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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, 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:

Aim 1: Test the hypothesis that 8 hours (1 night) of “high-dose” chronic intermittent hypoxia (IH-1) impairs AIH-induced pLTF and increases expression of phosphorylated p38 MAP kinase in cervical spinal motoneurons and microglia

*This aim was to be addressed in year 1 of the award by Major Task 3 (see below). This involved performing spinal injury surgeries and 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. Following these experiments tissues would be harvested, sectioned, and processed for analysis of p38 MAP kinase expression in cervical motoneurons and microglia. Due to delays in obtaining University and DoD administrative approvals, recruitment and training of necessary staff was delayed, and thus, some aspects of Major Task 3 are still underway as described below in detail.

Aim 2: Test the hypothesis that prolonged “high-dose” chronic intermittent hypoxia (IH-28; 15 episodes per hour, 28 days) 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 IH-28?

Aim 4: Does intraspinal p38 MAPK inhibition restore/enhance pLTF following IH-28?

The following goals and tasks were proposed in the approved SOW for Year 1:

Task 1: Complete administrative requirements (Obtain University of Florida IACUC approval)

- Obtained in May 2017

Task 2: Complete administrative requirements (Obtain ACURO approval)

- Obtained in November 2017

*Milestone #1: Obtain UF and DoD Animal Use Approvals - **Accomplished**

Task 3: Quantify effect of IH-1 on pLTF & cervical p38 MAP Kinase in rats with chronic cSCI (**Aim 1**)

- Subtask 3a: Perform spinal injury surgeries (n=20) and shams (n=20)
 - Proposed dates: Months 5-9
 - Actual date of completion: August 2018
- Subtask 3b: Expose rats to IH-1 (n=20) or normoxia (n=20) at 12 weeks post-injury
 - Proposed dates: Months 8-12
 - Actual date of start: September 2018
 - Anticipated date of completion: December 2018
- Subtask 3c: Perform terminal neurophysiology experiment to assess the impact of IH-1 on AIH-induced pLTF and phrenic motor output (Months 8-12)
 - Proposed dates: Months 8-12
 - Actual date of start: September 2018
 - Anticipated date of completion: December 2018
- Subtask 3d: Perfuse and harvest tissues from rats from subtask 3c to quantify phosphorylated p38MAP Kinase
 - Proposed dates: Months 8-12
 - Actual date of start: September 2018
 - Anticipated date of completion: December 2018
- Subtask 3e: Section cervical spinal tissues (Months 8-12)
 - Proposed dates: Months 8-12
 - Actual date of start: September 2018
 - Anticipated date of completion: December 2018
- Subtask 3f: Process cervical spinal cord tissues for total and phosphorylated p38 MAP kinase in and around retrogradely labeled phrenic motoneurons (Month 12)
 - Proposed dates: Month 12
 - Anticipated date of completion: December/January 2018

