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

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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.

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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

Aim 2

Major Task 2: Experiments for Groups 2, 3 and 4 (8-weeks training). Four animals will be trained at a time in a step-wise manner (mix of all groups) as there are varying train/non-train periods. Again, each training period consists of one hour of stepping.	
Subtask 1: Pre-injury baseline assessments (once a week for two weeks): 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)	20-34
Subtask 2: Contusion injuries then post-injury assessment at 7 days and 14 days post-SCI: • Locomotor assessment using 21-point BBB scale • Total feces pellet count and total feces weight in a 24-hour period (metabolic cages - CLAMS)	20-34
Subtask 3: 8 weeks of daily step training on a treadmill beginning two weeks post-injury (weekly assessments). • Locomotor assessment using 21-point BBB scale • Total feces pellet count and total feces weight in a 24-hour period (metabolic cages - CLAMS)	20-34
Subtask 4: Terminal assessments • 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	20-34
Milestone(s) Achieved: completion of all remaining Aim 2 groups of rats (36 for an Aim 2 total 60)	
Major Task 3: Immunohistochemistry and Western Blot for analyses of combined data for the five groups of 12 rats (60 rats total), for lesion reconstructions, and for preparation of manuscripts for publication.	32-36

Research Accomplishments:

Aim 1 is completed for collection of data, including terminal assessments of intracolonic pressure recordings and anal sphincter EMG's. All tissue analyses and lesion histology are complete. The first manuscript was published in Journal of Neurotrauma (Sept. 15, 2020). Three more manuscripts are in preparation from the Aim 1 groups of animals. One manuscript contains the physiological data from functional assessments. A methods figure showing the groups from that manuscript is provided below (Figure 1). The Results section is in progress. The second two manuscripts relate to RT-PCR and immunohistochemistry data on rectal tissue from rats along the same timeline. An example of data contained in one figure is provided below (Figure 2).

