Award Number:
Contract #: W81XWH-14-1-0483
Grant #: SC130298

TITLE:
"Recovery of Breathing and Forelimb Function after Prolonged Exposure to Repetitive Acute Intermittent Hypoxia".
PRINCIPAL INVESTIGATOR: Gordon S. Mitchell, PhD

CONTRACTING ORGANIZATION: University of Florida
Gainesville, FL 32611

REPORT DATE: FEBRUARY 2019

TYPE OF REPORT: FINAL

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

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
FEBRUARY 2019	FINAL	1SEP2014 - 28 NOV 2018

4. TITLE AND SUBTITLE

"Recovery of Breathing and Forelimb Function after Prolonged Exposure to Repetitive Acute Intermittent Hypoxia".

5a. CONTRACT NUMBER
W81XWH-14-1-0483

5b. GRANT NUMBER
SC 130298

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Gordon S. Mitchell, PhD and Gillian Muir, DVM/PhD

gsmitche@phhp.ufl.edu
Gillian.muir@usask.ca

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Florida
PO Box 115500
Gainesville, FL 32611

8. PERFORMING ORGANIZATION REPORT NUMBER

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

The fundamental goal of this project is to test the efficacy and safety of prolonged repetitive exposure to acute intermittent hypoxia (rAIH; 10 episodes per day, 3 to 4 days per week, 3 to 6 months) in a rodent model of chronic, incomplete cervical spinal injury (C_2 spinal hemisection in rats; C2HS). In this collaborative project (Florida and Saskatoon, Canada), we are exploring the impact of prolonged rAIH on both respiratory (Florida) and limb function (Canada), and on markers of neuro-cognitive and cardiovascular safety (Florida). Three specific aims were proposed: **Aim 1**: Test the hypothesis that prolonged rAIH elicits robust and prolonged improvement of breathing capacity after chronic C2HS; **Aim 2**: Test the hypothesis that prolonged rAIH in combination with task specific training elicits robust and prolonged improvement of voluntary forelimb function after chronic C2HS; and **Aim 3**: Test the hypothesis that prolonged rAIH has no significant impact on hippocampal cell survival or systemic blood pressure. These pre-clinical studies are an essential "next-step" in our efforts to translate rAIH as a therapeutic modality to restore respiratory and non-respiratory motor function in patients with chronic, incomplete SCI.

15. SUBJECT TERMS
Spinal cord injury, treatment, intermittent hypoxia, prolonged, rats, safety, efficacy.

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	13	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

Abstract:

Neural plasticity is a significant contributor to motor recovery following SCI. Acute intermittent hypoxia (AIH) triggers spinal motor plasticity, strengthening synapses onto respiratory motor neurons by a mechanism known as long-term facilitation. Hypoxia stimulates the carotid body chemoreceptors, indirectly activating brainstem raphe neurons to release serotonin near their targets, including spinal motor neurons that underlie breathing and limb movements. Intermittent serotonin receptor activation on spinal, respiratory motor neurons stimulates new protein synthesis that underlies plasticity, including new synthesis of brain derived neurotrophic factor (BDNF). By repeatedly activating this mechanism (7 days), we discovered it is possible to improve diminished breathing capacity after cervical SCI.

Repetitive AIH also elicits plasticity in non-respiratory, somatic motor systems, restoring limb function after chronic, incomplete SCI. In specific, daily AIH for 7 days elicits long-lasting improvements in horizontal ladder walking in rats with chronic cervical SCI; further, even a single AIH exposure increases ankle strength, and daily AIH for 5 days with combined walking training improves over-ground walking in humans with chronic, incomplete SCI.

We now know that very modest but prolonged protocols of repetitive AIH elicit profound neurochemical plasticity in respiratory and non-respiratory motor neurons. Because of its promise as a therapeutic tool, we propose to test the efficacy and safety of this modest repetitive AIH protocol to treat motor deficits caused by cervical SCI. We will use a sham-controlled, repeated-measures experimental design. An essential element in the translation of our findings that short repetitive AIH protocols (5-7 days) improve motor function following SCI is to determine if these effects can be enhanced by prolonged repetitive AIH exposure. We hypothesize that prolonged, (4x per week, 3 months) repetitive AIH elicits greater and longer-lasting recovery of respiratory motor function. Repetitive AIH is most effective at restoring walking ability when paired with walking training. Thus, we propose that prolonged repetitive AIH combined with task specific training elicit more robust and longer-lasting recovery of forelimb function in rats with chronic SCI. We will assess key molecules necessary for IH-induced respiratory motor plasticity within or near the relevant motor neurons, including serotonin, BDNF and TrkB. Although we demonstrated that brief repetitive AIH does not cause detectable pathology, it is essential to know if prolonged AIH protocols cross a threshold to pathology. Thus, we will assess the impact of prolonged repetitive AIH on systemic blood pressure and brain (hippocampal) pathology.

