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TITLE: Chronic Intermittent Hypoxia-Induced Neuroinflammation Undermines Respiratory Motor Plasticity After Chronic Incomplete Cervical Spinal Cord Injury

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14. ABSTRACT

The goal of this project is to determine the impact of chronic intermittent hypoxia, a primary feature of sleep apnea, on respiratory recovery and plasticity following chronic cervical spinal cord injury (SCI). Prior work has shown that chronic intermittent hypoxia can trigger neuroinflammation and impair expression of respiratory plasticity. This this work has important clinical applications in SCI, since the prevalence of sleep apnea is much higher in than the uninjured population. Thus it raises concern that individuals with chronic SCI who also suffer from sleep apnea may have limited potential for plasticity and recovery of breathing function, if underlying inflammation is not addressed. Major accomplishments from this reporting period include: 1) obtaining institutional approvals; 2) recruitment and training of study personnel, which resulted in completion of a related preliminary study which showed dose-dependence effects of intermittent hypoxia on expression of respiratory plasticity and markers of spinal neuroinflammation; and 3) completion of spinal injury and sham surgeries (and subsequent aging of animals to a chronic post-surgical time point) required for aim 1. Terminal neurophysiology and tissue processing for aim 1 are planned for the early part of the upcoming reporting cycle. Work from this first year provides the first direct evidence of a dose effect of intermittent hypoxia, which ranges from therapeutic at low doses, to pathologic at higher doses. Upcoming studies are the first assessments of high-dose, chronic intermittent hypoxia on respiratory plasticity and recovery of phrenic output in chronic spinal cord injury. Success of this project can lead to a significant paradigm shift in current approaches to managing respiratory dysfunction after SCI.

15. SUBJECT TERMS
Respiration, cervical spinal cord injury, sleep apnea, chronic intermittent hypoxia, contusion, inflammation, neuroplasticity, phrenic long term facilitation

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1. INTRODUCTION:

Cervical spinal cord injury disrupts neural pathways to respiratory motoneurons, paralyzing respiratory muscles and impairing breathing capacity. Despite advances in medical management of cervical SCI, respiratory dysfunction remains the leading cause of morbidity and mortality. Repetitive exposure to low-dose, acute intermittent hypoxia (AIH) is a promising therapeutic approach to restore breathing capacity by harnessing intrinsic mechanisms of spinal plasticity. However, factors such as inflammation undermine the therapeutic potential of AIH-based therapies. An underappreciated consequence of SCI is a dramatic increase in sleep disordered breathing (SDB) with associated chronic exposure to high-doses of intermittent hypoxia (CIH). In fact, estimates predict that nearly 80% of individuals with cervical SCI have SDB. The clinical characterization of the severity of SDB is based on the number of apneic episodes and/or hypoxic events experienced by patients during each hour of sleep. The high doses of CIH that are characteristic of SDB (>15 hypoxic episodes/hour) induce systemic and neural inflammation, and we have previously showed that a single night of CIH impairs a form of respiratory motor plasticity known as phrenic long-term facilitation. The impact of CIH on plasticity and recovery of respiratory capacity in rats with chronic cervical SCI has not been shown. This has important clinical implications since it raises concern that individuals with chronic SCI who also suffer from SDB may have limited potential for plasticity and recovery, if the underlying systemic and neural inflammation are not addressed. Furthermore, by understanding the mechanisms by which CIH-induced inflammation impairs subsequent responses to rAIH, we may develop strategies to restore therapeutic efficacy of such interventions in this population. Success of this project can lead to a significant paradigm shift in current approaches to managing respiratory dysfunction following cervical SCI. Knowledge obtained from this study may also have broader implications, since almost all neurological and neurodegenerative disorders are characterized by disproportionately high numbers of individuals with SDB.

2. KEYWORDS:

Respiration
Cervical spinal cord injury
Sleep disordered breathing
Sleep apnea
Chronic intermittent hypoxia
Mid-cervical contusion
Inflammation
Neuroinflammation
Neuroplasticity
Phrenic Neurogram
Phrenic long term facilitation

3. ACCOMPLISHMENTS:
What were the major goals of the project?

The following Specific Aims were proposed for this project in intact rats and rats with chronic cSCI:

Aims 1a and 1b: Test the hypothesis that 8 hours (1 night) of "high-dose" chronic intermittent hypoxia (IH-1; 15 hypoxic episodes per hour, 8hrs/day) impairs AIH-induced pLTF (**Aim 1a**) and increases expression of phosphorylated p38 MAP kinase in cervical spinal motoneurons and microglia (**Aim 1b**).

*In the original SOW, aims 1a and 1b were to be addressed in years 1 and 2 of the award by Major Task 3 (see below). Accomplishing these aims involved performing spinal injury surgeries and associated post-operative animal care, then allowing SCI and sham rats to age to 12 weeks post-injury before exposing them to a single night of "high-dose" chronic intermittent hypoxia. Terminal neurophysiology would occur the following day, where baseline and maximal phrenic output, as well as a measure of spinal respiratory plasticity known as phrenic long-term facilitation (pLTF) would be assessed (**Aim 1a**). Following these experiments tissues would be harvested, sectioned, and processed for analysis of p38 MAP kinase expression in cervical motoneurons and microglia (**Aim 1b**). Initial delays in obtaining University and DoD administrative approvals, as well as time required for recruitment and training of necessary staff, delayed the start of these experiments by several months. In addition, we experienced some unforeseen issues during the second half of data collection for this study (detailed below and in our recent quarterly reports) which required that we repeat a considerable number of experiments needed to complete this aim. We were able to complete these repeat experiments in the second half of year 2, rounding out the majority of subtasks related to Major Task 3 with the exception of some tissue processing and analysis (subtasks 3f and g) which is currently ongoing.

Aim 2: Test the hypothesis that prolonged (days-weeks) "high-dose" chronic intermittent hypoxia (15 episodes per hour, 8hrs/day) impairs AIH-induced pLTF and increases expression of phosphorylated p38 MAP kinase in cervical spinal motoneurons and microglia
Aim 3: Does systemic ketoprofen restore/enhance pLTF following prolonged chronic intermittent hypoxia?
Aim 4: Does p38 MAPK inhibition restore/enhance pLTF following prolonged chronic intermittent hypoxia?

*Initiation of experiments related to aims 2-4 will take place this quarter to include initiation of spinal cord injury surgeries, chronic IH exposures and terminal neurophysiology experiments. Several aspects of these aims will be conducted simultaneously in order to maximize efficiency and minimize redundancy of control groups.

