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TITLE:	Combinatorial Treatment for Reach-and Grasp Recovery in Chronic Contusive Cervical SCI
PRINCIPAL INVESTIGATOR:	Martin Oudega, PhD
CONTRACTING ORGANIZATION:	University of Miami Miller School of Medicine
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our main goals in this first year of our proposal was to get the required approvals from IACUC and ACURO to conduct the proposed studies, set-up the hypoxia device from Therapeutiq LLC for which money was requested and granted, get familiarized with this new device, and conduct the first study as part of our overall investigation of the role of increased (extrinsic and intrinsic) axon plasticity on AIH/training-mediated functional recovery. We have accomplished all of these goals successfully in this first year. The obtained *in vivo* results show that the frequency of hypoxia treatment affects aspects of some forelimb function in rats with a chronic cervical contusion. The results have been presented at the Society for Neuroscience meeting in October 2019.

15. SUBJECT TERMS

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1. Introduction

Our granted proposal had three Specific Aims. In Specific Aim 1, we proposed to examine approaches to increase the aftereffects of AIH/training on reach-and-grasp recovery in adult rats with chronic contusive cervical spinal cord injury (cSCI). In Specific Aim 2, we planned to examine the effects of AIH/training on CST sprouting and synapse formation in the chronically contused cervical spinal cord in adult rats. In Specific Aim 3, we intended to examine the effect of extended AIH/training treatment on reach-and-grasp recovery in adult rats with chronic contusive cSCI. Our approach was to use a clinically relevant adult rat model of contusive cSCI to test the hypothesis that the effect of AIH/training on motor recovery in chronic contusive cSCI is restricted, at least in part, by limited axonal plasticity. We planned to enhance axonal plasticity extrinsically, using cABC treatment, and intrinsically, using AIH at higher frequency. On the calendar, of the 36 months during the three-year proposal, months 1-16 were scheduled for Specific Aim 1, months 17-29 for Specific Aim 2, and months 30-36 for Specific Aim 3.

2. Keywords

Spinal cord injury, contusion, chronic, axon growth, plasticity, hypoxia, chondroitinase ABC, corticospinal tract, sprouting, synaptogenesis.

3. Accomplishments

Following our Statement of Work:

Months 1-3: Local IACUC Approval.
The IACUC protocol for the proposed animal studies was written and approval was acquired from the University of Miami IACUC and IBC committees. IACUC #: 18-103, accompanied by IBC #: 18-067. This was achieved within the scheduled time.

Months 1-3: HRPO/ACURO Approval.
The ACORP protocol was written and approval was acquired. ACURO Log #: SC170326.w001. This was achieved within the scheduled time.

In addition to writing IACUC and ACURO protocols and get them approved, during these first months we also ordered and received all parts of the budgeted hypoxia device from Therapeutics, LLC. The equipment arrived in four separate deliveries spread out over 4 months. We assembled the device and installed it in an allocated room in the Animal Core Facility. We made ourselves familiar with the device. We tested and calibrated the device so that the period it takes from 21% to 10.5% oxygen (desaturation) is between 20-30 sec (see Fig. 1). This is important for proper timing of hypoxia and normoxia cycles. We are able to reach these numbers, which remain stable beyond the typical 100 min of treatment time.

Fig. 1. Front panel of the controller of the Therapeutic hypoxia device (**A**). We assembled the parts into the functioning device and then got familiar with, tested, and calibrated the device to be able to switch from 21% to 10.5% (i.e., 50% oxygen level) within 20-30 sec (**B**).

Month 4-16: Subtask 1.1 and 1.2.
As part of Specific Aim 1, there were two subtasks. In subtask 1.1, months 4-10, we planned to examine the effects of cABC treatment on AIH/training-mediated reach-and-grasp recovery. In subtask 1.2, months 11-16, we planned to examine the effects of AIH frequency on AIH/training-mediated reach-and-grasp recovery. Because due to ordering issues there would have been a delay in receiving the cABC, we started with subtask 1.2. The subtasks in Specific Aim 1 are independent and this switch had no affect on the outcome of the subtasks.

