Protocol

1. Project Title

Effects of acute intermittent hypoxia on sensory function in healthy adults

2. Investigator(s):

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3. Abstract:

Exposure to acute intermittent hypoxia (AIH) has safely induced facilitation of motor output in human studies. Despite this study, the affects of AIH on sensory function has not been established. We will use a commercially-available hypoxicator that can be programmed to achieve a targeted level of arterial oxygen saturation. We plan to compare 3 previously published protocols that have generated motor facilitation (and a sham condition) to characterize and effects on sensory function in healthy adults. Primary outcomes will be measures of sensory function (thresholds and tolerance).

4. Background:

Brief intermittent episodes of hypoxia (or acute intermittent hypoxia [AIH]) induce plasticity in muscle motor neurons [1]. AIH activates carotid chemo afferents that stimulate episodic serotonin release [2, 3] which subsequently strengthens synaptic input and motor output of motor nuclei [3-8]. The persistent increase in phrenic nerve burst amplitude following repeated exposure to AIH is termed long-term facilitation (LTF) [9]. For example, increases in breathing capacity as demonstrated by a significant increase in minute volume has been demonstrated after exposure to AIH in unanesthesized and anesthetized rats [10, 11].

AIH has been demonstrated to be safe in healthy persons and has been routinely used in training athletes for many years. Similarly AIH is safe for people with neurological dysfunction (eg patients with chronic spinal cord injury (SCI)). In these patients, a single exposure to IH increased ankle plantar flexion torque by more than 80% [12]. Significant improvements in 10 meter walk times and 6 minute walk distances have been seen in patients with chronic SCI when IH was combined with walking at maximal sustainable exertion for 30 minutes [13].

Despite this promising work, the effect of AIH on sensory function has not been determined in humans, making this proposal a logical progression of the promising work done to date in improving motor function.

5. Specific Aims:

Our aim is to characterize how exposure to AIH protocols shown to enhance motor function impacts sensory function.

6. Research Plan:

Participants who meet the eligibility criteria and provide written consent to participate will be enrolled. This includes a pregnancy test for female volunteers that will be performed on the first day. Each participant will complete 3 different protocols of AIH delivery based on protocols derived from the literature that have been used to enhance motor function as well as a sham condition. Each delivery and testing session will last approximately 2 hours and be separated by at least 24 hours.

Inclusion criteria

Male and female participants between the ages of 18-40 years will be eligible to participate in the study unless they meet any of the following criteria.

Exclusion criteria

a. Diagnosis of cardiovascular disease (Hypertension, arrhythmias, coronary artery disease, congenital and valvar heart diseases)
b. Diagnosis of neuromuscular disease
c. Diagnosis of any neurological disease
d. Presence of concurrent medical illness including infection, fractures
e. Diagnosis of obstructive sleep apnea
f. Diagnosis of obstructive/restrictive lung disease
g. Diagnosis of exercise induced asthma
h. FEV1/FVC<80% and/or FVC<80% of predicted value indicating airway obstruction
i. Subjects on prednisolone therapy or selective serotonin reuptake inhibitor (SSRI) therapy will be excluded from the study as these pharmacological agents are known to amplify the effects of IH[22, 23]
j. Diagnosis of epilepsy or history of seizures and attention deficit disorders
k. Pregnancy
l. Diabetes
m. History of coagulation disorders
n. History of chronic pain
o. Body mass index(BMI)> 35kg/m2
p. Subjects on prescription medicines such as beta blockers and other drugs that are prescribed in any of the exclusionary disorders listed above.

Intervention

We will use a commercially-available hypoxicator that can be programmed to achieve a targeted level of arterial oxygen saturation. The fraction of inspired oxygen (FiO2) during the hypoxic episodes will be adjusted to maintain pulse oximetry (SPO2) between 80%-

90%. In our experience, this can be achieved with a FiO2 in the range of 9%-11 %. FiO2 levels of 9% and 11% correspond to O2 concentrations in room air at altitudes of 6500 meters and 5000 meters respectively, both altitudes humans are exposed to while on trekking expeditions and also have permanent human habitation [29, 30]. We will use alternating phases of hypoxia (FiO2 :9%-11%) and normoxic or hyperoxic oxygen saturation with an FiO2 between 22%-38%,based on the previous protocols used in human studies of motor function.

A. 15 bouts of hypoxia for 1 minute, normoxia to hyperoxia 1 minute (Trumbower et al [12])
B. 15 bouts of hypoxia for 2 minutes, normoxia to hyperoxia 1 minute (Hayes et al [13])
C. 8 bouts of hypoxia for 2 minutes, normoxia to hyperoxia 1 minute (Tester et al [14])
D. Sham hyperoxia (normoxia) for 2 minutes, normoxia to hyperoxia 1 minute

The order of the protocol will be randomly assigned for each participant who will remain blinded to the actual protocol they are receiving. All measures will be collected by a research assistant blinded to protocol assignment.

Outcomes

We propose to collect standardized measures of sensory function. Measures will assessed at the hand and foot at baseline, immediately post- and every 10 minutes for 60 minutes after the AIH protocol.

Tactile:

Semmes-Weinstein filaments will be used to assess sensory thresholds. The patient in will be in sitting position with shoes and socks removed. Starting with the weakest plastic kept perpendicular to the skin surface force will slowly be applied until there is a slight bend in the filament. The participant will indicate if they feel pressure. If not, the next filament is used and testing proceeds until the thinnest fiber that the participant can perceive is reached.