The following goals and tasks were proposed in the approved SOW:
Task 1: Complete administrative requirements (Obtain University of Florida IACUC approval) - **COMPLETE**
Task 2: Complete administrative requirements (Obtain ACURO approval) - **COMPLETE**
*Milestone #1: Obtain UF and DoD Animal Use Approvals - **COMPLETE**
Task 3: Quantify the effect of IH-1 on pLTF (**Aim 1a**) & cervical p38 MAP Kinase in rats with chronic cSCI (**Aim 1b**):

- Subtask 3a: Perform cervical spinal cord injury surgeries - **COMPLETE**
- Subtask 3b: Expose rats to IH-1 or normoxia at 12 weeks post-injury or equivalent time point in controls - **COMPLETE**
- Subtask 3c: Perform terminal neurophysiology to assess the impact of IH-1 on phrenic output and pLTF - **COMPLETE**
- Subtask 3d: Perfuse and harvest tissues from rats from subtask 3c to quantify phosphorylated p38MAP Kinase – **COMPLETE**
- Subtask 3e: section cervical spinal cord tissues – **COMPLETE**
- Subtaslk 3f: process cervical spinal cord tissues for p38 MAP Kinase in and around phrenic motoneurons – **ONGOING**
- Subtask 3g: Quantify p38 MAP Kinase expression in and around phrenic motoneurons – **ONGOING**

*Milestone #2a: Understand the impact of IH-1 on pLTF in rats with chronic cSCI (Aim 1a) – **COMPLETE**
*Milestone #2b: Understand the impact of IH-1 on expression of phosphorylated p38 MAP Kinase in the cervical spinal cord of rats with chronic cSCI (Aim 1b) – **ONGOING**
Task 4: Quantify the impact of prolonged CIH on pLTF and cervical p38 MAP Kinase expression in rats with and without chronic cSCI - **UPCOMING**
*Milestone #3: Understand the impact of prolonged CIH on pLTF and p38 MAP Kinase expression in rats with and without chronic cSCI.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) **Major Activities:**
    a. Completion of spinal cord injury surgeries, intermittent hypoxia exposures and terminal neurophysiology studies required to complete Aim 1a (Subtasks 3a, 3b and 3c)
    b. Completion of tissue harvest, sectioning, and initiation of immunostaining of cervical spinal cord tissues from experimental animals required to complete Aim 1b (Subtasks 3d, 3e and 3f)
    c. Presentation of neurophysiology findings concerning the impact of IH-1 on phrenic capacity and plasticity in rats with chronic cSCI (Aim 1a) at the annual Experimental Biology Conference
    d. Submitted manuscript concerning recently completed complimentary study investigating the impact of different intermittent hypoxia doses/protocols on phrenic capacity and plasticity in intact rats (details below): *this study was not funded directly by this grant but enabled us to train personnel on the necessary methods required for completing the proposed studies including CIH exposures, terminal neurophysiology, tissue processing, imaging, and quantification of immunohistochemistry.
2) **Specific objectives:**
    a. Quantify the impact of IH-1 on pLTF in rats with chronic cSCI (Aim 1a): For Aim 1a, we proposed to assess the impact of IH-1 (vs normoxia) on phrenic motor output and plasticity in rats with chronic mid-cervical contusion. Rats were aged to 12-weeks post-injury prior to exposure. As noted in our recent quarterly reports, we had originally planned to complete all required experiments for Aim 1a earlier this year, however data collected from a period spanning several months from late 2018 to early 2019 was inconsistent with data collected prior in the earlier part of 2018. These results were considerably different from our prior data and happened to coincide with the initiation of major construction adjacent to our laboratory. Following a series of control experiment in uninjured rats not exposed to intermittent hypoxia, we determined that indeed this construction was having significant impact on our studies, likely related to significant disruption in sleep patterns and stress due to construction related vibration. This construction was completed in the spring of 2019, and following its completion, we were again able to elicit results that were consistent with our prior work. Unfortunately, this unforeseen issue necessitated that we repeat a considerable number of experiments related to Aim 1a, as noted in our recent quarterly reports. We completed the necessary repeat experiments in the spring and summer of 2019 and the results of these experiments were presented at the Experimental Biology conference this past spring. A manuscript concerning these experiments is currently being prepared for submission to a peer reviewed journal later this year. Methodology used to complete this objective includes:
        i. Mid-cervical Spinal Cord Contusion Injury: Mid-cervical spinal cord contusions were made using the Infinite Horizon pneumatic impactor. A C3/4 laminectomy was performed on anesthetized rats to expose the cervical spinal cord. A 200kD impact force was delivered between the C3 and C4 dorsal roots. If the animal went into respiratory arrest, it was intubated, mechanically ventilated and oxygen saturation was measured until the animal could be weaned from the ventilator. The overlying muscle and skin were closed with suture and stainless steel clips and rats were given post-operative analgesia, fluids and nutritional supplementation until adequate eating and drinking has resumed. Rats were returned to their home cage until exposures begin at 12 weeks post-injury.
        ii. Intermittent Hypoxia Exposure: At 12 weeks post-injury, rats underwent exposure to either IH-1 or Nx-1. During exposures, rats were housed in custom exposure cages which utilized computer-controlled mass flow controllers to enable precise titration and cycling of inspired gases. While undergoing exposures, rats continued to receive food and water ad libitum and were housed with a 12/12 hour light/dark cycle. Rats were exposed to 8 hours of either:

1. normoxia (Nx1): 21% O2, 8 hrs/day; AHI equivalent = 0
2. high-dose chronic intermittent hypoxia (IH1-2/2): 2 min. hypoxic episodes, 2 min. normoxic intervals, 8hrs/day; 120 total hypoxic episodes; AHI equivalent = 15

iii. Terminal Neurophysiology: The day after IH1 or Nx1 exposures were completed, terminal phrenic nerve recordings were conducted in urethane anesthetized, paralyzed, vagotomized and ventilated rats using a dorsal approach and custom-made, silver wire suction electrodes. A femoral catheter was placed for periodic arterial blood sampling. Baseline arterial PCO2 was set 2-3 mmHg above the CO2 recruitment threshold and baseline phrenic output is assessed over a stable 20 min. period. Three 5-min bouts of hypoxia were administered (10.5% O_2 with 5 min. normoxic intervals), and phrenic nerve activity was recorded for 60 minutes after the final bout of hypoxia. This 60 min. value was compared with baseline activity to determine the extent of phrenic motor plasticity (phrenic long term facilitation).

b. Quantify the impact of IH-1 on cervical p38 MAP Kinase expression in rats with chronic cervical spinal cord injury exposed to IH-1 (versus normoxia) (Aim 1b).

