AWARD NUMBER: W81XWH-18-1-0675

TITLE: Effects of Activity-Dependent Plasticity on Bowel Function After Spinal Cord Injury

PRINCIPAL INVESTIGATOR: Dr. Charles Hubscher

CONTRACTING ORGANIZATION: University of Louisville
Louisville, KY 40202-3805

REPORT DATE: October 2019

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2019	Annual	30SEP2018 - 29SEP2019

4. TITLE AND SUBTITLE

Effects of Activity-Dependent Plasticity on Bowel Function After Spinal Cord Injury

5a. CONTRACT NUMBER
W81XWH-18-1-0675

5b. GRANT NUMBER
SC170098

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Charles Hubscher, Ph.D.

E-Mail: chhubs01@louisville.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Louisville
2301 S. 3rd St. Louisville,
KY 40208-1838

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development 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

Bowel complications after spinal cord injury (SCI) have been repeatedly rated as a primary concern by this population. In most surveys, bowel concerns rate as higher priority than being able to walk again. There currently exists an insufficient amount of research on the topic of basic bowel function after SCI in animals and therefore a lack of an appropriate model to develop treatments to improve function. The major impairment of quality of life caused by bowel complications must therefore stimulate research so that mechanisms can be understood, and treatments developed. The current proposal represents an attempt to fill a gap the literature regarding the effect of SCI on colonic motility and anorectal dynamics in rats. To develop quantifiable outcome measures in an experimental SCI rodent animal model for future use by SCI investigators, the current experiments are designed to collect baseline outcome data on colonic motility and rectal/anal sphincter dynamics at multiple time-points after incomplete SCI (Aim 1) and demonstrate utility by assessing the impact of locomotor training (Aim 2; based on evidence from our ongoing human studies), a widely used rehabilitation strategy which has shown non-locomotor benefits, including improvements in urological function (Hubscher Lab; in both humans and in a rodent contusion model). The anorectal manometry methods that will be employed mimic those used in humans to determine descending bowel dysfunction and will therefore be ideally suited to promote future translation of the basic science bowel outcome data to the clinic.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1) Introduction

Bowel complications after spinal cord injury (SCI) have been repeatedly rated as a primary concern by this population. In most surveys, urinary and bowel concerns rate as higher priority than being able to walk again. There currently exists an insufficient amount of research on the topic of basic bowel function after SCI in animals and therefore a lack of an appropriate model to develop treatments to improve function. The major impairment of quality of life caused by bowel complications must therefore stimulate research so that mechanisms can be understood, and more effective treatments developed to address this significant problem in the SCI population. Although bowel dysfunction is highly prevalent in humans after SCI, a clinically relevant rat model with quantifiable outcome measures to enable systematic examination of underlying mechanisms and/or therapeutic interventions has not been developed. To develop quantifiable outcome measures in an experimental SCI rodent animal model for future use by SCI investigators, Aim 1 experiments are designed to collect baseline outcome data on colonic motility and rectal/anal sphincter dynamics at multiple time-points post-incomplete SCI. Assessments of bowel function will include weekly collection of feces over a 24-hour period using metabolic cages, terminal experiments at different time end-points that will include quantitative measures of colonic motility (frequency, amplitude and duration of colonic contractions) and rectal/anal sphincter dynamics (EMG responses to movement of a balloon the size of a fecal pellet), and subsequent colon tissue evaluation of various neurochemical markers associated with bowel function that will include α2-adrenergic receptors, Substance P, and Vasoactive Intestinal Peptide. It is hypothesized that all measured aspects of bowel function will diminish across acute (3 days), and sub-acute (7 and 14 days) time-points and then level off at chronic (28, 42 and 84 days) time points. In Aim 2, the impact of activity-based training on bowel function will be examined (per Aim 1) with time manipulations that address a key unanswered question regarding the maintenance of benefits of this widely used rehabilitation strategy. Aim 2 is based upon evidence of significant improvements in bowel function following locomotor training (LT) in our ongoing human studies as well as improvements in urological function in both humans and in a rodent contusion model. The anorectal manometry methods that will be employed mimic those used in humans to determine descending bowel dysfunction and will therefore be ideally suited to promote future translation of the basic science bowel outcome data to the clinic.