Controlled restoration of spinal motor neuron function is essential to restore automatic and volitional movements following incomplete SCI (eg. breathing and walking). Currently, no effective therapeutic options are available for persons with chronic spinal injury. Prolonged exposure to repetitive AIH is a highly novel means to promote spinal plasticity, and has considerable potential to be a simple, safe and effective means of promoting functional recovery of breathing, walking and possibly other motor behaviors. Our proposal is the essence of translational research since we use highly novel discoveries in rodent models of SCI to guide pre-clinical trials in humans with chronic, incomplete SCI. The rodent work outlined in this proposal is intended to establish a framework for future human clinical trials using long term repetitive AIH with/without combinatorial therapies. Thus the goal of our study is to test the **efficacy and safety** of a novel treatment strategy in a rodent model of chronic spinal cord injury. It is imperative to know if the benefits of this treatment are enhanced and are safe with long exposures. This research is based on more than 20 years of animal research, investigating the ability of intermittent hypoxia to induce neuroplasticity in the spinal cord, and follows our first explorations demonstrating remarkable ability to recovery leg strength and walking ability in humans with chronic incomplete injuries.

Three specific aims were proposed:

Aim 1: Test the hypothesis that prolonged repetitive AIH elicits robust and prolonged improvement of breathing capacity after chronic SCI. We predicted that 3 month AIH

exposures (4 times per week; 10, 5 min hypoxic episodes, 5 min intervals per day) elicit robust recovery of breathing ability in rats with chronic C2-hemisections (2 months).

Aim 2: Test the hypothesis that prolonged repetitive AIH combined with task specific training elicits robust/prolonged improvement of voluntary forelimb function with chronic SCI. Since repetitive AIH is most effective at restoring over-ground walking ability when paired with locomotor training, we paired 3 months of AIH with locomotor training to elicit more robust recovery of forelimb function. After C2-hemisection, multiple indicators of forelimb function were assessed, including reach-to-grasp performance. Forelimb muscles were injected with cholera toxin and tissues harvested to assess plasticity-related molecules in identified motor neurons.

Aim 3: Test the hypothesis that prolonged repetitive AIH does not adversely affect hippocampal cell survival or systemic blood pressure. It is essential to know if prolonged repetitive AIH crosses a threshold, leading to the onset of significant pathology.

Project Status:

Overall summary: To summarize essential findings from this grant: 1) Repetitive AIH for 3 months (10 episodes per day, 4x per week) had disappointing effects on breathing ability (although we did not see persistent deficits from the injury model studied); 2) **Repetitive AIH paired with rehabilitation elicited profound improvement in reach/grasp ability in rats with chronic SCI**; and 3) there was no evidence for hypertension or hippocampal pathology (or pathology in numerous other tissues). Thus, prolonged repetitive AIH appears to have great promise to restore forelimb function in a safe manner. We are preparing 2 manuscripts for publication: 1) documentation that prolonged repetitive AIH causes no detectable pathology; and 2) prolonged repetitive AIH improves reach/grasp performance with chronic spinal injury.

Aims 1 & 3: Accomplishments from work at the University of Florida

This portion of the progress report provides updates associated with Aims 1 & 3 of this project.

Milestone #2 –Understand efficacy of prolonged, repetitive AIH for recovery of breathing function. Understand changes in key molecules in spinal respiratory and non-respiratory motor neurons.

Essential findings: Despite difficulties, we observed what we believe to be consistent results, demonstrating that the specific repetitive AIH protocol used in this study has minimal impact on breathing ability in rats with chronic C2-hemisection. This conclusion is based on convergent lines evidence from this and another recently completed study: 1) in this study, the ability to increase breathing during maximal chemoreflex activation was unaffected by either injury or repetitive AIH (Figure 1); and 2) in a recently published study of chronic spinal hemisection, daily AIH (7 days) had minimal impact on breathing capacity or respiratory muscle EMG acitivity unless the rats were pretreated with an adenosine 2A receptor antagonist prior to AIH sessions (Navarette-Opazo et al., 2017). Disappointing effects on breathing in this study (Figure 2) are still being evaluated, and may be due to: 1) the minimal breathing deficit resulting from the chronic, incomplete C2 hemisection injury model studied here (ie. there is nothing to recover from, Figure 2); 2) the AIH protocol was not optimal in animals with chronic injuries (possibly due to accumulation of adenosine during hypoxic episodes, which is known to undermine AIH-induced respiratory motor plasticity; Navarette-Opazo et al., 2017); and/or 3) a need to pair AIH with task specific training (as with forelimb function). We are now investigating these issues.

Status of specific tasks in the Statement of Work:

Task 1. Quantify effects of repetitive AIH on breathing in rats with chronic SCI.

