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Jen Botezat December 9, 2013

Repetitive Intermittent Hypoxia Improves Hand Function in Humans With Incomplete Spinal Cord Injury

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Abstract

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Spinal cord injury severs connections between the brain and spinal cord, radically impairing mobility below the level of injury. One promising strategy to increase mobility following SCI is by episodic exposures to low oxygen (repetitive acute intermittent hypoxia (rAIH)). In persons with chronic, incomplete spinal cord injury (iSCI), rAIH breathing increases lower limb muscle activation and overground walking ability. Yet whether comparable rAIH-induced recovery of hand function occurs in persons with cervical injuries remains unknown.

A randomized, double-blind, crossover design was used to test the hypothesis that rAIH (fifteen 90-second low oxygen episodes per day, 1 minute intervals; 5 consecutive days) improves hand function in persons with chronic, cervical iSCI. Six SCI subjects (6 men, 44±11 years old) received rAIH breathing. Hand function was assessed at baseline, after the first and fifth intervention days, and at follow-up (within a week from end of intervention). Primary outcomes were hand dexterity, strength, speed, and maximal opening. Using electromyograms, we also assessed magnitude of muscle activity in two hand flexors and two extensors, as well as level of co-activity between these muscles, during hand opening. Results were compared with those obtained when subjects received a sham intervention (fifteen 90-second normoxia episodes, 1 minute intervals; 5 consecutive days).

We found rAIH safe and effective at improving hand function in persons with chronic iSCI. At rAIH follow-up, participants exhibited increased manual dexterity ($p=0.001$), pinch strength ($p=0.04$), and dominant hand speed ($p=0.01$), as compared to sham. Although maximal hand opening remained similar to baseline at follow-up, finger extensor activity approached significance ($p=0.05$) in rAIH versus sham. The ratio of flexor and extensor activity indicated increased extensor contribution to hand opening ($p<0.001$) after rAIH, a strategy more reminiscent of able-bodied muscle activation. Grip strength and non-dominant hand speed did not increase significantly at rAIH follow-up over sham. Sham did not elicit changes relative to baseline in any outcome measure. We conclude that rAIH may be a useful adjuvant to current rehabilitation therapies for people with longstanding hand impairments due to chronic, cervical iSCI.

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Abstract

Spinal cord injury severs connections between the brain and spinal cord, radically impairing mobility below the level of injury. One promising strategy to increase mobility following SCI is by episodic exposures to low oxygen (repetitive acute intermittent hypoxia (rAIH)). In persons with chronic, incomplete spinal cord injury (iSCI), rAIH breathing increases lower limb muscle activation and overground walking ability. Yet whether comparable rAIH-induced recovery of hand function occurs in persons with cervical injuries remains unknown.

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reminiscent of able-bodied muscle activation. Grip strength and non-dominant hand speed did not increase significantly at rAIH follow-up over sham. Sham did not elicit changes relative to baseline in any outcome measure. We conclude that rAIH may be a useful adjuvant to current rehabilitation therapies for people with longstanding hand impairments due to chronic, cervical iSCI.

Introduction

Spinal cord injury (SCI) radically impairs mobility by disrupting communication pathways between the brain and spinal cord. While some spontaneous functional recovery occurs in the first 6 months after injury (Ditunno, 1999), these gains are limited and inadequate to restore normal function (McDonald et al., 2002). Moreover, only a few SCI therapies address persistent motor deficits (Houle and Reier, 1988; Jin et al., 2000; Coumans et al., 2001; Edgerton et al., 2006), with no cure yet developed (Houle and Tessler, 2003). There is therefore a paucity of rehabilitation options for people with chronic impairments due to SCI (chronic SCI). These people, many of whom are young adults, will live with challenges posed by their injury for many years (Furlan & Fehlings, 2009). Since most spinal cord injuries are chronic and their number continues to rise (National Spinal Cord Injury Statistical Center (NSCISC), 2013), research focused on safe, cost-effective therapies to promote long-term functional independence and quality of life is vital.

A major roadblock to independence in chronic, cervical SCI is loss of hand function. Optimal hand use permits a wide repertoire of motions including hand opening, grasping, pinching, and whole hand translation. These motions allow able-bodied individuals to perform basic social and self-care tasks (e.g., shaking hands, reaching for a doorknob, buttoning a shirt, and feeding). People with hand impairments due to cervical SCI have extreme difficulty performing such daily activities and must resort to caregiver assistance, often at a steep cost (Ditunno et al., 2000; Harvey et al., 2001). Indeed, surveys of people with cervical SCI show that regaining hand function is their greatest priority (Hanson and Franklin, 1976; Anderson, 2004) and the main factor that could improve quality of life (Snoek et al., 2004). Thus, therapies that improve hand function, particularly when there is little hope of additional spontaneous recovery,

will profoundly benefit a large percentage of the SCI population.

Few viable therapies presently exist to restore hand function in chronic, cervical SCI. Surgical nerve transfers elicit some manual function improvements (Mackinnon et al., 2012), but these procedures remain a last resort due to their invasiveness. Surface electrical stimulation of peripheral nerves also improves hand function (Beekhuizen et al., 2008), but the effect diminishes quickly after cessation of stimulation training (Conforto et al., 2002). Another motor training approach, massed practice, relies on repetitive performance of functional tasks (Hoffman and Field-Fote, 2010). Yet in SCI, three weeks of massed practice produced only minor hand function increases (Beekhuizen et al., 2008). Thus, there is a critical need for new therapies to elicit meaningful, long-term recovery of hand function.

One promising means to noninvasively facilitate motor recovery in chronic injuries is by repeat exposures to mild episodes of low oxygen (repetitive acute intermittent hypoxia, rAIH). In rodents with motor incomplete injuries, rAIH enhances neural plasticity in spared spinal pathways long after most spontaneous recovery has taken place (Golder and Mitchell, 2005; Vinit et al, 2009). Repetitive AIH is typically administered over 5-10 consecutive days in sessions lasting less than one hour a day. Each session consists of 10-20 episodes of low oxygen breathing (Fio_2= 0.09-0.11) interspersed with 1-5 minute intervals of room air breathing (Fio_2= 0.21). The physiological effect initiated by hypoxic exposure is fundamentally pattern sensitive. As long as the hypoxia is intermittent, protocols with moderately varying severity and duration of hypoxic exposures elicit a similar effect (Mahamed and Mitchell, 2007). Intermittent hypoxia is also commonly administered acutely (that is, for a short time period each day over several days). This limitation is necessary, because severe, chronic intermittent hypoxia protocols (e.g. 72 episodes per night, 7 nights; Fuller et al., 2003) elicit adverse effects. In rodents, such effects

include hypertension (Prabhakar and Kumar, 2004), learning deficits, and hippocampal cell death (Gozal and Kheirandish-Gozal, 2007). In contrast, rAIH has not been linked to adverse events or morbidity in animal or clinical studies (Lovett-Barr et al., 2012; Hayes et al., 2013).

The enhancement of spinal plasticity by rAIH was first detailed in the respiratory system of chronically injured rats (Bach and Mitchell, 1996; Baker and Mitchell, 1999). In this neural network, rAIH triggers a signaling cascade that evidently increases phrenic motor neuron output and thereby, breathing capacity (Fuller et al., 2000; Mahamed and Mitchell, 2007). Since rAIH breathing is broadly reminiscent of breathing in obstructive sleep apnea, the functional effect triggered by rAIH is thought to be compensatory, leading to respiratory stability despite disruptive stimuli in the form of apneas (Mahamed and Mitchell, 2007). Initially, hypoxia stimulates carotid body chemoreceptors (Morris et al., 1996), which are sensitive to the partial pressure of oxygen. The carotid chemoafferents respond by activating serotonergic neurons in the raphe nuclei (Bach and Mitchell, 1996). Serotonin episodically released by raphe neurons activates 5-HT receptors on spinal phrenic motoneurons (Baker-Hermann and Mitchell, 2002), triggering *de novo* intracellular synthesis of brain-derived neurotrophic factor (BDNF) within 15 minutes of hypoxia onset (Baker-Hermann et al., 2004). Newly synthesized BDNF activates its high-affinity receptor, tyrosine kinase type 2 (TrkB), which enhances phrenic motoneuron sensitivity to glutamate signaling from respiratory pre-motoneurons. This enhanced sensitivity leads to long-lasting (>1 hour) increases in phrenic motoneuron output, termed long-term facilitation (LTF; Fuller et al., 2000). LTF is manifested functionally as increased breathing capacity, the product of breathing frequency and tidal volume (Golder and Mitchell, 2005; Lovett-Barr et al., 2012; Olson et al., 2001).

Interestingly, rAIH also induces LTF-dependent plasticity in nonrespiratory motor neurons (Satriotomo et al., 2009). This outcome may be foreseeable due to broad similarities between respiratory and nonrespiratory motor systems (e.g. innervation of striated muscles, alpha motor neurons, volitional and autonomic control, serotonergic innervation). Particularly, rAIH (10 hypoxic episodes per day, 7 days) improves forelimb function in chronic, spinal-injured rats, while also increasing expression of BDNF and phosphorylated TrkB in somatic motor nuclei (Lovett-Barr et al., 2012). These concurrent functional and molecular changes suggest that rAIH promotes functional recovery by triggering similar mechanisms in somatic and phrenic motoneurons. Indeed, first-in-human studies show that in chronic SCI, one day of intermittent hypoxia (15 episodes) increases lower limb muscle activation (Trumbower et al., 2012) and over several days (repetitive; 15 episodes per day, 5 days), improves overground walking ability (Hayes et al., 2013).

Similar rAIH-induced potentiation of motor output may occur in the upper limbs of people with cervical injuries and elicit functional changes. However, no studies have addressed this possibility. Accordingly, the goal of this investigation was to quantify the effects of an rAIH protocol (15 episodes per day, 5 days) on volitional hand movement in persons with spinal cord injury. Particularly, we tested the hypothesis that rAIH improves hand function in people with chronic, cervical iSCI. We quantified six hand function measures in six chronic, cervical iSCI subjects before, during, and after rAIH. Results were compared with those obtained when subjects received a five-day sham intervention, repetitive SHAM (rSHAM), consisting of 15 daily room air episodes.

Clinical measures were hand dexterity (Box and Blocks Test), muscle strength (grip and pinch dynamometers), and hand speed (Jebsen Taylor Hand Function Test). Using 3-dimension

motion tracking cameras, we also assessed static maximal aperture, defined as the distance between the tips of the thumb and forefinger when subjects opened their hand as widely as possible. During maximal aperture production, electromyograms (EMGs) of two extensors and two flexors responsible for finger and wrist movement were recorded. While able-bodied individuals attain maximal aperture by robust extensor activation and simultaneous flexor inhibition, individuals with SCI produce decreased maximal aperture due to diminished extensor and increased flexor activation, which opposes hand opening (Stahl et al., 2012). Concurrent extensor and flexor activation (co-activity), as seen in SCI, may lead to joint stiffness (Baratta et al., 1988; Solomonow et al., 1988; Hortobagyi and DeVita, 2000). Accordingly, we tested whether rAIH alleviates these maladaptive effects of SCI using EMG recordings. We expected that rAIH would increase extensor activity, consistent with the theory that rAIH enhances motor output. We also measured flexor activity to ensure that the abnormal activation of this muscle group in SCI was not augmented by rAIH. We expected flexor/extensor co-activity to decrease following rAIH, allowing predominantly extensors to contribute to hand opening, with less opposition from flexor antagonists. We further add to existing knowledge about the safety of intermittent hypoxia use in humans. Our study supports the use of rAIH to improve hand function in people with chronic, cervical iSCI.

Methods

The Emory University Institutional Review Board approved this study. All participants gave written informed consent. The study was registered with ClinicalTrials.gov (NCT01272336).

Study Design

The design was single-center, double-blind, randomized, crossover, and counter-balanced (Figure 1). All experiments were performed at Emory University Center for Rehabilitation Medicine in Atlanta. The study consisted of two intervention rounds of 5 days each, separated by a washout period of 2 weeks. Intervention order was determined with a list randomizer and concealed from participants and clinical evaluators. Participants were not able to consistently distinguish between interventions.

Subjects

Six spinally injured subjects completed this study (6 men; mean age 44±11yrs; mean time post-SCI 18±16yrs; Table 1). Eligible individuals had spinal cord injury that was chronic (>1 year post-injury), cervical (between levels C5 and C7), and incomplete (American Spinal Injury Association (ASIA) Impairment Scale C or D; Raad, 2011). Included subjects were between the ages of 18 and 65, able to follow verbal and visual commands, demonstrated independent breathing, no joint contractures, and possessed no contraindications to electromyogram testing or passive limb movement. Ineligible subjects had progressive SCI, brain injury, previous diagnosis of obstructive sleep apnea (apnea-hypopnea index > 30; ASAA, 2013), or concurrent physical therapy sessions, to control for the possible effect of additional active processes on the nervous system. Subjects were also excluded if they were pregnant, had concurrent cardiovascular

disease, unhealed decubiti, infection, ossification, recurrent autonomic dysreflexia, and history of cardiac or pulmonary complications, due to the unknown effects of intermittent hypoxia in these conditions. Included subjects were required to maintain prescribed medications and not take serotonin-related antidepressants. We tested the most impaired hand in all outcome measures, determined by the upper extremity with the lowest ASIA motor score at initial assessment (Table 1).

Experiment Setup

Subjects received breathing interventions and performed all assessments seated upright at a table (18 cm from xiphoid process, 5 cm below xiphoid) in an adjustable chair (Figure 2). For breathing interventions, we used a hypoxic generator (Model HYP-123, Hypoxico Inc, New York, New York) to produce isocapnic oxygen mixtures of Fio_2=0.09 or Fio_2=0.21, as needed (Figure 2B; described previously in Trumbower et al., 2012). Oxygen reduction was balanced by an increase in percent nitrogen within the mixture. Subjects inhaled the gas mixtures through a non-rebreathing mask to prevent inhalation of room air or exhaled gas. Oxygen concentration was continuously tracked to ensure that inspired oxygen fraction was accurate (OM-25RME; Maxtec Inc.). The rAIH intervention lasted 37.5 minutes per day (fifteen 90-sec hypoxic episodes at Fio_2 = 0.09, 1 minute intervals) for 5 consecutive days (D1-D5). The 5-day rSHAM intervention was identical in design, except that Fio_2 was held at 0.21 (normoxia). Intervention design is depicted in Figure 3.

Safety Monitoring

We monitored subjects for adverse responses to intervention, including headaches, pain, lightheadedness, dizziness, altered vision, respiratory distress, cyanosis, spasms, or unexpected

changes in function. To guard against potential autonomic dysreflexia or detrimental effects of low oxygen, we ensured that all subjects maintained a heart rate between 40BPM and 160BPM, and systolic blood pressure between 85mmHg and 160mmHg (Table 2). Oxyhemoglobin saturation levels were also monitored for safety and remained above 75% throughout the interventions.

Protocols

Data collection

To determine the effects of rAIH on hand function, each subject received a five-day rAIH and a five-day rSHAM intervention, in random order to reduce carryover effect (Figure 1). Hand function was quantified by clinical, kinematic, and electromyogram assessments at baseline (BL; up to three days before start of intervention on Day 1), immediately after intervention on Day 1 (D1post), immediately after intervention on Day 5 (D5post), and on a follow up day (F) within 4 days after Day 5 (Figure 3).

Clinical assessment. A blinded evaluator assessed manual dexterity, strength, and speed using four clinical tests of high intrarater and interrater reliability (Figure 4; Table 3). Box & Blocks Test (Patterson Medical Holdings, Inc.) quantified manual dexterity (Platz et al., 2005) by tracking how many blocks subjects could transfer one-by-one across a partition in 60 seconds. Grip and Pinch Strength Tests (hydraulic hand dynamometers; Hand Evaluation Set 10-533, Baseline®, Irvington, New York) gauged hand strength (Burns et al., 2005; Herbison et al., 1996) when subjects squeezed or pinched the respective dynamometers. We obtained 3 grip and 3 pinch force measurements and calculated the average of the 3 trials. Jebsen-Taylor Hand Function Test (Sammons Preston, Bolingbrook, IL) consisted of seven timed sub-tasks, and the

amount of time subjects required to complete these tasks provided a measure of hand speed (Jebsen et al., 1969).

Maximal aperture. We tracked static maximal aperture of subjects, defined as the displacement between the tips of the thumb and forefinger when subjects opened their hand as widely as possible. Initially, subjects rested their hand on top of a baseball-sized wooden ball (diameter 7.62 cm) with elbow resting on the table (Figure 2A). Two infrared LED markers were attached to the tips of the index finger and thumb. The position of these two markers in space was tracked with a 0.1-mm resolution by two 3-dimension motion capture cameras (Optotrak 3020 and Certus; Northern Digital, Waterloo, Ontario). On red-to-green "go" signal from a traffic light, triggered by evaluators (Figure 2A), subjects opened their hand as widely as possible for 3 seconds, with verbal encouragement. Optotrak cameras collected marker position data at 100 Hz from 0.01 seconds before onset of the "go" signal until the end of the trial. More than ten back-to-back maximal aperture trials were recorded. We kept a trial during data collection if more than 95% of the data from each position marker was visible during data collection.