2) Keywords Bowel, spinal cord injury, anorectal manometry, locomotor training, exercise, external anal sphincter, colon, colonic motility

3) Accomplishments

Major Goals:

Aim 1: To determine the impact of SCI and time post-injury on colonic motility and anorectal EMG using a clinically relevant rat contusion model. Additionally, tissue will be collected from the colon and spinal cord to assess potential injury-induced changes of several established targets involved in colonic motility including: α2-adrenergic receptors, Substance P (SP), and Vasoactive Intestinal Peptide (VIP). Baseline motility and EMG values as well as neurochemical profiles will be obtained from a laminectomy sham SCI control group for comparison.

Aim 2: To determine the impact of locomotor training (60 minutes daily, 5 times per week of quadrupedal stepping on a treadmill) on bowel function (terminal outcome studies after 8-week

train/non-train period per Aim 1) using a clinically relevant incomplete contusion injury. Maintenance will be addressed using multiple groups that include the following: Group 1 – 8 weeks LT; Group 2 - 4 weeks LT then a 4-week non-training period; Group 3 – 4 weeks LT, 3 weeks non-training, then resume LT for 5 sessions (1 week); Group 4 – 4 weeks LT, 4 weeks non-training but with 1 60-minute session per week of LT; and Group 5 – 8 weeks non-training. Tissue will be collected from the colon and spinal cord for all groups after terminal motility/anorectal EMG assessments to examine potential differences in several established candidate systems (per Aim 1).

Table 1: SOW

Research-Specific Tasks:

Specific Aim 1: To determine the impact of SCI and time post-injury on colonic motility and anorectal EMG using a clinically relevant rat contusion model. Additionally, tissue will be collected from the colon and spinal cord to assess potential injury-induced changes of several established targets involved in colonic motility including: α2-adrenergic receptors, Substance P (SP), and Vasoactive Intestinal Peptide (VIP). Baseline motility and EMG values as well as neurochemical profiles will be obtained from a laminectomy sham SCI control group for comparison.	
Major Task 1: IACUC and ACURO approvals	
IACUC approval process for animal studies	1
ACURO regulatory review and approval processes for rat studies	2-3
Major Task 2: Experiments for Aim 1 acute and subacute groups of rats – n=12 for 3-day survival (SCI acute group); n=12 for 7-day survival (SCI sub-acute group); n=12 for 14-day survival (SCI sub-acute group); n=12 for 3-day survival (sham acute group). Six groups of 8 rats (two 3-day sham, two 3-day SCI, two 7-day SCI, two 14-day SCI) will be done at three-week intervals for a total of 48 rats in 4 months.	
Subtask 1: Pre-injury baseline assessments (two measures in one week): • Locomotor assessment using 21-point BBB scale • Total feces pellet count and weight in a 24-hour period (metabolic cages - CLAMS)	4-7
Subtask 2: Contusion injuries then post-injury assessment on the day prior to terminal study (group dependent – 2, 6 or 13 days post-injury): • Locomotor assessment using 21-point BBB scale • Total feces pellet count and total feces weight in a 24-hour period (metabolic cages - CLAMS)	4-7
Subtask 3: Terminal assessments (group dependent – 3, 7 or 14 days post-injury)	4-7

• intracolonic pressure recordings (amplitude, duration, and frequency) of the distal colon and rectum to a balloon stimulus • EMG responses of the anal sphincter to passage of a pellet sized balloon	
Milestone(s) Achieved: completion of 4 groups of 12 rats per group (total 48) – all Aim 1 acute/sub-acute groups	
Major Task 3: Experiments for Aim 1 chronic groups of rats – n=12 for 28-day survival (SCI chronic group); n=12 for 42-day survival (SCI chronic group); n=12 for 84-day survival (SCI chronic group); n=12 for 42-day survival (sham chronic group). Six groups of 8 rats (two 28-day SCI, two 42-day SCI, two 84-day SCI, two 42-day sham) will be done at spaced time-periods for a total of 48 rats in 6 months.	
Subtask 1: Pre-injury baseline assessments (two measures in one week): • Locomotor assessment using 21-point BBB scale • Total feces pellet count and weight in a 24-hour period (metabolic cages - CLAMS)	8-12
Subtask 2: Contusion injuries then post-injury assessment once a week until terminal study (group dependent – 28, 42 or 84 days post-injury): • Locomotor assessment using 21-point BBB scale • Total feces pellet count and total feces weight in a 24-hour period (metabolic cages - CLAMS)	8-12
Subtask 3: Terminal assessments (group dependent – 28, 42 or 84 days post-injury): • intracolonic pressure recordings (amplitude, duration, and frequency) of the distal colon and rectum to a balloon stimulus • EMG responses of the anal sphincter to passage of a pellet sized balloon	8-12
Milestone(s) Achieved: completion of 4 groups of 12 rats per group (total 48) – all Aim 1 chronic groups.	