*Subtask 1a***.** Prepared documents for animal use approval. Done

*Subtask 1b***.** Breathing assessments of naïve rats prior to performing cervical spinal injuries. Done. Since the new instrumentation used to collect these data was defective, all early time-point data were lost. After repairs, we were able to collect plethysmography data from all rats (total 59 from the different groups), enabling cross-sectional analysis at the study end-point (**Figure 1**).

*Subtask 1c***.** Initiate repetitive AIH treatment and assess breathing capacity. Done with assessment of breathing capacity immediately post-repetitive AIH.

Subtask 1d. Breathing assessments at 4 and 8 weeks after repetitive AIH. Not done since data indicated no benefit immediately post-repetitive AIH.

*Subtask 1e***.** Assess phrenic motor output and arterial blood pressure in acute neurophysiology experiments. Perfuse rats. Done. Unfortunately, only the blood pressure data were usable (Figure 3) due to investigator effects in neurophysiology experiments.

*Subtask 1f***:** Determine if repetitive AIH increases expression of key plasticity molecules in spinal motor nuclei. Serotonin done. Others proposed in process.

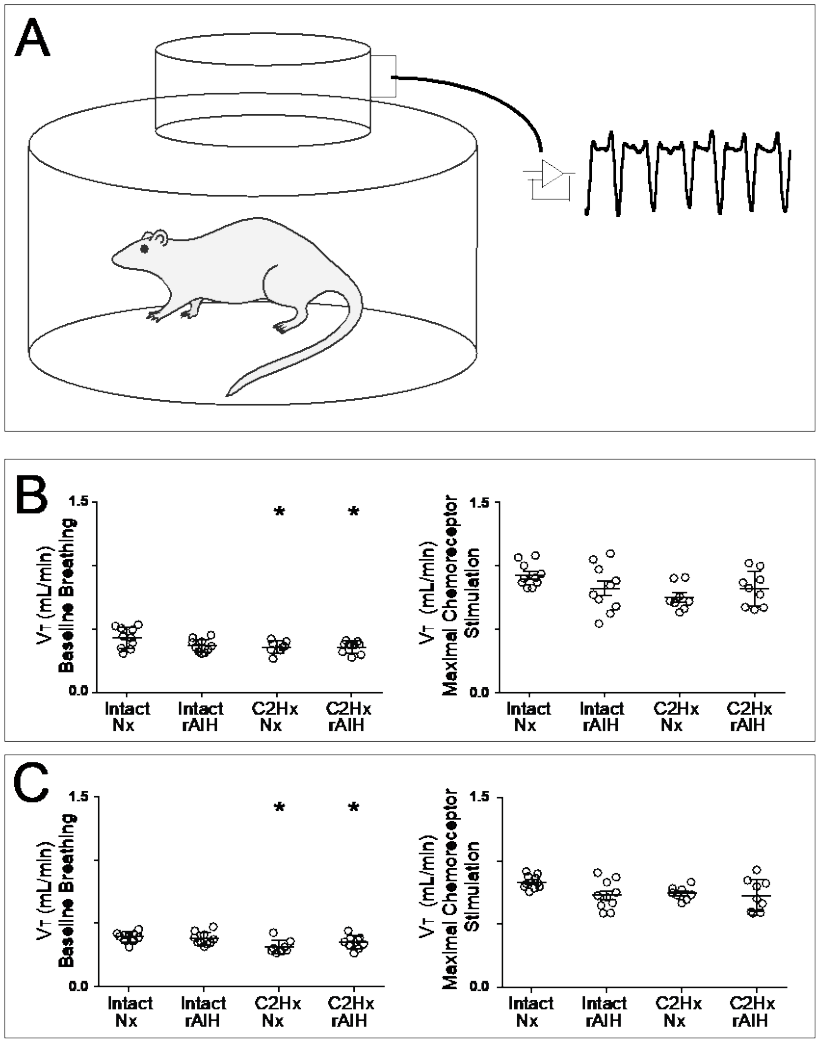

Figure 1: Ventilatory capacity measured with whole body plethysmography. ***A*****,** Ventilatory capacity was assessed in awake, spontaneously breathing rats prior to and following 3 months of Nx or rAIH exposure **(*A*)**. Prior to exposure, baseline tidal volumes (mL/min) were reduced in spinal cord injured animals compared with spinal intact animals, but no differences were observed between groups during maximal chemoreceptor stimulated breathing **(*B*)**. Following rAIH, baseline tidal volumes were reduced in spinal cord injured rats, but no effect of rAIH was evident. No effect of injury or exposure was evidence for maximal chemoreceptor stimulated breathing **(*C*)**. Thus, we found little evidence that rAIH improved breathing in this study, although there was no persistent ventilatory deficit to recovery from, likely due to the modest nature of the injuries performed in this study (see Figure 2). *Significantly different from intact; $p < 0.05$.

