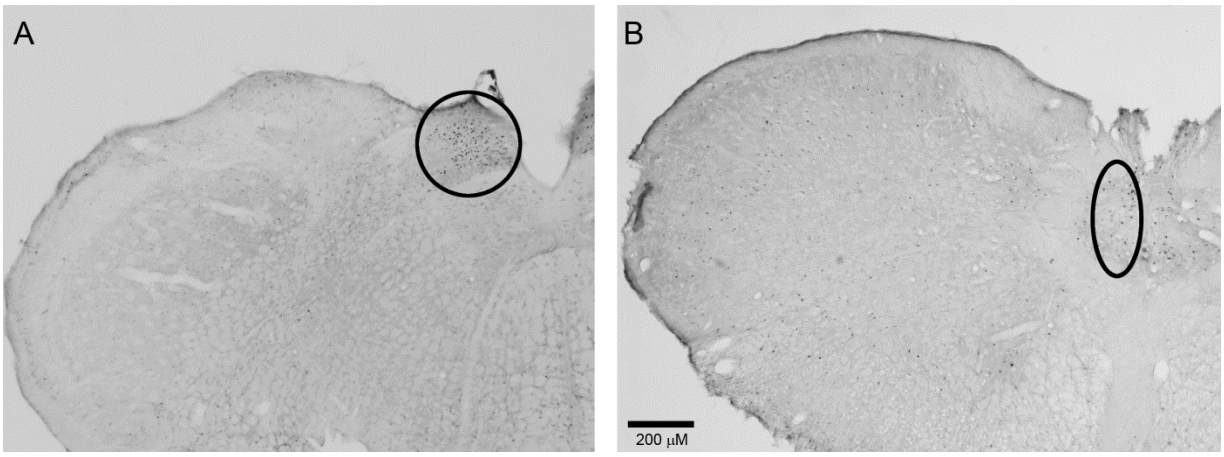


Fig. 2. Activation of dorsal vagal complex neurons by glucose. The DVC (circled region of interest) of a control rat (A) shows marked c-Fos positive neurons; an indication of neuronal activation in response to glucose. The DVC of an SCI rats (b) shows only a small region of c-Fos immunoreactivity; indicating an attenuation of neuronal activation in response to glucose.

Subtask 7: Behavioral studies. Baseline (prior to SCI, and related to Aim 1) behavioral tests for the effect of diet and SG surgery has been started in a larger set of rats using the brief-access taste testing protocol. The first set of data established taste-preference profiles in rats prior to SCI. The next phase will determine the effects of 2) high fat diet, and 2) SCI or control surgery on taste behaviors.

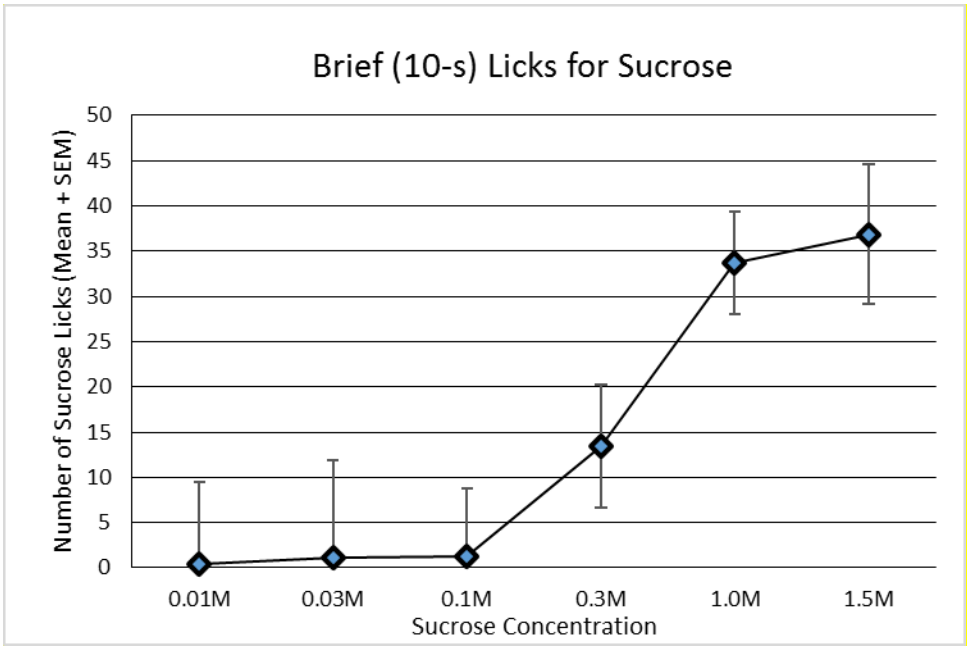


Fig. 3. Sucrose Preference Profile. Brief lick responses for various concentrations of sucrose using automated gustometers in male rats (n=6) prior to development of obesity and SCI. This experiment established normal taste preferences for sweet taste, thus provide a baseline for the proposed studies (SCI in Aim 1, and SCI+SG in Aim 2).