i. p38 MAP Kinase Immunohistochemistry Methods: Phrenic motoneurons were identified via retrograde labeling with Cholera toxin B fragment (CTB) by intrapleural injection (12 uL; o.5% in sterile saline, 5th intercostal space, 6mm depth). Following terminal experiments, rats were perfused (4% paraformaldehyde), the spinal cords removed, post-fixed, and cryoprotected, and 40um transverse sections are prepared from the C3-C6 segment using a freezing microtome. Sections were incubated overnight in primary antibodies for CD11B (microglia), phosphorylated p38 MAP Kinase, and CTB (phrenic motoneurons) followed by incubation with fluorescent secondary antibodies for visualization. Tissues are then imaged using a Keyence BZ-X700 microscope (ongoing) and digital micrographs (20x) will be analyzed and densitometry performed using a custom Matlab script to assess the amount of phosphorylated p38 MAP Kinase in and around the phrenic motor nucleus (upcoming).

c. Completion of a separate, but related study concerning the impact of intermittent hypoxia "dose" on phrenic capacity and plasticity in intact rats: While training study staff in experimental methods, gearing up for performing chronic intermittent hypoxia exposures in chronically injured rats, we conducted a separate, but complimentary study comparing the effects of different "doses" of intermittent hypoxia on phrenic output and plasticity (phrenic long-term facilitation) in intact rats. The goal of this study was to evaluate different "doses" of intermittent hypoxia ranging from low doses (which are often considered therapeutic) with an apnea-hypopnea index (AHI; # of apneas per hour of sleep) equivalent of 1.25 (diagnosed as none-minimal sleep apnea) to higher doses (considered pathologic) with an AHI equivalents of 6 (diagnosed as mild sleep apnea) or 15 (diagnosed as moderate sleep apnea). Understanding the impact of IH dose on plasticity and motor output will help guide future studies aimed at maximizing functional recovery and plasticity without inducing concurrent pathology.

i. Intermittent Hypoxia Exposure Methods: As described above, rats underwent exposure to intermittent hypoxia or normoxia using our custom in-cage gas delivery system. Rats are exposed to 7 days of either:

1. normoxia (NX7): 21% O2, 8 hrs/day; AHI equivalent = 0
2. low-dose, daily acute intermittent hypoxia (dAIH7): 10, 5-min. hypoxic episodes (10.5% O2), 5 min. normoxic intervals; AHI equivalent = 1.25
3. moderate-dose chronic intermittent hypoxia (IH7-5/5): 5 min. hypoxic episodes, 5 min. normoxic intervals, 8 hrs/day; 48 total hypoxic episodes; AHI equivalent = 6
4. high-dose chronic intermittent hypoxia (IH7-2/2): 2 min. hypoxic episodes, 2 min. normoxic intervals, 8hrs/day; 120 total hypoxic episodes; AHI equivalent = 15

ii. Terminal Neurophysiology Methods: Terminal neurophysiology was performed as described above. Briefly, the day after exposures were completed, terminal phrenic nerve recordings were conducted using an anesthetized rat preparation. Phrenic output was assessed over a stable baseline and at 60 min. after an acute intermittent hypoxia protocol.

iii. Phosphorylated p38 and ERK MAP Kinase Immunohistochemistry Methods: Tissues were sectioned and processed as described above. Briefly, phrenic motoneurons were identified via retrograde labeling with Cholera toxin B fragment (CTB) by intrapleural injection. Following terminal experiments, rats were perfused, the spinal cords removed, post-fixed, and cryoprotected, and 40um transverse sections are prepared from the C3-C6 segment using a freezing microtome. Sections were incubated overnight in primary antibodies to CTB (phrenic motoneurons) and either phosphorylated p38 MAP Kinase or ERK MAP Kinase, followed by incubation with fluorescent secondary antibodies for visualization. Tissues were then imaged using a Keyence BZ-X700 microscope and digital micrographs (20x) were analyzed and using a custom Matlab script to assess the amount of phosphorylated p38 and ERK MAP Kinase in and around the phrenic motor nucleus.

3) Significant results or key outcomes:

a. **El Chami M, Holland AE, Santiago JV, Mitchell GS, Gonzalez-Rothi EJ. "Chronic Intermittent Hypoxia and Respiratory Motor Plasticity after Cervical Spinal Cord Contusion" – manuscript in preparation for submission to *J Neurotrauma*.** As noted above, we recently completed the neurophysiology experiments for Aim 1a. Results from these experiments were presented at the Experimental Biology conference this past spring and are currently being prepared as a manuscript for submission to a peer reviewed journal later this year. In brief, the goal of this study was to explore the impact of a single night of "high-dose" chronic intermittent hypoxia (simulating the number of hypoxic episodes per hour experienced by an individual with moderate sleep apnea over the course of a night; 15 episodes per hour, 8 hours) on phrenic motor output and expression of plasticity in rats with chronic cervical contusion injury. These experiments are clinically relevant in the field of spinal cord injury given the prevalence of sleep apnea/sleep disordered breathing in the spinal cord injured population (estimates near 80% in individuals with cervical SCI). We previously showed that 8 hours of "high dose" chronic intermittent hypoxia (IH-1; 15 episodes per hour) completely abolished expression of phrenic motor plasticity in intact rats via a mechanism that requires systemic and neural inflammation. However, given the tremendous body of work highlighting that the injured spinal cord is a "new spinal cord", it was essential to explore the impact of CIH in rats with chronic cervical contusion. We demonstrated that IH-1 had no impact on phrenic motor capacity (fig. 1), but significantly blunted expression of phrenic motor plasticity (pLTF) in rats with chronic cervical spinal cord contusion (cSCC; fig 2).

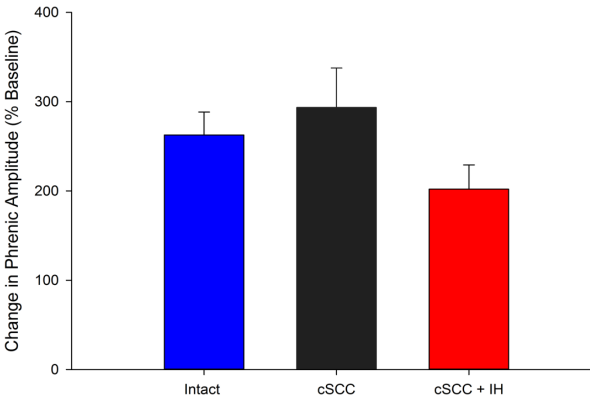

Figure 1. Phrenic motor capacity. Phrenic capacity (maximal chemoreflex activation; % baseline) was similar between intact and cSCC. A slight, but non-significant trend towards reduced capacity was observed in cSCC rats exposed to IH1 (p=0.242)