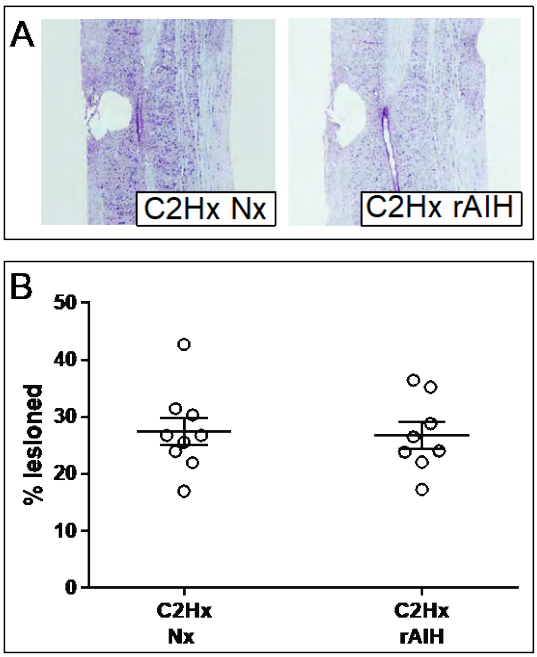

Figure 2: **Reconstructed C2 spinal injuries**. In these studies, a single knife cut was made, creating incomplete lesions of the ipsilateral spinal cord. **A**. representative micrographs of the injury site, with (right) and without repetitive AIH (left). **B**. The lesion area expressed as a percentage of the total spinal cord cross-sectional area: 1) was approximately 30% of spinal cross-sectional area; and 2) lesion area was similar in rats treated with normoxia and repetitive AIH. Values are individual data points and means ± SEM.

Milestone #4 –Understand impact of prolonged rAIH on hippocampal pathology and systemic blood pressure.

Essential findings: There was no evidence for pathology in blood pressure (Figure 3), hippocampus (Figures 4 & 5), aorta (Figure 6) heart (not shown), aorta (not shown), kidney (not shown), liver (not shown) or cancellous bone (Figure 7). These are critical findings since it speaks to the safety profile of repetitive AIH as a therapeutic modality. The critical importance of safety verification was reinforced by a workshop organized by the PI and sponsored by the Craig H. Neilsen Foundation to create a "Road Map to Clinical Translation" for repetitive acute intermittent hypoxia. This workshop consisted of clinical trialists, medical device engineers, pulmonary clinicians, rehabilitation clinicians, and researchers investigating AIH in animal models and humans with chronic SCI. Based on their recommendations, we harvested other tissues (described above) for blinded pathology assessments. Complete.

Task 3: Test effects of repetitive AIH on blood pressure and hippocampal cell survival.

*Subtask 3a***.** Quantify the effect of prolonged repetitive AIH on blood pressure. (Note-the same rat groups are in all sub-tasks within Aims 1 & 3; 59 were completed. Done

*Subtask 3b***:** Quantify repetitive AIH effects on hippocampal cells. (Note-the same rat groups are in all sub-tasks within Aims 1 & 3; 59 rats completed). Done. We also analyzed numerous other tissues from throughout the body for possible pathology.

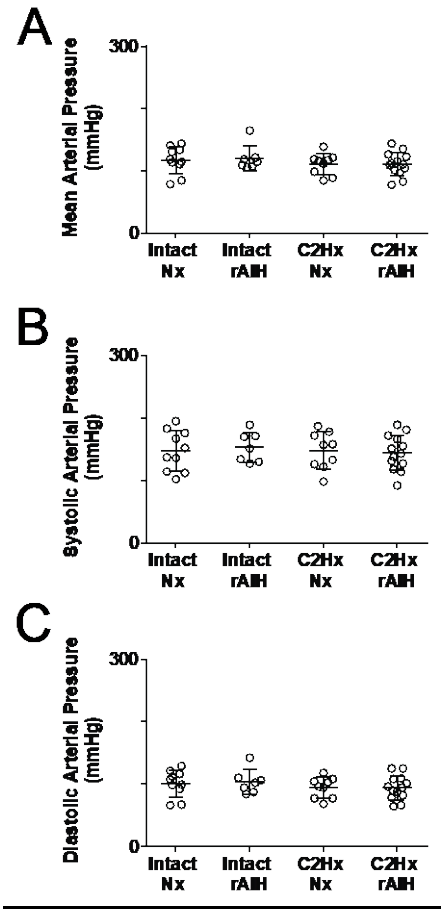

Figure 3: Blood Pressure. Mean arterial (***A***), systolic (***B***), and diastolic (***C***) blood pressure (mmHg) recorded from the femoral artery following 3 months of Nx or rAIH exposure in rats with and without chronic C2Hx injury. Long-term delivery of rAIH did not cause hypertension in intact or chronically injured rats. Thus, neither rAIH nor cervical spinal hemisection impacted systemic arterial pressure in urethane anesthetized rats. Values are individual data points as well as means ± SEM.