Subtask 1.2: Examine effects of AIH frequency on AIH/training-mediated reach-and-grasp recovery.

Introduction: AIH can cause increases in function in both animal models of SCI and in humans with SCI. Attempts to optimize the dose, within the previously defined "safe" range, to maximize AIH-mediated functional recovery after SCI have not been reported. Frequency of neuromodulation may affect the outcome with higher frequencies eliciting stronger aftereffects. We hypothesized that the limited effect of AIH treatment on motor function recovery after SCI is in part due to sub-optimal frequency of AIH exposures. We designed a study to evaluate the effects of a range of frequencies of hypoxia/normoxia-cycles on clinically relevant functional outcome measures (reaching and grasping and grip strength) in an adult rat model of chronic contusive cSCI.

Study design (Fig. 2): In our study, adult female Sprague Dawley rats (200-250g; n=31) were pre-trained in the Montoya staircase for reach and grasp behavior before they received a C5 contusion (unilateral; right side) with a force of 175 kDyn using the Infinite Horizon impactor. AIH treatment was given at different frequencies but with the same total exposure time to hypoxia and normoxia using the Therapeutiq Research hypoxia chambers and regulator. Normoxia (21% O2)/hypoxia (10% O2) cycles were 2.5/2.5, 5/5, or 10/10 min or continuous normoxia. Treatment time was 100 min total once a day for five consecutive days. Note that one week prior to starting the treatment, rats were re-acclimated in the Montoya staircase and base line measures were taken. After treatment, rats were tested in the Montoya staircase for the effects of the treatment on reach and grasp behavior. In this experiment, our survival rate was 93.5% (2 rats died during or shortly after the SCI surgery). The results were statistically analyzed using Kruskal Wallace with Bonferroni correction using SPSS v24 and significance set at $p<0.05$.

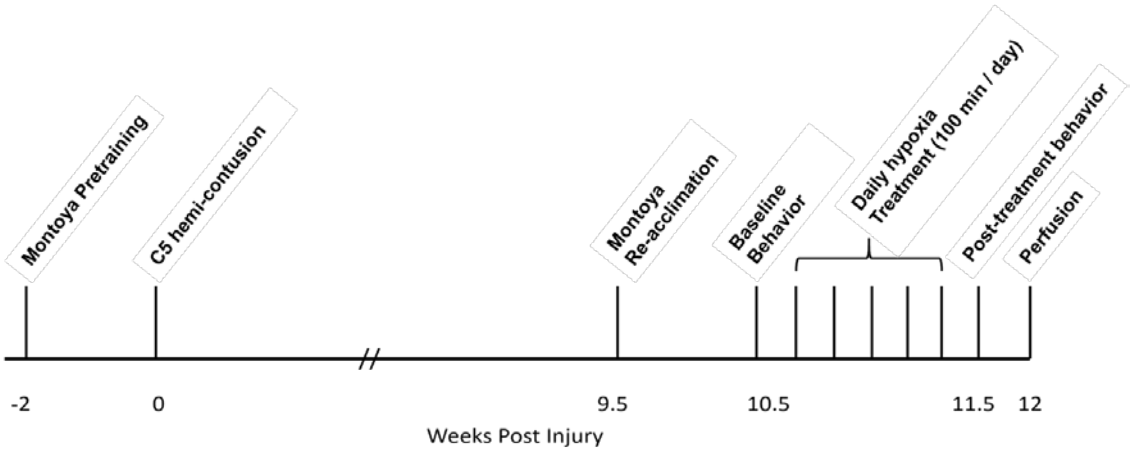


Fig. 2. Schematic representation of the study activities in time before and after contusive cervical SCI.

Results: We acquired stable hypoxia/normoxia cycles during the 100 min of treatment with normoxia (Fig. 3A), 2.5/2.5 min (Fig. 3B), 5/5 min (Fig. 3C), and 10/10 min (Fig. 3D). The Therapeutiq hypoxia device we use for our treatment has specialized cage tops to provide optimal exposure of the rats to

the cycles (Fig. 3E). After treatment, rats were tested for reach and grasp function in Montoya staircases (Fig. 3F).