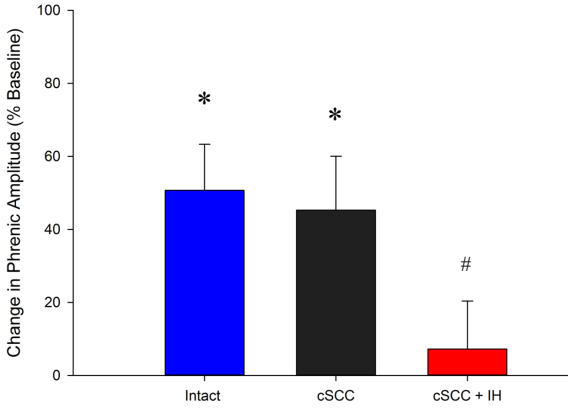

Figure 2. Phrenic motor plasticity. Expression of pLTF was observed in intact rats and rats with cSCC, but not in rats with cSCC exposed to IH-1. The extent of pLTF was similar between intact and cSCC, but reduced in cSCC+IH1-2/2 ($p<0.05$). *differs from baseline; #differs from intact rats.

b. **Gonzalez-Rothi EJ, Perim RR, Tadjalli A, Allen LL, Mitchell GS. "Dose Dependent Effects of Intermittent Hypoxia on Phrenic Long Term Facilitation" - manuscript submitted to *Experimental Neurology*.** As noted above, we completed a complimentary study while laboratory personnel were being trained on necessary methods and we were gearing up for initiation of long-term chronic intermittent hypoxia exposures in chronically injured rats. Results from this study were presented at the Oxford Conference on Modelling and Control of Breathing, the first annual Therapeutic Intermittent Hypoxia Conference, and Experimental Biology, and a manuscript was recently submitted to *Experimental Neurology*. In brief, the goal of this study was to directly compare different "doses" of intermittent hypoxia, ranging from what is considered therapeutic (<15 episodes per day), to what is considered pathologic (as observed in individuals with sleep apnea). The premise of this study was based on conflicting reports concerning the impact of different levels of hypoxia oh respiratory motor plasticity, ranging from enhancement of plasticity complete abolishment. However, differing protocols, durations, and time of day limited our ability to directly compare these different "doses". Thus, the design of this study entailed intact animals being exposed to different "doses" of intermittent hypoxia for seven days during their "rest phase", as depicted below (fig 1). Following the exposure period, rats underwent terminal neurophysiology experiments to assess phrenic motor output and phrenic long term facilitation. Our findings indicate a complex relationship between IH dose and phrenic output and plasticity. On one hand, all doses of IH enhanced phrenic motor output similarly under baseline, eupneic conditions (fig 2). But dose-dependent effects of intermittent hypoxia preconditioning on phrenic plasticity were observed, with low doses (dAIH7; 15 episodes per day) enhancing plasticity and high doses (IH7-2/2; 15 episodes per hour, 8 hours per day) abolishing it (fig 3). Following terminal experiments, spinal cord tissues were processed for quantification of phosphorylated p38 and pERK MAP kinases in and around phrenic motoneurons and cervical microglia, which also demonstrated dose dependent effects with low-doses (dAIH7) reducing phospho-p38 in phrenic motoneurons and high-doses (IH7-2/2) increasing phrenic phospho-ERK expression (figs 4 and 5).

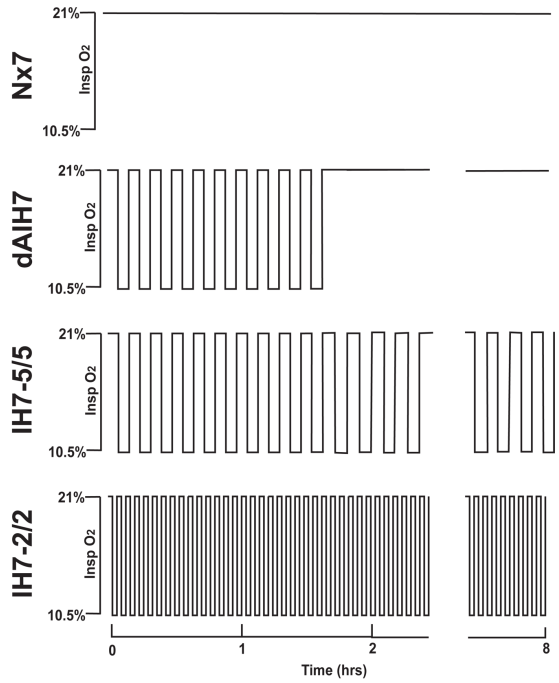

Figure 1: IH Preconditioning Protocols of varying "doses". Schematic diagram depicts Nx7, dAIH7, IH7-5/5, and IH5-2/2 preconditioning protocols, which were conducted daily for 7 days. Nx7 consisted of 8 hours of normoxia (21% O2), dAIH7 consisted of 10, 5-minute episodes of 10.5% O2 with 5 minute normoxic intervals; IH7-5/5 consisted of 8 hours of 5-minute hypoxic episodes (10.5% O2) with 5-minute normoxic intervals. and IH7-2/2 consisted of 8 hours of 2-minute hypoxic episodes (10.5% O2) with 2-minute normoxic intervals.