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. Obtaining institutional approvals (UF IACUC and DoD ACURO) – **accomplished**
- b. Recruitment and training of personnel – **accomplished**
- c. Completion of spinal cord injury surgeries (n=20) and shams (n=20) for Aim 1 - **accomplished**
- d. Aging of injured and sham rats to 12 weeks post-surgery for chronic SCI study (Aim 1) - **accomplished**
- e. Completed a complimentary study while gearing up for the proposed neurophysiology experiments (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. **Recruitment and training of study personnel:** We recruited a post-doctoral fellow for this study with expertise in clinical research studies exploring the effects of intermittent hypoxia on respiratory plasticity in humans with sleep apnea and spinal cord injury. Although his background was highly relevant in terms of subject matter, he had no prior experience working with animal models, thus a major objective in the first reporting period was training him in the required methods, including spinal cord contusion surgeries, animal handling and post-operative care, intermittent hypoxia exposures, terminal neurophysiology, intrathecal catheters and drug delivery methods, intracardiac perfusions and tissue harvesting. A research technician was also hired for this study. She worked as an undergraduate research assistant in the laboratory prior to her hire, thus she was experienced with some of the necessary procedures. However, she did receive additional advanced training in tissue processing, immunohistochemistry, microscopy and image analysis.
- b. **Spinal cord injury surgeries (specific aim 1):** For aim 1, we proposed assessing the impact of IH-1 (versus normoxia) on phrenic motor output and plasticity in rats with and without chronic mid-cervical contusion. These surgeries were completed in the first year of the award and the rats were allowed to age to 12-weeks post-surgery prior to exposure. Exposures and terminal experiments will occur in the first part of the second year of the award.
 - i. **Mid-cervical Spinal Cord Contusion Injury Methods:** Mid-cervical 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.
- c. **Completion of a separate, but related study:** While training study staff in experimental methods, gearing up for performing long term intermittent hypoxia exposures (>1 day), and allowing experimental rats for aim 1 to age to chronic time point, 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:** Rats were housed in custom exposure cages which utilized computer-controlled mass flow controllers to enable careful titration and cycling of inspired gases. Rats received food and water ad libitum and were housed with a 12/12 hour light/dark cycle. Rats were 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:** The day after 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 was assessed over a stable 20 minute period. Three 5-min bouts of hypoxia (10.5%; 5 min. normoxic intervals) were performed, and phrenic nerve activity was recorded 60 minutes after the final bout of hypoxia. This 60 minute value was compared with baseline activity to determine the extent of phrenic motor plasticity (phrenic long term facilitation).
- iii. **p38 MAP Kinase Immunohistochemistry Methods:** Phrenic motoneurons were identified via retrograde labeling with Cholera toxin B fragment (CTB) by intrapleural injection (12 uL; 0.5% in sterile saline, 5th intercostal space, 6mmdepth). Following terminal experiments, rats were perfused (4% paraformaldehyde), the spinal cords removed, post-fixed, and cryoprotected, and 40um transverse sections were 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 were then imaged using a Keyence BZ-X700 microscope and digital micrographs (20x) were 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.

3) **Significant results or key outcomes:**

- a. **Gonzalez-Rothi EJ, Perim RR, Tadjalli A, Allen LL, Mitchell GS. “Dose Dependent Effects of Intermittent Hypoxia on Phrenic Long Term Facilitation” - manuscript in preparation for submission to *Experimental Neurology*.** As noted above, we completed a complimentary study while newly hired laboratory personnel were being trained on necessary methods, while we were gearing up for initiation of long-term chronic intermittent hypoxia exposures, and while rats for aim 1 were aging to the chronic post-injury time point. The results of this study were presented at the Oxford Conference on Modelling and Control of Breathing and at the first annual Therapeutic Intermittent Hypoxia Conference and a manuscript is currently in preparation for submission to *Experimental Neurology* this fall. In brief, the goal of this study was to directly compare different “doses” of intermittent hypoxia, ranging from what is considered therapeutic, 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 on 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 detailed above. Following the exposure period, rats underwent terminal neurophysiology experiments to assess phrenic motor output and phrenic long term facilitation (Figure 1). 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 (Figure 1A). But dose-dependent effects of hypoxia preconditioning on phrenic plasticity were observed, with low doses (dAIH7) enhancing plasticity and high doses (IH7-2/2) abolishing it. Following terminal experiments, spinal cord tissues were processed for quantification of phosphorylated p38 MAP kinase in and around phrenic motoneurons and cervical microglia (Figure 2), which also demonstrated dose dependent effects with low-doses (dAIH7) reducing phospho-p38 in phrenic motoneurons and high-doses (IH7-2/2) increasing its expression.

