PROTOCOL TITLE:
Safety and Cognitive Effects of Acute Intermittent Hypoxia-Induced Neuroplasticity in Traumatic Brain Injury

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Principal Investigator:
Jordan Grafman, Ph.D.
Director, Brain Injury Research
Chief, Cognitive Neuroscience Lab
Think + Speak Lab
Shirley Ryan AbilityLab

Professor, Department of Physical Medicine & Rehabilitation, Neurology, Cognitive Neurology and Alzheimer's Center, Department of Psychiatry, Feinberg School of Medicine & Department of Psychology, Weinberg College of Arts and Sciences
Northwestern University

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Institutional Review Board Protocol

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Name: Jordan Grafman, Ph.D.
Director, Brain Injury Research
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Shirley Ryan AbilityLab

Professor, Department of Physical Medicine & Rehabilitation, Neurology, Cognitive Neurology and Alzheimer's Center, Department of Psychiatry, Feinberg School of Medicine & Department of Psychology, Weinberg College of Arts and Sciences
Northwestern University

Telephone Number: 312-238-1495
Email Address: jgrafman@northwestern.edu
E-Mail Address: jgrafman@sralab.org

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October 3, 2022

STUDY SUMMARY:

Investigational Agent(s) (Drugs or Devices)	N/A
IND / IDE / HDE #	
Indicate Special Population(s)	☐ Children ☐ Children who are wards of the state ☐ Adults Unable to Consent ☐ Cognitively Impaired Adults ☐ Neonates of Uncertain Viability ☐ Pregnant Women ☐ Prisoners (or other detained/paroled individuals) ☐ Students/Employees
Sample Size	16
Funding Source	NIH grant 1 R21 NS114815-01A1
Indicate the type of consent to be obtained	☒ Written ☐ Verbal/Waiver of Documentation of Informed Consent ☐ Waiver of HIPAA Authorization ☐ Waiver/Alteration of Consent Process
Site	☐ Lead Site (For A Multiple Site Research Study) ☐ Data Coordinating Center (DCC)

Research Related Radiation Exposure	☐Yes ☒ No
DSMB / DMC / IDMC	☒Yes ☐No

OBJECTIVES:

Aim 1: To establish safety boundaries for administering acute intermittent hypoxia therapy in subjects with traumatic brain injury (TBI), in order to provide guidance for future therapeutic hypoxia interventions.

This is a Phase I safety study that has as a primary goal to determine whether there are any negative effects of administering acute intermittent hypoxia (AIH) to patients who suffered a mild to moderate traumatic brain injury. We will administer hypoxia dose-escalation exposures with continual assessment of the effects of these modest hypoxia exposures in individuals with traumatic brain injury. We hypothesize that we will not see any adverse events related to the hypoxia exposures consistent with the reports of ongoing studies with spinal cord injured (SCI) and stroke patients.

Aim 2: To collect preliminary data about the potential efficacy of this approach in improving cognitive processing, as a prelude to a more extensive clinical trial.

We plan to measure memory/cognitive processing and motor control at baseline and post-treatment along with a brief exam following each AIH exposure sequence. We hypothesize that similar to motor improvements reported in ongoing stroke and SCI studies using AIH, we will see stable or improved cognitive learning and motor control after AIH sessions.

BACKGROUND:

Of the 1,000,000+ people who experience a TBI every year in the US, only a small percentage of those with moderate to severe TBI will achieve full recovery. While current rehabilitation therapies promote a variety of sensory, motor and cognitive skills, none directly address the unique potential of the brain to reorganize following injury. The goal of this project is to explore the effects of a novel therapy, acute intermittent hypoxia (AIH). During this therapy, individuals receive brief bouts of reduced oxygen levels by inhalation through a face mask. (This is akin to climbing to the top of a tall mountain). In brief exposures, AIH is known to trigger the release of specific proteins that help the brain adapt to oxygen reductions and thereby facilitate neuroplasticity. Published results in people with incomplete spinal cord injury have shown that AIH enhances muscle strength and coordination rather quickly. We now aim to study the effects of AIH in TBI survivors.

AIH has most often been studied in patients with spinal cord injury. In the past five years, the main findings relevant to the use of AIH in persons with incomplete spinal cord injury (SCI) are:

1. The neuroplasticity mechanisms linked to AIH described initially in animal models appear to be present and active in humans.
2. In persons with incomplete SCI, the use of AIH alone (15 60-second episodes of 9% oxygen) can augment muscle strength and joint torque at the ankle. The effects of AIH are often dramatic, increasing voluntary strength in ankle muscles by 30-40% within 90-120 minutes.

3. Repeated (daily) acute intermittent hypoxia (dAIH) exposure combined with conventional rehabilitation therapy such as over ground walking can greatly improve walking speed and endurance beyond that achieved with either intervention applied separately.

Recent work has also demonstrated the effectiveness of dAIH to enhance spinal motor plasticity related to respiration in SCI patients. (Tester, Fuller et al. 2011, Yokhana, Gerst et al. 2012) A randomized, triple-blind, two-arm parallel clinical trial was recently done in Chile (Navarrete-Opazo, Alcayaga et al. 2016) This study compared the effects of a 4-week protocol of dAIH combined with body weight-supported treadmill training on walking in persons with incomplete SCI. Subjects received dAIH or sham dAIH combined with 45 minutes of training for 5 consecutive days, followed by dAIH or sham dAIH 3 times per week for 3 additional weeks. They found that walking recovery was significantly better after dAIH + training, compared to training alone. Moreover, additional 3 times per week dAIH prolonged or enhanced daily IH-induced walking speed and endurance improvements up to 5 weeks post dAIH. In addition, this protocol of dAIH does not elicit any visual or verbal memory impairment (Navarrete-Opazo, Alcayaga et al. 2016).

TBI is a leading cause of long-term disability worldwide. Despite the spontaneous recovery that occurs following mild TBI, young and older people experiencing complex mild-to-severe TBI show substantial residual impairments, imposing difficulties in everyday life including challenges in returning to the same level of work or schooling. These burdens are likely to increase in coming decades, due to a rapidly aging population at risk for TBI along with the stable risk of experiencing a TBI in younger people. Accordingly, new interventions to alleviate impairment in TBI survivors are urgently needed. ***The development and testing of one such novel intervention, termed Acute Intermittent Hypoxia (AIH), is the primary focus of this NINDS funded R21 study.***

Our aim is to answer questions related to safety and preliminary efficacy of AIH in TBI survivors.

- First, we will establish whether brief reductions in inhaled oxygen concentration can be safely tolerated in TBI survivors. A research associate will closely monitor subjects for any adverse events and immediately report any such events to the resident on-call (ROC) and the independent study physician monitor and then to the IRB.

- The second aim is to establish whether there are any effects of AIH on memory, cognition, and motor control.

Subjects will be monitored closely for any adverse events during these experiments. Data will be analyzed to determine if there is an improvement in key outcomes at any dose level.

STUDY ENDPOINTS:
Primary study endpoint: Safety will be assessed through the continuing review of the clinical/neurological status over the duration of the study including post-session assessment of mood, episodic memory, and motor coordination. This study is intended to determine if AIH can be safely tolerated in patients with TBI as it has been in patients with spinal cord injury and stroke. Therefore, all adverse events will be reviewed for safety and study continuation by the medical monitor. It will be determined if a specific subject should continue the experimental hypoxia protocol or if the study should be terminated.

Secondary outcome: Each subject will also serve as his/her control; imaging and neurobehavioral data collected at baseline will be compared to the imaging and neurobehavioral data collected post-treatment. Clinical outcomes evaluated at baseline and post-treatment include The Repeatable Battery for the Assessment of Neuropsychological Status, Finger Tapping Test, Grooved Pegboard Test, California Verbal Learning Test, Serial Reaction Time Test, Word Fluency, Trail Making Test, the Effort Expenditure for Rewards Task, and Beck Depression Inventory. These tests evaluate a broad range of cognitive abilities, memory, response times, language production, executive functions, and mood.

Imaging outcomes: The 3T-MRI protocol duration will be one hour and will be done prior to initiating our tests at baseline, and at the follow-up visit. The MRI will be a structural /resting state MRI looking at a change in structure or brain network function from pre- to post-treatment. The sequence of the MRI will be the type typically used in the standard of care for a TBI evaluation and measuring resting state and is described in more detail below under Procedures.

STUDY INTERVENTION(S)/INVESTIGATIONAL AGENT(S):
Hypoxic Experimental Therapy: The hypoxic stimulus will be implemented through a facemask. Hypoxia sessions will consist of a baseline breathing normoxic (containing normal concentration of oxygen), ambient air, followed by 15 cycles of hypoxia for up to 60 seconds, interspersed with up to 90-second normoxic episodes.

All conditioning sessions will be supervised by a research associate, who will be:
1. Assisting the lab techs to complete a pregnancy test (in females) and a resting EKG prior to the hypoxia session.
2. Monitoring the SpO2, blood pressure and pulse rate throughout the hypoxia session. If there are concerns, the medical monitor will review.