- **What opportunities for training and professional development has the project provided?**

  Lisa Willing has received additional training in properly identifying the dorsal vagal complex for histological analysis. She has validated the c-Fos training protocol that was necessitated by the original antibody supplies no longer being available. She has secured a new vendor and run dilution optimization for the new antibody.

- **How were the results disseminated to communities of interest?**

  Data has not yet been presented at a scientific forum.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  - In addition to a hire of a post doc and his/her training with electrophysiological recordings, during the next reporting period we plan to complete all sub-aims of Aim 1 and a-c of Aim 2 (see table in #3 above).

- **IMPACT:** Complications of SCI include reduced gastrointestinal integrity and motility (ileus), poor energy homeostasis and an increased risk for obesity. It is unknown how bariatric surgical intervention for the able-bodied population translates similarly to the SCI population. Our neurophysiological study of the beneficial mechanisms of SG in an animal model of SCI is singular, novel, and likely to provide significant evidence-based data for applying this intervention to persons with SCI.
- **What was the impact on the development of the principal discipline(s) of the project?**

  Nothing to Report.
- **What was the impact on other disciplines?**

  Nothing to Report.
- **What was the impact on technology transfer?**

  Nothing to Report.
- **What was the impact on society beyond science and technology?**

  Nothing to Report.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

  Nothing to Report.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - We encountered delays with the project due to a later approval of the ACURO *(Approved on 12/05/17)*
- **Changes that had a significant impact on expenditures**
  - Due to the later start of the actual animal experiments we have had a delay in hiring a post doc, therefore carrying over funds originally budgeted for the postdoc salary in year 1. This delay, however, did not change the scope of the project or caused substantial delays in meeting the objectives by performing the task by technicians. An official

advertisement for the post doctoral position has been posted and the search in progress for a targeted start date of January 1, 2019. Link to Job on Job Website: https://psu.jobs/job/80768

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations** Nothing to Report.

Journal publications. Nothing to Report.

Books or other non-periodical, one-time publications. Nothing to Report.

Other publications, conference papers, and presentations. Nothing to Report.

Website(s) or other Internet site(s) Nothing to Report.

- **Technologies or techniques.** Nothing to Report.

Inventions, patent applications, and/or licenses. Nothing to Report.

Other Products. Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name: Andras Hajnal
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: no change
Contribution to Project: Directing the project, coordinating with the Holmes lab, directly supervising GI surgeries and behavioral studies.

Name: Gregory Holmes
Project Role: Co-I
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: no change
Contribution to Project: Supervision or performance of SCI surgeries and vagal nerve recordings

Name: Lisa Willing
Project Role: Research Technician
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 month
Contribution to Project: Performance of SCI surgeries, post-operative care, data management and archiving

Name: Nelli Horvath
Project Role: Research Technician
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 4
Contribution to Project: Animal care, GI surgeries, behavioral tests, data and lab management for the experiments run in the Hajnal lab.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Yes**

- **PI: Hajnal**
  Nothing to Report

- **CO-I: Holmes Other Support Change**

  Closed

  295319 Craig H. Neilsen Foundation PI: Holmes 07/01/2014-06/30/2018

  Nutrient-vascular dysregulation and gastrointestinal health after SCI

  The objective of this project is to investigate intestinal blood flow and barrier permeability following enteral feeding of common pharmaconutrient formulations in an animal model of spinal cord injury.

- **What other organizations were involved as partners?**
  - Nothing to Report.
    - **Location of Organization:** *(if foreign location list country)*
    - **Partner's contribution to the projec**t *(identify one or more)*
      - **Financial support;**
      - **In-kind support** *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;
      - **Facilities** *(e.g., project staff use the partner's facilities for project activities)*;
      - **Collaboration** *(e.g., partner's staff work with project staff on the project)*;
      - **Personnel exchanges** *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*; and
      - **Other.**

        SPECIAL REPORTING REQUIREMENTS
- **COLLABORATIVE AWARDS:**
- **QUAD CHARTS:** See attached..