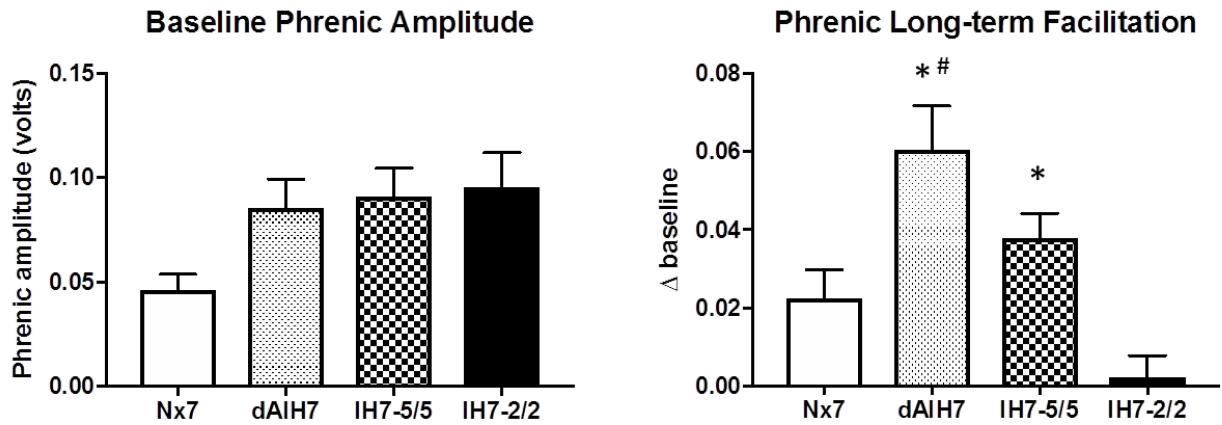


Figure 1. A) Impact of IH dose on baseline phrenic amplitude ($p=0.058$) and B) phrenic long term facilitation (Δ baseline; $p<0.0001$). *different from IH7-2/2 ($p<0.05$); #different from Nx7 ($p<0.05$)

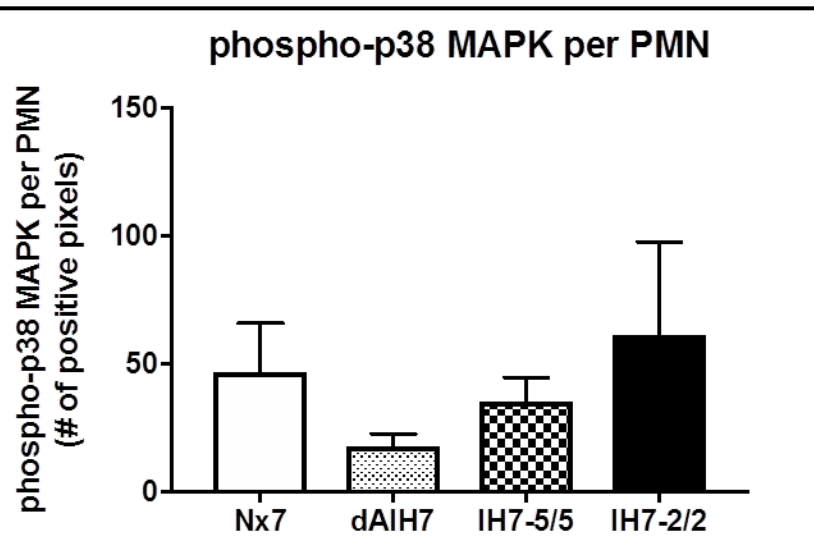


Figure 2. Impact of IH “dose” on expression of phosphorylated p38 MAP kinase per phrenic motoneuron.

- 4) **Goals not met:** None - Although the start of the study was delayed for four months due to delays in obtaining institutional approvals, receipt of award, and recruitment and training of personnel, all goals proposed for the first 8 months of the study were met.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