PROCEDURES INVOLVED:

Each of the Hypoxia sessions consist of a baseline during which participants breathe normoxic, ambient air, followed by 15 cycles of hypoxia for up to 60 seconds, interspersed with up to 90-second normoxic episodes.

Outcome and Monitoring Assessments are listed below followed by details describing the intervention and outcome measures.

Visit 1: Prescreening and Baseline; Length of Visit: estimated 6 hours

- MRI (no task)
- Medical history
- Demographic data
- Current medications/comorbidities
- TMS screening and neuronavigated motor mapping (to locate the hand motor area of the brain that will be stimulated by TMS in Visits 2 and 5)
- Pregnancy test for women of child-bearing potential (WOCBP)
- Resting 12-lead EKG
- Repeatable Battery for the Assessment of Neuropsychological Status (RBANS),
- State-Trait Anxiety Inventory (STAI),
- Ohio State University TBI (OSU TBI) Identification Method interview,
- Finger Tapping Test,
- Grooved Pegboard Test,
- California Verbal Learning Test (CVLT-II),
- Serial Reaction Time Task (SRTT),
- Word Fluency Test,
- Trail Making Test (TMT) – Parts A & B,
- Effort Expenditure for Rewards Task (EEfRT), and
- Beck Depression Inventory (BDI-II).

Visit 2: 21% “normal oxygen”; Intervention Day 1; Length of visit: estimated 2 hours

- Hypoxia session: 30 mins
- Outcome assessments plus transcranial magnetic stimulation (TMS) and electromyographic (EMG) measurement
- Monitoring assessments

Visit 3: 17% oxygen; Intervention Day 4; Length of visit: estimated 2 hours

- Hypoxia session: 30 mins
- Outcome assessments
- Monitoring assessments

Visit 4: 13% oxygen; Intervention Day 8; Length of visit: estimated 2 hours

- Hypoxia session: 30 mins
- Outcome assessments
- Monitoring assessments

Visit 5: 9% oxygen; Intervention Day 12; Length of visit: estimated 2 hours

- Hypoxia session: 30 mins
- Outcome assessments plus transcranial magnetic stimulation (TMS) and electromyographic (EMG) measurement

- Monitoring assessments

Visit 6: Post-Treatment Evaluation; Post-intervention Day 16; Length of visit: estimated 4 hours

- MRI (no task)
- Repeatable Battery for the Assessment of Neuropsychological Status (RBANS),
- Finger Tapping Test,
- Grooved Pegboard Test,
- California Verbal Learning Test (CVLT-II),
- Serial Reaction Time Task (SRTT),
- Word Fluency,
- Trail Making Test (TMT) – Parts A & B,
- the Effort Expenditure for Rewards Task (EEfRT), and
- Beck Depression Inventory (BDI-II).

Note: The time interval between interventions is subject to modification, depending on the staff and equipment availability, as well as the patient's scheduling preferences. The above visit lengths are our best time estimations given the procedures involved in each. The actual amount of time for each visit will vary from participant to participant, to account for variations including waiting room time, transport time from one procedure to another, and other timing aspects of the study that cannot, by nature, be set.

Intervention procedures: During each of the four AIH sessions, study participants will undergo a single session of AIH, consisting of 15 periods of hypoxia, lasting up to 60 seconds, alternating with up to 90 seconds of normoxia (21% O2), for a total of up to 35 minutes. AIH will be applied by directing gas flow to a reservoir bag connected via plastic tubing to a non-re-breathing facemask/respiratory valve system while the participants are in a seated upright position. Defined gas mixtures will be delivered by manual adjustment of one-way valves attached to a hypoxia generator (HYP123, Hypoxico Inc.). An oxygen monitor will continuously measure and record the fraction of inspired oxygen delivered to the subject (MAX-250E, Maxtec Inc.).

Inspired fraction of oxygen (FiO2) of the gas mixture will be individually adjusted using the valve settings to reach the target SpO2. The gas mixtures administered during the four sessions will be 21% O2 (target SpO2 = 95%), 17% O2 (target SpO2 = 92%), 13% O2 (target SpO2 = 87%) and 9% O2 (target SpO2 = 82%).

The Shirley Ryan AbilityLab and study co-investigators have extensive experience with this protocol and have administered it to both healthy, spinal cord injury, and stroke individuals. As the individuals get hypoxic gas for only a brief period, i.e., up to 60 seconds, the risks associated are minimal. To put into perspective, SpO2 of 82% is akin to oxygen levels at the top of a tall mountain.

All conditioning sessions will be supervised by a research associate, who will be: 1) assisting in administering the EKG and the pregnancy test prior to the first hypoxia

session (if applicable), 2) monitoring SpO2 levels, blood pressure and pulse rate throughout the session. If there are concerns, the medical monitor will be notified and will review the concerns. If there is an unforeseen alteration in the cardiopulmonary parameters being assessed, the experiment will be stopped and appropriate care will be provided by the ROC and the medical monitor, and the emergency medical team at the Shirley Ryan AbilityLab will be promptly notified. We will resume normoxia immediately should the participant develop complaints, while awaiting the arrival of the ROC, via the "ROC Stat" procedure at the Shirley Ryan AbilityLab, in conjunction with the hospital Security department and announced immediately via the emergency loudspeaker system at the hospital.

Any and all adverse events will be documented and appropriately reported, as required by the Institutional Review Board.

Outcome assessments:

- MRI – evaluates brain structure and examines correlations between brain regions' functional activity at rest. Magnetic resonance imaging (MRI) will be used to determine whether there are alterations in the brain structure or resting state functional connectivity pre- to post-treatment. Subjects will have a total of two MRIs in the Shirley Ryan AbilityLab Siemens 3T machine. The structural MRI will be reviewed by a physician to confirm the extent of brain damage due to TBI at baseline, to certify the results were collected and reviewed correctly, and to compare the post-treatment scan to the baseline one.
- Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) – The brief test battery evaluates various aspects of cognition and memory and has two psychometrically equated versions.
- State-Trait Anxiety Inventory (STAI) – This self-report scale measures the intensity of anxiety.
- Ohio State University TBI (OSU TBI) Identification Method interview – OSU TBI is a standardized procedure for eliciting a person's lifetime history of TBI through a 3-5-minute structured interview.
- Finger Tapping Test – This test measures the rate of finger presses in order to assess simple motor coordination. Five to ten ten-second trials per hand are administered.
- Grooved Pegboard Test – this task measures motor coordination requiring the participant to rotate a peg with a groove in it to fit in a grooved hole. There are 25 pegs and holes and each hand is tested once.
- California Verbal Learning Test (CVLT-II) – this is a multi-trial word learning test that includes recall and recognition measures, as well as recording recall improvement across 12 trials.
- Serial Reaction Time Task (SRTT) – this is a procedural learning task. Participants are asked to press a key that is below a marker (filled circle) appearing on a monitor. They are generally unaware that there is a repeated sequence. Implicit learning is measured as the difference between the average

time required to respond to repeated sequences vs random presentations of the markers on the screen.

- Word Fluency – participants are asked to produce as many words as possible that begin with a specific letter (F,A,S) or a category (e.g., animal names). This is a simple measure of the ability to retrieve words from semantic memory.
- Trail Making Test (TMT) – Parts A & B – Trail Making Part A requires participants to draw a line between circles containing numbers in ascending order (e.g., 1-2-3…etc.). Part B requires participants to alternate drawing a line between ascending letters and numbers (e.g., 1-A-2-B…etc.). The key measures are the time required to complete and the number of errors done in Part A and Part B. This test measures various aspects of cognition including attention, visual search, motor coordination, reasoning, and task-switching (executive function).
- Effort Expenditure for Rewards Task (EEfRT) – the EEfRT measures the trade-off between the likelihood of the reward and the amount of effort required to procure the reward. This trade-off is considered a measure of motivation.
- Beck Depression Inventory (BDI-II) – this self-report scale measures the amount of depressive symptoms. The total score across the items contained in the Inventory is indicative of depression severity. A score of 14 and above indicates the participant is experiencing depression.
- Rey Auditory Verbal Learning Test (RAVLT) – Five presentations of a 15-word list (list A) are given, each followed by attempted recall. This is followed by a 15-word interference list (list B), followed by recall of list A. Delayed recall and recognition are tested.
- VAM-S – a visual analogue mood scale
- TMS: Transcranial magnetic stimulation with targeted neuronavigation (based on individual MRI scans) will be delivered using one of the two available set-ups: (1) MagPro X100 with MagOption stimulator and a C-B60 figure-of-eight coil (MagVenture, Farum, Denmark), Localite neuronavigation system (St Augustin, Germany); (2) Nexstim eXimia NBS stimulator, coil and neuronavigation system (Helsinki, Finland). TMS will be delivered to the optimal scalp position for activation of the target muscle in TBI patients. The optimal scalp position will be determined by moving the coil in small steps along the hand representation of the primary motor cortex to find the region where the largest MEP can be evoked with the minimum intensity in the targeted muscle. The muscle to be activated will be the first dorsal interosseous of the dominant hand. We will measure the resting motor threshold (minimum intensity required to induce MEPs greater than 50 μV in 5/10 consecutive trials at rest). We will collect 20 MEPs at an intensity 20% above the resting motor threshold.