Pain sensitivity:

Participants will undergo quantitative sensory testing (QST) to determine pain sensitivity to thermal and pressure stimuli, as per protocols in our preliminary studies.

Study participants will undergo standard psychophysical pain testing using a contact thermode to deliver the evoked, thermal pain stimuli. The stimuli are to be applied by a research assistant who ensures proper thermode application and the range of stimulus intensities to be used (40-51°C) will be presented beforehand in ascending one-degree steps to each subject. We have found this procedure to be useful because it familiarizes subjects with the stimulus range, tends to obviate range effects in psychophysical scaling, and helps alleviate subject anxiety about the upper limit of stimulus intensities to be used. In order to standardize the scaling instructions and to clarify the distinction between the sensory intensity and affective dimensions of pain, a standardized instructional set will be used for all subjects. The research assistant will record numeric rating scale (NRS) response to each stimulus. The NRS will consist of a scale whose endpoints are

designated as '0 - no pain sensation' and '100 - the most intense pain sensation imaginable'. In order to standardize the scaling instructions, standard instructions will be used for all participants.

Threshold and Tolerance. A continuous heat stimulus will be delivered to the subjects' dominant arm. The stimulus started at 35°C and will be increased at a rate of 0.5°C with subjects terminating the stimulus when the temperature reached pain threshold ("when the sensation first transitions from heat to pain") and tolerance ("when the sensation becomes so strong you want to remove it from your skin"). These procedures will be repeated three times and the average threshold and tolerance will be calculated.

Threshold and tolerance measures will also be collected using mechanical pressure applied using a Fischer Dolorimeter (Pain Diagnostics, Great Neck, NY). The tip of the dolorimeter is equipped with a rubber foot-plate of 1-cm diameter. During Dolorimeter testing, force is slowly increased until the subject indicates that the sensation changes from pressure to pain. Subjects are asked to rate any pain they are experiencing using a numeric rating scale (NRS) anchored at 0 (no pain sensation at all) and 100 (worst pain imaginable). Dolorimetry measures are made in the hand (1st dorsal interosseus muscle) and between the first and second toe on the dorsal aspect of the foot.

Temporal summation. A train of 6 heat pulses will be applied to the glabrous skin of the foot. To ensure temporal summation, an inter-stimulus interval of 2 seconds will be used with temperatures starting at 39°C and increasing to 50°C (the TSA medoc maintains 10°C/sec rate in this range which is sufficient for maintaining the desired inter-stimulus interval). The participants will be asked to rate the magnitude of their delayed (second) pain sensation following each heat pulse. These response ratings are believed to be primarily C-fiber mediated.35,36

We will test effects protocol using a two way (protocol and time) analysis of covariance with sensory measures as the dependent variables. Demographic variables that show an association with the sensory measures in our sample (eg gender differences have mixed evidence related to differences in sensory report) will be included as covariates.

7. Possible Discomforts and Risks:

Evoked pain: Risk of injury with this testing is minimal in that they are widely used and safe procedures. While pain is produced, risk to the individual is minimal, because 1) the pain is transient in nature and generally subsides immediately after the procedure; 2) subjects are instructed that they may stop any procedure at any time with no adverse consequences; 3) the level of pain experienced by subjects is below their tolerance level; 4) this pain will only last as long as the stimulus is applied (which is under the control of the study participant); 5) thermal stimulus intensity will not cause any damage to the study participant's skin. Some study participants will feel pain after the painful stimulus is removed, but no long-lasting pain perception (i.e. longer than 90s) is expected from quantitative sensory testing. Similarly a slight risk of burn is involved using thermal stimuli. The thermal stimulator

(Medoc TSA) has a built in shut of threshold that prevents the delivery of temperatures over 51 deg C. Our group has completed more than 1000 testing sessions in the past 5 years without any adverse events.

Acute intermittent hypoxia: Our experiment is modeled from past human studies that have delivered similar doses of hypoxia that was considered safe and sufficient to elicit an increase in physiological response [12, 13, 14]. Moderate reductions in inspired oxygen can cause light- headedness, dizziness, reduced vision, and euphoria. We will continuously monitor the subject's heart rate, respiratory rate, and oxygen saturation (SPO2) to ensure that the administered oxygen levels remain within the targeted range (80- 85%). In an unlikely circumstance where in the heart rate drops below 50 BPM or rises more than 150 BPM and/or systolic blood pressure drops below 85mm Hg or increases beyond 150mmHg, IH intervention will be stopped. If SPO2 falls below 75% or if subjects complain of light headedness, dizziness and reduced vision, the IH intervention will be terminated and supplemental O2 will be provided. In addition, the hypoxicator has a built in, feedback based, safety mechanism. In a circumstance of desaturation below a pre-set threshold of 80%, the hypoxicator switches to deliver hyperoxic air to increase saturation above the minimum set threshold of 80%.

8. Possible Benefits:

There is no direct benefit expected for any of the participants. Potential future benefits to the public include an increased knowledge of the effects of IH, which may eventually lead to the future treatments for neuromotor diseases.

9. Conflict of Interest:

We declare no conflicts of interest.

References

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