Research Accomplishments:

Major Task 2 is completed for collection of data, including terminal assessments of intracolonic pressure recordings and anal sphincter EMG's. The total count of animals completed for Aim 1 acute and subacute groups is 51. Analysis of bowel data (preliminary findings – for acute 3-day group - follow Table 2) and histology of lesions (see completed animals to date in Table 3) are almost complete. For Major Task 3, 28-day, 42 -day and 84-day group rats have now been completed (49 rats; see Table 2 below) including some of the lesion histology (see 84-day group in Table 3 below). The remaining analysis and histology are in progress (ALL animal experiments completed in Year 1 per SOW). A manuscript will be prepared and submitted during Year 2.

Table 2: . Injury data and Baseline Assessments

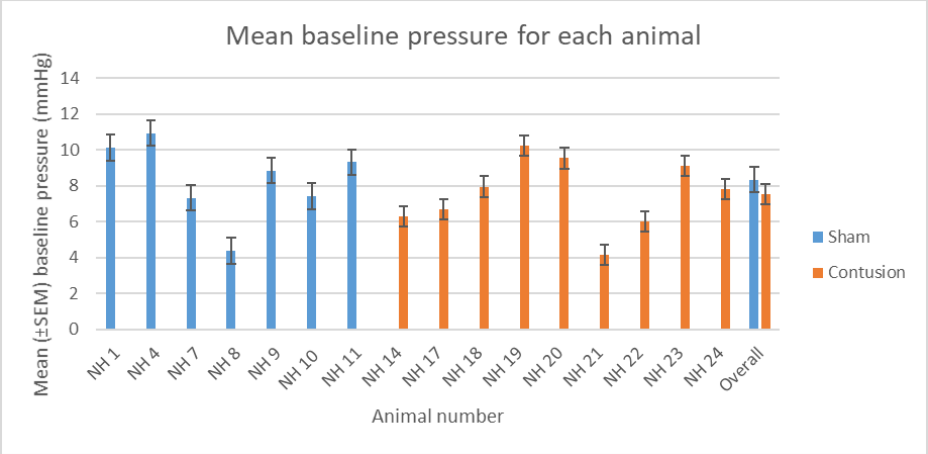
Animal Number	Bladder Volume (ml)	Injury Force (kdyne)	Displacement (um)	Survival Days	Injury Level
1	0	Sham	Sham	3	T9
2	0	Sham	Sham	3	T9
3	0	Sham	Sham	3	T9
4	0	Sham	Sham	3	T9
5	0	Sham	Sham	3	T9
6	0	Sham	Sham	3	T9
7	0	Sham	Sham	3	T9
8	0	Sham	Sham	3	T9
9	0	Sham	Sham	3	T9
10	0	Sham	Sham	3	T9
11	0	Sham	Sham	3	T9
13	Term Res	228	1464	3	T9
14	Term Res	217	1428	3	T9
15	Term Res	217	1128	3	T9
16	Term Res	216	1340	3	T9
17	Term Res	218	1287	3	T9
18	Term Res	270	162	3	T9
19	Term Res	217	1305	3	T9
20	Term Res	222	1340	3	T9
21	Term Res	226	1552	3	T9
22	Term Res	216	1340	3	T9
23	Term Res	219	1305	3	T9
24	Term Res	215	1552	3	T9
25	0	Sham	Sham	7	T9
26	0	Sham	Sham	7	T9
27	0	Sham	Sham	7	T9
28	0	Sham	Sham	7	T9
29	0	Sham	Sham	7	T9
30	4.1	296	1781	7	T9
31	4.7	215	705	7	T9
32	4.4	282	1605	7	T9
33	4.8	219	1375	84	T9