<u>Monitoring assessments (carried out throughout the hypoxia administration)</u>:

- Vitals: Blood pressure guidelines for participation include systolic blood pressure between 90-160mmHg, diastolic blood pressure between 60-100mmHg before and during each hypoxia session; SpO2 and pulse rate
- Symptom checklist: A simple yes/no subjective checklist will be asked and repeated to the participant at each time point: 2, 6, 14, 24 and 30 mins from the beginning of the hypoxia session.

Subjective Symptom checklist:

Symptom	*Yes*	*No*
Chest pain		
Shortness of breath		
Lightheadedness		
Neck pain		
Dizziness		
Arm pain (left side) for cardiac symptoms		
Sweaty/feeling warm		
Sensory changes: new signs of numbness		
Increased weakness		

Other outcome assessments immediately following the hypoxia administration include:

- Motor coordination (Finger Tapping Test, Grooved Pegboard Test), mood assessment (VAM-S), and word-list learning (RAVLT)

Other outcome assessment before and after hypoxia sessions #1 (Visit 2) and #4 (Visit 5):

- Single-pulse assessment for motor cortical functioning using transcranial magnetic stimulation and electromyographic recording

Transcranial Magnetic Stimulation (TMS) and electromyography (EMG): TMS sends a magnetic pulse to the brain and stimulates brain activity. A wire TMS coil will be placed over the participant's head. The TMS coil will be held to the head with a custom coil holder. To limit head movement, the head will be secured to the headrest with a soft Velcro strap. When the TMS is activated, this coil will generate a magnetic pulse over a specified area of the brain that is responsible for hand movement. When the pulse occurs, the participant will hear a click or a snapping sound, and may feel a pulling sensation on their skin underneath the area where the coil is placed. They may also feel a light twitch of the muscles in their arm, face or leg. During Visit 1, we will do motor mapping, that is, we will use neuronavigation to find the area of the brain that activates the target hand muscle. During Visits 2 and 5 we will apply a series of TMS pulses to this brain area to evoke EMG responses from the target muscle. During the EMG recording, the participant will be comfortably positioned so that their arms are supported by a testing device. Recording electrodes will be attached to the surface of the skin over the target hand muscle to record its activity.

Exclusion criteria to TMS participation for TBI patients:

- Uncontrolled medical problems including pulmonary, cardiovascular or orthopedic disease
- Premorbid, ongoing major depression or psychosis, altered cognitive status
- History of stroke
- Metal in head (e.g., surgical clips, shrapnel)
- History of seizures or epilepsy diagnosis
- Receiving drugs acting primarily on the central nervous system, which lower the seizure threshold such as antipsychotic drugs (chlorpromazine, clozapine) or tricyclic antidepressants
- Surgery to the head
- Any non-TBIrelated neurological diseases
- Illnesses that may have caused brain injury
- Unexplained frequent or severe headaches
- Pregnancy in females
- Implanted devices (e.g., pacemakers, medical pumps, brain stimulators)

Patients will not be eligible to participate in the study if they are not eligible to undergo MRI or TMS.

Blood draw. A blood draw from WOCBP will be carried out once, as part of health screening routines during Visit 1. A serum pregnancy test requires a sterile needle to be inserted into a vein to collect a 1-5 ml (0.2-1 tsp) blood sample.

DATA AND SPECIMEN BANKING

Data from the study (e.g., assessment and outcome variables) will be stored in a password-protected database, REDCap, which is managed by Northwestern University. If needed, an Excel or SPSS file will be managed with password protection; located on the secure network at the Shirley Ryan AbilityLab. Upon enrollment, each subject will be assigned an identifier number such that the data do not contain identifiable private information; the data will only be coded with the identifier number. Thus, there is no identifiable data. The data will be uploaded into REDCap or an Excel/SPSS file. Only authorized personnel of this study (IRB-approved) will have direct access. Once all patient data has been collected and uploaded, the study investigators and statisticians will analyze the data and provide reports, which will be used to publish relevant findings. Names of participants will not be used in study reports. We will retain study documentation including all case report forms, data correction forms or queries, source documents, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms) for at least three years after the completion and final study report of this investigational study.

SHARING RESULTS WITH PARTICIPANTS

This is not a blinded study; all subjects will each be given the same treatment.

Subjects will be provided with a phone number to call if they have any questions about the study treatment they received or the results of the clinical trial. It is not anticipated

that any information, unless an adverse event occurs, will need to be shared with primary care or other physicians.

STUDY TIMELINES

Participants will be initially screened over the phone or in person to ensure eligibility based on the established inclusion and exclusion criteria. We anticipate that each participant will take about 2-4 weeks to complete the study procedures/testing (depending on scheduling and study staff availability). We anticipate a total of 2 years to enroll, complete data collection and complete the primary analyses.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

- Aged 18-65 years
- A first time, mild to moderate traumatic brain injury (TBI) confirmed by medical records
- When available, a Glasgow Coma Scale score between 9-15
- Able to use a keyboard
- Able to understand and communicate in English
- Able to consent independently
- Able to leave a research visit with a companion/group transportation
- Women of child-bearing age must be comfortable confirming a negative pregnancy prior to participating in the study
- Must not be involved in any other research intervention study testing neurobehavioral functioning

Exclusion criteria:

- Other neurological diagnoses or a diagnosis of severe psychiatric disorder (e.g., psychosis) or a reported childhood learning disability
- Severe aphasia, preventing subject from understanding the protocol and giving written consent
- Pre-existing hypoxic pulmonary disease
- Severe hypertension (>160/100)
- Medically documented history of obstructive lung diseases [e.g., Chronic obstructive pulmonary disease (COPD) or significant asthma]
- Ischemic cardiac disease
- Ineligible to undergo MRI or TMS

VULNERABLE POPULATIONS

N/A

The participants will be post-traumatic brain injury patients living in the community and be independent without significant cognitive impairment.

PARTICIPANT POPULATION(S)

The total recruitment goal is 16 subjects.

RECRUITMENT METHODS

Potential participants will be identified through a variety of means, including: medical records, physician contacts, recruitment flyers within the Shirley Ryan AbilityLab and other Northwestern-affiliated hospital and websites (e.g., https://www.feinberg.northwestern.edu/research/clinical-research/trials/, text of the recruitment ad attached), surrounding community hospitals, the Clinical Research Registry (NU IRB # STU00212893; see Authorization Letter in Supporting Documents), the Center for Rehabilitation Outcomes Research (CROR) research registry (NU IRB # STU00012894; see Letter of Support uploaded in Supporting Documents), ClinicalTrials.gov (Identifier: NCT04890639) and community outreach presentations.

Shirley Ryan AbilityLab Research Registries: Subjects will be recruited through the Clinical Neuroscience Research Registry. The database is maintained through combined efforts of the Shirley Ryan AbilityLab and Northwestern University Department of Physical Therapy. It includes up to 1000 individuals with various neurologic conditions, including traumatic brain injury. Researchers are able to modify their search criteria based on level of injury, severity of injury, and primary means of mobility. Patients will also be recruited through the Shirley Ryan AbilityLab's electronic medical records and through the PM&R clinic physicians.

Flyers: These will be primarily distributed through the Shirley Ryan AbilityLab hospital and websites, surrounding community hospitals, and at the surrounding Chicagoland support groups for individuals having suffered a TBI.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

- Payment will be provided through ClinCard.
- Each participant will be compensated $20 per hour for participation in the study. Partial hours completed will be compensated at a prorated rate of $5 per 15 minutes.

During the Covid-19 pandemic, we will reimburse round-trip private transportation cost within the Chicago metropolitan area, including driver tip (e.g., accessible Uber, Lyft, taxi, parking fees etc.), for visits to and from the Shirley Ryan AbilityLab. A receipt is required for full reimbursement of transportation cost.

WITHDRAWAL OF PARTICIPANTS

Subjects may withdraw from participation in the study at any time. Any data collected up to the time of withdrawal will be retained for study purposes.

Participants may withdraw from the study at any time at their own request or they may be removed at the discretion of the investigator for safety, behavioral or administrative reasons.
The reason(s) for discontinuation will be documented and may include:

- Participant voluntarily withdraws from treatment;
- Participant withdraws consent;
- Participant is unable to comply with protocol requirements;
- Researcher staff decides that continuation with the study would not be in the best interest of the participant.

Subjects will be considered as not evaluable if they choose to drop out voluntarily and will be replaced. However, if an adverse event is experienced and it is deemed that the subject must be removed from the research study for safety, that subject with not be replaced. This determination will be made by the medical monitor and will be reflected in subjects' research record in the data file used.