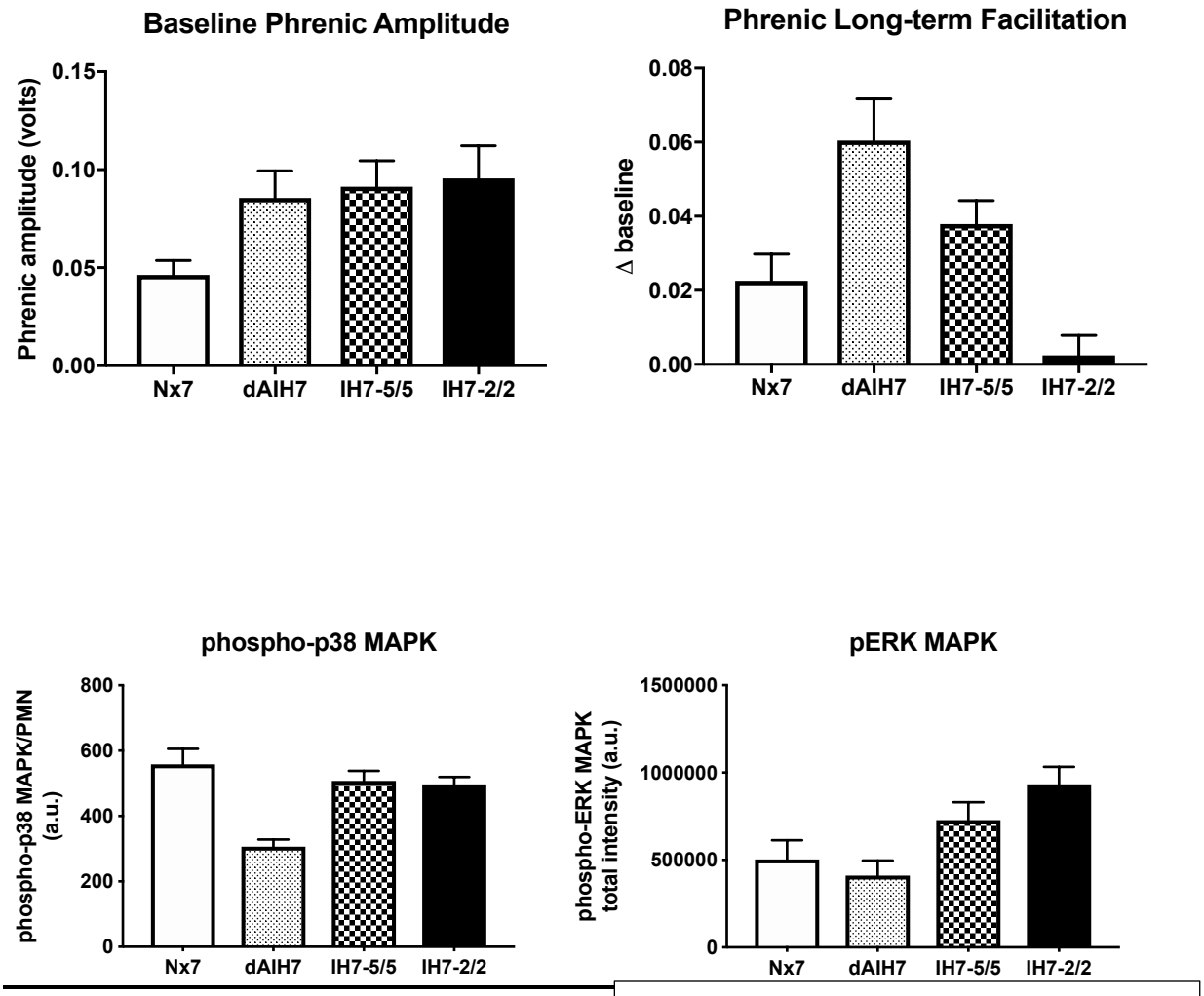

Figure 5: Phosphorylated ERK MAP Kinase expression in phrenic motoneurons. Average intensity of phosphorylated ERK MAP Kinase labeling in retrogradely labeled phrenic motoneurons in the cervical spinal cord depicts increased expression of phospho-ERK following and IH7-2/2.

4) **Goals not met:** None - Although the completion of Aim 1 was delayed initially due to delays in obtaining institutional approvals, receipt of award, and recruitment and training of personnel and then further delayed due to disruptions related to construction that necessitated repeating a large number of experiments, our goals related to aim 1 have been largely met and a manuscript associated with this work is in preparation. In addition, we have submitted an associated manuscript for peer review that was conducted while study personnel were being trained and animals were being aged to a chronic post-injury time point. This study provides important information concerning the impact of different IH "doses" on phrenic output and expression of plasticity, which is an important question that must be addressed before "low-dose" IH can be used clinically for therapeutic benefit and that will provide insights into the impact of "high-dose" IH (resembling that experienced by individuals with sleep apnea) on respiratory outcomes and the potential to express plasticity within the respiratory motor system. We anticipate no further delays/complications and progress towards completion of aims 2-4 should progress smoothly from this point forward.

What opportunities for training and professional development has the project provided?
Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

PI Dr. Gonzalez-Rothi attended national and international conferences including the American Physiological Society's annual Experimental Biology conference, the American Physical Therapy Association's annual Combined Sections Meeting, and the first annual Intermittent Hypoxia Symposium, which brought together some of the world's experts in both the therapeutic and pathological consequences of intermittent hypoxia. In addition to her active participation in the American Physiological Society, the Society for Neuroscience and the American Physical Therapy Association, Dr. Gonzalez-Rothi is also an active member of the Center for Respiratory Research and Rehabilitation (CRRR) at the University of Florida. The CRRR brings together researchers from across the UF campus in a collaborative effort to advance the understanding and treatment of neuromuscular disorders that compromise breathing. The CRRR organizes weekly journal clubs focused on plasticity, modulation and the neural control of breathing, as well as coordinating and sponsoring monthly respiratory-themed seminars and annual conferences related to intermittent hypoxia and the control of breathing. Of particular note, the CRRR regularly hosts world-renowned experts in the fields of sleep apnea, respiratory plasticity, spinal cord injury, and inflammation, including Dr. Jerry Dempsey (respiratory plasticity and chronic intermittent hypoxia), Dr. Barbara Morgan (chronic intermittent hypoxia), Dr. Warren Allilain (spinal injury and respiratory plasticity), Dr. Susan Harkema (spinal injury), Dr. David Berlowitz (sleep apnea and spinal injury), Dr. David Gozal, (sleep apnea), Dr. Phil Popovich (inflammation and spinal injury), and Dr. Tracy Baker (respiratory plasticity and sleep apnea). Lastly, Dr. Gonzalez- Rothi is an active member of the McKnight Brain Institute at the University of Florida, which brings together more than 300 multidisciplinary faculty from across the UF campus with expertise in neuroscience and neuromedicine to advance our understanding of how the brain works and how various diseases alter brain function.

The postdoctoral fellow and research technicians associated with this grant are also active participants in activities sponsored by the CRRR, the McKnight Brain Institute, and the American Physiological Society. Dr. El Chami has received significant one-on-one training from PI Dr. Gonzalez-Rothi as well as collaborators Dr. Gordon Mitchell, David Fuller and Kristi Streeter in spinal cord injury surgical methods, post-operative animal care and handling, use of the chronic intermittent hypoxia exposure system, terminal neurophysiology experiments (phrenic neurograms, intrathecal and femoral catheter placement, etc), perfusions and tissue processing and data acquisition and analysis. Research technicians Ashley Holland and Juliet Santiago volunteered as undergraduate research assistants in Dr. Gonzalez-Rothi's laboratory before becoming research technicians, thus were already proficient in many of the necessary techniques. They also received one-on-one training in assisting with spinal injury surgeries and advanced training in tissue processing, microscopy and data analysis. They have also presented work from their undergraduate honors theses and more recently, aspects of this project at the Experimental Biology Conference over the past two years.