34	4.2	288	1569	84	T9
35	4.3	267	1640	84	T9
36	4.9	219	1440	7	T9
37	3	130	793	7	T9
38	4	220	1252	7	T9
39	6.2	223	1393	84	T9
40	3.1	221	1516	84	T9
41	2.3	217	1499	84	T9
42	4.4	292	1534	7	T9
43	3.8	279	1446	7	T9
44	3.5	217	1516	7	T9
45	5.4	214	1411	84	T9
46	3.8	221	1199	84	T9
47	4.2	322	1693	84	T9
48	3.3	230	1287	7	T9
49	2.9	217	1305	7	T9
50	4.4	221	1305	7	T9
51	3	220	1799	84	T9
52	4.4	215	1411	84	T9
53	5.3	221	1428	84	T9
54	1.4	217	1234	14	T9
55	4.4	216	1340	14	T9
56	4.1	224	1252	14	T9
57	4.9	223	1340	14	T9
58	4.5	225	1305	14	T9
59	2.5	224	1181	14	T9
66	2.9	224	1552	14	T9
67	2.3	222	1428	14	T9
69	3	230	1569	14	T9
70	3.5	224	1234	14	T9
71	2.9	221	1428	14	T9
72	0	Sham	Sham	42	T9
73	0	Sham	Sham	42	T9
74	0	Sham	Sham	42	T9
78	0	Sham	Sham	42	T9

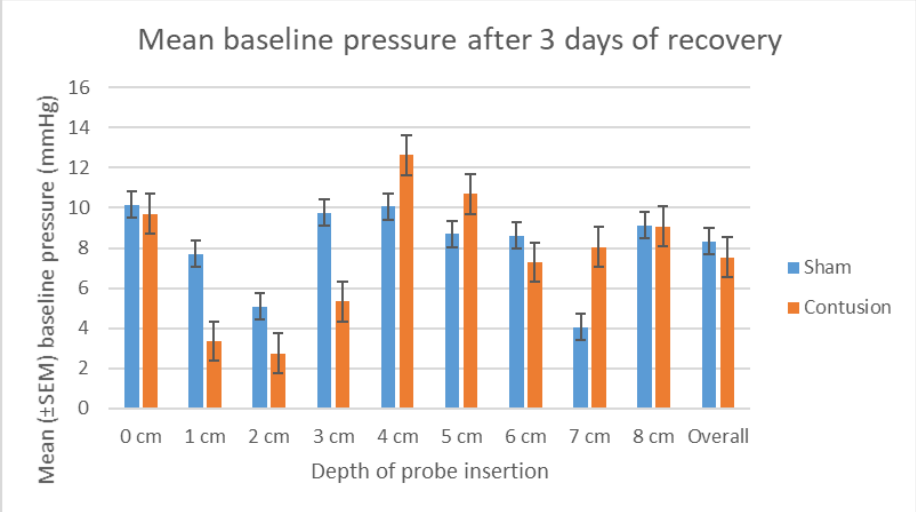
79	0	Sham	Sham	42	T9
80	0	Sham	Sham	42	T9
87	0	Sham	Sham	42	T9
88	0	Sham	Sham	42	T9
89	0	Sham	Sham	42	T9
93	0	Sham	Sham	42	T9
94	0	Sham	Sham	42	T9
95	0	Sham	Sham	42	T9
109	3.2	217	1569	42	T9
110	2.4	300	1464	42	T9
112	1.6	220	1393	42	T9
113	3.1	312	1534	42	T9
114	0.9	257	1446	42	T9
115	0.1	222	1393	42	T9
116	0.8	225	1393	42	T9
117	3.5	226	1516	42	T9
118	1.9	222	1605	42	T9
119	1.1	220	1569	42	T9
120	2.6	219	1481	28	T9
121	3.3	215	1428	28	T9
123	1.3	220	1569	28	T9
124	4.6	217	1499	28	T9
125	4.2	220	1234	28	T9
132	2.7	217	1270	28	T9
133	3.4	226	1428	28	T9
134	3.8	222	1340	28	T9
138	3.2	215	1358	28	T9
139	5.1	248	1740	28	T9
140	4.3	216	1322	28	T9
150	4.6	224	1481	42	T9
151	5	219	1464	42	T9
152	4.1	223	1199	42	T9
153	2.8	223	1322	42	T9
154	4.4	231	1446	42	T9
155	3.1	218	1234	42	T9

Major Task 2 Data Analysis Summary To-Date

Evaluation of the variability between animals showed no overall differences in the mean baseline pressure between the groups. Dixon's test for a single outlier was used to exclude one sham animal that had a mean baseline pressure over 2 standard deviations above the sham mean.



Even though there were no overall differences between the groups in mean baseline pressure, our analysis shows that at certain depths of insertion there appear to be differences between injured and uninjured animals. Sham animals have a higher mean baseline pressure at 1, 2, and 3 cm depths which indicates a disruption in the ability of the colon to maintain tone in the caudal portion of the rectum. This portion of the rectum is active in the late stages of motility and moves material toward the anus for defecation. A failure to maintain proper tone of this musculature could impair defecation and lead to fecal retention. Furthermore, at 4, 5, and 7 cm it appears that there is increased tone after injury. This is consistent with data from chronically injured animals and may indicate the onset of a phenotype that persists into the chronic phase of recovery. Increased tone of the rectum is also seen in humans after injury and is related to functional obstructions of the bowel and reduced motility.