Subject accrual will stop at any point in the study if two or more subjects (out of the 16) experience an adverse effect (AE) at any of the dose levels following baseline.

RISKS TO PARTICIPANTS

Potential risks related to acute intermittent hypoxia, although rare, may include headaches, lightheadedness, dizziness, altered vision, respiratory distress, cyanosis, increase in breathing rate, sweating, muscle spasms and fainting. There can be alterations in the field of vision, a sensation of numbness or tingling and feelings of euphoria. An additional risk to wearing a mask in severely claustrophobic individuals is discomfort, anxiety or panic.

In case of a sudden emergency, the ROC (Resident on Call) will be summoned via the hospital's loudspeaker to attend to the participant. We will resume normoxia immediately should the participant develop complaints, while awaiting the arrival of the ROC, via the "ROC Stat" procedure at the Shirley Ryan AbilityLab, in conjunction with the hospital Security department and announced immediately via the emergency loudspeaker system at the hospital.

There are two major risk groups that could be determined, 1. cerebral or 2. cardiac, described in more detail below.

(1) A non-silent cardiac event will require the ROC to be called to the clinic room for immediate and urgent attention for the chest pain experienced. The medical monitor will determine the next steps for the safety of the subject, which may include a visit to the emergency room located at Northwestern Memorial Hospital.

(2) A cerebral adverse event is experienced through a change in vision and/or changes in the neurological assessment. The medical monitor will be informed of this outcome and will provide advice accordingly.

In terms of research-related risks, participants may become frustrated, tired, or bored during the testing. While undergoing the MRI, the participant may experience mild stress from lying still in a noisy machine in an enclosed space. Our study team is trained to respond to indications of unease with support and comfort. If the participant is unable to lie still or if they seem distressed by the scanning, the study will be stopped immediately. We will immediately stop all of the testing, including neuropsychological tests and MRI scanning, at the request of the participant.

The following items may interfere with the MRI scan and can be potentially hazardous: cardiac pacemaker, aneurysm clip(s), implanted insulin/drug pump, neurostimulator (TENS unit), biostimulator/bone growth stimulator, hearing aid/cochlear implant, Gianturco coil (embolus coil), vascular clip(s), surgical clip or staple(s), heart valve prosthesis, Greenfield vena cava filter, middle ear implant, shrapnel or bullet, wire sutures, tattooed eyeliner, dental items held in place by a magnet, other implanted items not mentioned, intraventricular shunt, wire mesh, artificial limb or joint, orthopedic items (i.e., pins, rods, screws, nails, clips, plates, wire, etc.), certain tattoos, dentures, dental braces, or any type of removable dental items. The SRALab Safety Questionnaire (see “MRI_Screening_Form”) submitted as a Supporting Document with this protocol is a screen for the presence of the above-listed items. If the SRALab radiology staff deems any of the items checked by the participant to be hazardous, they will not be allowed to have an MRI.

MRIs are considered to be safe and have been used for many years. It is a completely non-invasive procedure. No needles, chemicals, or radiation are used. Instead, a powerful magnet and radio waves are used to create images of the participant’s brain. There is no evidence that the magnetic field used by the scanner has any harmful effects. MRI scanning has been performed for over 20 years, but nonetheless, there could be unknown risks. In our MRI work performed so far, there have been no significant adverse events reported. These procedures have also been safely used in participants in previous studies and are commonplace at major medical centers worldwide.

TMS may cause temporary discomfort to some individuals due to the sensations of tingling or twitching in the scalp, jaw or face, as well as twitches in the stimulated muscles. Seizures are a possible, but an extremely rare risk associated with undergoing TMS.

The possible risks associated with blood drawing are pain, bleeding, a rare risk of fainting, bruising, infection and/or hematoma (blood clot under the skin) at the injection site.

All adverse events occurring to participants enrolled in this protocol will be reported according to the Northwestern University IRB standard procedure.

POTENTIAL BENEFITS TO PARTICIPANTS

There may be no direct benefit for participants enrolled in this study.

DATA MANAGEMENT AND CONFIDENTIALITY

All case report forms will be kept in study binders for each subject. Each subject will only be identified by subject number (subject ID). Study binders will be kept in a locked cabinet on the 16th or 25th floors of the Shirley Ryan AbilityLab. The 16th floor is not a public access floor. The 25th floor is where our research lab and offices are located and are secured when unoccupied.

Statistical Considerations for AE detection

Trial Design. Participants enrolled in this study will undergo within-subject dose escalation of hypoxia. The planned dose levels are standard O2 content (21%, dose level d0), 17% (dose level d1), 13% (dose level d2) and 9% (dose level d3) O2 content. Each participant will initially be tested at dose level d0 (baseline). At the second study visit, the participant will be treated at the next dose level (d1), and, if no qualifying adverse events (AEs) occur, dose level will be further escalated to d2 at the third visit. Similarly, if no qualifying AEs occur at visit 3, the participant will be treated at dose level d3 at the last visit. Participants who experience adverse events or cannot tolerate treatment at dose level d0 will be excluded from the study. Participant accrual will stop if at any point in the study 2 or more participants experience an AE at any of the dose levels d1, d2 or d3. AIH will be considered unsafe if 2 or more participants experience an AE at any dose level.

Sample Size Considerations: We used simulation studies to determine operating characteristics for detecting excessive AEs for this trial. Under the null hypothesis, we assume that the AE rate is extremely low, $HH0$:$pp1$ =$pp2$ = $pp3$ = 0.01, where $ppjj$ corresponds to the probability of AE at dose level $ddjj$. We further assume that $ppjj$ are uncorrelated under $HH0$. Under the alternative hypothesis, we consider three types of dose-toxicity curves: (I) $pp2$ = $pp1$ +0.025, $pp3$ = $pp1$ + 0.025, (II) $pp2$ = $pp1$ +0.025,$pp3$ = $pp1$ +0.050, and (III) $pp2$ = $pp1$ +0.050, $pp3$ = $pp1$ + 0.050. We considered $pp1$ ranging between 0.05 and 0.125. Under the alternative hypothesis, subjects' outcomes (AE, yes or no) at the three dose levels were simulated as correlated binary data, assuming within-subject correlation between AE occurrence at the various dose levels is $\rho\rho$ = 0.3. R=10,000 trials were simulated under each scenario using the "bindata" package in R. For each simulated trial, we calculated the number of AEs occurring among all participants at any dose level, and hypoxia treatment was declared unsafe if ≥ 2 participants in a given trial experienced an AE at any dose level. Type I error rate ($\alpha\alpha$-level) was calculated as the proportion of all R=10,000 trials simulated under $HH0$ that were declared unsafe. Power was calculated as the proportion of all trials under a particular alternative hypothesis scenario that were declared unsafe. We considered sample size n=14 or 16. Under $HH0$, the probability of declaring hypoxia unsafe (Type I error) was 0.063 when n=14 and 0.081 when n=16. Power curves for the three types of dose-toxicity curves are presented in the Figure below. For example, for a dose-toxicity curve of type III when {$pp1$ = 0.08,$pp2$ = 0.13,$pp3$ = 0.13} under the alternative hypothesis, we will have 87.6% power with n=14 and 91.6% power when n=16. More generally, when n=14, we will have >80% power under all dose-toxicity scenarios with

$pp1$ ≥ 0.08. When n=16, we will have >80% power to under all dose-toxicity scenarios when $pp1$ ≥ 0.07.
Aim 2: To collect preliminary data about the potential efficacy of this approach in improving neurobehavioral functions, as a prelude to a more extensive clinical trial. We plan to use a standard set of memory and cognitive tasks and neuroimaging at baseline and after completion of the AIH trial as well as an abbreviated evaluation immediately after each session for finger tapping, motor coordination, mood, and word-list recall along with single-pulse evaluations before and after AIH sessions 1 and 4 respectively.
Statistical Analysis of Efficacy: AIH efficacy will be assessed at each O2 dose level for all subjects, as continuous outcomes (e.g., finger tapping speed). Data will be analyzed using generalized linear mixed models with repeated measures, with the outcome measure as the response variable, and dose level as a categorical predictor to allow for a nonlinear dose-response relationship. Baseline measurements will be adjusted for in the model. Within-subject correlation between multiple doses will be accounted for with an appropriate variance-covariance structure (e.g. compound symmetry or autoregressive of order 1, AR(1)). Model fit and assumptions will be checked using model diagnostic techniques for mixed models, and the outcome variable may be transformed to satisfy the normality assumption. Mixed models with repeated measures have been shown to be robust when data are normally distributed even with small sample sizes. Post-hoc tests will be used to determine whether there is an improvement in outcome relative to baseline at any dose level, and whether there is a difference between dose levels. Because of the small sample size, these analyses will be exploratory. Analysis will be done using PROC MIXED in SAS, R, MATLAB, SPSS or other available statistical software package.