Muscle activity. To assess upper extremity muscle activity, we obtained surface electromyogram (EMG) recordings (bipolar electrodes; Model DE-2.1; Delsys USA, Inc, Boston, Massachusetts) during maximum aperture trials. Seven muscles ranging the shoulder, elbow, and wrist were recorded: anterior deltoid, biceps brachii, triceps brachii, flexor carpi ulnaris, extensor carpi ularis, extensor digitorum, and flexor digitorum. Each session began by recording maximal voluntary contractions (MVCs) of the muscles using 5 isometric joint tests (10 seconds of data collection per test): shoulder adduction, elbow flexion and extension, wrist flexion and extension. MVC data were obtained by manually resisting the respective joints in

turn, and were used to normalize EMG signals from each muscle. The maximal activity of each muscle across the five joint tests was labeled the muscle's MVC value for that session. During maximal aperture trials, EMG data was collected in synchrony with marker position data. A common clock was used to synchronize this data collection. All EMG signals were amplified 1000 times using a Bagnoli-16 system with a bandwidth of 20 to 450 Hz. A 16-bit analog-to-digital converter (NI-PCI-6289; National Instruments) was used to sample the filtered EMG signals at 2500 Hz.

Data processing

Of the seven muscles for which EMG data were collected, this study examines signals from four only: extensor carpi ularis (ECU), extensor digitorum (ED), flexor carpi ulnaris (FCU), and flexor digitorum (FD). We used this subset of muscles because they most directly regulate movement of the hand. All data were processed post-hoc using custom code in Matlab (Mathworks, Inc, Natick, Massachusetts). EMG signals were post-hoc demeaned, rectified, and low pass filtered using fourth order Butterworth filters with zero phase lag. Position marker data, collected at 100 Hz, was interpolated to 2500 Hz to match the sampling rate of the EMG system. We removed trials from analysis if position marker data was missing and if the EMG activity or maximal aperture were outlier values.

We defined maximal aperture as the maximum distance between thumb and index finger position markers during each maximal aperture trial. This value was averaged across all trials. EMG signals of each muscle during maximal aperture were normalized to the muscle's MVC for that session to obtain an EMG measure relative to maximal volitional muscle activation. Muscle activity contributing to maximal aperture was defined as a 100-ms time window of the normalized EMG signal occurring 100 ms before subjects attained maximal aperture. The EMG

data within this time window was averaged to provide a numerical value of muscle activity. This value was averaged across EMG trials. We tested the effect of rAIH on muscle activity magnitude in the four muscles of interest by obtaining muscle activity values at baseline (BL), D1post, D5post, and F (Figure 3). Particularly, changes from BL during rAIH and rSHAM were used to compare the two interventions: D1post – BL; D5post – BL; and F – BL (Figure 10).

We defined muscle co-activity as the ratio of flexor and extensor activity contributing to maximal aperture production. We used the muscle activity values obtained from EMG to quantify co-activity of flexors (FCU, FD) and extensors (ECU, ED) for maximal aperture by the formula: (Extensor EMG activity – Flexor EMG activity) / (Extensor EMG activity + Flexor EMG activity). We separately calculated hand co-activity (FD versus ED), wrist co-activity (FCU versus ECU), and total co-activity (FCU and FD versus ECU and ED). In the range of numeric values produced by the formula (-1 to 1), a co-activity value of 0 signified equal flexor and extensor activity; positive values corresponded to greater extensor than flexor activity, and negative values indicated greater flexor activity. We assessed the effect of rAIH and rSHAM on co-activity by comparing co-activity changes from BL for both interventions: D1post – BL; D5post – BL; and F – BL (Figure 11).

Statistical analysis

A repeated-measure, randomized crossover statistical design was used to test the hypothesis that 5 days of rAIH improves hand function, as determined by 6 outcome measures. All statistical analyses were performed in SPSS® 21 (IBM Inc.) using a linear mixed effects model. This model was used to handle correlated data arising from repeated measures of the same subjects over multiple days. The model also accounted for missing data points inadvertently accumulated during data collection. Fixed main effects were intervention (rAIH or

rSHAM) and day (D1, D5, and F1) and random effects were subjects. Repeated measures were hand dexterity, strength, speed, maximal aperture, magnitude of EMG activity, and flexor/extensor co-activity. In all repeated measures, collected data was normally distributed (Shapiro-Wilk Test, $p>0.05$), and no difference in variance was observed between the two intervention groups (Levene Test, $p>0.05$). These tests were performed to ensure that all assumptions of the mixed model analysis were met.

Statistical power was calculated to determine the likelihood of detecting significant intervention effects and thereby avoid false-negative errors. We obtained power using means and variability of prior, unpublished data for each tested outcome measure in SCI. We estimated that 6 subjects would provide approximately 56% power to detect a clinically important difference (6 blocks; Lang et al., 2013) in Box and Blocks performance between rAIH and rSHAM interventions at a 0.05 significant level. The relatively low power obtained for this and other outcome measures (Table 2) arises largely from the small sample size (n=6), which is typical of pilot studies (e.g. Beekhuizen and Field-Fote, 2005).

To account for large inter-subject variation in motor performance, differences relative to baseline of all repeated measures at D1, D5, and F were used in analyses. Potential statistical error due to repeated post-hoc comparisons was addressed using Bonferroni-corrected p-values. Results were considered significant at $p<0.05$ and reported as mean ± 1 standard error (SE). Where listed, confidence intervals (CI) are 95%.

Results

Subjects exposed to rAIH showed significant increases in hand dexterity, expressed as a greater number of blocks transferred in Box and Blocks Test (BBT) versus baseline (BL; Figure 5). Improvements were pronounced at D5 (95% CI 0.4-6 blocks p=0.001) and F (CI 0.04-6.0 blocks p=0.006), as compared to rSHAM. After five days of rAIH, BBT performance improved by 6.4±2.9% and remained similar at F (follow-up; 6.1±3.2%). Changes due to rSHAM were not significantly different from zero on all three testing days.

Pinch strength increased after rAIH, expressed as change in force production (kg) relative to baseline (Figure 6). Changes were significant at D1 (CI 0.001, 0.6 kg p=0.010) and F (CI -0.1, 0.6 kg p=0.040 n=5), as compared to rSHAM (Figure 6). Pinch force increased over BL after the first day of rAIH (7.6±4.6%) and remained elevated on the fifth intervention day (CI 0.03, 0.6 kg 6.8±4.7%). Pinch strength changes due to rSHAM did not differ significantly from zero. Grip strength did not change appreciably after rSHAM or rAIH, remaining similar to baseline at D1, D5, and F (Figure 7).

Hand speed, expressed as reduced time to complete the JTHFT relative to baseline, increased in the dominant hand following rAIH (Figure 8). The speed increase was evident at D1 (p=0.017) and F (p=0.01 n=5), as compared to rSHAM (Figure 8A-B). At rAIH follow-up, time to complete the test decreased significantly from baseline (CI -15.7, -3.3s), corresponding to a 14.5±5.7% hand speed increase. Speed changes due to rSHAM did not differ significantly from zero. In the non-dominant hand, speed did not change between rAIH and rSHAM at D1, D5, or F (Figure 8C-D). However, time spent on the test decreased significantly from baseline at the rAIH follow-up (CI -44.3, -6.4s). Of note, inter-subject variation in JTHFT performance was much

greater in the non-dominant than the dominant hand (Figure 8 B,D). Performance in both hands combined is noted in Figure 8E-F.

Maximal aperture (in mm) increased following rAIH (Figure 9). The increase was significant after five days of rAIH (CI 3.0, 16.0 mm p=0.031 n=5), as compared to rSHAM, and corresponded to a 6.4±1.9% increase over BL. Changes from baseline due to rSHAM were not significantly different from zero on all three testing days. Corresponding EMG activity, expressed as change in normalized EMG compared to baseline (BL; Figure 3), increased in extensors and decreased in flexors during maximal aperture production after rAIH (Figure 10). Particularly, after five days of rAIH, muscle activity increased in extensor carpi ulnaris (CI -0.13, 0.42 p=0.031) and extensor digitorum (CI 0.1, 0.4 p=0.033), as compared to rSHAM. Increased activity persisted in extensor digitorum at rAIH follow-up (CI -0.05, 0.26 p=0.05]. In contrast, flexor carpi ulnaris exhibited significantly reduced activity from baseline at rAIH follow-up (CI -0.78, -0.03). EMG activity in flexor digitorum did not differ significantly from baseline after rAIH or rSHAM (Figure 10E).

Co-activity values approached more positive values in rAIH versus rSHAM, indicating greater extensor than flexor activation during maximal aperture. After five days of rAIH, hand co-activity (ED versus FD) was significantly more positive (CI 0.1, 0.6 p=0.029) and persisted at follow-up (Figure 11A), indicating a greater ratio of extensor digitorum activity relative to flexor digitorum. Wrist co-activity (ECU versus FCU) was also significantly more positive (CI -0.1, 0.5 p=0.006), as compared to rSHAM, and persisted at follow-up (Figure 11C). Greater extensor activation is a strategy that corresponds more closely to able-bodied muscle activation. Co-activity was not significantly affected by rSHAM at any of the three assessed time points. Figure 11E shows the total flexor/extensor co-activity change (FCU and FD versus ECU and ED).

Subjects tolerated rAIH breathing without discomfort or adverse events during and after the intervention. We recorded blood pressure at baseline and throughout both interventions (5-minute intervals). Heart rate and oxyhemoglobin concentration were recorded continuously with a pulse oximeter (GE Dash 4000 Monitor, GE Healthcare, Smith Medical Inc.). We found no significant difference in systolic/diastolic blood pressure or heart rate relative to baseline during or after rAIH (Table 2). Oxyhemoglobin saturation levels dropped following rAIH by about 10% (Table 2), as compared to baseline. This drop is a common feature of intermittent hypoxia protocols and was not linked to adverse outcomes. Motor and cognitive function also did not differ from baseline following rAIH, as gauged by 1) Spinal Cord Independence Measure, and 2) Mini-Mental State Examination. These results align with previous clinical studies showing that moderate rAIH protocols are safe in humans (Trumbower et al., 2012; Hayes et al., 2013; Serebrovskaya et al., 2008).

Figures and Tables

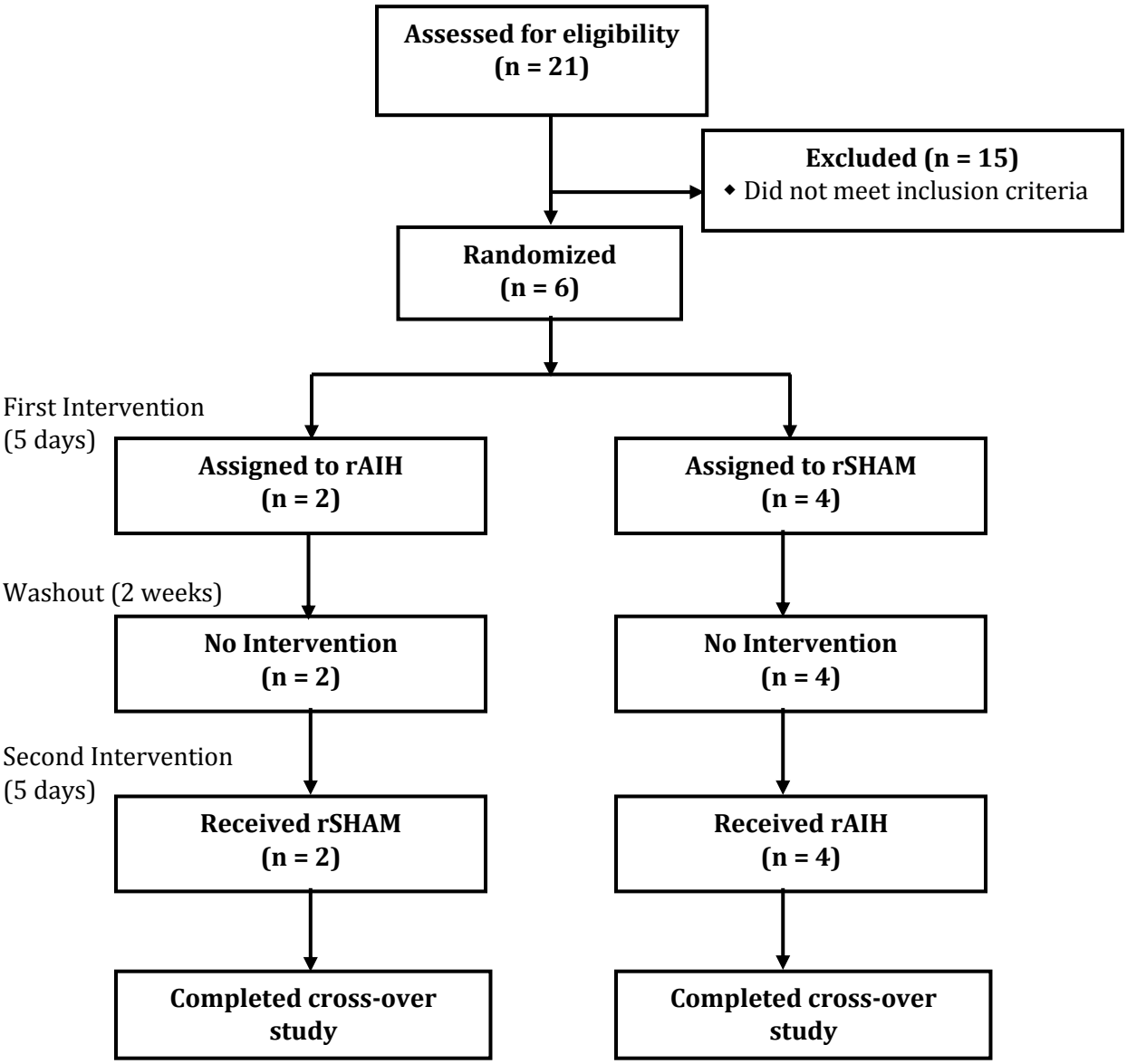


Figure 1. Consort Diagram. Of the 21 assessed subjects, 6 met all inclusion criteria. Two of these subjects were randomly assigned to receive repetitive acute intermittent hypoxia (rAIH) first, followed by repetitive SHAM (rSHAM). Four subjects received rSHAM first. Subjects were split into uneven groups (n=2 and n=4), because this was the division generated by the utilized computer number randomizer. Intervention order was varied to control for the possible effects of order on functional outcomes (e.g. task learning during rSHAM first might increase performance during rAIH).

Subject	1	2	3	4	5	6	Mean (SD)	Range
Age	54	28	44	32	48	56	44 (11)	28-56
Gender	M	M	M	M	M	M	M	M
Years since injury	39	8	7	2	15	38	18 (16)	2-39
Injury level	C5-C6	C5-C7	C6	C6-C7	C5-C6	C5-C6		C5-C7
ASIA	C	D	C	D	D	D		C-D
Dominant Hand	R	R	R	R	R	R		
Tested Hand	R	R	R	R	R	L		
Hand Motor Score (out of 25)	15	22	22	22	17	22		
Medication	Lipitor	Valium	Gabatine Colace Oxybutynin	Baclofen	N/A	Nucynta Provigil		
Assistive Devices	W/C	W/C Crutches	W/C	W/C	Cane	Crutches Cane		

Table 1. Subject demographics. All subjects were right-dominant males with chronic, cervical, incomplete spinal cord injury. While in five subjects, the dominant hand was also the most impaired (as gauged by ASIA motor score), Subject 6 had almost normal function in his dominant hand and thus his left (L) hand was assessed for motor improvements. Subjects continued taking their prescribed medications, above, consistently for the duration of data collection. These medications are not thought to significantly affect physiological processes acted upon by rAIH when taken in a consistent manner.

Abbreviations: SD, standard deviation; M, male; C, cervical; ASIA, American Spinal Cord Injury Association Impairment Scale; N/A, not applicable; W/C, wheelchair; rAIH, repetitive acute intermittent hypoxia.