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report." Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The following goals and tasks are planned for the next reporting period:

Specific Aim 1b: Does IH-1 alter expression of phosphorylated p38 MAP Kinase in the cervical spinal cord of rats with chronic cSCI?
Task 3: Quantify effect of IH-1 on cervical p38 MAP Kinase in rats with chronic cSCI

- Subtask 3f: Process cervical spinal cord tissues for p38 MAP kinase expression
- Subtask 3g: Image and Quantify cervical spinal cord tissues to determine the impact of IH-1 on p38 MAP Kinase expression in the cervical spinal cord

Specific Aim 2: Does prolonged "high-dose" CIH impair expression of AIH-induced pLTF and alter expression of phosphorylated p38 MAP Kinase in the cervical spinal cord of rats with chronic cSCI?
Task 4: Quantify impact of prolonged CIH on pLTF and cervical p38 MAP Kinase expression in rats with chronic cSCI

- Subtask 4a: perform spinal injury surgeries
- Subtask 4b: Expose rats to prolonged, "high-dose" CIH vs normoxia, beginning 8 weeks post-injury
- Subtask 4c: perform terminal neurophysiology experiments to assess the impact of prolonged CIH on pLTF
- Subtask 4d: perfuse and harvest tissues from rats from subtask 4c to quantify p38 MAP kinase

Specific Aim 3: Does systemic ketoprofen restore/enhance expression of pLTF in rats with chronic cervical SCI exposed to prolonged CIH?
Task 5: Quantify impact of systemic anti-inflammatory administration on expression of pLTF in rats with chronic cervical SCI exposed to prolonged, "high-dose" CIH.

- Subtask 4a: perform spinal injury surgeries
- Subtask 4b: Expose rats to prolonged, "high-dose" CIH, beginning 8 weeks post-injury
- Subtask 4c: perform terminal neurophysiology experiments to assess the impact of systemic ketoprofen on expression of pLTF in rats with chronic cervical SCI exposed to prolonged, "high dose" CIH

Specific Aim 4: Does inhibition of p38 MAP Kinase restore/enhance expression of pLTF rats with chronic cervical SCI exposed to prolonged CIH?
Task 6: Quantify impact of cervical p38 MAP Kinase inhibition on expression of pLTF in rats with chronic cervical SCI exposed to prolonged, "high-dose" CIH.

- Subtask 4a: perform spinal injury surgeries
- Subtask 4b: Expose rats to prolonged, "high-dose" CIH, beginning 8 weeks post-injury
- Subtask 4c: perform terminal neurophysiology experiments to assess the impact of cervical p38 MAP Kinase on expression of pLTF in rats with chronic cervical SCI exposed to prolonged, "high-dose" CIH

Milestones:

- Abstract submission/conference presentation concerning the impact of prolonged chronic intermittent hypoxia on phrenic output and plasticity in rats with chronic cervical contusion and the impact of both systemic and targeted administration of treatments to mitigate inflammatory processes
- Manuscript preparation and submission concerning prolonged CIH in chronic cSCI and potential treatments to mitigate its effects.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Sleep apnea is a common disorder that affects a large segment of the population in the US. One of the primary features of sleep apnea is frequent and repeated bouts of low/no oxygen entering the airways (also known as chronic intermittent hypoxia). However, in very low doses (fewer than 15 episodes per day, intermittent hypoxia has also been shown to have a therapeutic benefit, as it has been shown to improve breathing function, arm and leg strength, and walking function in people with spinal cord injury. Thus we sought to investigate the impact of various doses of intermittent hypoxia (low, medium, and high dose) on breathing function, which represents a critical step in translating low therapeutic intermittent hypoxia to clinical practice. As with any drug or therapy, it is important to understand both how much is enough to get a desired effect, as well as how much is too much and may cause further problems. The results of our recent study show that while all of the doses studied increased baseline breathing, the higher doses led to the development of inflammation in the spinal cord, and abolished therapeutically-induced increases in in breathing output (respiratory plasticity). Thus it is clear that to maximize functional gains without inducing negative consequences, doses of intermittent hypoxia should be in the low to moderate range.

Additionally, understanding the impact of chronic intermittent hypoxia on respiratory recovery and plasticity in chronic spinal cord injury will address an important clinical question, and has the potential to dramatically alter our approach for medical management and rehabilitation of individuals with spinal cord injury. In particular, understanding how chronic intermittent hypoxia/sleep apnea impairs respiratory plasticity and recovery will enable the targeted development of therapies to treat underlying cause (likely neuroinflammation). The findings from this study may show that it may be important to treat individuals with spinal cord injury for their sleep apnea and the underlying neuroinflammation it causes prior to physical and/or respiratory therapy in order to maximize the potential for plasticity and functional gains. Indeed, our recently completed study confirms our hypothesis that chronic intermittent hypoxia does indeed impair expression of respiratory motor plasticity in chronic spinal cord injury. In our upcoming experiments, we are exploring the potential for mitigating these effects using anti-inflammatory treatments. These experiments will provide meaningful insights regarding whether individuals with chronic spinal cord injury who also have sleep apnea should be treated with anti-inflammatories prior to participating in rehabilitation interventions in order to maximize the potential for plasticity and functional recovery.

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

As documented previously, delays in obtaining ACURO approval delayed the receipt of funding and the start of this project until December 2017. Consequently, recruitment, hiring and training of study personnel was also delayed. Although the post-doctoral fellow who was hired for this study had extensive clinical research experience working with individuals with spinal cord injury and sleep apnea, he had no prior experience with animal research, experimental models of spinal cord injury, or neurophysiology, thus there was significant time and emphasis placed on learning/training during the first part of the award period. In addition, as described in our recent quarterly reports, halfway through data collection for aim 1, our experimental results were all at once quite different from data collected during the first part of data collection for this aim. After running a number of control experiments in intact, unexposed rats, we identified that the discrepancy in data outcomes coincided with the initiation of major construction and roof replacement occurring adjacent to our laboratory. Based on anecdotal reports from our group, we determined that the considerable vibrations and loud noises generated by this ongoing construction led to physiological disruptions that impaired our ability to interpret data we were acquiring. Data collected during the period of time that this construction was ongoing were not able to be included, thus we had to repeat injuries, exposures and terminal experiments in order to accomplish study objectives. The bulk of construction was completed in the spring of 2019, at which point we were able to generate additional animals to repeat the experiments required to complete data collection for this aim, which were completed this summer (Aim 1a), with spinal cord tissue processing ongoing (Aim 1b). Lastly, as reported to Dr. Fontaine earlier this year, PI Dr. Gonzalez-Rothi had a baby in May 2019 and was on maternity leave over the summer. Progress towards the proposed work continued in her absence and she has since returned full time from leave. Despite these delays and complications, we were quite productive during the first two years of this award, completing a separate but related study comparing the impact of IH dose on phrenic output and spinal plasticity in intact rats, which has been submitted for peer review. In addition, the experiments that were recently completed for Aim 1a have been drafted into an initial manuscript concerning the effects of IH-1 on phrenic capacity and plasticity in rats with chronic cervical spinal cord injury. Pending the results of the immunohistochemical assessments that are ongoing concerning the impact of IH-1 on p38 MAP Kinase expression in phrenic motoneurons, this manuscript will be submitted for peer review. Although delayed, we anticipate no further issues completing the proposed experiments, but will be conducting aspects of Aims 2-4 simultaneously over the 3rd year of this award in order to maximize efficiency and minimize redundancy of control groups. We anticipate no additional problems or delays.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