Research material will include questionnaires assessing inclusionary/exclusionary criteria, neurobehavioral tasks, orientation questions during the acute intermittent hypoxia sessions, outcome questionnaires, electromyographic recordings, and neuroimaging scans and datasets. All data will be used solely for the purposes of the research proposed herein. Personal medical records, results of previous laboratory, neuroimaging, neuropsychological testing, and information from attending physicians that are obtained will be held in secure files. Identifying information will not be included in any report or publication. All data will be labeled with a participant code only and will be kept on a password-protected server. Participants will be identified by a code number, and access to their names will only be available to the PI, the Co-Investigators, post-doctoral fellows, and the research assistants approved by the Northwestern University Institutional Review Board. A master list connecting the participant names with their participant ID number will be stored in an Excel file kept on an SRALab IT-supported, secure, password-protected server. All collected data will be used for research purposes only. The Principal Investigator (PI) will manage access to the project data, including identifiable private information. Only approved research team members will have access to the data. All paper files will be kept in a locked and secured file cabinet in the Cognitive Neuroscience Laboratory at the Shirley Ryan AbilityLab. All electronic data will be de-identified and kept on an SRALab, IT-supported, secure, password-protected server, with the exception of the original MRI scans. These scans will be stored on the SRALab, IT supported, secure, password-protected server, but by nature

could contain facial features, names, and birthdates in the metadata (this is standard for these types of files). Original MRI scans will additionally be stored on DVDs that will be locked in a drawer on the 25th floor, and accessible only to study team members.

Electronic data, including survey data, will be backed up in REDCap. While REDCap can be used to collect data in many environments (including compliance with 21 CFR Part 11, FISMA, HIPAA, and GDPR) and is specifically designed to support online and offline data capture for research studies and operations. (For further details, https://www.project-redcap.org)

Apart from the original scans and master list, identifying information will be stored separately from the de-identified experimental data. Only the PI and approved study members, who are listed on the IRB protocol, will have access to the data. Neuroimaging data will be uploaded to the Northwestern University Research Image Processing System (NURIPS), an online collaborative research environment for securely storing, managing, analyzing and sharing de-identified medical imaging, associated data (e.g. behavioral), and results from advanced customizable processing pipelines. NURIPS is supported by both NUIT and FSM-IT and takes advantage of the NU high performance computing cluster, Quest. NURIPS is a secure environment that supports the latest NU policy and procedures for encryption of data during transit and rest, provides granular project level access controls with varying permissions based on user groups, and allows non-NU collaborators access once they obtain an affiliate NetId. All data are backed up and have restore points that go back for 30 days. Users have access to common data analysis pipelines and the opportunity to create and share their own pipelines.

An encrypted SRALab IT-approved USB stick will be used to carry the anatomical MRI scan from the MRI lab to the TMS lab on the same floor of the SRALab hospital. Once a scan has been transported to the TMS lab computer for cortical reconstruction and properly stored on the SRALab server, it will be deleted from the USB stick. (The USB stick is intended as a temporary transport device only).

We are not collecting any urine or tissue specimens.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS

The PI has the ultimate responsibility for this study. Dr. Grafman will oversee the entire project with respect to study design, recruitment processes, study progress, participant safety, data interpretation and data reporting. An independent Medical Safety Monitor will review all adverse events and make recommendations to the participant and the PI.

Medical Monitor:

The medical monitor on this study is Dr. Elliot Roth, MD (Physical Medicine and Rehabilitation, Shirley Ryan AbilityLab). Responsibilities will include:

1. Observing recruitment and enrollment procedures and the consent process for individuals

2. Overseeing study interventions and interactions.
3. Reviewing monitoring plans.
4. Overseeing data matching, data collection, and analysis.
5. Placing the MRI order to determine the presence of a brain damage due to a TBI before the subject can be scheduled for intervention visits. A detailed analysis of the size and location of the damage will be done by a radiologist during data collection/analysis after all subject data has been collected.
6. Reviewing any report of an adverse event.

Additionally:
1. May discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research;
2. Shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report;
3. Shall have the responsibility to promptly report their observations and findings to the IRB.

The medical monitor will be placing the order for the research MRI. Once results of the MRI have been generated, the medical monitor will review and sign off on the images to confirm the results and for safety of the subject receiving hypoxia. If results generate safety concerns, then a radiologist on subscription with the Shirley Ryan AbilityLab will be contacted for assistance.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS
An ongoing dialogue will take place during the subject's introduction to the study and the informed consent process to ensure that the candidate fully understands the study, what is required, the risks and the benefits, and their rights as a participant. Only participants that are able to independently consent in English will be approached.

All information provided by participants will be de-identified.

Documentation will be stored within a locked cabinet within a specified specialty lab within the Shirley Ryan AbilityLab. Furthermore, all data stored electronically will be password-protected. Only authorized individuals will have access to files and data, as entered within the Northwestern IRB site.

COMPENSATION FOR RESEARCH-RELATED INJURY
N/A

ECONOMIC BURDEN TO PARTICIPANTS
There are no costs to participants for participating in this study.

CONSENT PROCESS

After potential subjects are identified, they will be invited to return to the Shirley Ryan AbilityLab for a research appointment to complete screening and consent. This appointment will be arranged and coordinated by the designated research associate for the study. The informed consent documentation will be reviewed with the participant and authorized research personnel during Visit 1, prior to any testing or interventions. The principal investigator/medical monitor will be available for any questions regarding the consent of the participant.

This process will be completed in a private space within the Shirley Ryan AbilityLab.

Participants will be encouraged to ask questions related to study purpose or procedures, or have family present. They will be provided a copy of the signed informed consent. Participants will be told that they are volunteers who can stop participation at any time without penalty. The consent process should take approximately 30 minutes. Individuals who are not yet adults, are not cognitively intact, unable to understand English or unable to consent will not be recruited for this study.

NON-ENGLISH SPEAKING PARTICIPANTS

Non-English speaking participants will not be recruited because the tests are standardized in English and all other study materials and study instructions will be given in English.

WAIVER OR ALTERATION OF CONSENT PROCESS

N/A

PROTECTED HEALTH INFORMATION (PHI AND HIPAA)

Protected health information will remain for the most part in the Cerner Research Medical Record database, which is used to maintain clinical medical records, and is accessible only by authorized personnel with electronic ID and password. Any other PHI will be in paper records (e.g. CRFs), which will include only the subject's study ID and not their names. Paper records will be stored on the 25th floor of the Shirley Ryan AbilityLab in the research offices which are secured when unoccupied. These records are kept in locked cabinets. All paper records will be kept for three years after the end of the study and then will be destroyed in accordance with the Shirley Ryan AbilityLab policies. We will have in the research database from this study information that characterizes participants including age, education level, sex of subject, but not any of the following information: name, geographic subdivision, telephone number, fax number, social security number, medical records numbers, health plan numbers, other account numbers, vehicle identifiers, photographs, biometrics or related identifiers, and the information generated from their research participation will not be put in any of their medical records.

SETTING

This single-site study will have all research procedures and visits take place at the Shirley Ryan AbilityLab, located at 355 E. Erie St, Chicago, IL 60611.

Statistical Analysis Plan Update

Date: February 12, 2025

Sample Size: While the initial target was to recruit 16 participants, we encountered challenges finding eligible volunteers who would be able to participate in six in-person research visits and were only able to enroll 12 participants, all of whom completed all six visits and whose data will be included in the analyses.

Statistical Analysis: To evaluate the efficacy of the AIH protocol and its effects on vital signs, motor function, cognition and mood we will implement the following analyses.

Vital signs data (HR, SpO2) will be preprocessed in MATLAB R2020a (MathWorks, Natick, MA). For each cycle in an AIH session, the highest HR and the lowest SpO2 readings will be identified; then, the average highest HR (max HR) and lowest SpO2 (min SpO2) across all cycles will be computed per session.

Across-session comparisons of behavioral performance on the motor and cognitive tests, vital signs (min HR, max SpO2) and motor evoked potentials (MEPs) will be conducted using IBM SPSS Statistics v. 29.0, with the significance level set at $\alpha = .05$. Normality of data will be assessed using the Shapiro-Wilk test ($\alpha = .05$). For normally distributed variables, means (*M*) and standard deviations (*SD*) will be calculated, and groups will be compared using a two-tailed paired-samples *t*-test or a repeated measures (RM) ANOVA. Mauchly's test will be used to check the RM ANOVA's assumption of sphericity; when violated ($p < .05$), the Greenhouse-Geisser correction will be applied. For non-normally distributed data, medians (*Mdn*) and interquartile ranges (*IQR*) will be calculated, and group comparisons will be performed using the Wilcoxon signed-rank test or the Friedman test. Effect sizes will be calculated as follows: Cohen's *d* for *t*-tests, *r* for Wilcoxon tests, partial eta squared (η_p^2) for RM ANOVA, and Kendall's *W* for Friedman tests. Post hoc tests for RM ANOVA and Friedman tests will be conducted using paired-samples *t*-tests and Wilcoxon signed-rank tests respectively, with multiple comparisons corrected using the false discovery rate (FDR) method (Benjamini & Hochberg, 1995), as implemented in the R function *p.adjust*. Data visualizations will be created using the ggplot2 package (Wickham, 2016) in R v. 3.6.3 (R Core Team, 2020).