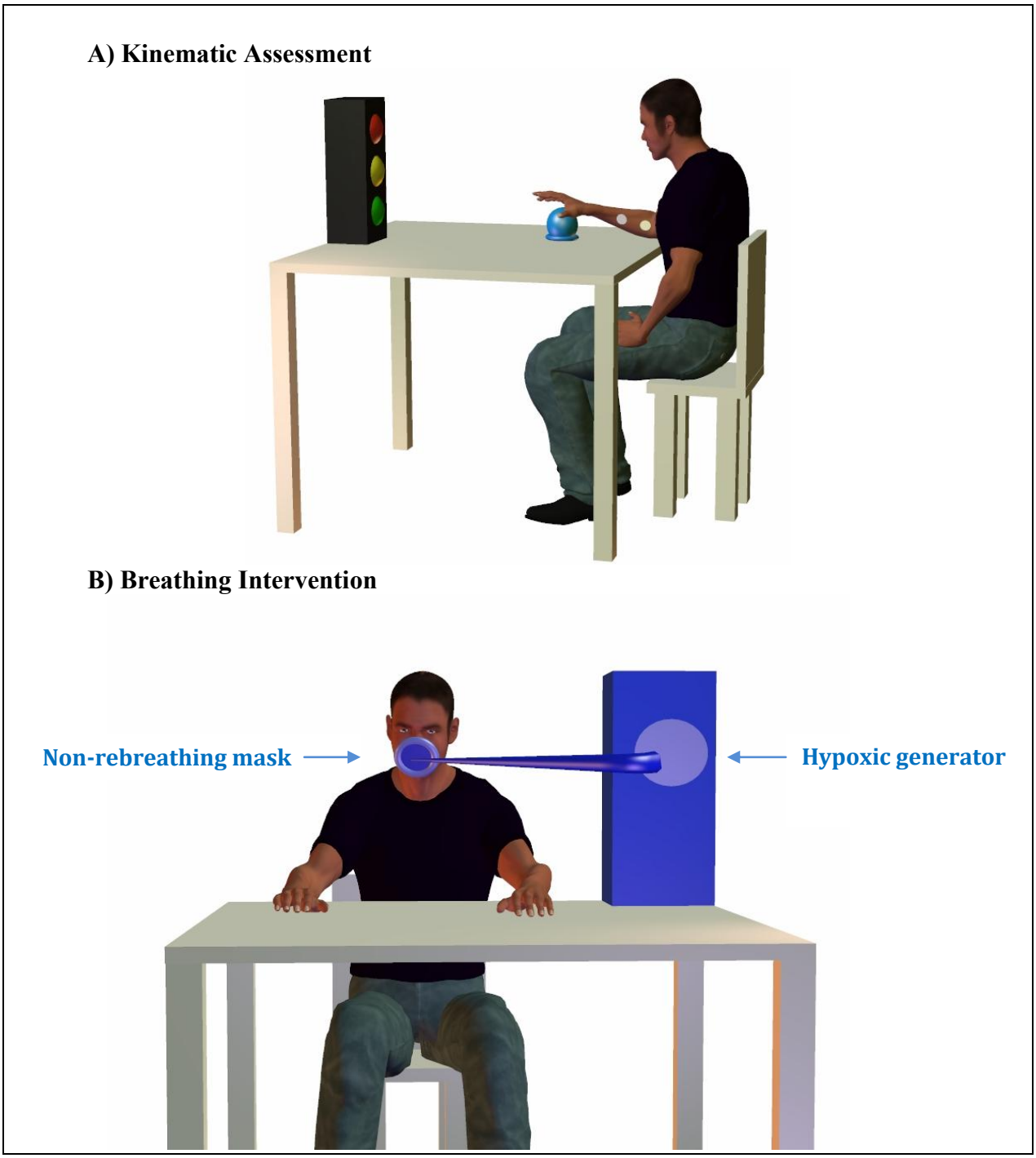


Figure 2. Experimental setup. **A)** Subject at assessment table in front of visual signal (traffic light). On red-to-green "go" signal, subject opens hand as quickly and widely as possible. Concurrent EMG recordings (white patches) and optical data from index finger and thumb markers (not visible) are recorded. **B)** Subject receives breathing interventions through mask from hypoxic generator supplying FiO_2=0.09 (repetitive acute intermittent hypoxia) or FiO_2=0.21 (repetitive SHAM), depending on the intervention round.

SAFETY INDICATORS	Days	Before rAIH Mean (SD)	Immediately After rAIH Mean (SD)	t-test p-value
Heart Rate (BPM)	1	62 (9)	65 (13)	>0.27
	2	73 (11)	67 (11)	
	3	68 (9)	72 (10)	
	4	72 (11)	65 (9)	
	5	64 (10)	67 (7)	
Blood Pressure Systolic (mm Hg)	1	113 (15)	102 (11)	> 0.21
	2	94 (22)	89 (23)	
	3	97 (24)	100 (12)	
	4	104 (32)	94 (15)	
	5	101 (18)	97 (21)	
Blood Pressure Diastolic (mm Hg)	1	67 (10)	68 (11)	> 0.37
	2	62 (14)	57 (15)	
	3	60 (13)	62 (12)	
	4	66 (21)	60 (11)	
	5	68 (13)	61 (14)	
Oxyhemoglobin saturation (%)	1	97 (4)	95 (3)	0.4
	2	96 (2)	93 (6)	0.3
	3	98 (0.9)	89 (10)	0.06
	4	95 (3)	92 (6)	0.4
	5	98 (1)	89 (8)	0.02

Table 2. Safety monitoring summary. We recorded heart rate, blood pressure, and oxyhemoglobin concentration before and immediately after rAIH. Before-intervention values were averaged across subjects. Changes due to rAIH were not significantly different from baseline for all safety indicators, except oxyhemoglobin concentration, which experienced a transient decrease of around 10%. This moderate decrease is typical of intermittent hypoxia protocols and was not linked to adverse outcomes in this or previous clinical studies.

Abbreviations: SD, standard deviation; BPM, beats per minute; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia (rAIH).

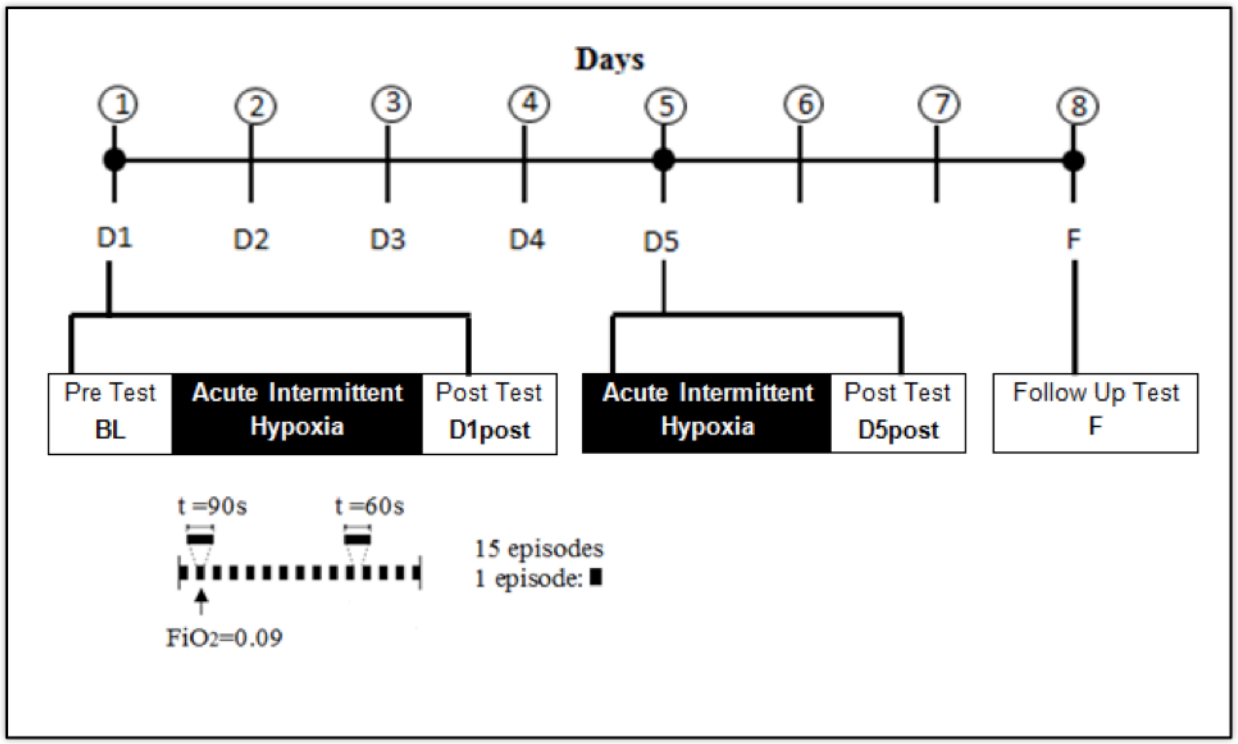


Figure 3. Repetitive acute intermittent hypoxia (rAIH) breathing and testing protocol. Repetitive AIH consists of 37.5-min (fifteen 90-second episodes of low oxygen at Fio_2 = 0.09, 1 minute intervals) for 5 consecutive days. Repetitive SHAM intervention consists of 37.5-min (fifteen 90-second episodes of normoxia at Fio_2 = 0.21, 1 minute intervals) for 5 consecutive days.

Abbreviations: BL, baseline (within 3 days before Day 1); D1-5, consecutive intervention days 1-5; D1post, immediately after intervention on Day 1; D5post, immediately after intervention of Day 5; F, follow-up (1-4 days after end of Day 5); FiO_2, fraction of inspired oxygen.

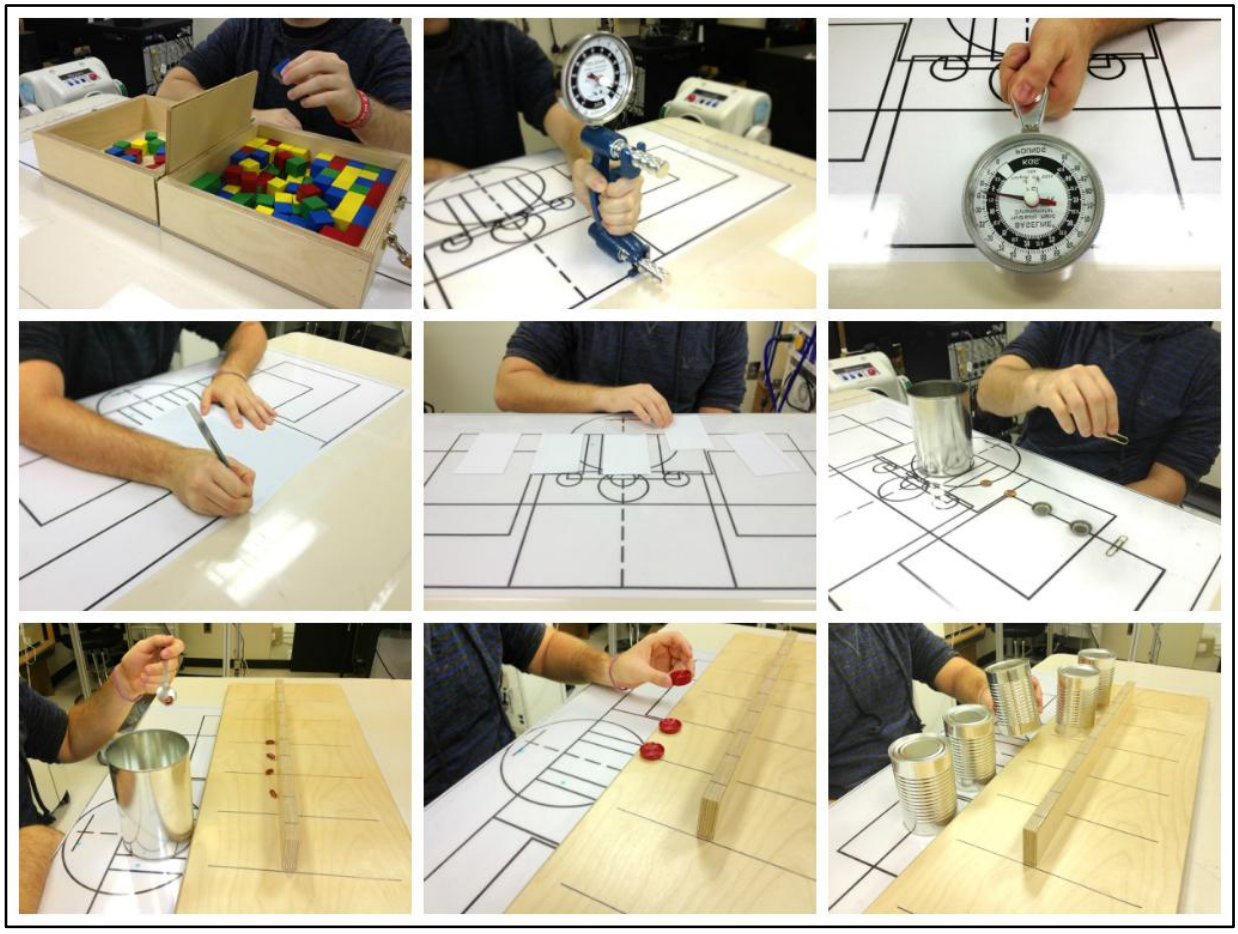


Figure 4. Clinical outcome measures. From left to right from top: 1) Box and Blocks Test, 2) Grip Strength Test, 3) Pinch Strength Test, 4) Jebsen Writing, 5) Jebsen Simulated Page Turning, 6) Jebsen Lifting Small Common Objects, 7) Jebsen Simulated Feeding, 8) Jebsen Stacking Checkers, 9) Jebsen Lifting Large Light/Heavy Objects.

SETUP	MCID	Power to detect MCID (n=6)
Box and Blocks Test of Manual Dexterity		
Subject seated before box, with hands on the table. Two layers of cubes fill left side of the partitioned box. Subject transfers blocks one-by-one across the partition. After 15 seconds of practice, subject has 60 seconds to transfer as many blocks as possible.	6 blocks[1]	56%
Grip Strength Test		
Subject grips dynamometer with palm and fingers quickly and forcefully. Maximum scale reading is recorded. Three trials, separated by 1-minute rest breaks.	5 kg, most affected limb[1]	40%
Pinch Strength Test		
Subject places gauge between thumb and index finger, pinching quickly and forcefully. Maximum scale reading is recorded. Three trials, separated by 1-minute rest breaks.	1.2 kg	52%
Jebsen-Taylor Hand Function Test		
The following tasks were timed. Subjects were instructed to complete them as quickly as possible, first with non-dominant hand, then with dominant. ***Writing:*** Subject uses standardized pen and copy paper to write an elementary sentence, typed on index card. ***Simulated Page Turning:*** Five index cards are placed in a row in front of subject. Subject turns over the cards one by one. ***Lifting Small Objects:*** Six objects (2 paper clips, 2 bottle caps, 2 US pennies) are arranged in a row by a coffee container. Subject picks up the objects one by one and tosses them into the container. ***Simulated Feeding:*** Five kidney beans are arranged in a row next to a coffee container. Subject uses regular teaspoon to scoop and drop the beans into the container one by one. ***Stacking Checkers:*** Four checkers are arranged in a row. Subject stacks checkers, one over the next. ***Lifting Large, Lightweight Objects:*** Five empty aluminum cans are placed in front of a wooden board. Subject places the cans one by one on the board. ***Lifting Large, Heavy Objects:*** Identical to previous, except that water-filled aluminum cans are used.	30 sec (7-task composite)	38%

Table 3. Clinical outcome measure descriptions. Statistical power to detect a minimal clinically important difference (MCID; minimum change necessary for meaningful functional recovery) is listed. MCID was determined in previous studies by subject quality-of-life questionnaires (references in Table 4).

[1] Data derived from individuals with limb paresis due to stroke

A)