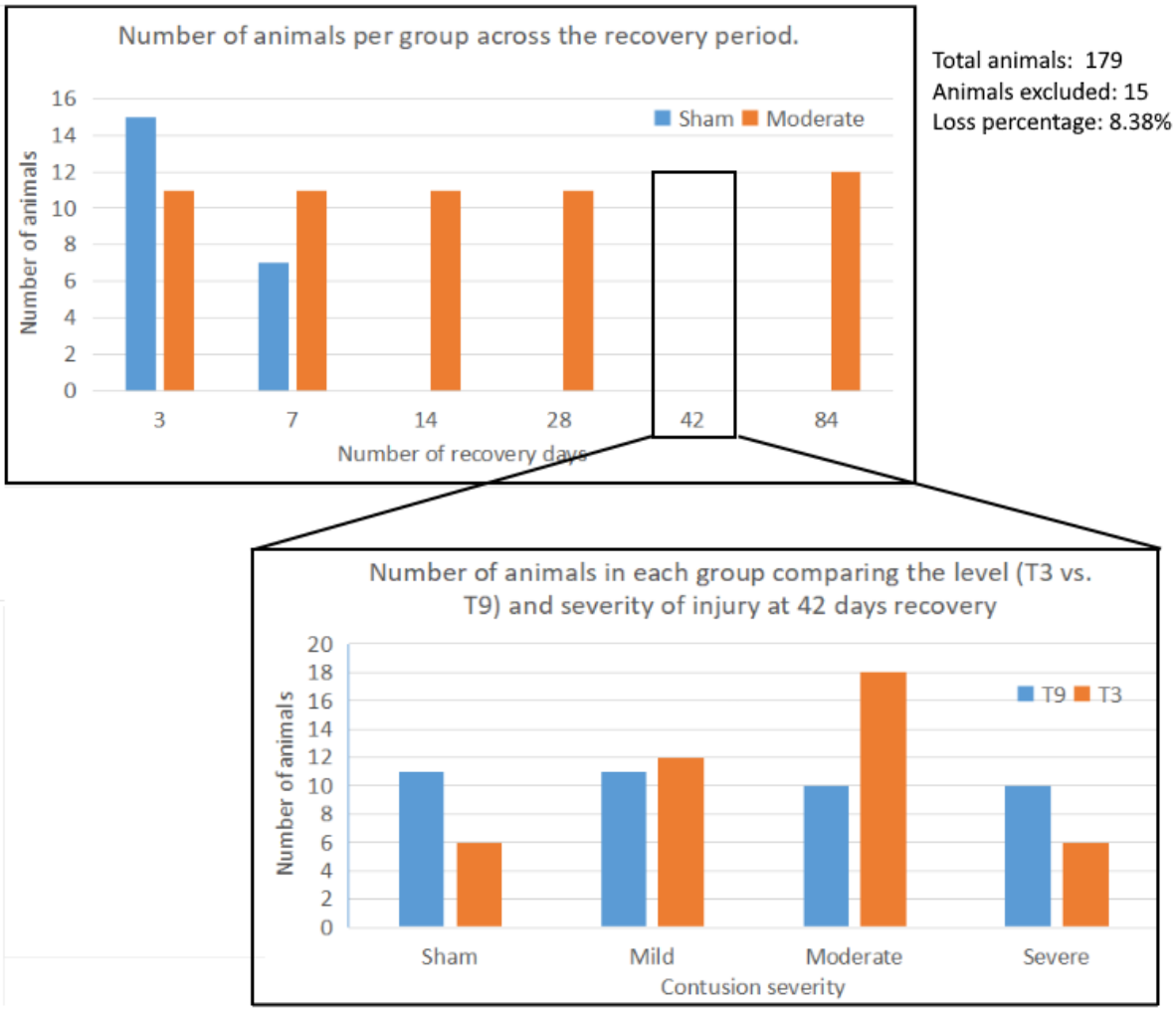

Figure 1. Aim 1 group N's for chronicity and severity of spinal contusion injuries experiments.

Rectal hyposensitivity (i.e. impaired or blunted rectal sensation) is found most commonly in patients with SCI or clinically documented neuropathy. Rectal hyposensitivity has been found in up to 67% of patients with complete SCI and hindgut dysfunction. Neurogenic cells (possible rectospinal neurons) which localize to the distal rectum secrete chemokines such as CCL21, MCP-1, CXCL9 which attract leukocytes such as $CD14^{+}CD16^{+}$ monocytes and lymphocytes such as CD4

T cells to the rectum causing inflammation and rectal tissue remodeling resulting in rectal hyposensitivity. A timeline for changes in chemokines is provided in Figure 2.

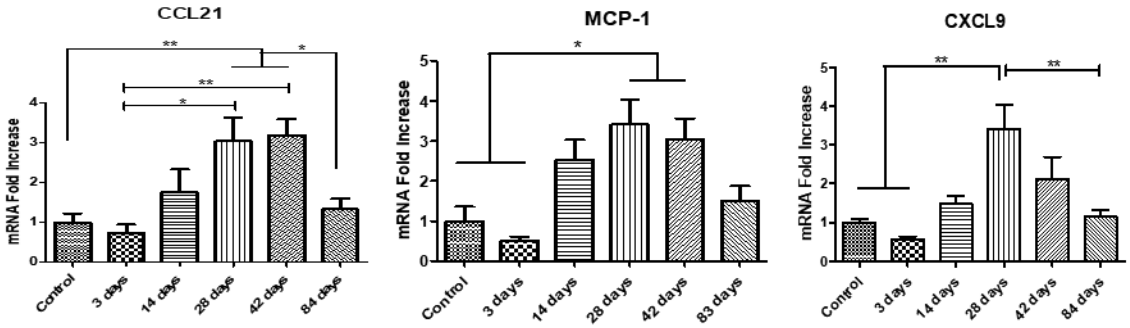

Figure 2 – RT-PCR results (n=48 rats in total) showing that chemokines such as CCL21, MCP-1, CXCL9 significantly increased after SCI at Day 28 to Day 42 in rectal tissue. PCR is repeated 3 times and one-way ANOVA with Bonferroni's post hoc test is used to compare group differences. Differences are considered significant at $p < 0.05$. * $p<0.05$, ** $p<0.01$.