References:

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological), 57*(1), 289–300. https://doi.org/10.1111/J.2517-6161.1995.TB02031.X

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis.* Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4

Informed Consent Form

Title of Research Study: **Safety and Cognitive Effects of Acute Intermittent Hypoxia-Induced Neuroplasticity in Traumatic Brain Injury.**

Principal Investigator: Jordan Grafman, Ph.D

Supported By: This research is supported by the National Institute of Neurological Disorders and Stroke.

Key Information:
The first few pages of this document include a summary of this study to help you decide whether or not to participate. Detailed information is provided after the summary.

Why am I being asked to take part in this research study?
We are asking you to take part in this research study because you are over the age of 18, have experienced a first-time traumatic brain injury, which was confirmed by a medical report. Additionally you speak and understand English with the ability to independently consent and are able to travel without a companion/group transportation.

What should I know about a research study?
- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- You can ask all the questions you want before you decide.

Why is this research being done?
The aim of this study is to answer questions related to the safety and effectiveness in improving cognition of a new intervention, acute intermittent hypoxia (AIH), in traumatic brain injury survivors. For the purposes of this study, hypoxia means reducing the amount of oxygen you will be breathing, using a specialized face mask which controls oxygen content in inhaled air.

In order to deliver the air with the different concentrations of oxygen, we will use the Hypoxico gas-mixing generator. It is considered an investigational medical device as used in this study, and is not approved by the US Food and Drug Administration (FDA).

First, we will establish whether brief and moderate reductions of oxygen in breathing air can be safely tolerated by people participating in this study. A clinician will closely monitor you for any negative medical occurrences also known as adverse events.

The second aim is to establish what effects AIH may have on cognitive processing. You will be closely monitored for any adverse events during these experiments. Data will be analyzed to determine if there is a change in cognitive performance at any of the oxygen dose levels.

How long will the research last and what will I need to do?
We expect that you will be in this research study for two to four weeks.
You will be asked to come in for six visits total (one screening visit, four treatment visits and one post-treatment follow-up appointment).

More detailed information about the study procedures can be found under the section **What happens if I say "Yes, I want to be in this research"?**

Is there any way being in this study could be bad for me?
There are two major risk outcomes that could be experienced; in the brain (cerebral) or in the heart (cardiac). These two risks are described in more detail below in the section, "**Detailed Risks: Is there any way being in this study could be bad for me**?". These risks are based on using the similar procedure in patients with spinal cord injury or stroke, which are conditions that include risk factors related to change in oxygen and normal brain blood flow, among others. There are no known risks related directly to AIH sessions used in mild-to-moderate patients with brain injury, although risks have been documented when AIH is used in patients with spinal cord injury and stroke, as mentioned previously and described further in Detailed Risks. While we do not anticipate the same degree of risk in patients with mild-to-moderate brain injury, we simply do not know the full range of risks at this time, when AIH is used specifically in patients with mild-to-moderate brain injury. This study will help us document any such risks in patients with mild-to-moderate brain injury. You will be screened before your participation to make sure that these types of events are unlikely, but a small possibility remains that either a cardiac or cerebral event might occur.

The research associate who is responsible for administering the acute intermittent hypoxia sessions will be frequently evaluating your medical and cognitive status during all sessions (including the baseline session) and in the event of an emergency will be trained to seek emergency medical staff. Prior studies at the Shirley Ryan AbilityLab with spinal cord injury and stroke patients receiving acute intermittent hypoxia sessions similar to our study reported no adverse events during their studies.

More detailed information about the risks of this study can be found under **"Is there any way being in this study could be bad for me? (Detailed Risks)"**

Will being in this study help me in any way?
There are no direct benefits to you from taking part in this research. We cannot promise any benefits to others from taking part in this research.

What happens if I do not want to be in this research?
Participation in research is completely voluntary. You decide whether or not to participate. If you choose not to participate, there will be no penalty to you or loss of benefit to which you are entitled.

Your alternative to participating in this research study is not to participate.

Detailed Information:
The rest of this document includes detailed information about this study (in addition to the information listed above).

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the Principal Investigator, Dr. Jordan Grafman at (312) 238-1495.

This research has been reviewed and approved by an Institutional Review Board (IRB). You may talk to them at (312) 503-9338 or irb@northwestern.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research participant.
- You want to get information or provide input about this research.

How many people will be studied?
We expect that 16 people will complete this research study.

What happens if I say "Yes, I want to be in this research"?
You will be asked to come to the Shirley Ryan AbilityLab for a total of 6 visits. The first visit is a baseline evaluation, that will include a health screening, MRI scans of your brain, TMS motor mapping, along with neuropsychological testing and questionnaires. The following four visits will include the acute intermittent hypoxia delivery, monitoring

assessments, and a series of brief outcome assessments; two of these four visits will also include TMS. The final visit is a post-treatment evaluation that will include another MRI scan along with neuropsychological testing and questionnaires.

Each visit is broken down with the exact tasks that will occur just below. A detailed explanation of the assessments is listed at the end of the consent.

Experimental Hypoxia Sessions:
Hypoxia will be administered via a specialized face mask, which controls oxygen content in inhaled air while breathing into a gas mixing device, called Hypoxico.The hypoxia administering unit will be manually adjusted to reach the target oxygen level (approximately 21%-normal oxygen, 17%, 13%, and 9%) for each of the four sessions. The acute intermittent hypoxia sessions consist of 15 cycles of hypoxia lasting for up to 60 seconds alternated with up to 90-second cycles of breathing ambient air. All sessions will be supervised by a trained research associate, with a resident-on-call ("ROC") physician present at the Shirley Ryan AbilityLab during the hypoxia administration, who will be reachable via the hospital's "ROC Stat" procedure.

PROCEDURES: research visits will all take place at the Shirley Ryan AbilityLab
Visit 1: Prescreening and Baseline. Length of visit: estimated 6 hours

- Discuss the research project, complete the informed consent process and confirm eligibility
- Magnetic resonance imaging (MRI). This study uses magnetic resonance imaging (MRI) to allow investigators to look at the brain structure and function. Magnetic resonance imaging is a technique that uses magnetic fields and radio waves to make images of the brain. The scanning session will include no-task MRI scans looking at brain structure and activity while you are at rest. During the session, you simply have to lie still in the scanner while we image your brain.
- Medical history
- Demographic data
- Current medications/comorbidities
- TMS screening and neuronavigated motor mapping (to locate the area of the brain that will be stimulated by TMS in Visits 2 and 5)
- Pregnancy test for women of childbearing potential via a blood draw
- Resting 12-lead EKG

Neurobehavioral assessments - Clinical outcomes measured at the baseline and post-treatment sessions:

The neurobehavioral assessment will include the following:

- Repeatable Battery for the Neuropsychological Assessment of Status (RBANS)
    - This brief assessment evaluates various aspects of cognition, including visuospatial perception, attention, memory, and language.
- State-Trait Anxiety Inventory (STAI)

  - This self-report scale measures the intensity of anxiety.
- Ohio State University TBI (OSU TBI) Identification Method interview
  - OSU TBI is a standardized procedure for getting a person's lifetime history of TBI through a 3-5-minute structured interview.
- Finger Tapping Test
  - This test measures the rate of finger presses. It is a measure of simple motor coordination. Five to ten ten-second trials per hand are administered.
- Grooved Pegboard Test
  - This task is to rotate a peg with a groove to fit in a grooved hole. It measures motor coordination. There are 25 pegs and holes. Each hand is tested once.
- California Verbal Learning Test (CVLT-II)
  - This is a word learning test including word recall and recognition measures.
- Serial Reaction Time Task (SRTT)
  - This task is to press a key that is below a marker appearing on a monitor. It measures attention and learning.
- Word Fluency
  - This task is to produce as many words as possible that begin with a specific letter or a category. It is a measure of the ability to retrieve words from semantic memory.
- Trail Making Test (TMT) – Parts A & B
  - This task is to draw a line between circles containing numbers and/or letters in ascending order. It measures various aspects of cognition including attention, visual search, motor coordination, reasoning, and task-switching.
- Effort Expenditure for Rewards Task (EEfRT)
  - This task is to make a series of choices between an easier, low-reward option and a harder, high-reward option. It is a measure of motivation.
- Beck Depression Inventory (BDI-II)
  - This scale measures the amount of self-reported depressive symptoms.

These tests provide a brief general evaluation of cognitive functions (RBANS), memory (CVLT-II, SRTT, Word Fluency), executive functions (TMT, EEfRT), and emotional wellbeing (BDI-II, STAI).