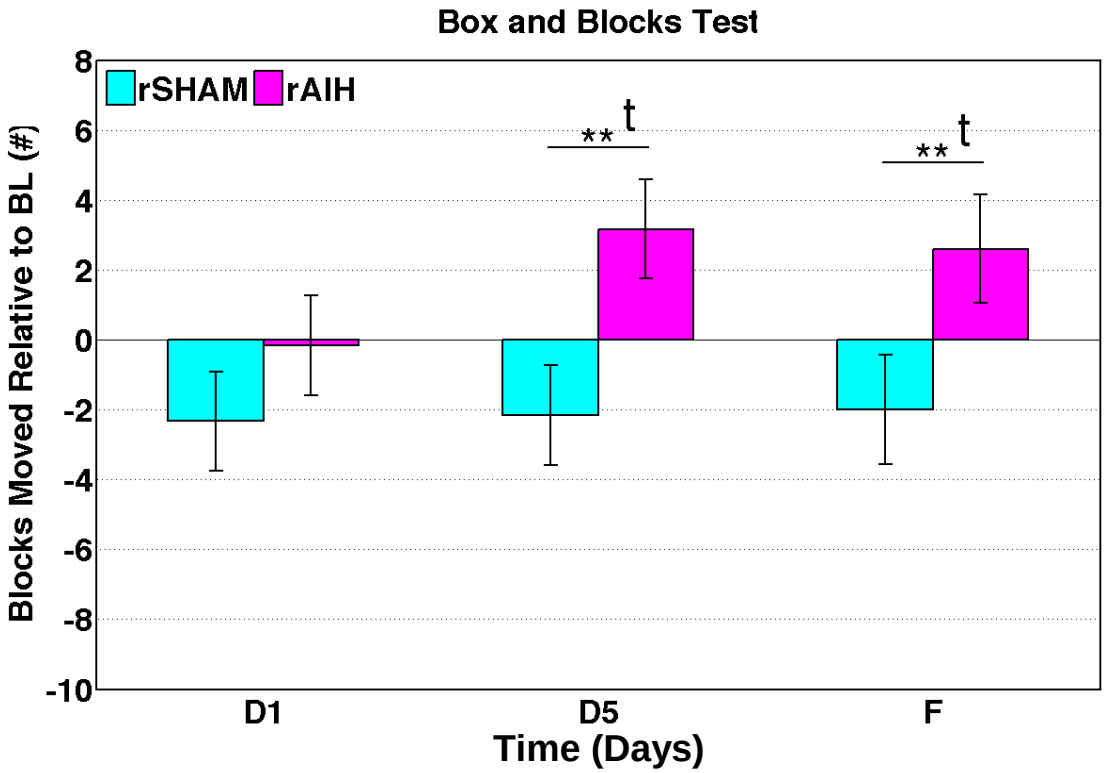
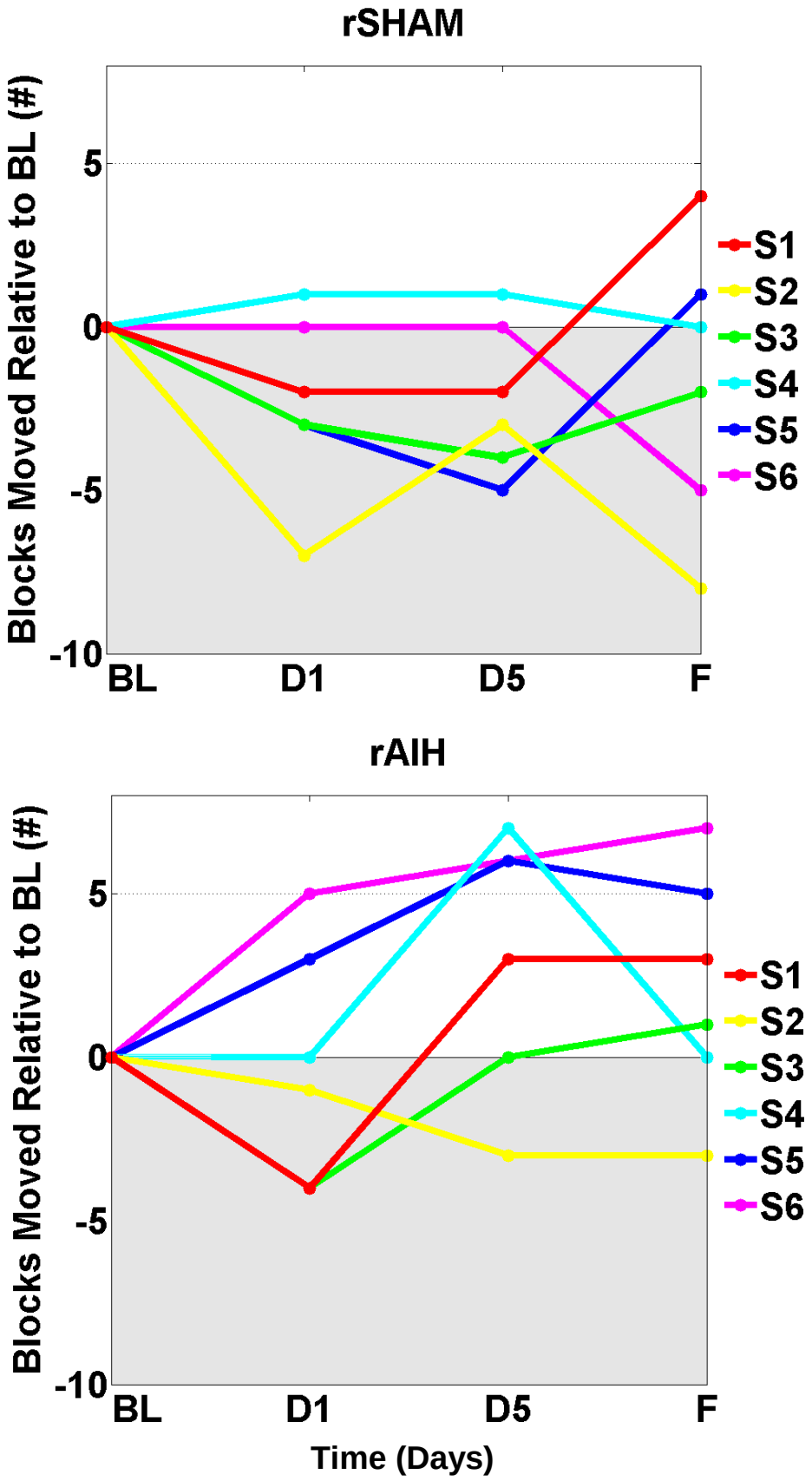


Figure 5. Box and Blocks Test outcomes. A) Average changes in test performance between rSHAM and rAIH. B) Performance changes by subject. Data are expressed as mean ± 1 SE. Comparisons are reported as changes from baseline (BL; recorded before intervention on Day 1): D1 = D1post – BL; D5 = D5post – BL; F = F – BL. ** $p \leq 0.01$, repeated measures linear mixed model; **t,** rAIH significant to baseline.

Abbreviations: D1post, after intervention on Day 1; D5post, after intervention on Day 5; F, follow-up after Day 5; S1-6, subjects; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia.

B)


rSHAM
Blocks Moved Relative to BL (#)
5
0
-5
-10
BL
D1
D5
F
S1
S2
S3
S4
S5
S6
rAIH
Blocks Moved Relative to BL (#)
5
0
-5
-10
BL
D1
D5
F
Time (Days)
S1
S2
S3
S4
S5
S6

A)

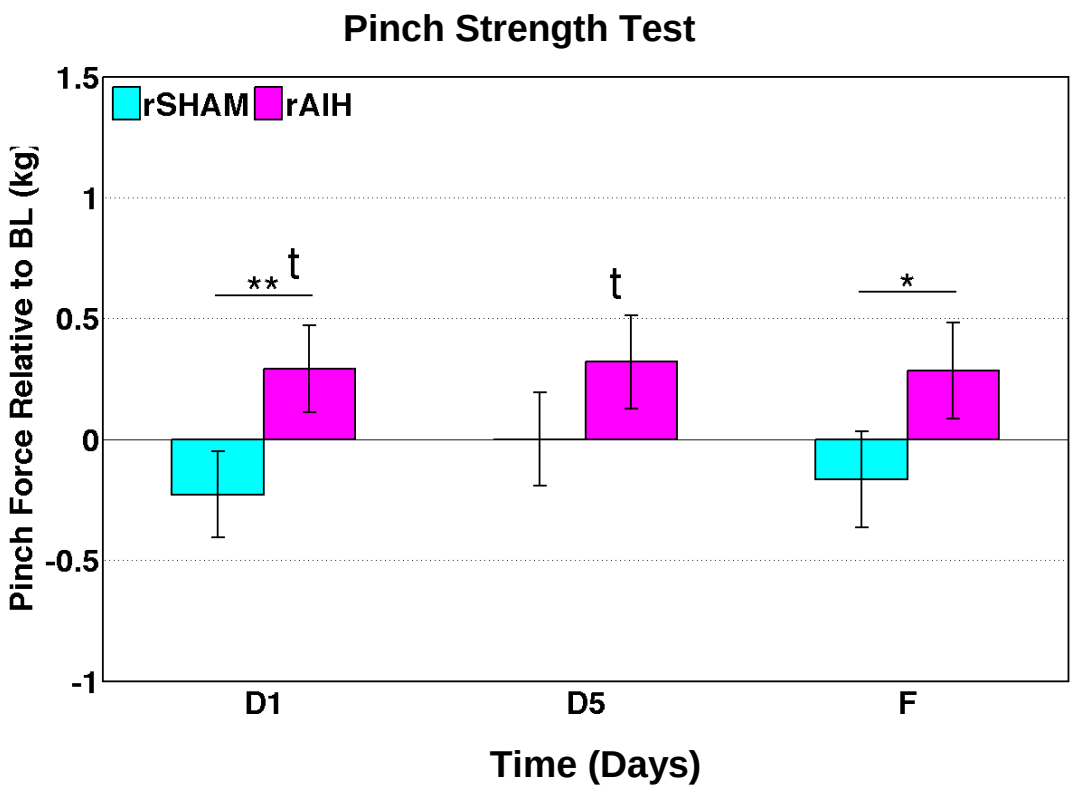
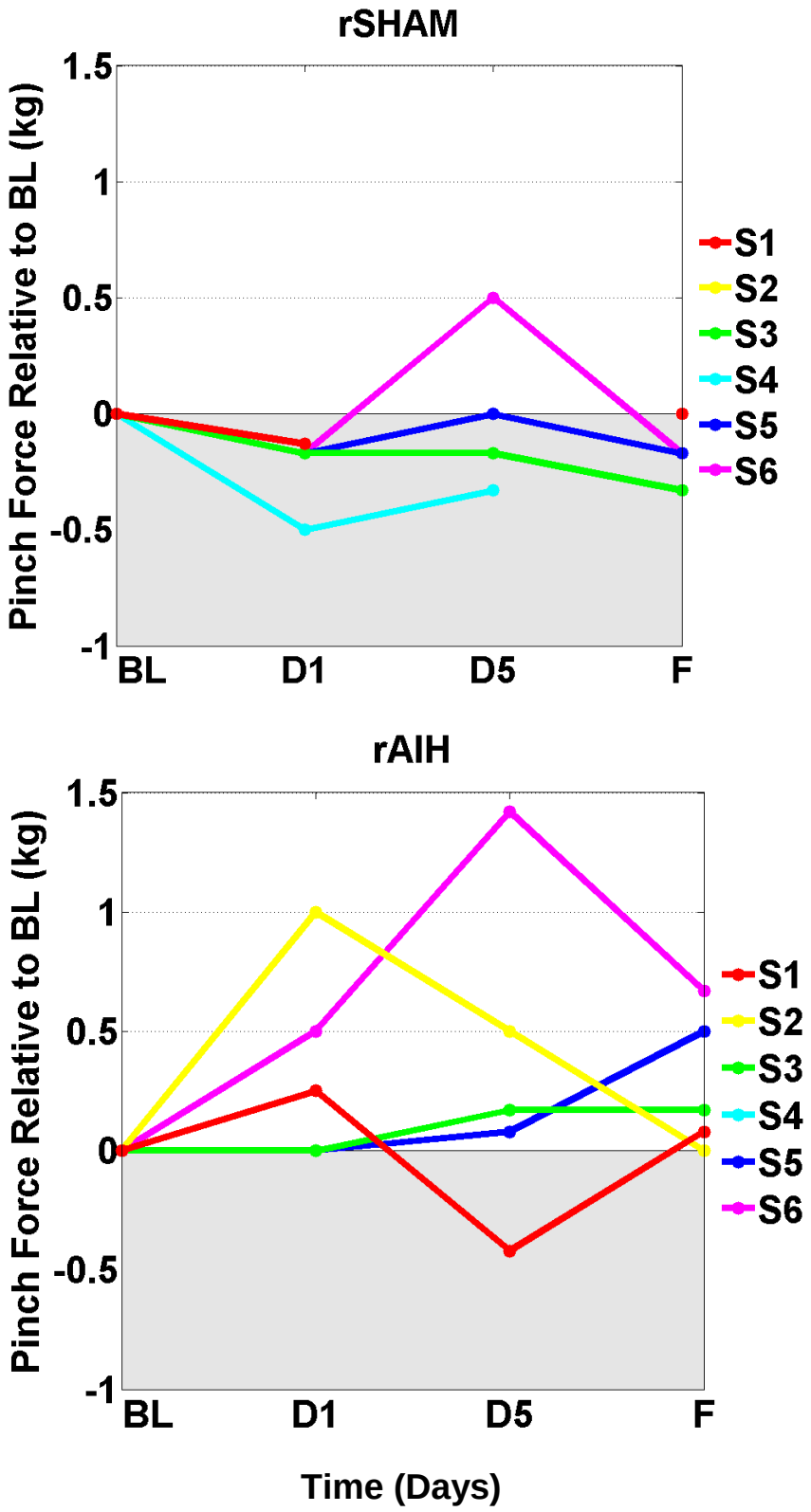


Figure 6. Pinch Strength outcomes. A) Average changes in pinch test performance between rSHAM and rAIH. B) Performance changes by subject. Data are expressed as mean ± 1 SE. Comparisons are reported as changes from baseline (BL; recorded before intervention on Day 1): D1 = D1post – BL; D5 = D5post – BL; F = F – BL. ** p≤0.01, *p≤0.05, repeated measures linear mixed model; **t,** rAIH significant to baseline.

Abbreviations: kg, kilograms; S1-6, subjects; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia; D1post, after intervention on Day 1; D5post, after intervention on Day 5; F, follow-up after Day 5.

B)


rSHAM
Pinch Force Relative to BL (kg)
1.5
1
0.5
0
-0.5
-1
BL
D1
D5
F
S1
S2
S3
S4
S5
S6
rAIH
Pinch Force Relative to BL (kg)
1.5
1
0.5
0
-0.5
-1
BL
D1
D5
F
S1
S2
S3
S4
S5
S6
Time (Days)

A)

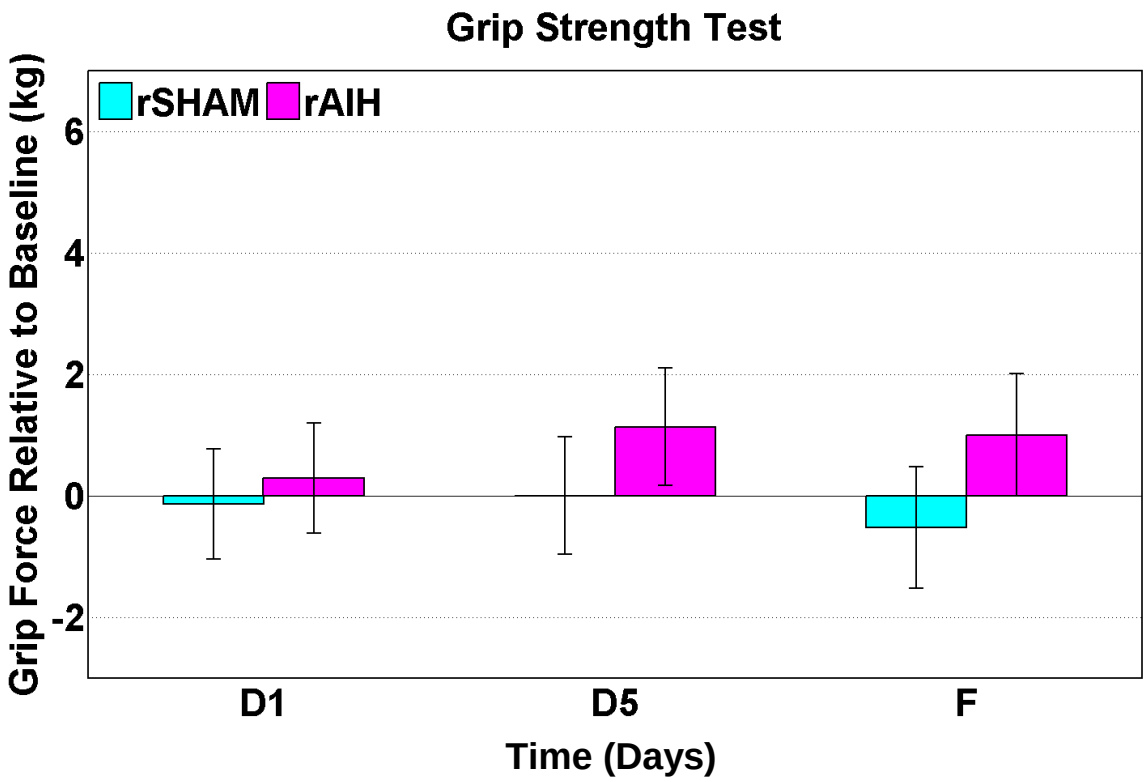
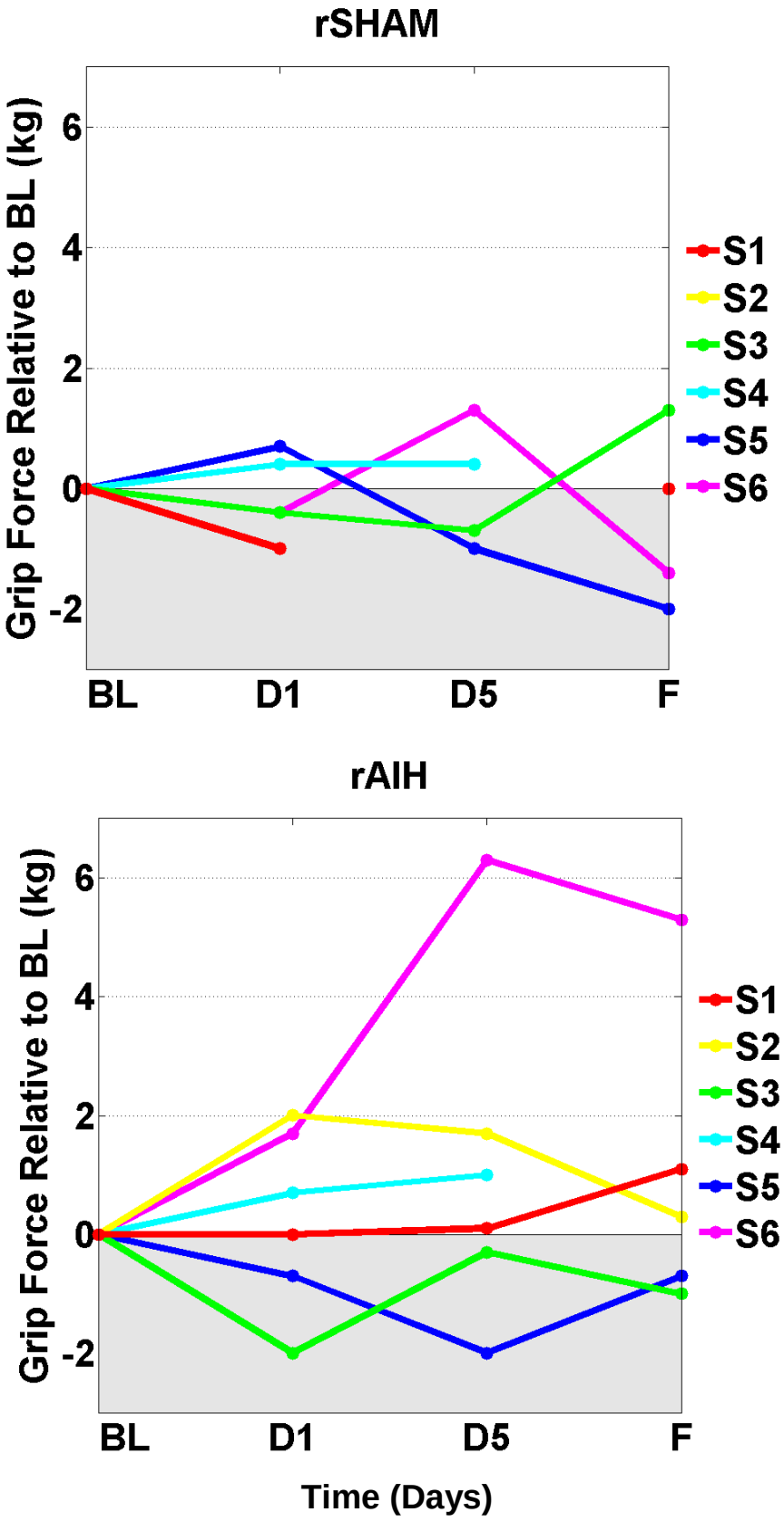


Figure 7. Grip Strength outcomes. A) Average changes in grip test performance between rSHAM and rAIH. B) Performance changes by subject. Data are expressed as mean ± 1 SE. Comparisons are reported as changes from baseline (BL; recorded before intervention on Day 1): D1 = D1post – BL; D5 = D5post – BL; F = F – BL.

