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TITLE: Combinatorial Treatment for Reach-and Grasp Recovery in Chronic Contusive Cervical SCI

PRINCIPAL INVESTIGATOR: Martin Oudega, PhD

CONTRACTING ORGANIZATION: Shirley Ryan AbilityLab,
355 E. Erie St
Chicago, IL 60611

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14. ABSTRACT Our main goals in this year were to (1) complete our experiments and prepare the data for a submission to a peer-reviewed journal, (2) move our lab and equipment from Miami, FL, to the Shirley Ryan Ability Lab, where the PI, Dr. Oudega had accepted a new position as per January 2020, (3) transfer the award administratively to the Shirley Ryan AbilityLab, (4) set up the lab and animal-related facilities, (5) write new IACUC protocols for the local committee at Northwestern University. We have accomplished all of these goals successfully in this last year. The lab and equipment were moved by the end of January. Lab set-up was started in February and then at first interrupted by COVID-19. Recently, the set-up was completed. Most time was spend to these moving and set-up efforts. Other time was spend to write animal protocols, and a manuscript for publication in a peer-reviewed journal. The manuscript is in its last phase of revisions befop0re submission.								
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1. Introduction

Our proposal had the following 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 effect 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 $p < 0.05$.

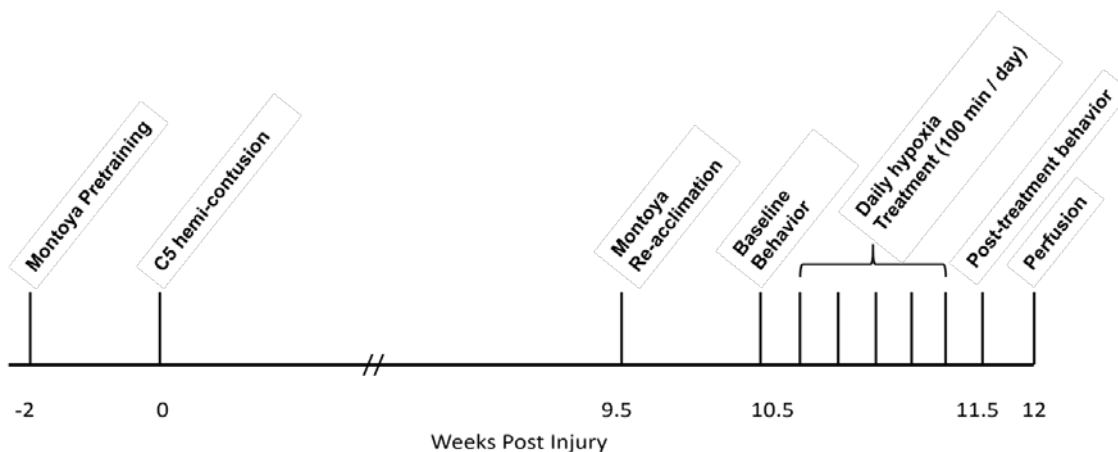


Fig. 2. Schematic representation of the study activities in time before and after contusive cervical SCI.

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).

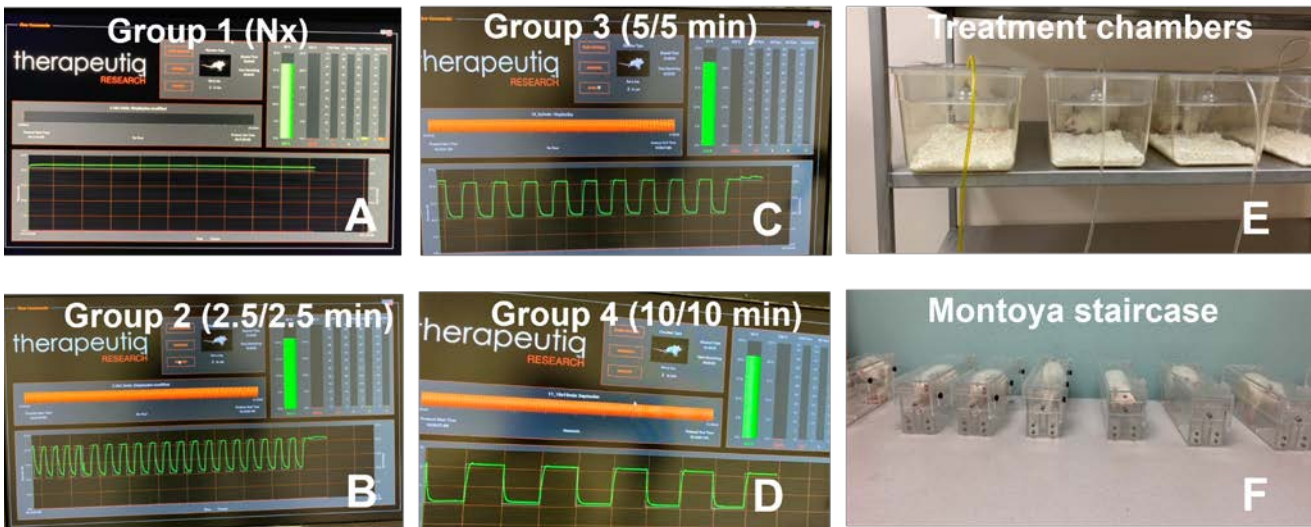


Fig. 3. AIH treatment and behavioral testing. The Therapeutiq Research hypoxia chamber was used to apply AIH at (A) normoxia, (B) 2.5/2.5 min cycles, (C) 5/5 min cycles, or (D) 10/10 min cycles once daily for five consecutive days. (E) rats in their treatment cage with specialized tops for optimal exposures.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