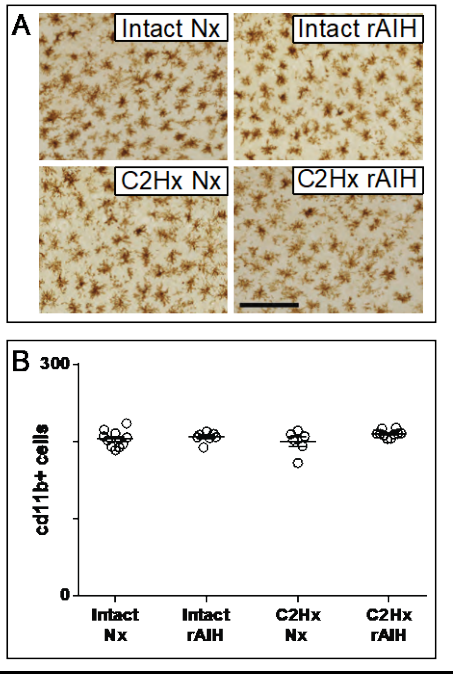

Figure 4: Microglial staining in hippocampus. Representative micrographs of CD11b immunostaining in coronal sections through the CA1 subfield of the hippocampus of intact and injured rats exposed to 3 months of either Nx or rAIH (***A***). Neither chronic spinal cord injury nor rAIH exposure increased the number of CD11b positive microglia in the hippocampus. Thus, rAIH did not activate microglia in the CNS. Values are individual data points as well as means ± SEM (***B***).

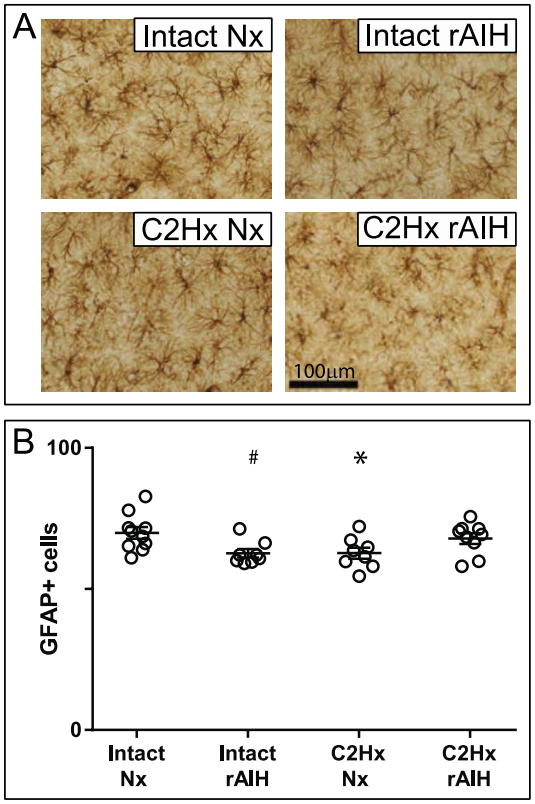

Figure 5: Hippocampal astroglia. Representative micrographs depicting GFAP immunostaining in coronal sections through the CA1 region of the hippocampus of intact and spinal cord injured rats exposed to 3 months of Nx or rAIH (***A***). The number of GFAP positive astrocytes was slightly reduced following long-term rAIH in spinal intact but not spinal injured rats (which was unchanged by rAIH; **B**). Thus, rAIH does not cause significant hypertension. *Significantly different from intact; # Significantly different from Nx. $p < 0.05$.