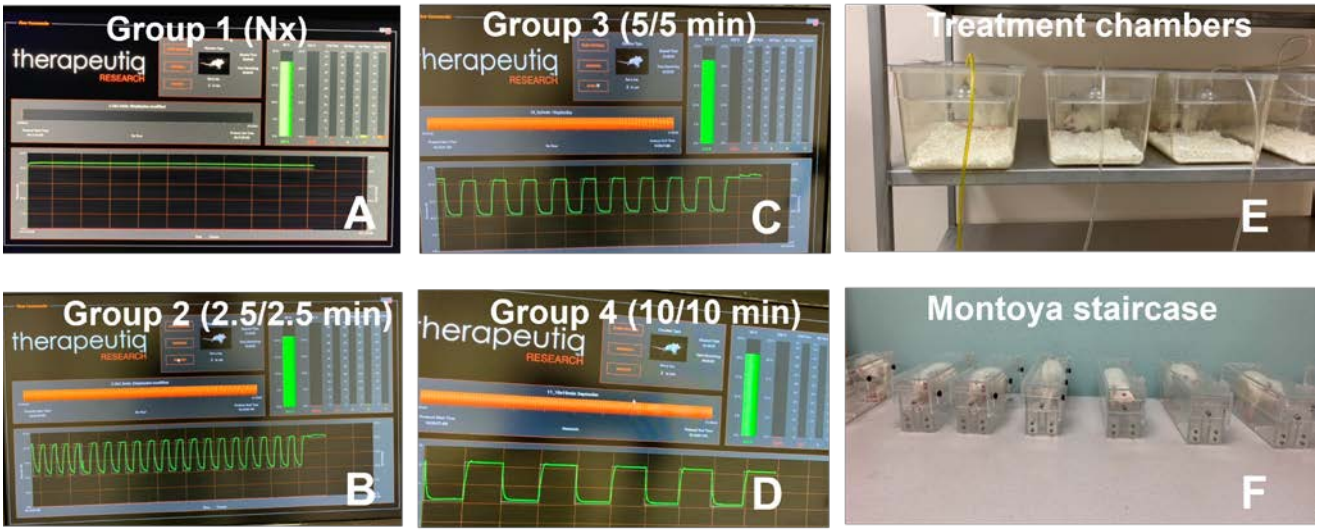


Fig. 3. AIH treatment and behavioral testing. The Therapeutiq Research hypoxia chamber was used to apply AIH at (**A**) normoxia, (**B**) 2.5/2.5 min cycles, (**C**) 5/5 min cycles, or (**D**) 10/10 min cycles once daily for five consecutive days. (**E**) rats in their treatment cage with specialized tops for optimal exposures. (**F**) rats during functional testing of reach and grasp in the Montoya staircase.

We examined the effects of our treatments on grip strength (Fig. 4) and reach and grasp function (Fig. 5). We found a statistically non-significant decrease in grip strength compared to base line measure with normoxia treatment, which was significantly increased with 5/5 min, but not with 2.5/2.5 min or 10/10 min, cycles of hypoxia/normoxia (Fig. 4).

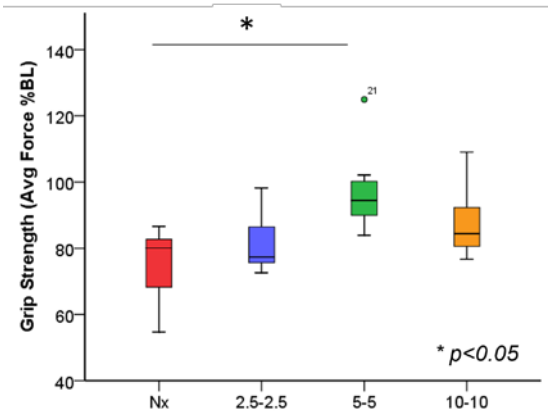


Fig. 4. Grip strength was decreased by 20% from baseline in rats that received normoxia. In rats that received AIH at 5/5 min frequency, but not other frequencies, grip strength was significantly increased to return back to baseline value. P < 0.05.