The mean contraction frequency of the colon is reduced after injury. Activity is less frequent at nearly all levels tested and suggests a colon that is less able to move material due to a lower speed

of motility. This may be due to spinal shock, which lasts about 7 days and thus should be resolved in later recovery group animals (analysis in progress).

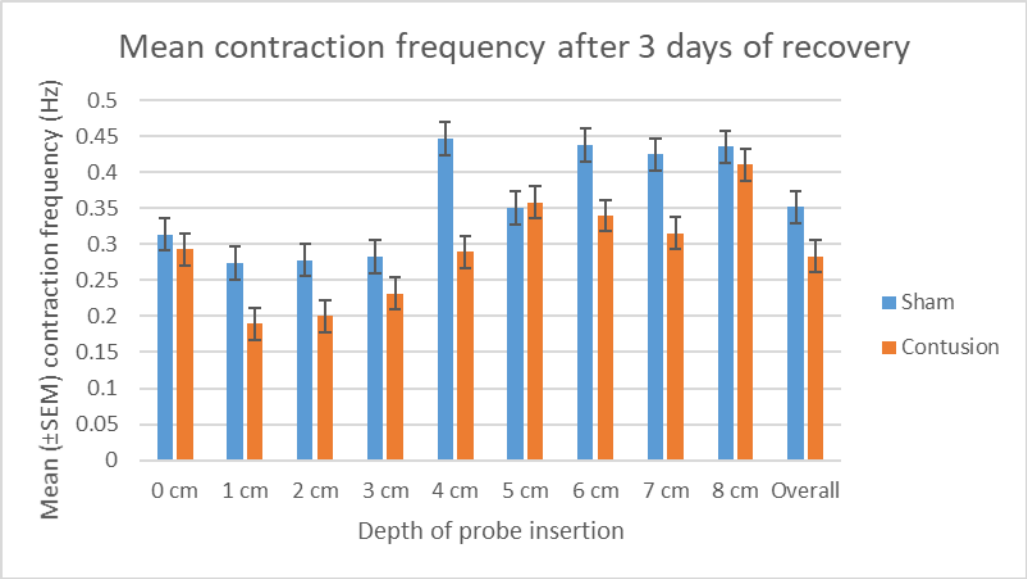


Table 3. Lesion Reconstruction Data (Aim 1, Task 3)

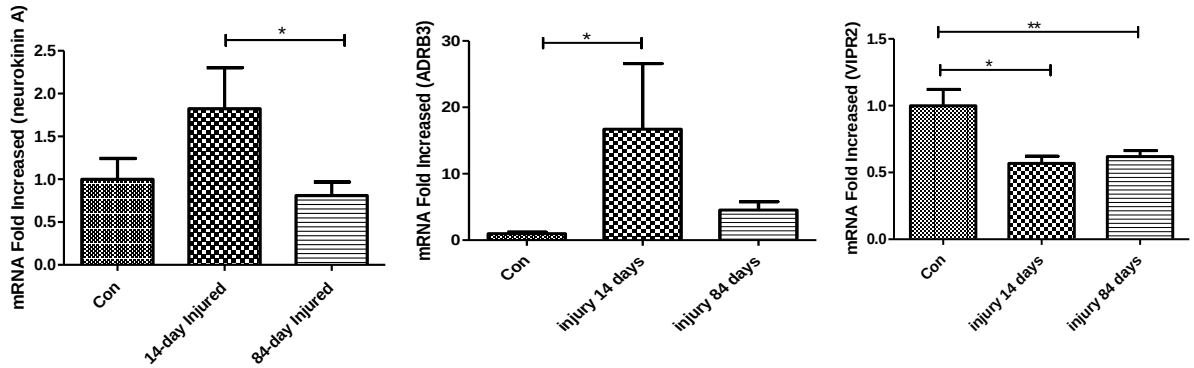
Animal Number	%WMS	%GMS
NH30	13.6316	57.4899
NH31	35.4496	78.157
NH32	19.7375	36.2967
NH33	7.522578	0
NH34	9.870375	0
NH35	6.573866	1.06883
NH36	20.2538	20.2751
NH37	41.4543	10.2515
NH38	20.7775	16.6698
NH39	10.01515	0
NH40	11.35605	0
NH41	13.54909	0
NH43	19.0252	10.9293
NH44	16.9462	32.2166
NH45	6.567837	0
NH46	17.10429	0.836398
NH47	15.15467	0
NH48	35.4244	37.9118
NH49	33.0622	24.2614
NH50	37.5568	13.5439