Abbreviations: kg, kilograms; S1-6, subjects; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia; D1post, after intervention on Day 1; D5post, after intervention on Day 5; F, follow-up after Day 5.

B)


rSHAM
Grip Force Relative to BL (kg)
6
4
2
0
-2
BL
D1
D5
F
S1
S2
S3
S4
S5
S6
rAIH
Grip Force Relative to BL (kg)
6
4
2
0
-2
BL
D1
D5
F
Time (Days)
S1
S2
S3
S4
S5
S6

A) Dominant Hand

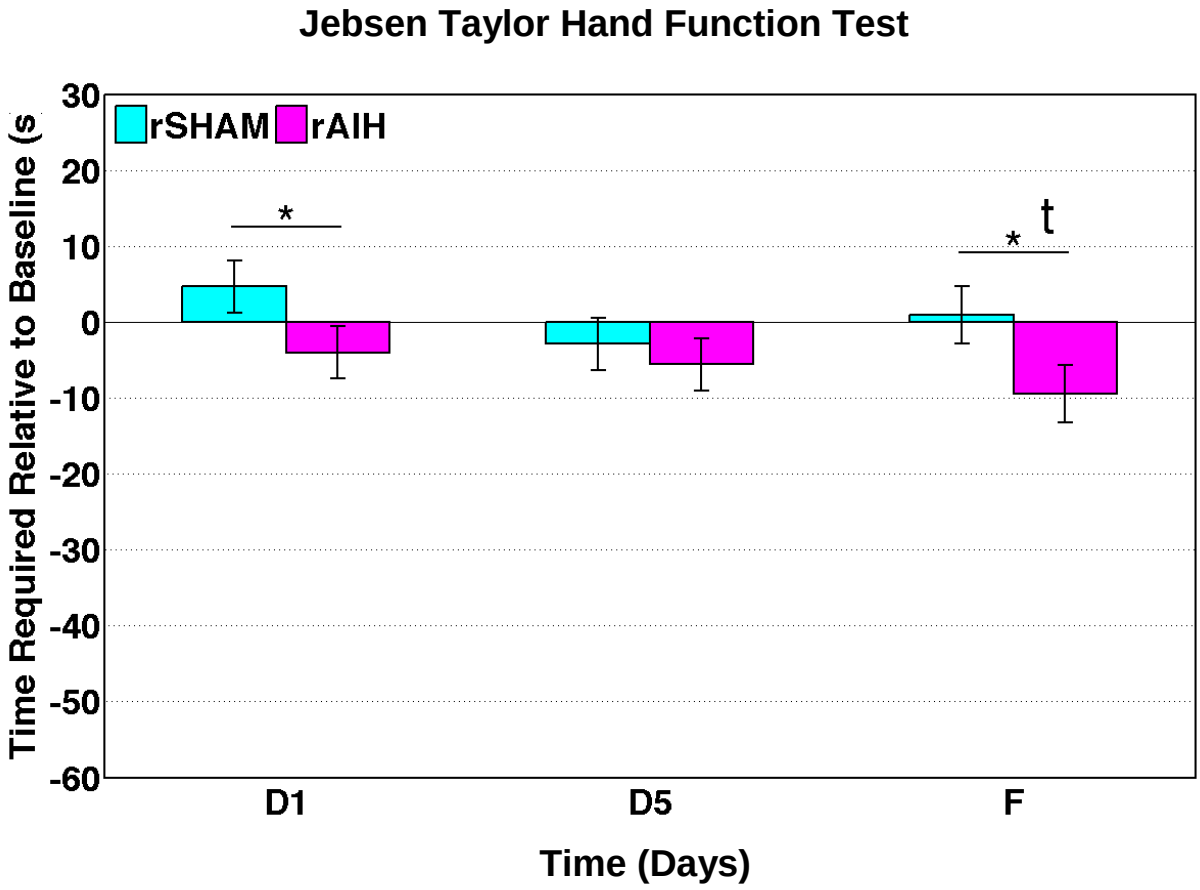
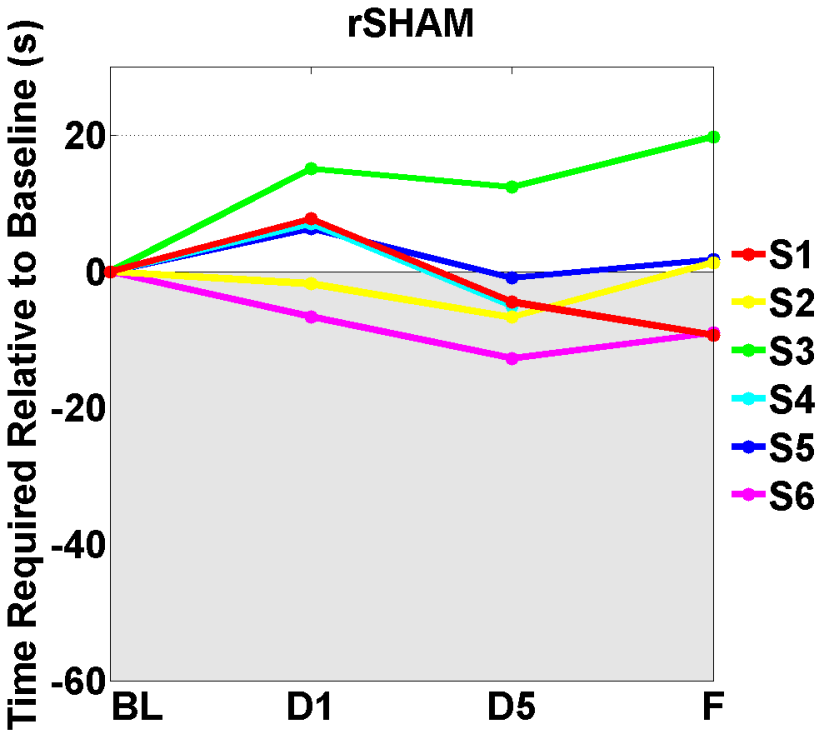
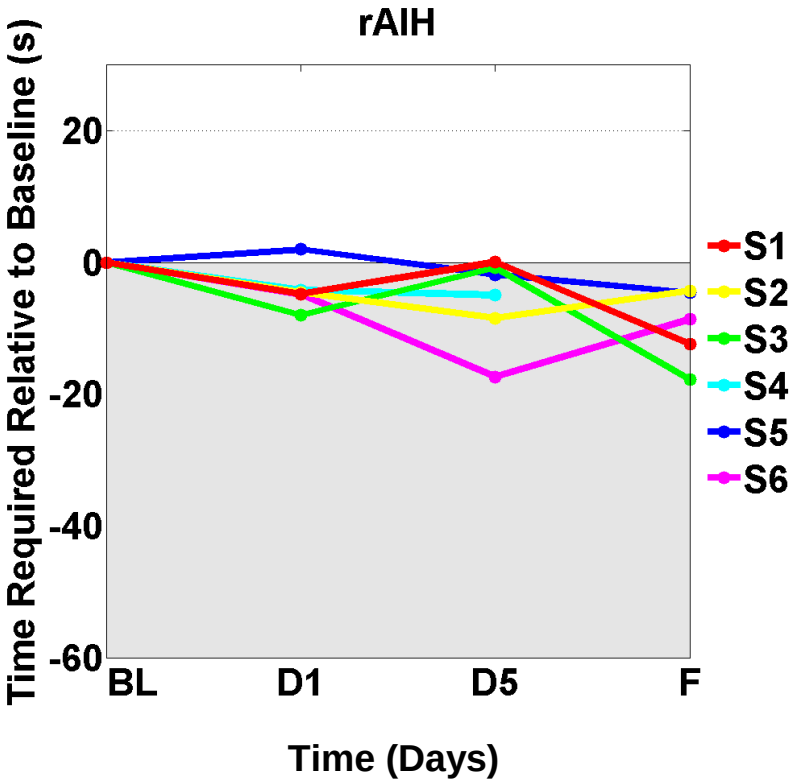


Figure 8. Jebsen Taylor Hand Function Test outcomes. **Lower values correspond to improved test performance. A)** Dominant hand performance changes. **B)** Dominant hand performance changes by subject**. C)** Non-dominant hand changes. **D)** Non-dominant changes by subject. **E)** Average changes for both hands combined. **F)** Changes for both hands combined by subject. Data are expressed as mean ± 1 SE. Comparisons are reported as changes from baseline (BL; recorded before intervention on Day 1): D1 = D1post – BL; D5 = D5post – BL; F = F – BL. *p≤0.05, repeated measures linear mixed model; **t,** rAIH significant to baseline.

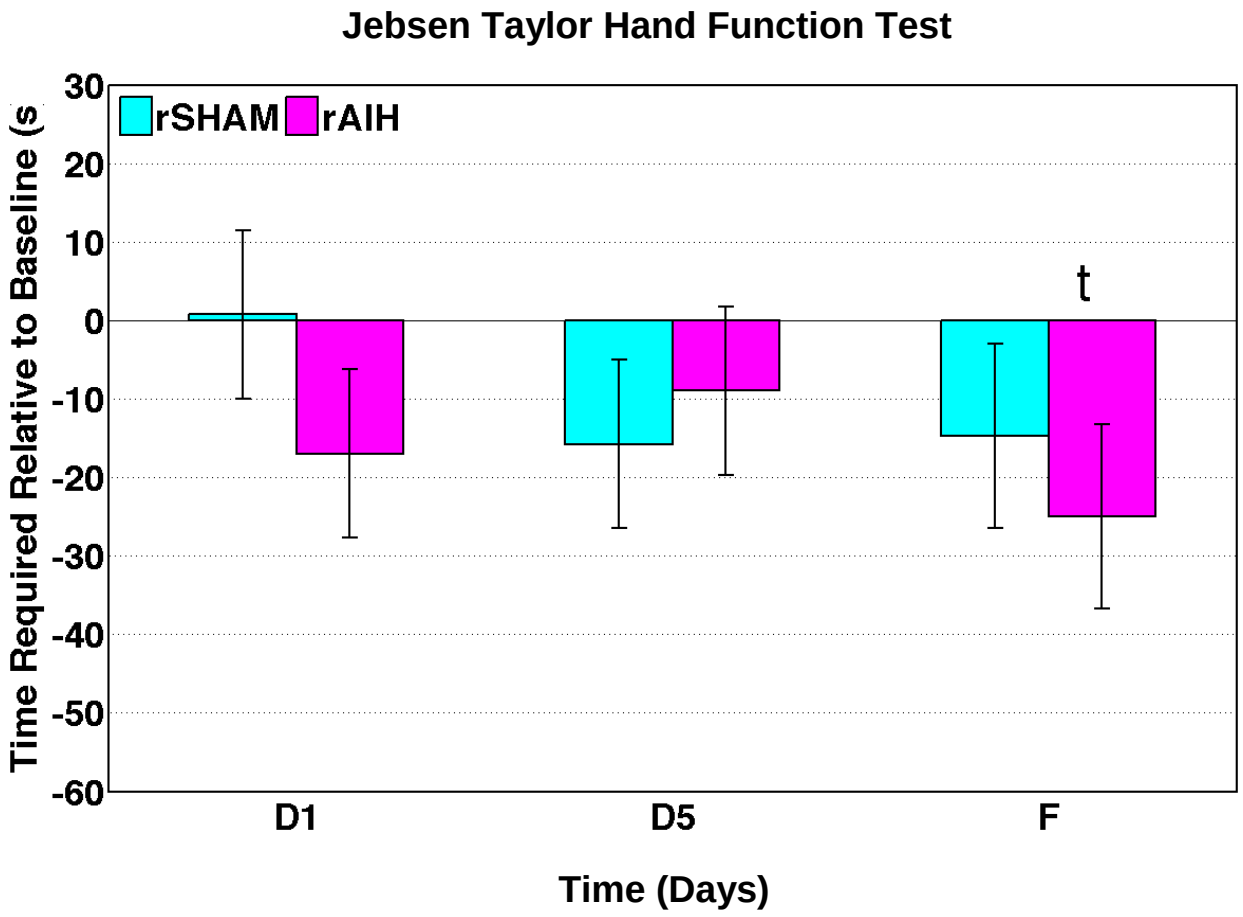
Abbreviations: S1-6, subjects; D1post, after intervention on Day 1; D5post, after intervention of Day 5; F, follow-up after Day 5; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia.