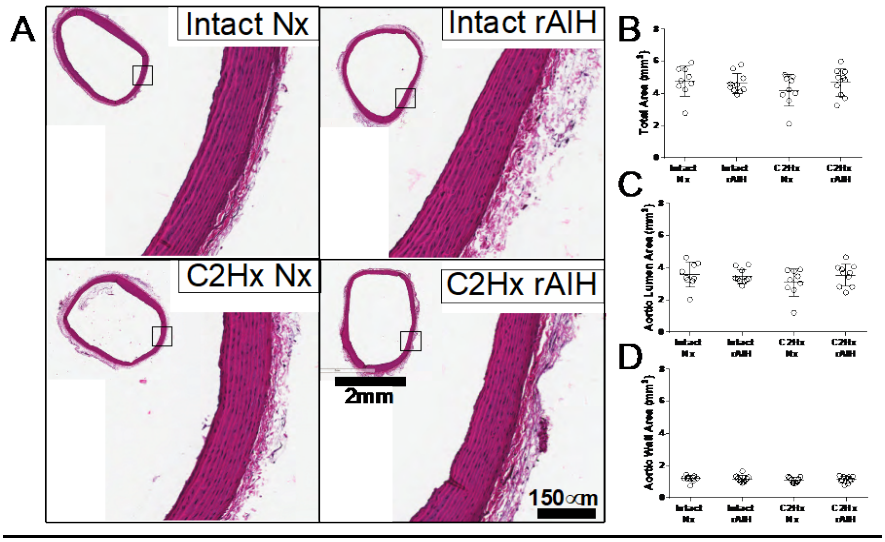

Figure 6: Ascending Aorta. Representative micrographs depict cross sections through ascending aorta of injured and uninjured rats exposed to 3 months of Nx or rAIH (***A***). Total aortic area (lumen + intima-media; mm^2; ***B***), intraluminal area (mm^2; ***C***), and area of the intima-media (mm^2; ***D***) were quantified. No evidence of vascular remodeling (increased wall thickness, lumen size) was observed with long-term rAIH. Neither rAIH nor C2Hx altered elastic fiber orientation or increased isubendothelial myxoid extracellular matrix. Values are individual data points as well as means ± SEM.

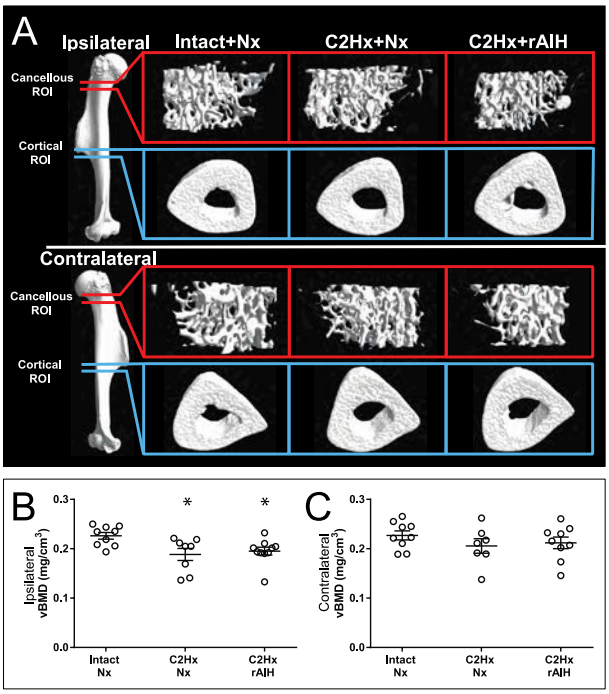

Figure 7: Cancellous volumetric bone density of proximal humerus measured via µCT. *A*, Representative 3D renderings of cancellous bone within ROI of the proximal humerus. Average volumetric bone density (mg/cm^3) of ipsilateral (***B***) and contralateral (***C***) humerus. Volumetric bone density was reduced ipsilateral to injury following C2Hx, but no effect of rAIH was evident. No changes in contralateral bone density were observed. Values are means ± SEM. * Significantly different from Intact Nx. $p < 0.05$.

All experimental work was completed as reported in quarterly progress reports. We completed tissue processing and have prepared a draft manuscript concerning safety and respiratory effects. This paper is the major outcome of this project from the UF site. Dr. Elisa Gonzalez-Rothi is the lead author, with Dr. G.S. Mitchell as senior author. Other reportable results include: safety data were reported at the Experimental Biology meeting as a poster, and an abstract was published (presented by Dr. E. Gonzalez-Rothi). The cardiac safety data were presented separately at the International Society for Neural Regeneration by technician K. Smith. The citations are:

- Gonzalez-Rothi EJ, Allen LL, Santiago-Moreno J, Ciesla MC, Asa ZA, Smith KN, Tadjalli A, Perim R, Santiago JV, Holland AE, Stefan KA, Ross A, Satriotomo I, Kelly MN, Simon AK, Poirier AE, Seven YB, Yarrow JF, and Mitchell GS. 2018. Long-term Delivery of "Low Dose" Repetitive Intermittent Hypoxia is Not Associated with Detectable Pathology. *FASEB Journal.* 32(1) - Experimental Biology 2018.
- Smith KN, Allen LL, Ciesla MC, Asa ZA, Santiago-Moreno JG, Tadjalli A, Perim R, Santiago JV, Holland AE, Stefan KA, Ross A, Satriotomo I, Simon AK, Poirier AS, Kelly MN, Seven YB, Gonzalez-Rothi EJ, and Mitchell GS. 2017. Safety Profile of Prolonged "Low Dose" Intermittent Hypoxia in Rats with Cervical Spinal Cord Injury. Poster at the International Symposium on Neural Regeneration and Repair 2017.