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 annual Experimental Biology Conference, San Diego CA, April 21-25, 2018, and the XIV Oxford Conference on Modeling and Control of Breathing, Oxford, UK, September 17-21, 2017. In addition, Dr. Gonzalez-Rothi is an active member of the Center for Respiratory Research and Rehabilitation (CRRR) at the University of Florida. The CRRR brings together researchers from throughout the UF campus in a collaborative effort to advance the understanding and treatment of neuromuscular disorders that compromise breathing. The CRRR organizes weekly “Respiratory Plasticity and Control” journal clubs, monthly respiratory-themed seminars featuring local, national, and international speakers, and hosted the first annual Therapeutic Intermittent Hypoxia conference this past spring. Of particular note, several world-renowned experts in sleep apnea, respiratory plasticity, spinal cord injury, and inflammation were visitors to the CRRR and Dr. Gonzalez-Rothi’s laboratory, including Dr. David Gozal (sleep apnea), Dr. David Berlowitz (sleep apnea in spinal cord injury), Jerry Dempsy (chronic intermittent hypoxia), Dr. Barbara Morgan (chronic intermittent hypoxia), Dr. Tracy Baker (Respiratory plasticity), and Dr. Phil Popovich (spinal cord injury and inflammation).

The postdoctoral fellow (Dr. El Chami) and research technician (Ashley Holland) associated with this grant are also active participants in all CRRR activities. Dr. El Chami has received significant one-on-one training from Dr. Gonzalez-Rothi 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. Ashley Holland volunteered as an undergraduate research assistant in my laboratory before becoming a research technician, thus she is already proficient in many of the necessary techniques. However, she has also received one-on-one training in spinal injury surgeries and advanced training in tissue processing, microscopy and data analysis. She also presented work from her undergraduate honors thesis at the Experimental Biology Conference this past spring.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

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 1: Does IH-1 impair AIH-induced pLTF and increase expression of phosphorylated p38 MAP Kinase in cervical spinal motoneurons and microglia in rats with chronic cSCI?

Task 3: Quantify effect of IH-1 on pLTF & cervical p38 MAPKinase in rats with chronic cSCI

- Subtask 3b: Expose rats to IH-1 (n=20) or normoxia (n=20) at 12 weeks post-injury
 - September –December 2018
- Subtask 3c: Perform terminal neurophysiology experiment to assess the impact of IH-1 on AIH-induced pLTF and phrenic motor output (Months 8-12)
 - September -December 2018
- Subtask 3d: Perfuse and harvest tissues from rats from subtask 3c to quantify phosphorylated p38MAP Kinase
 - September -December 2018
- Subtask 3e: Section cervical spinal tissues (Months 8-12)
 - September - December 2018
- Subtask 3f: Process cervical spinal cord tissues for total and phosphorylated p38 MAP kinase in and around retrogradely labeled phrenic motoneurons (Month 12)
 - January 2019
- Subtask 3g: Image and Quantify cervical spinal cord tissues to determine whether IH-1 increases expression of p38 MAP Kinase in the cervical spinal cord in rats with chronic cSCI.
 - February 2019-April 2019

Specific Aim 2: Does IH-28 impair AIH-induced pLTF and increase expression of phosphorylated p38 MAP Kinase in cervical spinal motoneurons and microglia in rats with chronic cSCI?

Task 4: Quantify impact of IH-28 on pLTF and cervical p38 MAP Kinase in rats with chronic cSCI

- Subtask 4a: perform spinal injury (n=20) and sham surgeries (n=20)
 - January-May 2019
- Subtask 4b: Expose rats to IH-28 (n=20) or normoxia (n=20), beginning 8 weeks post-injury
 - April 2019-August 2019
- Subtask 4c: perform terminal neurophysiology experiments to assess the impact of IH-28 on AIH-induced pLTF
 - April 2019-August 2019
- Subtask 4d: perfuse and harvest tissues from rats from subtask 4c to quantify p38 MAP kinase
 - April 2019-August 2019

Milestones:

- Abstract submission/conference presentation: “Impact of chronic intermittent hypoxia on phrenic output and plasticity in rats with chronic cervical contusion.” To be submitted for presentation at Experimental Biology 2019.
- Manuscript preparation and submission: “Intermittent hypoxia dose determines the impact of intermittent hypoxia on phrenic long term facilitation.” To be submitted to *Experimental Neurology*.