Mechanisms of gastrointestinal neuropeptide signaling in an animal model of SCI following weight loss surgery.
Log#: SC160171 **Task Title: Quarter Quad Progress - Studies have been initiated**
Award Number: W81XWH-17-1-0197

PI: Hajnal, A. **Org:** Pennsylvania State University **Award Amount:** $773,298

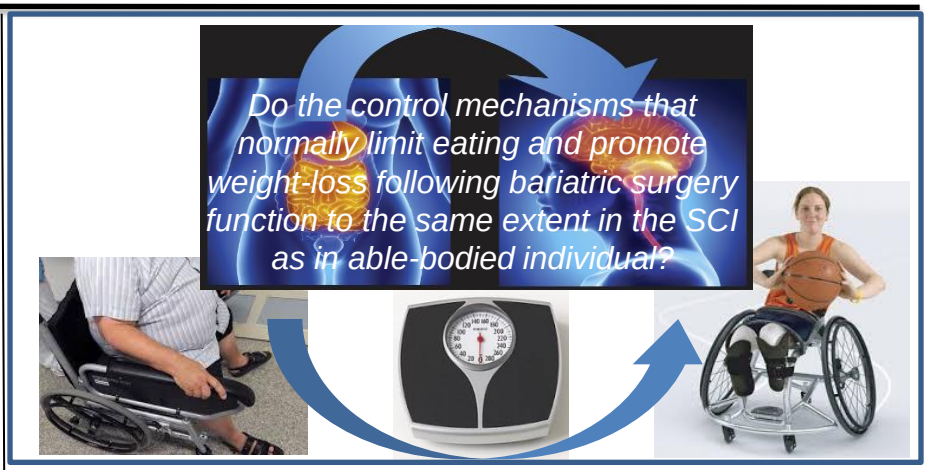
Study Aims and Approach

Approximately two-thirds of spinal cord injured (SCI) individuals become overweight or obese. Weight loss surgery (WLS), including sleeve gastrectomy (SG) and the Roux-en-Y gastric bypass (RYGB) surgery, is regarded as highly effective in the long-term treatment of obesity and remission of type 2 diabetes. Due to current knowledge gaps, it is unknown if obese individuals with SCI respond to WLS similarly to the able-bodied.

This proposal will combine GI physiology and animal models of WLS to explore the efficacy of SG to reverse the effects of obesity in an experimental model of SCI. Success of this proposal will develop evidence based strategies for the treatment of obesity following SCI.

• Specific Aim 1 will test the hypothesis that sensitivity of the gut-brain axis to nutrient-associated signaling is diminished in SCI rats, thus elevating the risk to developing obesity.

•Specific Aim 2 will test the hypothesis that SG intervention in obese SCI rats will restore sensitivity of the gut-brain axis to nutrient signaling and reduce adiposity.



Achievements: We have verified that obese rats with an SCI can tolerate the SG bariatric surgical procedure. Thus, the rodent model is a viable tool for addressing intervention strategies in individuals with SCI. We secured approvals of the animal protocols, hired and trained a technician. Nerve recording, histology and behavioral studies are in progress.

Timeline and Cost

Activities CY	17	18	19	
Preparation for the experiments Aim 1: Behavioral Studies				
Aim 1: Electrophysiological Studies				
Aim 2: Behavioral Studies				
Aim 2: Electrophysiological Studies				
Estimated Budget ($K)	**$231**	**$302**	**$240**	

Updated: July 31, 2018

Goals/Milestones

CY17 Goals – **A.** Full preparedness for the experiments
☑ Approval of animal techniques have been obtained (IACUC, ACURO)
☑ Hire and train new Technician (nerve recording training complete)
B. Demonstrate that nutrient-associated signaling reflects diminished
☑ Body weight, metabolism, and eating behaviors
☑ Vagal neuronal functions (currently in the second subset of 8 rats)

CY18 Goals – Studies related to Specific Aim 1 have been initiated:
☑ Continued refinement of nerve recording
☑ Histological studies (c-Fos) are initiated (second batch has started)
☑ Behavioral studies are ongoing to demonstrate that SG intervention in obese SCI rats will restore sensitivity of the gut-brain axis to nutrient signaling: Taste profiling started and established baseline functions.

CY19 Goal – Demonstrate that SG intervention in obese SCI rats will
☐ Restore vagal and taste neuronal functions
☐ and promotes healthy food choices and long-term weight maintenance.

Budget Expenditure to Date
Projected Expenditure Year 1: $93,260
Actual Expenditure (Including F&A): $137,201.54