Aim 2 studies examining the impact of activity-based training on bowel function is nearing completion. Three of the six training groups for Aim 2 (n=12 per group) were completed during Year 3. These groups include non-trained, 4week ON/3-4 week OFF/3-4 week ON training, and 4-week ON/3-week OFF/1-week ON training. Three final groups are being completed during the first half of the no-cost extension year. Groups in progress include 4 weeks ON/4 weeks OFF training, 8 weeks training, and 4 weeks ON then 1-day per week for 4-week group. The injury outcomes and assessment data for Year 3 animals are provided in Table 1. Lesion site reconstruction data completed to date are provided in Table 2. Initial metabolic cage feces collection data for the first completed group of rats (4week ON/3-4 week OFF/3-4 week ON training) is provided in Figure 3. All terminal study data will be analyzed once all the groups are completed during the second half of extension year.

Table 1: Injury data and Baseline Assessments

Animal Number	Injury Force (kdyne)	Displacement (um)	Bladder Volume (ml)	14 Day BBB
T37	214	1464	1.1	11--11
T39	218	1640	4.2	8--5
T40	216	1446	4.1	10--8
T41	218	1446	3.6	10--10
T42	223	1587	2.8	11--11
T44	226	1516	3.1	8--8
T45	224	1464	2.9	10--10
T47	219	1234	4.2	11--11
T48	219	1287	3.1	11--11
T50	217	1393	3.1	11--11
T52	217	952	0.6	12--12
T53	223	1217	3.8	11--11

T54	219	1428	2.8	11--11
T55	216	1322	3.7	8--8
T56	228	1393	3.9	10--8
T57	238	864	0.9	20--18
T58	235	934	0.7	12--12
T59	224	1287	0.8	11--11
T60	216	1481	4.7	10--10
T61	226	1234	5.1	11--11
T62	232	1516	4.3	8--8
T63	224	1499	2.3	8--8
T64	218	1481	3.8	7--1
T65	215	1393	2.8	10--9
T66	245	1534	3.1	8--8
T67	229	1658	4.4	11--10
T68	223	1516	5.1	8--2
T69	217	1111	1.4	11--11
T70	225	1534	5.3	8--5
T71	217	1358	0.5	13--13
T72	221	987	0.6	11--11

Table 2. Lesion Reconstruction Data (Aim 2)

Animal #	%WMS	%GMS
T25	13.1399	0
T26	28.33296	0
T28	9.59311	0
T30	17.08863	1.556183
T32	13.92991	0
T34	7.170696	0
T36	16.84029	0
T37	25.88205	0
T39	15.21118	0
T40	10.4195	0
T41	17.53142	0
T42	17.29817	0
T44	11.56054	0
T45	14.49326	0
T47	24.15257	0
T48	28.21793	2.75191
T50	20.63313	0
T52	39.34115	0
T53	22.28615	0