B) Jebsen Dominant Hand by Subject



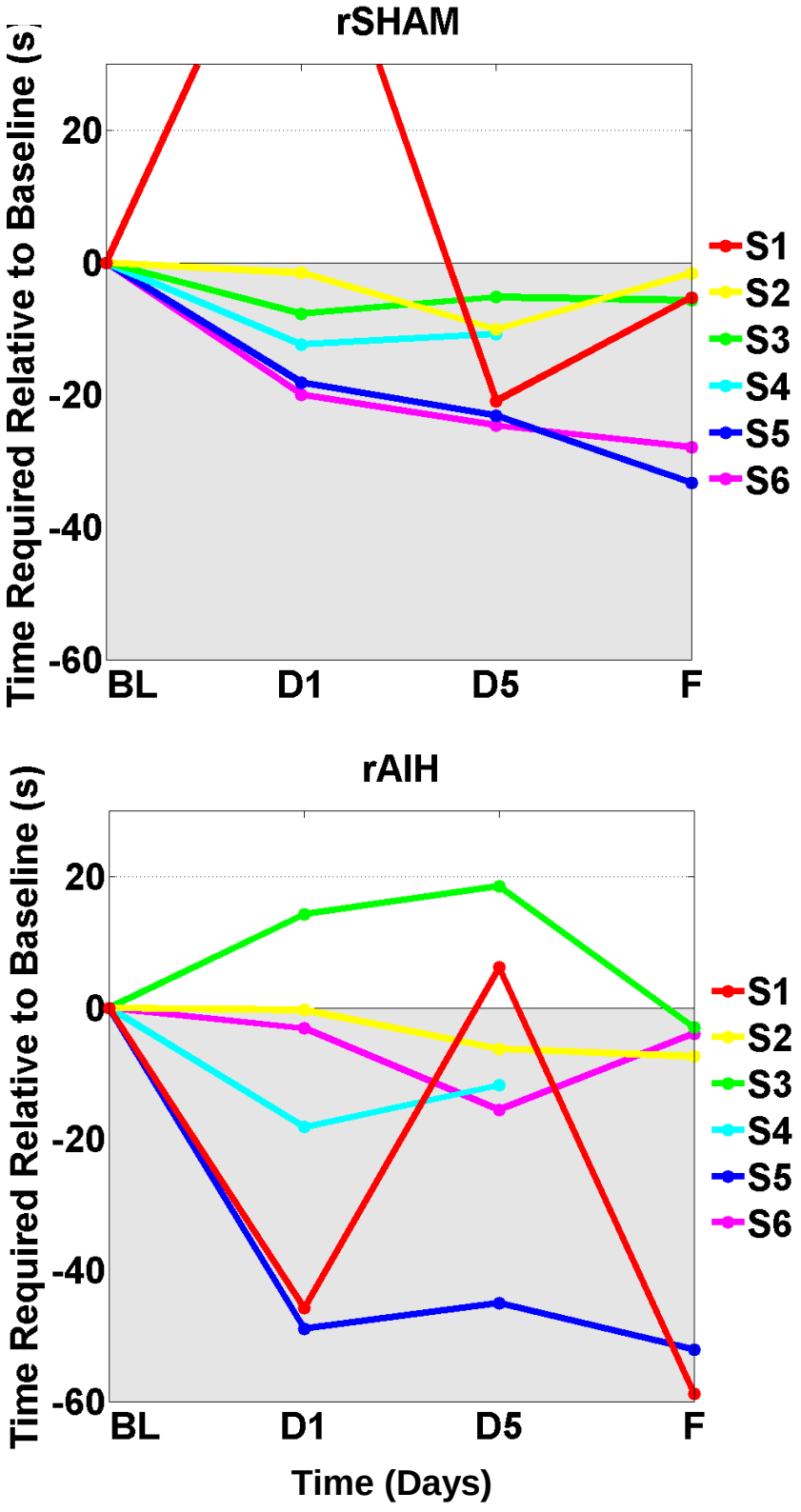


C) Non-Dominant Hand

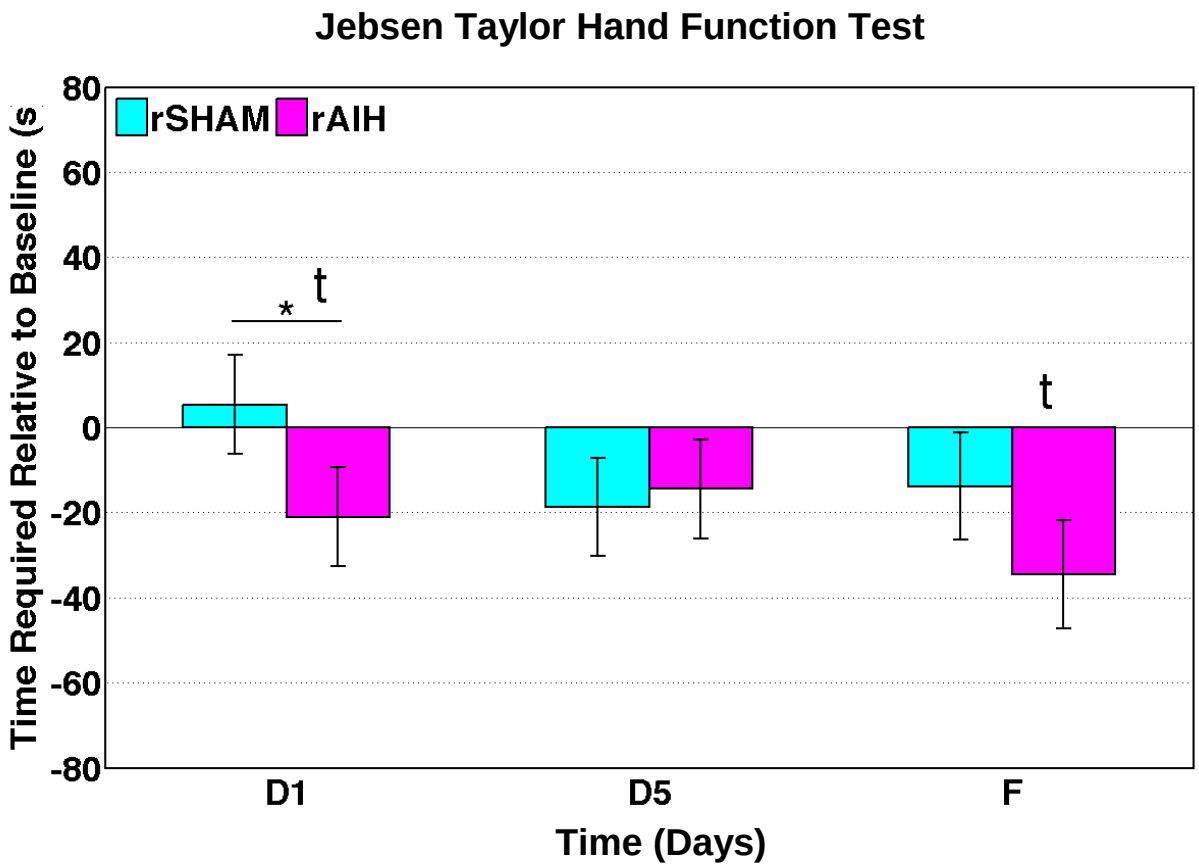


D) Jebsen Non-Dominant Hand by Subject

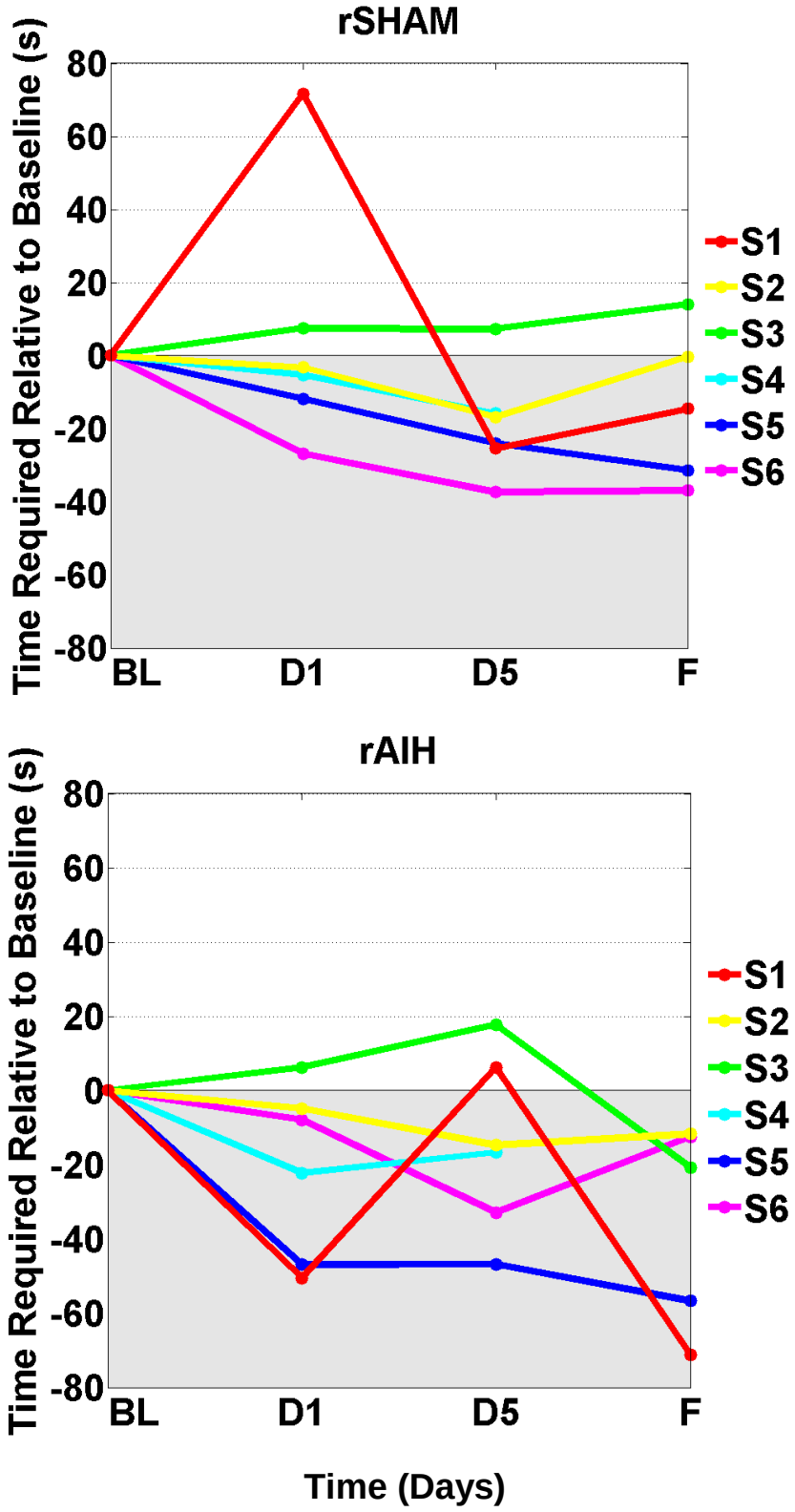
Datum omitted from figure:
S1, D1, 64s



E) Both Hands Combined



F) Jebsen Both Hands Combined by Subject



A)

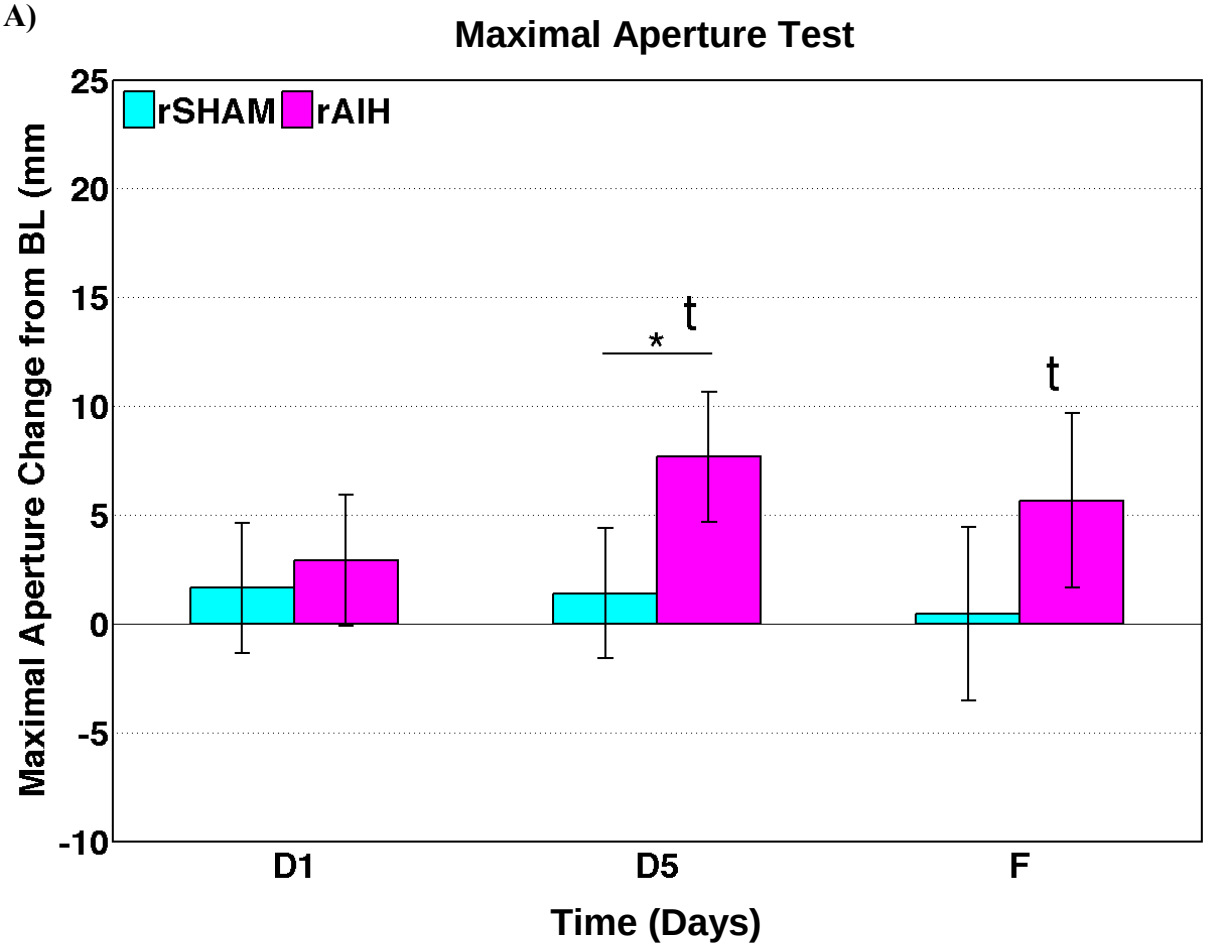
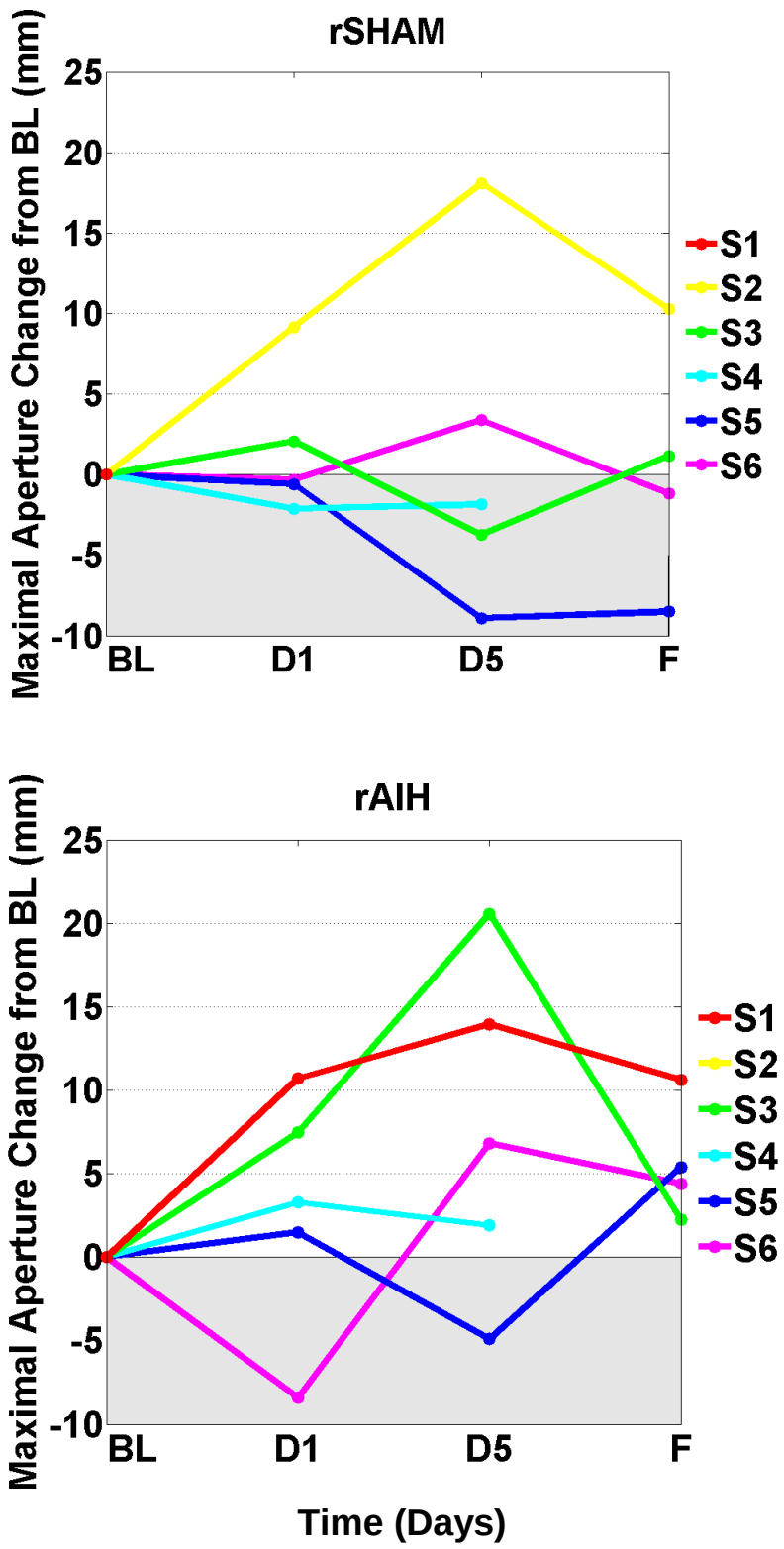


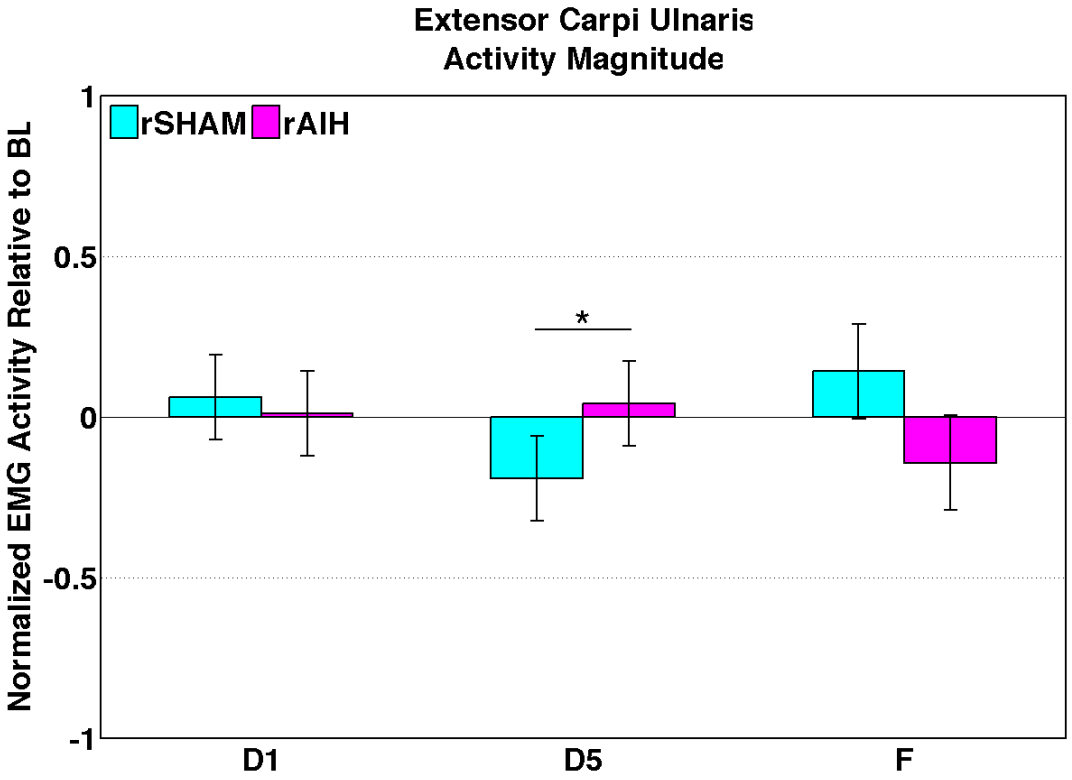
Figure 9. Maximal Aperture outcomes. A) Average changes in test performance between rSHAM and rAIH. B) Performance changes by subject. Data are expressed as mean ± 1 SE. Comparisons are reported as changes from baseline (BL; recorded before intervention on Day 1): D1 = D1post – BL; D5 = D5post – BL; F = F – BL. *p≤0.05, repeated measures linear mixed model; **t,** rAIH significant to baseline.