NH51	7.347615	0
NH52	7.581963	0
NH53	6.364211	0
NH54	39.3422	26.1223
NH55	17.4645	16.6023
NH56	27.0467	3.43129
NH57	35.3742	22.5222
NH58	18.7209	7.4783
NH59	14.9068	11.6592
NH66	10.4909	6.63528
NH67	6.99338	5.217
NH69	6.72825	5.28589
NH70	13.3247	6.13811
NH71	14.5983	12.3118

Preliminary Summary of Colon and Rectal Tissue assessment data.
(shams, 14-day SCI and 84-day SCI groups examined)

(1) For substance P, we did not find any significant differences across time for both distal colon and rectum.
(2) Neurokinin A was significant reduced for 84-day injured in mRNA level compared to 14-day injured in distal colon.
(3) For Adrenergic receptors ADRA2A we did not see significant changes in both distal colon and rectum in the rat SCI model. There was significantly increased levels of ADRB3 for 14-day injury in rectum compared to control.
(4) For VIP and PACAP there were no significant changes in RNA levels. There was however a significant increase for VIP receptor VIPR1 for 84 days after injury compared to controls in the distal colon. There was also a significant decrease for VIP receptor VIPR2 for both 14 day and 84-day animals compared to controls in rectum. Since VIPR1 is involved in smooth muscle relaxation, increased VIPR1 causes distal colon relaxation. Reduced PAC1 causes distal colon contraction.
(5) Thus, in chronic SCI, there is dysregulated activity for motility in distal colon for VIP/PACAP signaling. Since VIPR2 mediates the anti-inflammatory effects, the rectum may have reduced anti-inflammatory effects and vasodilation via VIP post-SCI.

Colon: Rectum:



Opportunities for training and professional development:

Training is being provided to a senior undergraduate Bioengineering co-op student who is assisting with the analysis of the manometry data. Likewise, funding of a postdoctoral associate and graduate student who are participating in the study have opportunities to attend local seminars and journal club in the Kentucky Spinal Cord Injury Research Center as part of their professional development.

Disseminated to communities of interest:

Nothing to report

Plans during the next reporting period to accomplish the goals:

Complete analysis of Aim 1 data and prepare for publication. Begin Aim 2 studies with activity-based training.

4) Impact

Impact on the development of the principal discipline(s) of the project:
Nothing to report

Impact on other disciplines:
Nothing to report

Impact on technology transfer:
Nothing to report

Impact on society beyond science and technology:
Nothing to report

5) Changes and/or Problems

None to report.

6) Products

Poster Presentation:

Hoey, R.F., Hubscher C.H. (2018) Development of a Comprehensive Protocol for Detecting Bowel Dysfunction after Spinal Cord Contusion in Wistar Rats. *2018 Abstract Viewer/Itinerary Planner.* San Diego CA: Society for Neuroscience.

Inventions, Patents and Licenses
None to report

7) Participants & other collaborating organizations

Individuals who have worked on the project:

Name:	Charles Hubscher, Ph.D.
Project Role:	P.I.
Effort:	11%
Contribution to Project:	Oversees all aspects of the project.
Name:	Robert Hoey, Ph.D.
Project Role:	Postdoc
Effort:	20%
Contribution to Project:	Anorectal manometry
Name:	James Armstrong
Project Role:	Senior Research Technician
Effort:	15%
Contribution to Project:	Involved with all aspects of the project, including care of animals.
Name:	Jason Fell
Project Role:	Research Technician
Effort:	15%
Contribution to Project:	Involved with animal training and terminal studies, perfusions, spinal lesion removal, reconstructions, and histological analyses.
Name:	Yun Zhou, Ph.D.
Project Role:	Research Associate
Effort:	50%
Contribution to Project:	Colon tissue assessments
Name:	Sai Sree Vangoor
Project Role:	Temporary worker (undergraduate student)
Effort:	100% (of a part-time position)
Contribution to Project:	Involved with manometry data analysis.

Changes in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period:
Nothing to report

Other organizations involved as partners:
Nothing to report

8) Special Reporting Requirements

Quad Chart is updated and submitted as an attachment.

9) Appendices

none