We will also administer two motor tasks (Finger Tapping Test and Grooved Pegboard Test) for comparison purposes with ongoing AIH studies in patients with spinal cord injury and stroke after each session along with a visual analogue scale so you can tell us how your mood is at the moment, and a brief word recall test using items from the Rey Auditory Verbal Learning Test.

Visit 2: 21% oxygen, that is, the same exposure as normal room air. Intervention Day 1, Length of visit: estimated 2 hours

- Hypoxia session with 21% oxygen: 30 minutes
- Outcome assessments – as above plus the measurement of motor evoked potentials (TMS)
- Monitoring assessments – taking your vital signs - We will measure your blood pressure, SpO2 (the amount of oxygen in your blood), and pulse rate during the hypoxia session.

Visit 3: 17% oxygen. Intervention Day 4, Length of visit: estimated 2 hours

- Hypoxia session with 17% oxygen: 30 minutes
- Outcome assessments – as above
- Monitoring assessments – as above

Visit 4: 13% oxygen. Intervention Day 8, Length of visit: estimated 2 hours

- Hypoxia session with 13% oxygen: 30 minutes
- Outcome assessments – as above
- Monitoring assessments – as above

Visit 5: 9% oxygen. Invervention Day 12, Length of visit: estimated 2 hours

- Hypoxia session with 9% oxygen: 30 minutes
- Outcome assessments – as above plus the measurement of motor evoked potentials (TMS)
- Monitoring assessments – as above

Visit 6: Post-Treatment Evaluation Day 16, Length of visit: estimated 4 hours

- Discuss the research project
- Magnetic resonance imaging (MRI), same as in Visit 1.
- Medical history – update baseline evaluation
- Current medications/comorbidities – update baseline evaluation
- Neurobehavioral assessments – see baseline evaluation (minus the STAI and OSU TBI assessments).

(Note: The time interval between interventions is subject to modification, depending on the staff and equipment availability, as well as your scheduling preferences. The above visit lengths are our best time estimations given the procedures involved in each. The actual amount of time for each visit will vary from participant to participant, to account for variations including waiting room time, transport time from one procedure to another, and other timing aspects of the study that cannot, by nature, be set.)

We would like to audio or video record the neurobehavioral assessment sessions during Visits 1 and 6. The recording is optional. If you agree to it, you will be asked to indicate so at the end of this consent form.

<u>Outcome assessments:</u>

Monitoring assessments (carried out throughout the hypoxia administration):

- Vitals: We will measure your blood pressure, SpO2 (the amount of oxygen in your blood), and pulse rate.
- Symptom checklist: A simple yes/no subjective checklist of physical symtpoms, such as chest pain, lightheadedness, etc., will be administered to you at 2, 6, 14, 24 and 30 minutes from the beginning of the hypoxia session.

Other outcome assessments before and afterAIH session #1 (Visit 2) and #4 (Visit 5):

- Single-pulse assessment of motor cortical functioning using transcranial magnetic stimulation and electromyographic recording

Transcranial Magnetic Stimulation (TMS) and electromyography (EMG): TMS sends a magnetic pulse into your brain and stimulates brain activity. A wire TMS coil will be placed over your head. The TMS coil will be held to your head with a custom coil holder. To limit head movement, your head will be secured to the headrest with a soft Velcro strap. When the TMS is activated, this coil will generate a magnetic pulse over a specified area of your brain that is responsible for hand movement. When the pulse occurs, you will hear a click or a snapping sound, and you may feel a pulling sensation on your skin underneath the area where the coil is placed. You may also feel a light twitch of the muscles in your arm, face or your leg. During Visit 1, we will do motor mapping, that is, we will use neuronavigation to find the area of your brain that activates the target muscle of your hand. During Visits 2 and 5 we will apply a series of TMS pulses to this brain area to evoke EMG responses from the target muscle. During the EMG recording, you will be comfortably positioned so that your arms are supported by a testing device. Recording electrodes will be attached to the surface of your skin over the target hand muscle to record its activity.

Exclusion criteria for TBI patients for TMS and EMG:

- Uncontrolled medical problems affecting the lungs (pulmonary diseases), the heart (cardiovascular diseases) or the muscuskeletal system (orthopedic diseases)
- Pre-injury, ongoing major depression or psychosis, altered cognitive status
- History of stroke
- Metal in head (e.g., surgical clips, shrapnel)
- History of seizures or epilepsy diagnosis
- Receiving drugs acting primarily on the central nervous system, which lower the seizure threshold such as antipsychotic drugs (chlorpromazine, clozapine) or tricyclic antidepressant
-
- Surgery to your head
- Any non-TBI-related neurological diseases
- Illnesses that may have caused brain injury
- Unexplained frequent or severe headaches
- Pregnancy in females
- Implanted devices (e.g., pacemakers, medical pumps, brain stimulators)

You will not be eligible to participate in the study if you are not eligible to undergo MRI or TMS.

What are my responsibilities if I take part in this research?
If you take part in this research, you will be responsible to arrive at the Shirley Ryan AbilityLab for all the scheduled visits and complete the research procedures with the staff.

What happens if I say "Yes", but I change my mind later?
You can leave the research at any time; it will not be held against you. If you decide to leave the research, contact the investigator.

Choosing not to be in this study or to stop being in this study will not result in any penalty to you or loss of benefit to which you are entitled. Specifically, your choice not to be in this study will not negatively affect your right to any present or future medical treatment.

Detailed Risks: Is there any way being in this study could be bad for me?

Cardiac adverse events could be silent (non-symptomatic) or non-silent (symptomatic).

In the event of a silent event subjects will be given a referral to a cardiologist at Northwestern Memorial Hospital to undergo more comprehensive assessments to confirm the severity of the adverse event.

A non-silent event will require the ROC (resident on-call) to be called to the clinic room for immediate and urgent attention for the chest pain experienced. The medical professional will decide the best care plan for you, which could include a trip to the emergency room.

A cerebral adverse event is experienced through a change in the neurological assessment, such as blurred vision or slurred speech. The Medical monitor will be informed of this outcome and will provide advice accordingly, which may result in being withdrawn from this research study for your own safety.

Breathing low levels of oxygen causes low levels of oxygen in the blood. Some of the side effects could include lightheaded sensation, dizziness, reduced vision, feeling short of breath, abnormally elevated heart rate, euphoria, and fainting. An additional risk to wearing a mask in individuals afraid of enclosed spaces (claustrophobic) is discomfort, anxiety or panic. We will monitor for these side effects, as well as monitor your vital signs to assure your safety during the session. In the event of an emergency, you will be withdrawn from the breathing apparatus, and the resident physician on-call will be notified for further management. There may also be other side effects that we cannot predict, or there may be risks that may hurt you in ways that are unknown. These may be a minor inconvenience or so severe as to cause death.

Some people cannot have an MRI because they have certain type of metal in their body. For instance, if you have a heart pacemaker, artificial heart valves, metal implants such as metal ear implants, bullet pieces, chemotherapy or insulin pumps or other metal such as metal clips or rings, you may not be allowed to have an MRI. If you are claustrophobic, lying in a small closed area inside a large magnetic tube during this test may feel uncomfortable. The MRI scanner makes loud banging noises while taking a measurement, so either ear plugs or specially designed headphones will be used to reduce the noise.

Some people experience temporary discomfort from undergoing TMS including mild headaches and/or tingling or twitching sensation in the scalp, jaw, or face. Seizures are a rare, but possible risk from undergoing TMS.

The primary risks associated with participation in neurobehavioral assessments are boredom, tiredness, and/or frustration. These risks are more likely to occur if you feel that you are not performing well on certain tasks. We will try to minimize these risks by offering you frequent rest breaks and providing positive feedback and encouragement.

A feeling of suffocation or breathlessness during Acute Intermittent Hypoxia is a rare but possible adverse event.

An additional research-related risk involves the use of your identifiable, personal information, and there is a chance that a loss of confidentiality could occur. The researchers have procedures in place to reduce the possibility of this happening. (See the section below titled: “**What happens to the information collected for the research?**”).

For any additional concerns, the number of the researcher is listed in the section **“Who can I talk to?”**

There may be some unknown or unanticipated risks or discomforts, because some of the procedures are relatively new and are attempts to advance medical knowledge. Every known precaution will be taken to ensure your personal safety and to minimize discomfort.

The possible risks associated with blood drawing are pain, bleeding, a rare risk of fainting, bruising, infection and/or hematoma (blood clot under the skin) at the injection site. If you develop bruising, this will go away after a couple of days and can be treated with warm compresses and/or medication.

What do I need to know about reproductive health and/or sexual activity if I am in this study?

The effect of this study’s breathing intervention on human sperm and eggs has not been studied. The effects on the developing fetus during breathing low oxygen levels during

pregnancy and the risk of birth defects are also unknown. Therefore, both men and women should not attempt pregnancy and women should not be pregnant or breast-feeding while taking part in this study.