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?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

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 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 impair 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.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

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

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. Despite these delays, we have been quite productive, completing a separate but related study comparing the impact of IH dose on phrenic output and spinal plasticity in intact rats. In addition, we have completed all of the milestones defined in our SOW for the first eight months of the proposed studies, which is consistent with the proposed timeline, if progress is assessed from the release of funds. Progress in the current reporting period includes generating chronic spinal injured and sham animals for specific aim 1. IH/normoxia exposures and terminal experiments on these animals will begin this month and are expected to be completed in late fall, consistent with our proposed timeline. 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.

Because of the extended period of time required to obtain institutional approvals and the subsequent delayed release of funds, hiring for this position was delayed, and the post-doctoral associate hired onto this position came from a clinical research laboratory and had not had prior experience in animal research models or electrophysiology. Thus in the early phases of the award period, a significant portion of time was dedicated to training in animal handling, spinal cord injury surgical methods, and procedures required for terminal neurophysiology experiments including anesthesia, tracheostomy and mechanical ventilation, arterial and venous catheterization, intrathecal catheterization, and bilateral phrenic neurograms. The delayed release of funding and the subsequent delay in initiating the proposed experiments have delayed but not altered the anticipated costs for completing the proposed objectives.

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. Episode frequency determines the impact of chronic intermittent hypoxia on phrenic long-term facilitation. In preparation for J Neurotrauma. (Federal support was 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.*

Invited Presentations:

1. Neuromedicine Seminar Series, University of Florida, Gainesville, FL. "Intermittent Hypoxia and Respiratory Motor Plasticity: Implications and Considerations for Rehabilitating the Injured Spinal Cord". June 11, 2018
2. 1st Annual International Therapeutic Intermittent Hypoxia Conference, University of Florida, Gainesville, FL. "Therapeutic Potential of Intermittent Hypoxia: A Matter of Dose". April 17, 2018.
3. Department of Human Physiology, University of Oregon, Eugene, OR. "Respiratory Motor Plasticity: Implications and Considerations for Rehabilitation the Injured Spinal Cord". December 1, 2017.

Conference Presentations:

1. Gonzalez-Rothi EJ, Perim RR, Tadjalli A, Allen LL, Mitchell GS. "Dose Dependent Effects of Intermittent Hypoxia on Phrenic Long Term Facilitation" (2017). XIV Oxfor Conference on Modelling and Control of Breathing (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

- **Other Products**

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 the institutional approval process, trained personnel, and has performed/assisted with spinal injury surgeries, post-operative animal care, intermittent hypoxia exposures and terminal neurophysiology experiments.*
Funding Support: *Dr. Gonzalez-Rothi has also received 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 “pending” awards from the Craig H. Neilsen Foundation and the National Institutes of Health are now active:

SCIRTS Pilot Research Grant

Gonzalez-Rothi (PI)	3.0 calendar	5/1/17-4/30/19
Craig H. Neilsen Foundation		\$299,826 total project

“Combinatorial Therapies to Treat Breathing Impairments After Cervical Spinal Cord Injury”
Description: The goal of this project is to investigate the impact of repetitive acute intermittent hypoxia on functional gains elicited by intraspinal electrical stimulation following incomplete cervical spinal cord injury.
Role: PI

NIH, 1 R01 HL139708-01

Fuller (PI)	0.96 calendar	8/1/18-7/31/23
National Institutes of Health		\$1,250,000 total project

“Ampakines and Respiratory Function After Spinal Cord Injury”
Description: The goal of this project is to investigate the impact of ampakines, a novel class of drugs that modulate AMPA-type glutamate receptor function, on plasticity and functional recovery after cervical 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 (IH-1) impairs AIH-induced pLTF and increases expression of phosphorylated p38 MAP Kinase in cervical motoneurons and microglia