Abbreviations: S1-6, subjects; D1post, after intervention on Day 1; D5post, after intervention of Day 5; F, follow-up after Day 5; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia.

B) Maximal Aperture by Subject



A)


Extensor Carpi Ulnaris
Activity Magnitude
rSHAM
rAIH
Normalized EMG Activity Relative to BL
1
0.5
0
-0.5
-1
*
D1
D5
F

B)

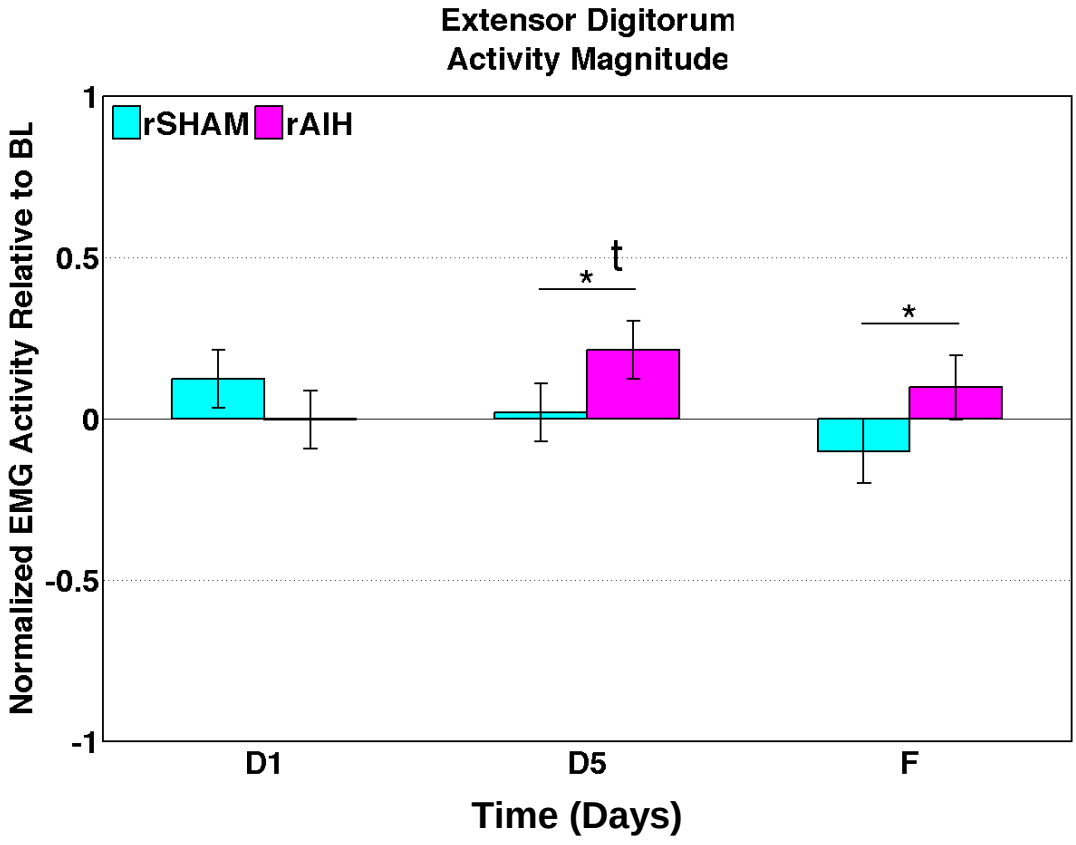
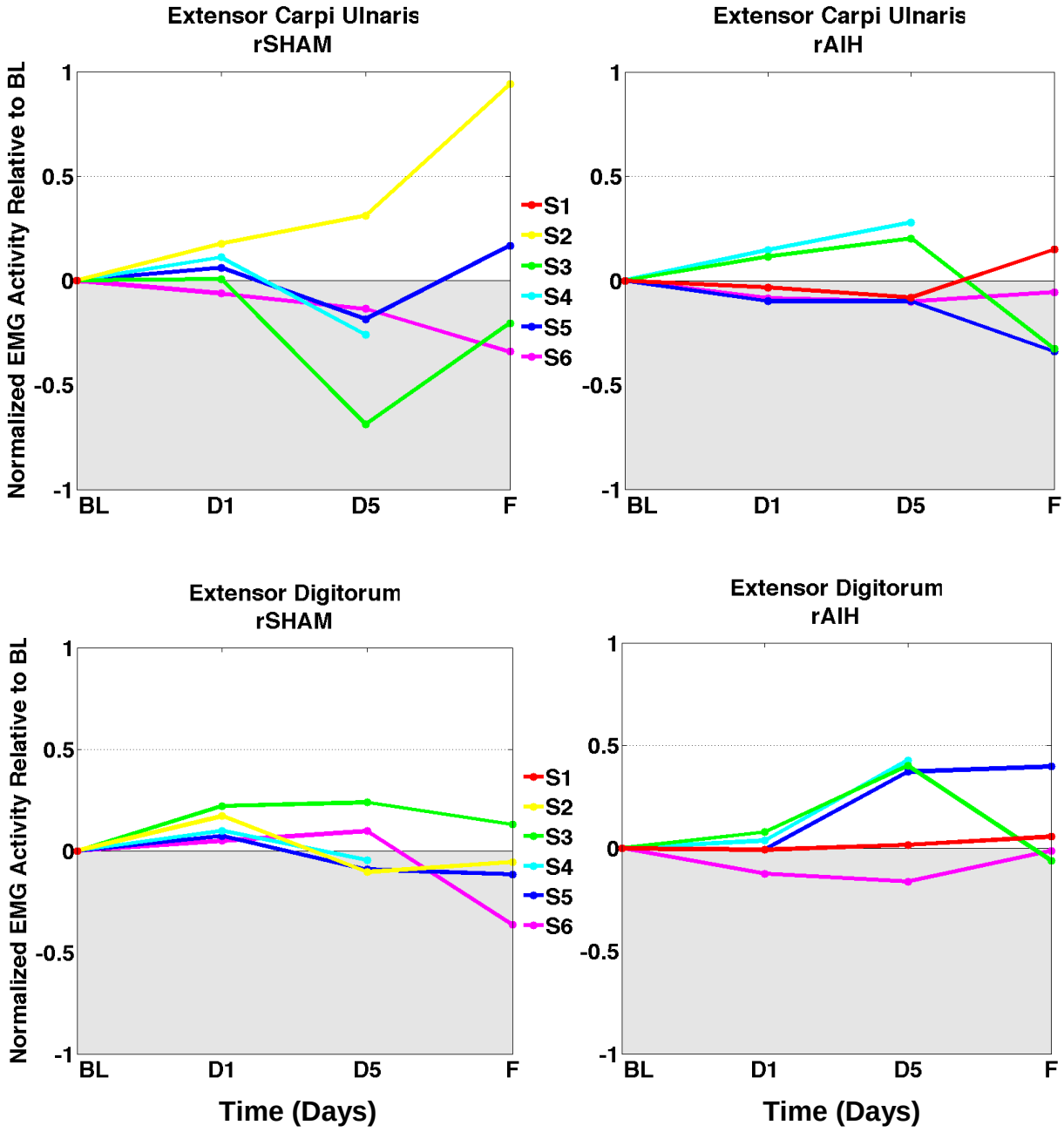
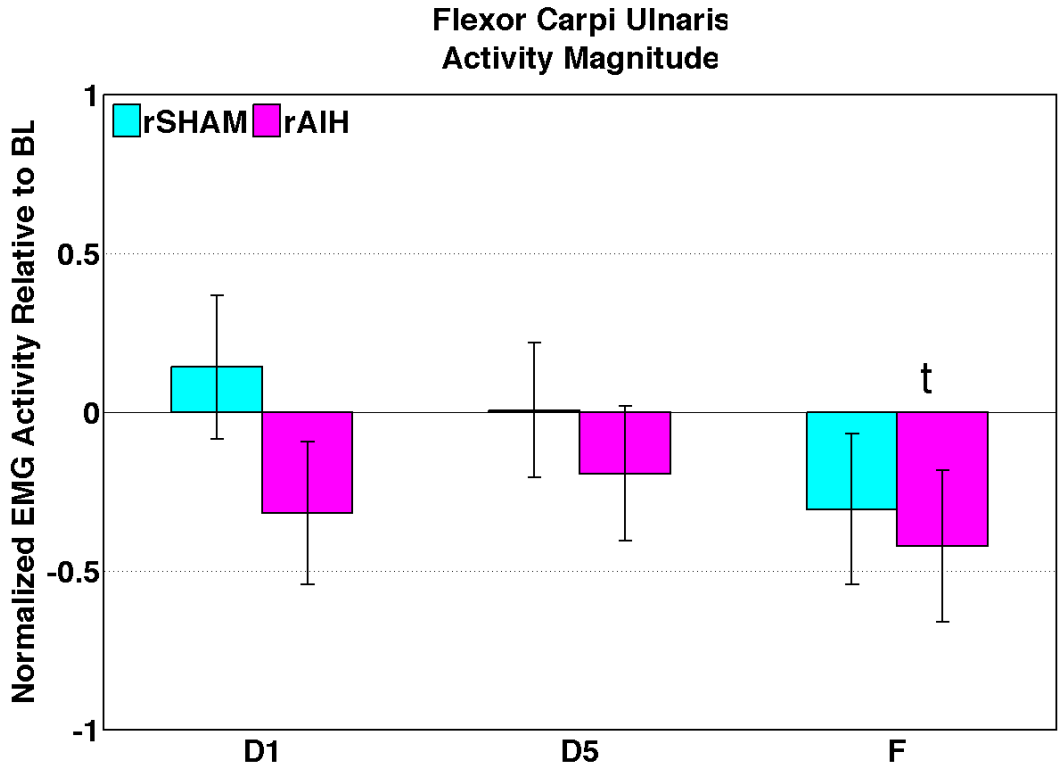

Extensor Digitorum
Activity Magnitude
rSHAM
rAIH
Normalized EMG Activity Relative to BL
1
0.5
0
-0.5
-1
t
*
*
D1
D5
F
Time (Days)

Figure 10. Flexor and extensor activity magnitude during maximal aperture. A) Extensor carpi ulnaris activity. B) Extensor digitorum activity. C) Extensor activity by subject. D) Flexor carpi ulnaris activity. E) Flexor digitorum activity. F) Flexor activity by subject. Data are expressed as mean ± 1 SE. Comparisons are reported as changes from baseline (BL; recorded before intervention on Day 1): D1 = D1post – BL; D5 = D5post – BL; F = F – BL. *p≤0.05, repeated measures linear mixed model; **t,** rAIH significant to baseline.
Abbreviations: S1-6, subjects; EMG, electromyogram; D1post, after intervention on Day 1; D5post, after intervention of Day 5; F, follow-up after Day 5; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia.

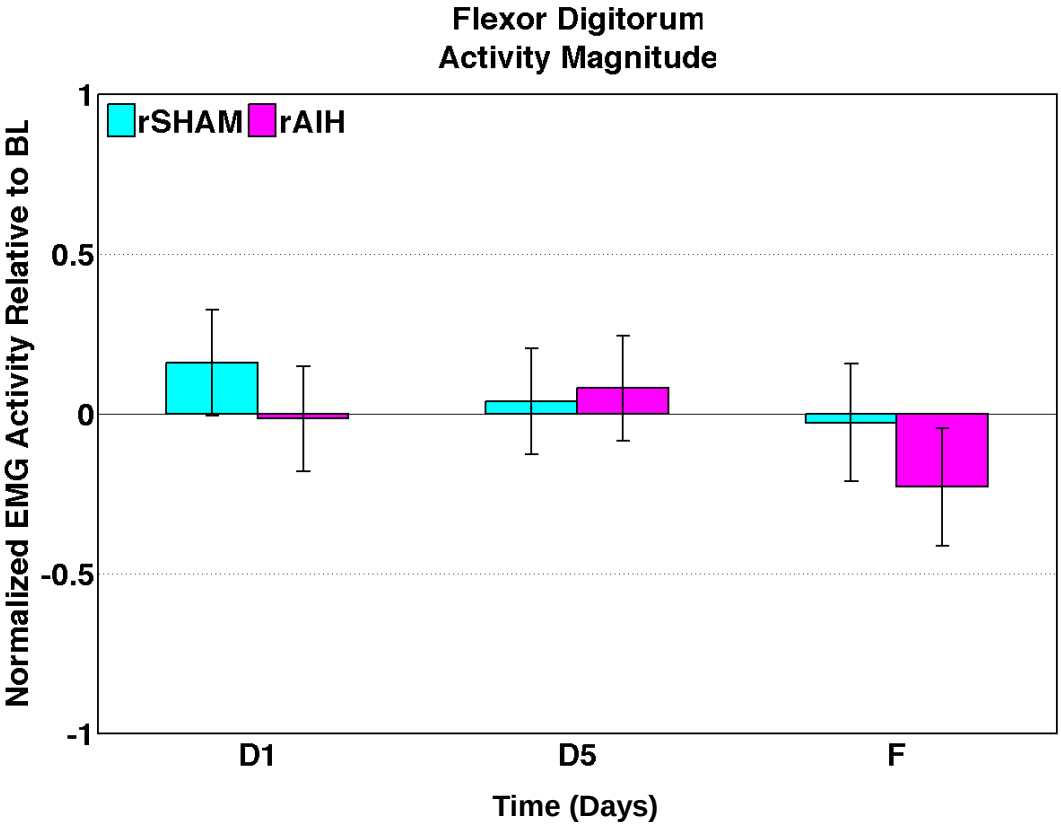
C) Extensors by subject



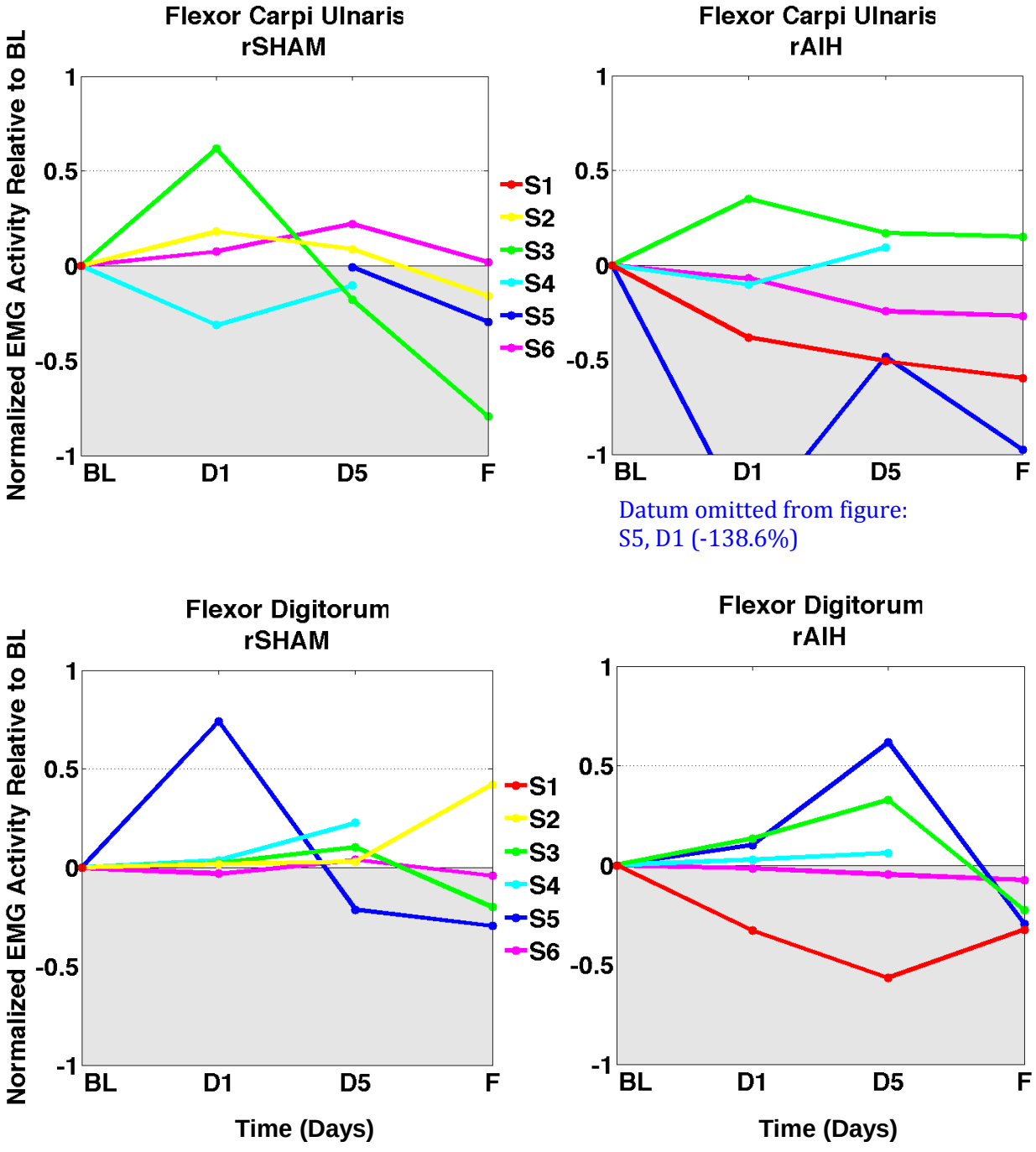
D)