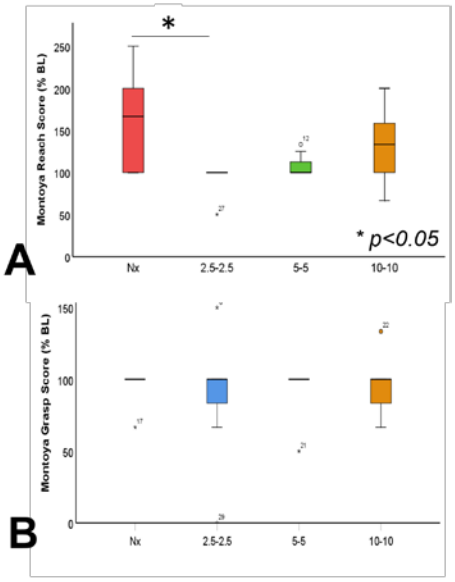


Fig. 5. Reach (i.e., touching stair and/or food pellet, no retrieval) was decreased from baseline in rats that received AIH at 2.5/2.5 min frequency (**A**), while grasp (i.e., retrieval of food pellet) was unaffected by AIH (**B**).

We found a statistically significant decrease in reach function with 2.5/2.5 min cycles of hypoxia/normoxia compared to normoxia treatment (Fig. 5A). With 5/5 min and 10/10 min cycles of hypoxia/normoxia, reach function was decreased non-significantly compared with normoxia (Fig. 5A). Grasp function was not affected by any of the treatments (Fig. 5B).

Summary: We found that AIH frequency increased grip strength, but not reaching nor grasping in rats with chronic cervical contusive SCI.

Conclusion 1: We conclude that frequency of AIH differentially influences its effects on forelimb functions in rats with chronic cervical contusive SCI. Cellular and molecular changes in the cervical spinal cord need to be examined in order to correlate and explain the obtained results to structural changes. Based on our results, we postulate that AIH frequency has differential effects on the axonal pathways and electrophysiological properties of their axons that are involved in forelimb functions and aspects thereof in chronic cervical contusive SCI.

Conclusion 2: AIH treatment may affect behavior in rats chronically injured to their cervical spinal cord, but it appears an accompanying intervention is needed to translate the effects into biologically significant changes in function.

Conclusion 3: Regarding the set Goals/Milestones for CY19, we have acquired approval from IACUC and ACURO, and studied the effects of frequency of AIH on forelimb function in rats with chronic cervical contusive SCI.

Planned next experiments: Following our proposed research plan we will investigate in the next year: 1) Effects of frequency on AIH combined with training; 2) Effects of cABC treatment on forelimb function; 3) Effects of combined AIH/training and cABC treatment on forelimb function.

4. Impact

The impact of our work so far is on the studies as they were described, and awarded, in our proposal. These first steps are imperative for the success of the proposed studies. We expect in time that our studies will deliver data that will allow us to: 1) better understand plasticity in the injured spinal cord, 2) determine the effect of hypoxia treatment on plasticity and on functional recovery, and 3) design clinically relevant strategies for promoting plasticity-based repair and recovery after SCI. The impact of these studies, if successful, will be tremendous on the population of people with SCI.

5. Changes/Problems

The major change is that the PI on the proposal, Dr. Martin Oudega, has accepted a position at the Shirley Ryan AbilityLab and Northwestern University in Chicago, IL. This has been communicated to our science officer, Sarah Fontaine. We are currently in the process to wrap up business in Miami and transfer to Chicago. Funds will be requested to be transferred to Chicago. This process has been initiated. Dr. Haggerty, the postdoctoral associate on this proposal has accepted another position. We are currently searching for a replacement but will have this occur at the time of our move.

6. Products

Abstract, presented at the Annual Society for Neuroscience meeting in October 2019 in Chicago.
Title: The relationship between frequency of acute intermittent hypoxia treatment and functional improvements in chronic cervical spinal cord injury.
Authors: Agnes E Haggerty, Natalia de la Oliva, Martin Oudega.

7. Participants & Other Collaborating Organizations

Name: Martin Oudega, PhD
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project: Dr. Oudega has written and revised protocols and acquired approval, supervised the assembly and familiarization with the hypoxia device, designed and supervised the first animal experiments with the device.