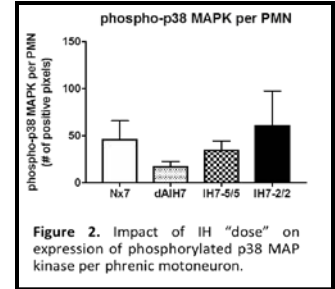
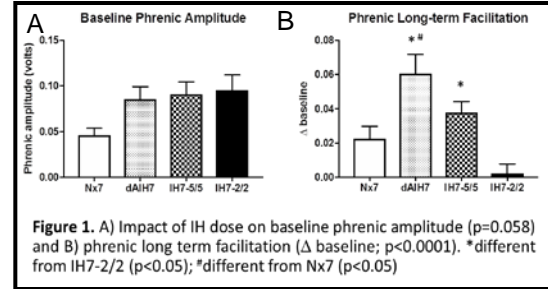
Aim 2: Test the hypothesis that prolonged “high-dose” chronic intermittent hypoxia (IH-28; 28 nights) impairs AIH-induced pLTF and increases phosphorylated p38 MAP kinase in cervical motoneurons and microglia

Aim 3: Does systemic ketoprofen restore/enhance pLTF following IH-28

Aim 4: Does intraspinal p38 MAP Kinase inhibition restore/enhance pLTF following IH-28?

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.



While training study personnel in required methods, performing cervical contusion injuries and allowing SCI and sham rats to age to chronic post-injury time point, we conducted a separate, but related study comparing the effects of different “doses” of intermittent hypoxia on phrenic motor output, phrenic long term facilitation, and p38 MAP kinase expression in phrenic motoneurons in intact rats. Rats were exposed to 7 days of either normoxia(Nx7), low-dose (therapeutic) daily acute intermittent hypoxia (dAIH-7), moderate dose chronic intermittent hypoxia (IH7-5/5; simulating mild sleep apnea), or high-dose chronic intermittent hypoxia (IH7-2/2; simulating moderate sleep apnea). All IH doses increased baseline phrenic output (Fig 1A). Dose-dependent effects on phrenic long-term facilitation (plasticity) were observed, with low dose dAIH7 enhancing plasticity, and high dose IH7-2/2, abolishing it (Fig 1B). In addition, we observed “dose” effects on p38 MAP kinase expression, with low doses reducing its expression and high doses increasing it (Fig 2). These data will be submitted as a manuscript this fall. In addition, all SCI and sham rats have been generated for aim 1 and have been aging to chronic post-injury time point. These rats will undergo IH/normoxia exposures and terminal neurophysiology experiments starting in September 2019.

Timeline and Cost

Activities	CY	17/18	18/19	19/20
Assess the impact of IH-1 on pLTF and spinal p38 MAP Kinase in cSCI rats (Aim 1)		█	█	
Asses the impact of IH-28 on pLTF and spinal p38 MAP kinase in cSCI (Aim 2)			█	█
Assess the impact of anti-inflammatories on pLTF after IH-28 in cSCI rats (Aim 3)				█
Asses the impact of p38 MAP Kinase inhibition on pLTF after IH-28 in cSCI rats (Aim 4)				█
Estimated Budget (\$499,999)		\$251k	\$257.5k	\$263.6k

Goals/Milestones

CY17/18 Goals

- Obtain UF IACUC and DoD ACURO approvals and final notice of award
- Hire personnel (post-doctoral associate and research technician)
- Train personnel in study methods
- Initiate and complete spinal cord injury and shams for aim 1
- Age sham and SCI rats to chronic time point (ongoing)
- Assess impact of IH “dose” on pLTF in intact rats (separate but related study conducted while training personnel and aging SCI rats)

CY18/19 Goal

- Assess impact of IH-1 on pLTF (starts 9/2018)
- Assess impact of IH-1 on p38 MAP kinase expression (starts 9/2018)
- Publish manuscript on IH “Dose”
- Submit abstract on IH-1 in SCI
- Spinal Injury and sham surgeries for aims 2 and 3 (IH-28 studies)
- Assess impact of IH-28 on phrenic plasticity

CY19/20 Goal

- Spinal injury and sham surgeries for aim 4
- Asses impact of anti-inflammatories on pLTF after IH-28
- Assess impact of p38 MAP Kinase inhibition on pLTF after IH-28