T54	43.3937	0
T55	21.11597	0
T56	15.63058	0
T57	75.66422	11.40259

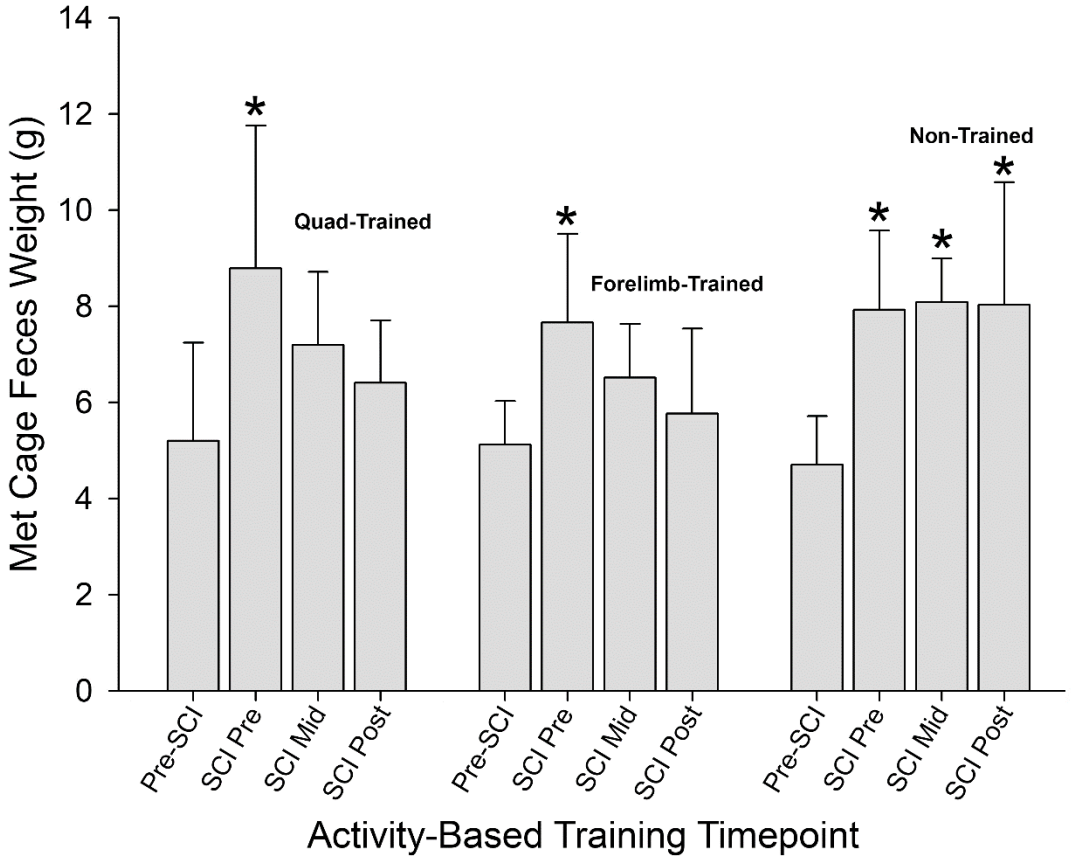

Figure 3: Data showing that both locomotor training (Quad-trained group) and exercise in general (forelimb-trained group) normalize defecation amount (based on feces weight) even with a 3–4-week gap in training. Note that the non-trained group maintained spinal cord injury (SCI) induced dysfunction across all time-points.

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:

Hoey, R.F., Hubscher C.H. (2020) Investigation of Bowel Function with Anorectal Manometry in a Rat Spinal Cord Contusion Model. J Neurotrauma, Sept 15;37:1871-982. PMID: 32515264

Plans during the next reporting period to accomplish the goals:

Complete publication of manuscripts from Aim 1 data and prepare activity-based training data (Aim 2) for publication.

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

The lab was closed due to COVID 19 for a 6-week period in April/May 2020 and a further delay occurred as the University did not allow purchasing of new animals for over a two-month period. As a result, we had to stop the first Aim 2 group mid-training. We resumed training upon the partial re-opening of the lab and have since completed the terminal studies on the first group. As Aim 2 deals with varying degrees of training and their impact on bowel function, we decided to use the interruption as an additional group (rather than not using them at all) and used the same timing for the second set of animals. We resumed the other groups with varying amounts of training starting in the latter part of the third quarter of Year 2. An extension at the end of the grant was requested and approved to complete the study. Hopefully COVID-19 will not impede progress any further. The University of Louisville has policies in place regarding the wearing of masks, maintaining physical distancing in buildings and in laboratories, and washing of the hands. Faculty, staff and students who come to campus are required to be vaccinated or tested on a regular basis.

6) Products

Publications:

Hoey, R.F., Hubscher C.H. (2020) Investigation of Bowel Function with Anorectal Manometry in a Rat Spinal Cord Contusion Model. J Neurotrauma, Sept 15;37:1871-982. PMID: 32515264

Hoey, RF., Medina-Aguiñaga, D., Fell, J., Hubscher, CH. Effect of chronicity, severity, and lesion level on bowel dysfunction in male rats after spinal contusion injury. In preparation.

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