As reported in our prior annual report, we did experience some administrative/institutional delays in the first year of this award that delayed the initial start date of this award, as well as a significant portion of time during the early phases of this award being devoted to training study personnel in animal handling, spinal cord injury surgical methods, and procedures required for terminal neurophysiology experiments (anesthesia, tracheostomy and mechanical ventilation, arterial and venous catheterization, intrathecal catheterization, and phrenic neurograms) and tissue processing/analysis. These early delays did not alter the anticipated costs for completing the proposed objectives, however the timeline for expenditures was pushed back accordingly. In the second year of the award, we had anticipated completing the first aim of the proposed objectives by early spring. However, as noted above and in our recent quarterly reports, significant construction in the laboratories next to and above ours as well as replacement of the roof just outside our lab created a significant disruption in our progress, which required repeating nearly half of the experiments required for completion of aim 1. This disruption caused considerable delays in completing aim 1 and also led to delays in initiating experiments for subsequent aims. However, since the construction was completed we have made substantial progress and were able to complete the proposed work for aim 1 and already have one manuscript submitted and a second manuscript in preparation. We anticipate starting on experiments related to aims 2-4 in the coming quarter and are confident that we will be able to complete the proposed scope of work without significant impact on expenditures.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  1. Gonzalez-Rothi EJ, Perim RR, Tadjalli A, Allen LL, Holland A, Santiago JV, Seven YB, Mitchell GS. Differential effects of intermittent hypoxia protocol on phrenic capacity and expression of plasticity. Submitted to Experimental Neurol. (Federal support acknowledged)
  2. El Chami M, Holland A, Santiago JV, Mitchell GS, Gonzalez-Rothi EJ. Chronic intermittent hypoxia and respiratory motor plasticity after cervical spinal cord contusion. In preparation for J Neurotrauma. (Federal support acknowledged)

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Conference Presentations:

1. Holland AE, Santiago JV, Allen LL, Seven YB, Asa Z, Ciesla MC, Simon AK, Perim RR, Tadjalli A, Mitchell GS, Gonzalez-Rothi EJ. "Impact of intermittent hypoxia protocol on phospho-p38 and phospho-ERK MAP Kinase expression in phrenic motoneurons" (2019). Experimental Biology (Poster).
2. El Chami M, Holland AE, Mitchell GS, Gonzalez-Rothi EJ. "Chronic intermittent hypoxia and respiratory motor plasticity after cervical spinal cord contusion" (2019). Experimental Biology (Poster).
3. Holland AE, Santiago JV, Allen LL, Seven YB, Asa Z, Ciesla MC, Simon AK, Perim RR, Tadjalli A, Mitchell GS, Gonzalez-Rothi EJ. "Impact of intermittent hypoxia protocol on phospho-p38 and phospho-ERK MAP Kinase expression in phrenic motoneurons" (2019). 10th Annual Neuromuscular Plasticity Symposium (Poster).
4. El Chami M, Holland AE, Mitchell GS, Gonzalez-Rothi EJ. "Chronic intermittent hypoxia and respiratory motor plasticity after cervical spinal cord contusion" (2019). 10th Annual Neuromuscular Plasticity Symposium (Poster).

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

- Nothing to report

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	*Elisa Gonzalez-Rothi*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-9833-5030*
Nearest person month worked:	*2.7*
Contribution to Project:	*Dr. Gonzalez-Rothi coordinated institutional approval process, trained personnel, performed/ assisted with spinal injury surgeries, post-operative animal care, intermittent hypoxia exposures, terminal neurophysiology experiments, data analysis and tissue processing and immunohistochemistry.*
Funding Support:	*Dr. Gonzalez-Rothi also has partial support from the Craig H. Neilsen Foundation and The National Institutes of Health.*
Name:	*Mohamad el Chami*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-8168-7681*
Nearest person month worked:	*8*
Contribution to Project:	*Mr. El Chami has performed spinal injury surgeries, post-operative animal care, intermittent hypoxia exposures and terminal neurophysiology experiments.*
Name:	*Ashley Holland*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-0372-3760*
Nearest person month worked:	*2*
Contribution to Project:	*Ms. Smith has assisted with spinal injury surgeries, performed animal care and intermittent hypoxia exposures, perfusions and tissue processing.*
Funding Support:	*Ms. Holland also receives partial support from the Craig H. Neilsen Foundation.*
Name:	*Amy Poirier*
Project Role:	*Laboratory Manager*
Nearest person month worked:	*1*
Contribution to Project:	*Ms. Poirier assisted with the institutional approval process and is responsible for the daily management of laboratory activities.*
Funding Support:	*Ms. Poirier is also partially funded by the Craig H. Neilsen Foundation and by funding sources from two other investigators.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Dr. Elisa Gonzalez-Rothi's previously "active" award from the National Institutes of Health is now closed:

NIH, Stimulating Peripheral Activity to Relieve Conditions (SPARC)
Bolser (PI) 2.4 calendar 9/1/16-7/30/19
Directors Commons Fund $299,826 total project
"Functional Mapping of Peripheral and Central Circuits for Airway Protection and Breathing"
Description: The goal of this project is to understand fundamental principles of modulation and plasticity in afferent pathways, brain networks and efferent systems controlling breathing and airway defense.
Role: Co-I

Dr. Elisa Gonzalez-Rothi's previously "pending" award from the National Institutes of Health is now active:

NIH, 1 R01 HL139708-01
Mitchell (PI) 2.4 calendar 9/1/19-8/31/24
National Institutes of Health $1,498,248 total project
"Optimizing respiratory plasticity with chronic cervical SCI"
Description: This project focuses on optimization of acute intermittent hypoxia protocols and mitigation of factors which undermine therapeutic efficacy of acute intermittent hypoxia to enhance functional recovery of breathing after chronic spinal cord injury.
Role: Co-I

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (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.*

Organization Name: University of Florida
Location: Gainesville, Florida
Contribution to the project: facilities

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Chronic intermittent hypoxia-induced neuroinflammation undermines respiratory motor plasticity after chronic incomplete cervical spinal cord injury