Name: Agnes Haggerty, PhD
Project Role: Postdoctoral Associate
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 9
Contribution to Project: Dr. Haggerty has helped writing and revising the animal protocols until approval was acquired, helped with the hypoxia device assembly and familiarization, and conducted the animal studies, gathered and started to analyze the data.

Name: Pantelis Tsoulfas, PhD
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project: Dr. Tsoulfas has helped writing and revising the animal protocols until approval was acquired, helped with the hypoxia device assembly and familiarization, and co-supervised the animal studies. Also started to produce viral vectors needed in the later parts of the study.

Name: Anne Kanaya
Project Role: Technical assistant
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project: Ms Kanaya has helped writing and revising the animal protocols until approval was acquired, and provided general help with all aspects of the animal studies.

8. Special Reporting Requirements

The Quad Chart is provided in **9. Appendices**

9. Appendices

Combinatorial treatment for reach-and-grasp recovery in chronic contusive cervical SCI

Funding Opportunity Number W81XWH-17-SCIRP-IIRA Pre-application C170326

PI: Oudega, Martin **Org: University of Miami** **Award Amount: $743,988**

Study Aims

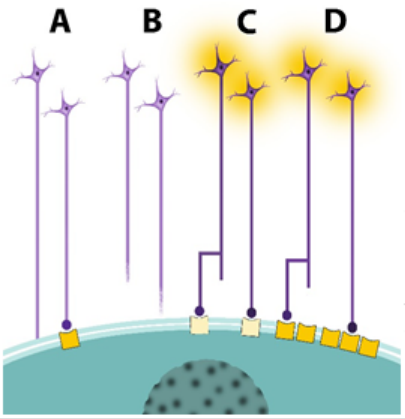
Specific Aim 1: : Examine approaches to increase the aftereffects of AIH/training on reach-and-grasp recovery in adult rats with chronic contusive cSCI.. .

Specific Aim 2: Examine the effects of AIH/training on CST sprouting and synapse formation in the chronically contused cervical spinal cord in adult rats.

Specific Aim 3: Examine the effect of extended AIH/training treatment on reach-and-grasp recovery in adult rats with chronic contusive cSCI.

Approach

Using a clinically relevant adult rat model of cervical spinal cord contusion we will test the hypothesis that that the effect of AIH/training on motor recovery in chronic contusive cSCI is restricted in part due to limited axonal plasticity. We will enhance axonal plasticity extrinsically, using concurrent cABC treatment, and intrinsically, using higher AIH frequency. We will study AIH and training effects on CST sprouting and synapse formation and examine the effects of extended AIH/training protocols. .



Schematic representation of the hypothesis. **(A)** Normal, uninjured, spinal cord with connections and passing axons; **(B)** SCI leads to axon damage and loss of synaptic connections; **(C)** AIH and training increase expression of plasticity-genes in CSMNs resulting in CST sprouting and synapse formation; **(D)** Training further supports the translation of structural effects into motor output.

Timeline and Cost

Activities CY	19	20	21
Approvals. Specific Aim 1: Examine ways to increase AIH/training aftereffects			
Specific Aim 2: Increase understanding of mechanism of AIH and training plasticity			
Specific Aim 3: Examine effect of extended AIH/training protocols			
Estimated Budget ($K)	**$276**	**$245**	**$223**

Updated: November 29, 2017

Goals/Milestones

CY19 Goals – Enhance effect of AIH/training on motor recovery
- Get approval IACUC and ACURO. Complete SA 1
- Established role of increased (extrinsic and intrinsic) axonal plasticity on AIH/training-mediated functional recovery
- Developed AIH/training protocols with enhanced recovery

CY20 Goals – Expand knowledge of axon plasticity of AIH and training
- Gathered quantitative data on CST axon sprouting/synapse formation and gene expression profiles in CSMNs and spinal inter- and motoneurons after AIH and/or training. Manuscript preparation.

CY21 Goal – Examine effect of extended AIH/training protocols
- Established role of length of AIH/training protocol in degree of functional recovery. Manuscript preparation.

Comments/Challenges/Issues/Concerns

Budget Expenditure to Date