Aim 2: Accomplishments from work at the Saskatchewan

This portion of the progress report provides updates associated with Aim 2 of this project.

Milestone #3 –Understand the efficacy of repetitive AIH for forelimb recovery after chronic SCI. Understand the impact of repetitive AIH on protein expression in forelimb motor neurons.

Essential findings: Despite delays outlined in the final section of this report, all functional data from Aim 2 were collected; work continues on immunohistochemical characterization of plasticity-related proteins in relevant motor pools. However, **the important positive finding of this grant project is that prolonged repetitive AIH paired with ladder walking triggered long lasting improvement of forelimb function (skilled reach/grasp task)**. This outcome is substantially different from the breathing data, possibly due to: 1) different properties/mechanisms of repetitive

AIH in this motor pool; and/or 2) the paired association of AIH and task specific training. Although data for breathing and forelimb function were collected at different sites (Florida versus Saskatoon), we made every effort to standardize AIH protocols across sites. Combined with evidence that prolonged repetitive AIH is safe (see Aim 3), we provide additional evidence that repetitive AIH represents a viable (simple and safe) therapeutic option to restore **limb function**.

Status of specific tasks in the Statement of Work:

Task 2. Quantify effects of repetitive AIH on voluntary limb function after chronic SCI.

*Subtask 2a***.** Done

*Subtask 2b***.** Measure limb function 2 months post-injury, during rAIH treatment and after final AIH treatment in first rat cluster. Done.

*Subtask 2c***.** Conditioning and limb function assessment in the 2nd cluster of naïve rats prior to spinal injuries (N=30 rats; 5 rats/task x 2 treatment x 3 tasks). Done.

*Subtask 2d***.** Measure limb function 1-month post-surgery, during rAIH and after final AIH treatment in a second rat cluster. Done.

*Subtask 2e***.** Perform spinal injuries and rAIH treatment for spinal protein analysis (N=10 rats; 5 rats/task x 2 groups). Done, imaging under way.

Subtask 2f. Quantify the expression of key proteins at regular time points during and after rAIH in AIH and SHAM animals. Imaging and analysis continue.

*Subtask 2g***.** Perform spinal injuries and AIH treatment for protein analysis in the 2nd cluster of naïve rats (N=10 rats; 5 rats/task x 2 groups). Done.

*Subtask 2h***.** Quantify expression of key proteins at time points during and after rAIH in 2nd cluster of rAIH and SHAM animals. Imaging and analysis continue.

This progress report provides updates associated with the Aim 2 of this project (initiated November 17, 2016; continued through November 30, 2018). Aim 2 of the project is carried on in Dr. Muir's laboratory at the University of Saskatchewan, and concerns the effects of prolonged repetitive acute intermittent hypoxia (rAIH) treatment on the recovery of forelimb function in rats with incomplete chronic cervical spinal cord injury. Here is a brief overview of our accomplishments in Aim 2:

Rat Cohorts: Three rat cohorts (n = 30) were completed in Aim 2. Each cohort followed the following pattern:

First 2-3 months: Rats were habituated and trained on specific behavioral tasks with a minimum of 2 tasks per cohort. After task training, hemisection at C2 was performed.

Next 2 months: Although behavioral task performance is reduced by SCI, partial spontaneous functional recovery occurs several weeks post-surgery. Thus, rats were initially studied 2 months post-surgery to focus on AIH treatment effects in **chronic SCI**. Spontaneous recovery of limb function was assessed prior to beginning treatment.

Next 3 months: Rats are treated with rAIH paired with locomotor training. Behavioral task performance was assessed weekly. One day after completion of AIH treatment, rats were euthanized, and spinal cord tissue collected and processed for analysis.

Next 3 months: Immunofluorescent/immunohistochemical staining of spinal cord samples is continuing to assess changes in key plasticity-related proteins in identified forelimb motor nuclei.

In essence, long term repetitive AIH treatment in rats with chronic SCI induced remarkable functional recovery of reach-to-grasp performance for at least 12 weeks after treatment initiation versus sham-treatment SCI rats (Figure 8). **These are exciting and unprecedented findings**.

We are confident in the robustness of these data, due to our use of random assignment of treatments, blinded assessments and use of multiple cohorts, which allowed replication of results.

We also analyzed performance on a skilled locomotor task and compared groups of experimental animals at regular intervals during the 3-month treatment regime. Data analysis of motor performance proceeded as expected, but is time-consuming and required frame-by-frame analysis of digital video. Data analysis and interpretation continue.