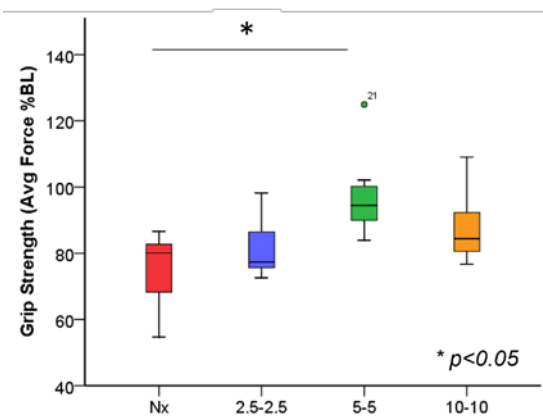


Fig. 4. Grip strength was decreased by 20% from baseline in rats that received normoxia. In rats that received AIH at 5/5 min frequency, but not other frequencies, grip strength was significantly increased to return back to baseline value. $P < 0.05$.

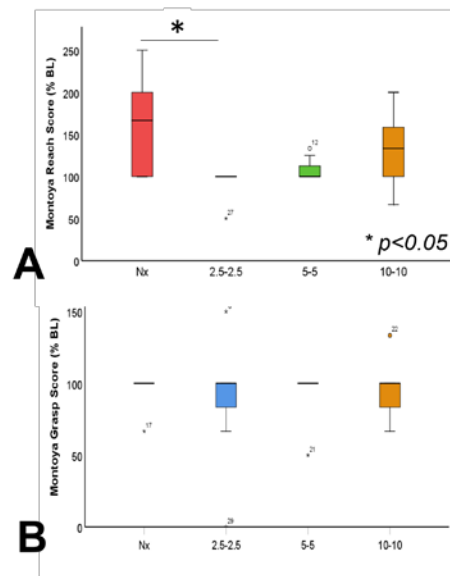


Fig. 5. Reach (i.e., touching stair and/or food pellet, no retrieval) was decreased from baseline in rats that received AIH at 2.5/2.5 min frequency (A), while grasp (i.e., retrieval of food pellet) was unaffected by AIH (B).

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.

We have all but some last revisions finalized a manuscript for submission to *Experimental Neurology*, a peer-reviewed journal with a readership interested in hypoxia, nervous tissue plasticity, and spinal cord injury.

Month 17-24: Move to Shirley Ryan AbilityLab, Transfer of Award, setting-up the lab.

All new goals for the time period of January 2020-October 2020 (month 17-24) have been met. The Award was relinquished by the University of Miami and subsequently transferred to The Shirley Ryan AbilityLab. The Award was active in May 2020 again. The laboratory and animal-related facilities have been set up. We are ready for our next planned experiments. These experiments are in accordance with our original research plan. In the next experiment we will investigate the effects of frequency on AIH combined with training. After that we will study effects of cABC treatment on forelimb function and 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. The steps taken in 2020 were necessary due to our move and award downtime. In our next experiments, 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. Our studies will contribute not only to our understanding of plasticity in the nervous tissue but also to the development of treatments based on hypoxia for people with spinal cord injury and other trauma that affects the CNS and impairs limb function.

5. Changes/Problems

The major change is that the PI on the proposal, Dr. Martin Oudega, now works at the Shirley Ryan AbilityLab and Northwestern University in Chicago, IL. This move caused some downtime with the award being relinquished and now again re-activated. Of course, this was a problem, but this is now behind us and our new set-up is ready.

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.

Manuscript to be submitted in October 2020:

Title: Effects of frequency in acute intermittent hypoxia treatment on forelimb function in chronic cervical spinal cord contusion.

Authors: Agnes E. Haggerty, Natalia de la Oliva, Ines Maldonado-Lasunción, Monica A Perez, Martin Oudega

7. Participants & Other Collaborating Organizations

Note that the Award had to be relinquished by the University of Miami and then awarded to the Shirley Ryan AbilityLab. This occurred between Jan – May 2020. In the information of participants below, these 4 months are included between brackets.

Name: Martin Oudega, PhD
 Project Role: PI
 Researcher Identifier (e.g. ORCID ID):
 Nearest person month worked: 12 (in 2019 and 2020, minus the 4 months)
 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: Pantelis Tsoulfas, PhD
 Project Role: Co-Investigator
 Researcher Identifier (e.g. ORCID ID):
 Nearest person month worked: 2 (in 2019 only)
 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: Ines Maldonado-Lasuncion
 Project Role: Technical assistant
 Researcher Identifier (e.g. ORCID ID):
 Nearest person month worked: 10 (only in 2020, minus the 4 months)
 Contribution to Project: Ines has helped and supported the move, lab set-up, writing protocols and manuscript. Ines will be conducting the next experiments.

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

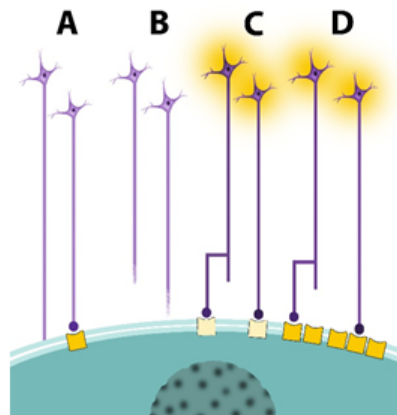
Specific Aim 1: Examine approaches to increase the aftereffects of AIH/training on reach-and-grasp recovery in adult rats with chronic contusive cSCI.

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 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