Spinal Cord Injury Research Program (SCIRP) – Investigator-Initiated Research Program
Funding Opportunity Number: W81XWH-16-SCIRP-IIRA (Award #: W81XWH-17-1-0315)

PI: Gonzalez-Rothi, Elisa J. **Org:** University of Florida **Award Amount:** $499,999

Study/Product Aim(s)

Aim 1: Test the hypothesis that 1 night of "high-dose" chronic intermittent hypoxia (CIH-1) impairs AIH-induced pLTF and increases expression of phosphorylated p38 MAP Kinase in cervical motoneurons and microglia

Aim 2: Test the hypothesis that prolonged "high-dose" CIH impairs AIH-induced pLTF and increases phosphorylated p38 MAP kinase in cervical motoneurons and microglia

Aim 3: Does systemic ketoprofen restore/enhance pLTF following prolonged CIH

Aim 4: Does intraspinal p38 MAP Kinase inhibition restore/enhance pLTF following prolonged CIH?

Approach

We will study the effects of chronic intermittent hypoxia (CIH) and associated neuroinflammation on a form of respiratory motor plasticity (pLTF; phrenic long term facilitation) in rats with chronic incomplete cervical spinal cord injury using an acute terminal neurophysiology preparation. We will investigate changes in a key molecule (p38 MAP kinase) that we believe may underlie these effects. And we will assess the impact of both systemic and targeted anti-inflammatory treatments on the expression of respiratory plasticity in injured and non-injured rats exposed to CIH.

Effects of IH-1 on respiratory output and plasticity in chronic cervical SCI: We recently completed neurophysiology experiments for Aim 1, concerning the impact of a single night of CIH on respiratory motor plasticity in chronic cervical spinal cord contusion (cSCC). As explained in detail in recent quarterly and annual progress reports, the findings from experiments conducted in late 2018/early 2019 were inconsistent with our prior findings. Specifically, we were unable to elicit respiratory plasticity in any animals, regardless of injury or exposure condition. These unexpected results corresponded with significant construction occurring in close proximity to our laboratory. This construction was completed in spring 2019, thus our major focus since then has been to repeat these experiments to enable completion of Aim 1. Our recently completed experiments are consistent with findings from our early studies (Figure 1), and neurophysiology experiments for Aim 1 have been completed, and cervical spinal cord tissues harvested and sectioned in preparation for assessment of p38 and pERK MAP Kinase expression in and around phrenic motoneurons, which will take place in the upcoming quarter. An an initial draft of this manuscript is in progress.

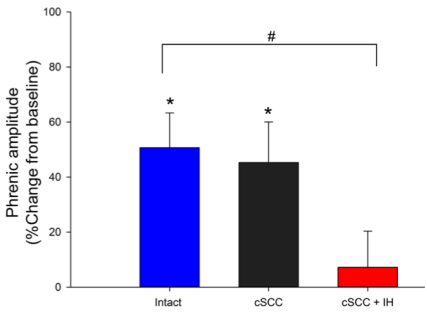

Fig.1 Expression of pLTF was similar between intact and cSCC, but reduced in cSCC+IH1-2/2 ($p<0/05$). *differs from baseline; #differs among groups

Effects of IH "dose" on respiratory output and plasticity in intact rats: We recently submitted a manuscript comparing the effects of different "doses " of intermittent hypoxia (IH) in intact rats. As noted in prior reports, this study was conducted while training study personnel and gearing up for our study concerning the effects of CIH in rats with chronic cSCI. In this study, rats were exposed to 7 days of either normoxia(Nx7), low-dose (therapeutic) daily acute IH (dAIH-7), moderate-dose chronic IH (IH7-5/5; simulating mild sleep apnea), or high-dose chronic IH (IH7-2/2; simulating moderate sleep apnea). All IH doses increased baseline phrenic output similarly, however dose-dependent effects on phrenic plasticity were observed, with low-dose dAIH7 enhancing, and high dose IH7-2/2, abolishing it. We also explored IH dose-related changes in expression of key molecules shown to either enhance (pERK) or impair (p38 MAP kinase) expression of respiratory motor plasticity. Contrary to our hypothesis, both p38 and pERK were reduced by low-dose dAIH7, whereas pERK expression was increased by IH7-2/2. Furthermore, unlike a single night of IH-2/2, p38 expression was not elevated by IH7-2/2, suggesting the increase in its expression with IH-1 is likely transient. This manuscript was submitted to Experimental Neurology.

Timeline and Cost

Activities CY	17/18	18/19	19/20
Assess impact of CIH-1 on pLTF and spinal p38 MAP Kinase in cSCI rats (Aim 1)			
Asses impact of prolonged CIH on pLTF and spinal p38 MAP kinase (Aim 2)			
Assess the impact of anti-inflammatories on pLTF after prolonged CIH (Aim 3)			
Asses the impact of p38 MAP Kinase inhibition on pLTF after prolonged CIH (Aim 4)			
Estimated Budget ($499,999)	**$251k**	**$257.5k**	**$263.6k**

Updated: September 10, 2019

Goals/Milestones

- ☑ Obtain UF IACUC and DoD ACURO approvals and final notice of award
- ☑ Hire and train personnel (post-doctoral associate and research technician)
- ☑ Complete spinal injury/sham surgeries & age to chronic time point for aim 1
- ☑ Assess impact of IH "dose" on pLTF in intact rats (related preliminary study conducted while training personnel and aging SCI rats)
- ☑ Prepare & submit abstract on IH "dose" on phrenic plasticity, p38 and pERK expression
- ☑ Prepare & submit abstract on effects of IH-1 in SCI based on preliminary results
- ☑ Prepare & submit manuscript on IH "Dose" (**completed**)
- ☑ Complete neurophysiology experiments concerning the impact of IH-1 on pLTF
- ☑ Perfuse, harvest and section cervical spinal tissues from intact rats and rats with chronic cervical spinal cord contusion (cSCC) exposed to one night of CIH (**completed**)
- ☐ Perform immunohistochemistry and microscopy to assess expression of p38 and pERK MAP kinase in intact rats and rats with chronic cSCC (**in progress**)
- ☐ Prepare & submit manuscript concerning the impact of IH-1 on pLTF and p38/pERK MAP kinase expression in intact rats and rats with cSCC (**in progress**)
- ☐ Spinal Injury and sham surgeries for aims 2-4 (prolonged IH studies)
- ☐ Assess impact of prolonged IH on phrenic plasticity (**in progress**)
- ☐ Spinal injury and sham surgeries for aim 4
- ☐ Asses impact of anti-inflammatories/p38 MAPK inhibition after prolonged IH (**in progress**)