Tissue samples were collected from all rats which completed the 3-month treatment protocol and are being processed for immunohistochemistry staining to investigate the differences in protein expression in identified regions of the brain and spinal cord after 3 months of treatment in both groups of animals. We continue to treat 2 rat cohorts from which tissue will be harvested to investigate differences in protein expression at earlier timepoints, namely in the 2nd and 4th weeks after treatment onset. These timepoints correspond to the timepoints at which we first noted the significant effect of rAIH on reach-to-grasp capabilities in rAIH-treated animals. We will compare differences in protein expression in brain and spinal cord between rAIH- and control-treated animals at 2, 4 and 12 weeks after treatment onset to determine whether ongoing treatment maintains differential protein expression between rAIH- and control-treated SCI animals. These analyses are continuing at a slow rate due to inadequate funding. We are also processing tissue from the injury site in all rats which completed the 3-month treatment protocol.

We have begun work on a manuscript reporting the effects of prolonged rAIH on forelimb function. This paper will be a major outcome of this project. Dr. Behzad Toosi (postdoctoral associate) will serve as lead author on this manuscript, with G. Muir as its senior author.

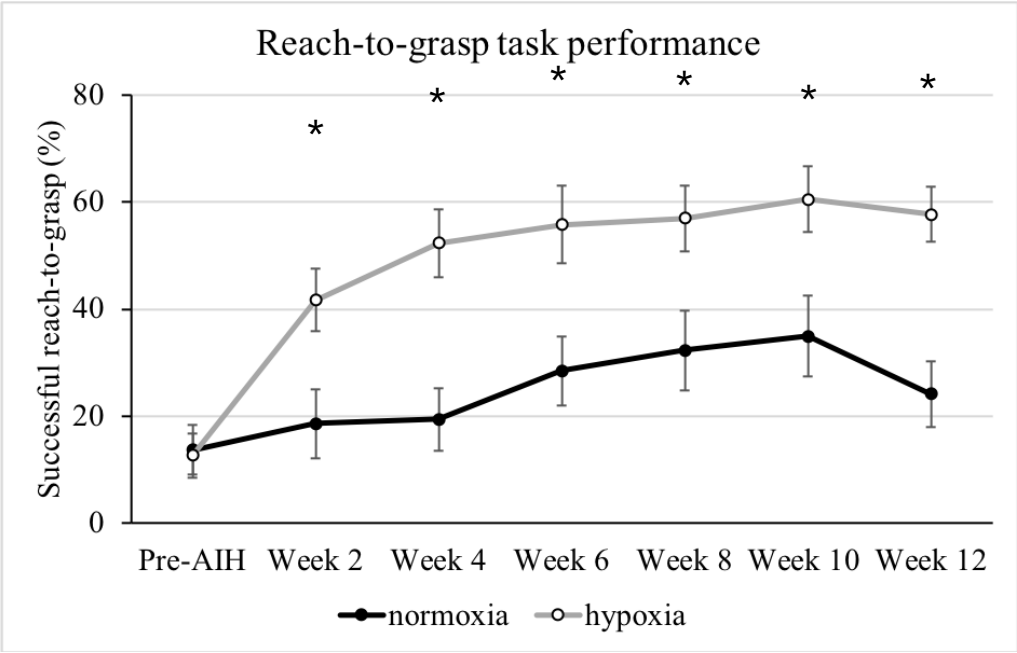

Figure 8. *Long term AIH treatment improves reach-to-grasp performance in chronic SCI rats:* Success on a single pellet reaching task in rats with cervical hemisection treated with AIH (hypoxia) and rehabilitative training was greater starting at 2 wk after the onset of treatment compared to the success in rats with cervical hemisection receiving control (normoxia) treatment and rehabilitative training. Hypoxia/normoxia treatment and rehabilitative training were administered 4 x/wk x 12 wks, *p<0.05, n = 16 (normoxia), n = 17 (hypoxia).

Reportable Outcomes
None

Future Plans
The main work left is to submit manuscripts for publication.

Problems/Issues encountered during study (slowing progress):
Mitchell's move from the University of Wisconsin to the University of Florida delayed the project start date. A no cost extension was necessary to enable completion of the work, particularly at the Saskatchewan site (Aim 2). We lost data from a defective plethysmograph system but successfully retrieved final measurements on 4 rat groups after repetitive AIH. Since we split nerve recording studies between 2 well-trained postdocs, and there was an unexpected investigator effect, we are not be able to publish these data. Regardless we assessed blood pressure across groups from these studies, providing evidence that prolonged repetitive AIH does not cause hypertension. We experienced delays due to loss of microscope availability due to Hurricane Irma, which temporarily shut down UF and leaked water into our laboratory.

At the Saskatchewan site, there were several delays: 1) transfer of funds from UW to UF, and then from UF to the University of Saskatchewan; 2) recruitment and training of postdoc to complete the studies; and 3) the first rat cohort was terminated due to pinworm infection. Problems were resolved with help of a no cost extension.

At both sites, work continues although funding for this project has expired. The pace is of course slower without dedicated funding, but we are committed to complete analyses and manuscript submission for publication.