If you are sexually active, both men and women should use at least one effective means of birth control while participating in this research study. According to the World Health Organization and the United States Center for Disease Control and Prevention, the most effective forms of birth control include complete abstinence, surgical sterilization (both male and female), intrauterine devices (IUDs), and the contraceptive implant. The next most effective forms of birth control include injectable, oral contraceptive pills, the contraceptive ring, or the contraceptive patch. Acceptable but least effective methods of birth control include male condoms (with or without spermicide) and female condoms. If you or your partner become pregnant while participating in this research study or for a week after you complete the study, it is important that you tell the study doctor or other research team member immediately. You may be required to stop participation in this study; however, other treatment options will be discussed with you at that time if necessary.

If you or your partner [are/is] considered to be postmenopausal, you are not required to use contraception while participating in this research study. Postmenopausal women rarely become pregnant.

Will it cost me anything to participate in this research study?
Taking part in this research study will be of no cost to you. All visits are entirely research-related and will not result in a cost to you or your insurance.

What happens to the information collected for the research?
Efforts will be made to limit the use and disclosure of your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the following:

- IRB and other representatives of this institution
- The study sponsor, monitors, auditors
- The Northwestern University Office for Research Integrity
- The US Office of Research Integrity (ORI)
- The US Office for the Protection of Human Research Protections (OHRP)
- The US Food and Drug Administration (FDA)

Any of these entities may be granted direct access to your medical records to conduct and oversee the research. By signing this document, you are authorizing this access. We may publish the results of this research. However, we will keep your name and other identifying information confidential.

If we learn about current or ongoing child [or elder] abuse or neglect, we may be required or permitted by law or policy to report this information to authorities.

Data Sharing
De-identified data from this study may be shared with the research community at large to advance science and health. We will remove or code any personal information that could identify you before files are shared with other researchers to ensure that, by current scientific standards and known methods, no one will be able to identify you from the information we share. Despite these measures, we cannot guarantee anonymity of your personal data.

Can I be removed from the research without my OK?
The person in charge of the research study, Jordan Grafman, Ph.D., can remove you from the research study without your approval. Possible reasons for removal include inability to comply with study requirements and/or appointments.

We will tell you about any new information that may affect your health, welfare, or choice to stay in the research.

What else do I need to know?
If you become ill or are injured as a result of this study (medications, devices or procedures), you should seek medical treatment through your doctor or treatment center of choice. You should promptly tell the study doctor about any illness or injury.

The hospital [university, researchers] will not pay for medical care required because of a bad outcome resulting from your participation in this research study. This does not keep you from seeking to be paid back for care required because of a bad outcome.

You will receive $20 per hour, that includes compensation for the treatment sessions, the baseline and the post-treatment evaluations. Any partial hours completed will be compensated at a prorated rate of $5 per 15 minutes. The Shirley Ryan AbilityLab will issue you a ClinCard, which is a specially designed debit card for clinical research. Once a visit that qualifies for compensation is completed, funds will be approved and loaded onto your card. The funds will be available within 1 day after being loaded and can be used at your discretion. You will be issued one card for the duration of your participation. If your card is lost or stolen, please call (866) 952-3795 or ask a coordinator for a replacement ClinCard.

Fees are incurred if used at an ATM (fees vary by location). However, if the card is used for in-store or online purchases via credit or debit, there are no associated fees and no expiration date.

Please be advised: Inactivity on the card for more than 3 months will incur a monthly fee. However, as long as there is activity on the card within 3 months (funds are added or a transaction is completed), the month period will reset and no monthly fee will be applied.

If you do incur a monthly fee, please contact Greenphire Support at the number on the back of your card and they will reverse the fee. See “Tips for Using the Attached ClinCard” for more information.

The Finance Department at the Shirley Ryan AbilityLab will be provided with your information, including your Social Security Number, in order to issue payment for your study participation. Study payments are considered taxable income and reportable to the Internal Revenue Service (IRS). An IRS Form 1099 will be sent to you if your total payments are $600 or more in a calendar year.

You may be given access to new inventions that are being developed by the investigator, the study sponsor, or other people involved in the study. Certain laws can make it harder to obtain legal protection for a new invention shared with a study participant, unless the study participant agrees to keep information about the invention confidential. You agree to keep confidential information you may receive about new inventions, such as new drugs, new devices, or new methods.

A description of this clinical trial will be available on https://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health (NIH). The researchers can use this Certificate to legally refuse to disclose information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings; for example, if there is a court subpoena. The researchers will use the Certificate to resist any demands for information that would identify you. You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, medical care provider, or other person obtains your written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

HIPAA Authorization

We are committed to respect your privacy and to keep your personal information confidential. When choosing to take part in this study, you are giving us the permission to use your personal health information that includes health information in your medical records and information that can identify you. For example, personal health information may include your name, address, phone number or social security number. Your health information we may collect and use for this research includes:

- Results of physical and other medical examinations related to your TBI
- Medical history from self-report and medical records
- Lab tests, or certain health information indicating or relating to a particular condition, as well as diaries and questionnaires
- Records about study medication or drugs
- Certain demographic information like age, sex, education level

The following clinical providers may give the researchers information about you: all current and previous health care providers, including but not limited to the Shirley Ryan AbilityLab (SRALAB), Northwestern Medical Group (NMG), Northwestern Memorial Hospital (NMH), and Northwestern Lake Forest Hospital (NLFH).

Once we have the health information listed above, we may share some of this information with the following offices or entities outside of Northwestern University and its clinical partners (or affiliates): the Northwestern University Institutional Review Board Office and Office for Research Integrity; the US Office of Research Integrity; the US Office for Human Research Protections; the US Food and Drug Administration.

Any research information shared with outside entities will not contain your name, address, telephone or social security number or any other personal identifier unless disclosure of the identifier is necessary for review by such parties or is required by law or University policy [except that such information may be viewed by the Study sponsor and its partners or contractors at the Principal Investigator's office].

The following entities may receive your health information:

- Authorized members of the Northwestern University and the Shirley Ryan AbilityLab workforce, who may need to see your information, such as administrative staff members from the Office for Research, Office for Research Integrity and members of the Institutional Review Board.
- Clinical affiliates, including but not limited to the Shirley Ryan AbilityLab (SRALAB), Northwestern Medical Group (NMG), Northwestern Memorial Hospital (NMH), Northwestern Lake Forest Hospital (NLFH), and the Ann & Robert H. Lurie Children's Hospital of Chicago (Lurie Children's). Your participation in this clinical trial may be tracked in an electronic database and

may be seen by investigators running other trials that you are enrolled in and by your healthcare providers.
- Clinical affiliates, including but not limited to Northwestern Medical Group (NMG), Northwestern Memorial Hospital (NMH), and Northwestern Lake Forest Hospital (NLFH), for purposes including, but not limited to, the affiliate's provision of care to you and/or the affiliate's scheduling of appointments and/or billing activities.
- Other University research centers and University contractors who are also working on the study.
- Government agencies and public health authorities, such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (DHHS).

Those persons who get your health information may not be required by Federal privacy laws (such as the Privacy Rule) to protect it. Some of those persons may be able to share your information with others without your separate permission.

The results of this study may also be used for teaching, publications, or for presentation at scientific meetings.

Unless you revoke your consent, it will expire on January 1st, 2024.

Although you may revoke consent to participation in this research at any time and in any format, you must revoke authorization for use or disclosure of your health information in writing. To revoke your authorization, write to:

PI's Name: Jordan Grafman, Ph.D.
Address: Cognitive Neuroscience Lab
Think and Speak Lab, 25th Floor
Shirley Ryan AbilityLab
355 E. Erie Street
Chicago, Illinois 60611-5146

You do not have to authorize the use or disclosure of your health information; however, you will not be allowed to take part in this research study unless you do so. If you do not authorize the use or disclosure of your health information, it will not affect your treatment by health care providers, or the payment or enrollment in any healthcare plans, or affect your eligibility for benefits.

Optional Elements:
The following research activities are optional, meaning that you do not have to agree to them in order to participate in the research study. Please indicate your willingness to participate in these optional activities by placing your initials next to each activity.

I agree	disagree	
_____	_____	The researcher may audio or video record me to aid with data analysis. The researcher will not share these recordings with anyone outside of the immediate study team.
_____	_____	The researcher may audio or video record me for use in scholarly presentations or publications. These recordings will be permanently available in print or electronic format (i.e., the internet). My identity may be shared as part of this activity, although the researcher will attempt to limit such identification.
_____	_____	The researcher may contact me in the future to see whether I am interested in participating in other research studies by the Principal Investigator of this study.

Signature Block for Capable Adult:

Your signature documents your permission to take part in this research. You will be provided a copy of this signed document.

_____ _____
Signature of Participant Date

_____
Printed Name of Participant

_____ _____
Signature of Person Obtaining Consent Date

_____
Printed Name of Person Obtaining Consent