Flexor Carpi Ulnaris
Activity Magnitude
rSHAM
rAIH
Normalized EMG Activity Relative to BL
1
0.5
0
-0.5
-1
t
D1
D5
F

E)


Flexor Digitorum
Activity Magnitude
rSHAM
rAIH
Normalized EMG Activity Relative to BL
1
0.5
0
-0.5
-1
D1
D5
F
Time (Days)

F) Flexors by subject



A)

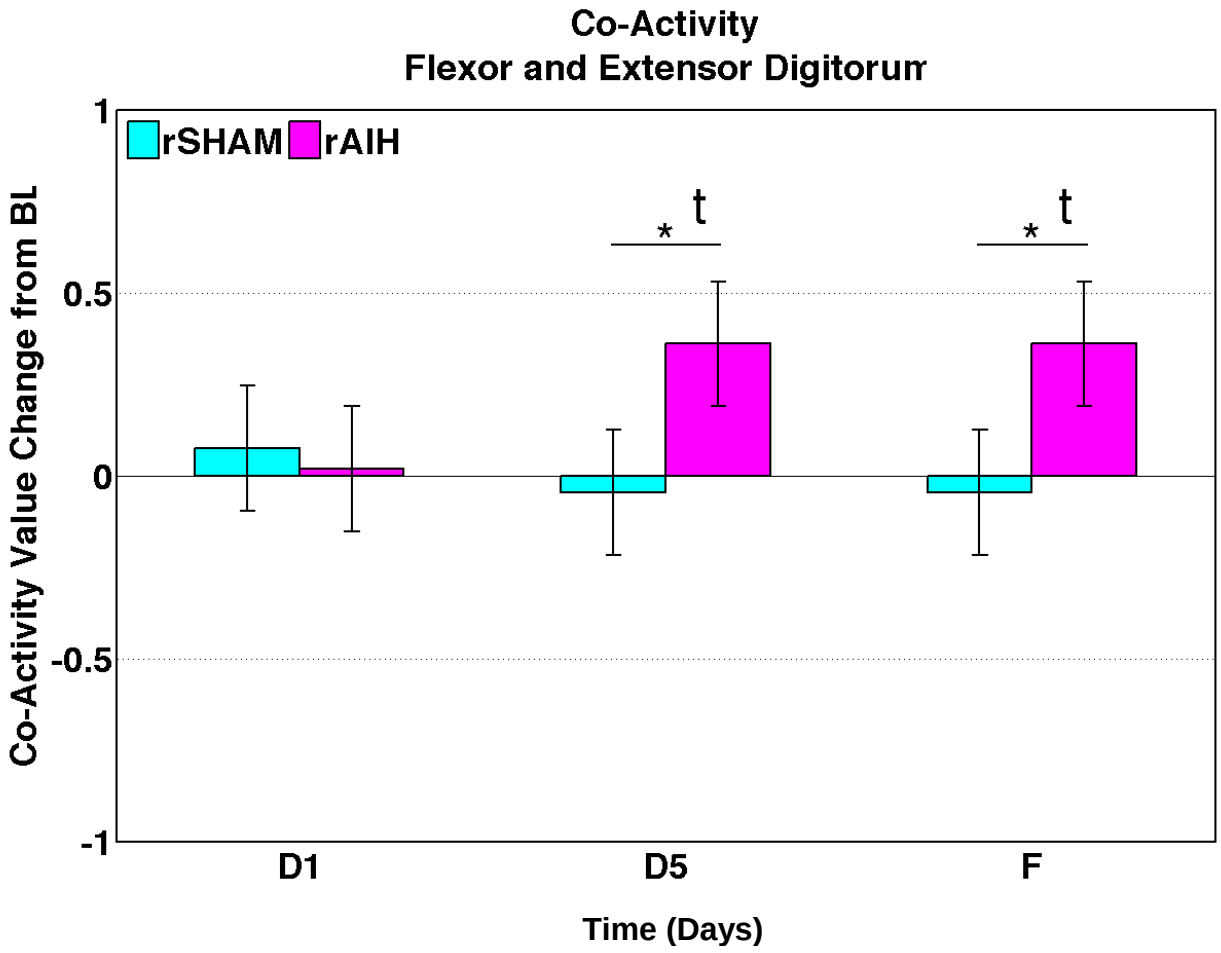
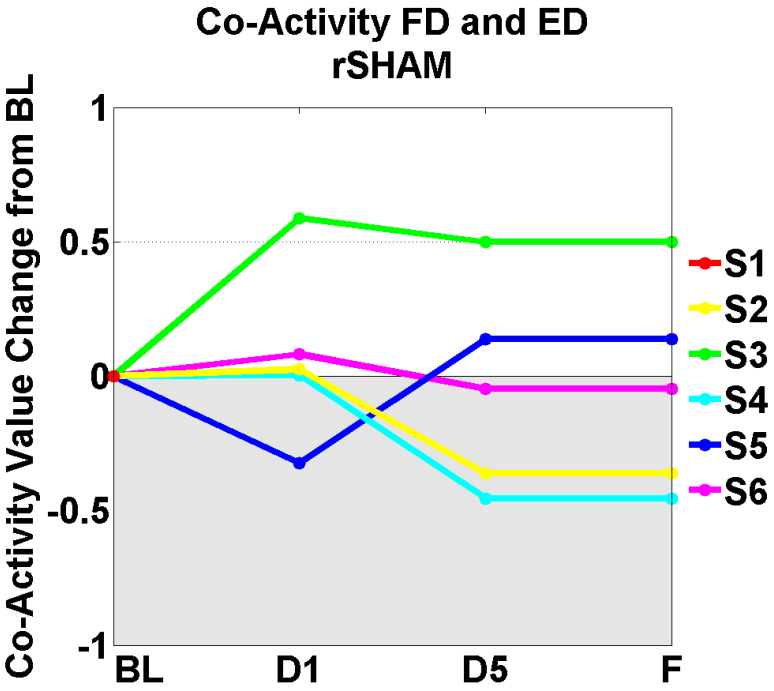
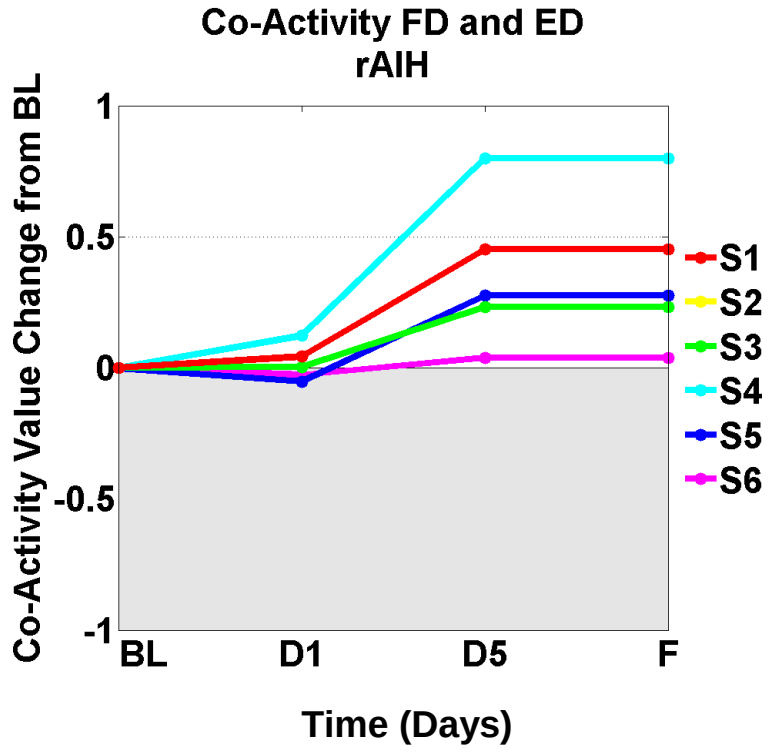


Figure 11. Flexor and extensor co-activity outcomes. A) Co-Activity of Flexor and Extensor Digitorum (FD and ED). B) Co-Activity of FD and ED by subject. C) Co-Activity of Flexor and Extensor Carpi Ulnaris (FCU and ECU). D) Co-Activity of FCU and ECU by subject. E) Overall Co-Activity of Flexors and Extensors. E) Overall Co-Activity by subject. Data are expressed as mean ± 1 SE. Comparisons are reported as changes from baseline (BL; recorded before intervention on Day 1): D1 = D1post – BL; D5 = D5post – BL; F = F – BL. *p≤0.05, repeated measures linear mixed model; **t,** rAIH significant to baseline.

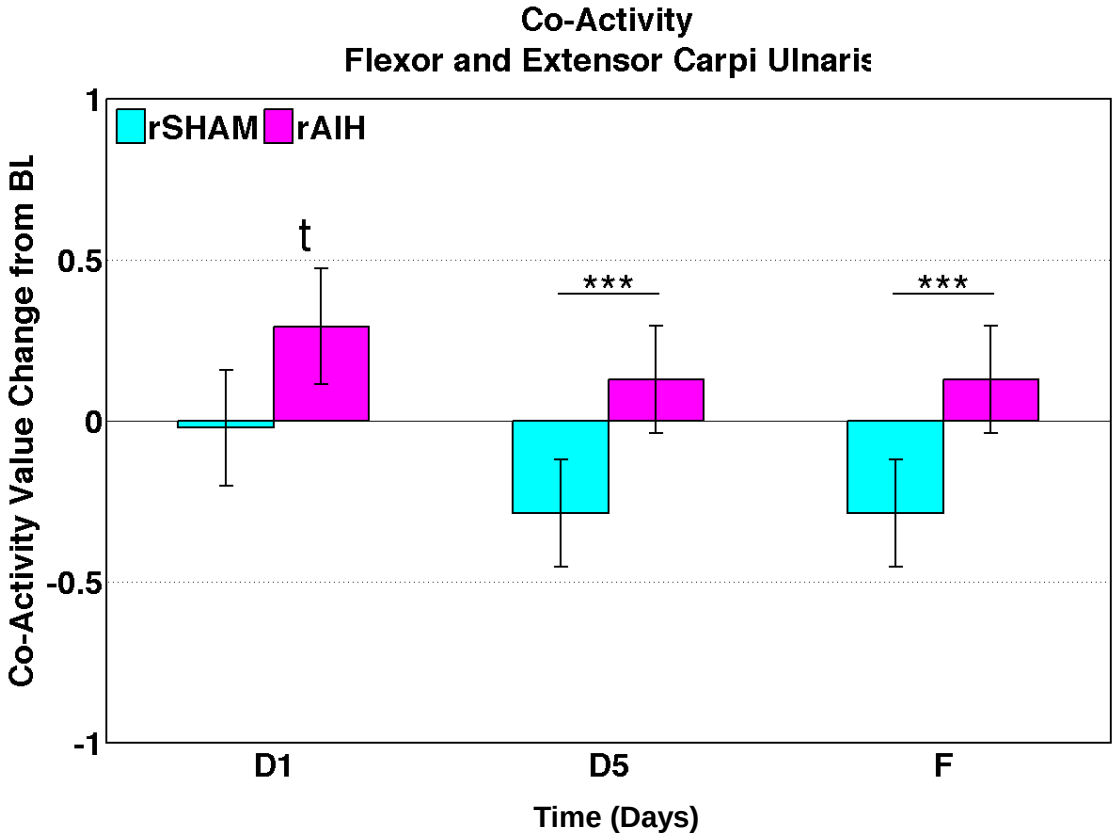
Abbreviations: BL, baseline; S1-6, subjects; FD, flexor digitorum; ED, extensor digitorum; FCU, flexor carpi ulnaris; ECU, extensor carpi ulnaris; D1post, after intervention on Day 1; D5post, after intervention of Day 5; F, follow-up after Day 5; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia.

B)

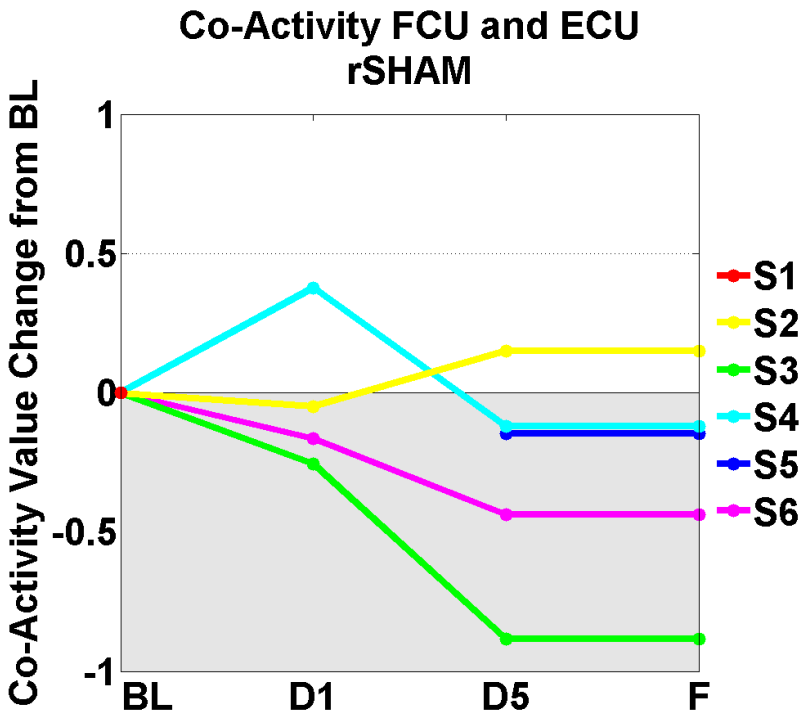

Co-Activity FD and ED
rSHAM
Co-Activity Value Change from BL
1
0.5
0
-0.5
-1
BL
D1
D5
F
S1
S2
S3
S4
S5
S6


Co-Activity FD and ED
rAIH
Co-Activity Value Change from BL
1
0.5
0
-0.5
-1
BL
D1
D5
F
Time (Days)
S1
S2
S3
S4
S5
S6

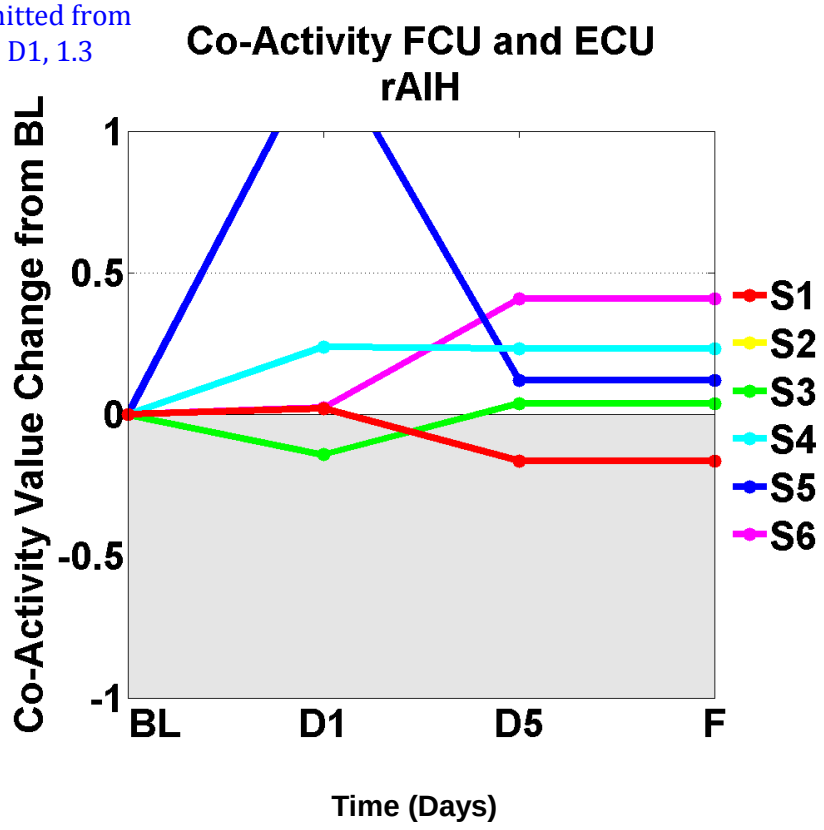
C)


Co-Activity
Flexor and Extensor Carpi Ulnaris
rSHAM
rAIH
t
***
***
1
0.5
0
-0.5
-1
Co-Activity Value Change from BL
D1
D5
F
Time (Days)

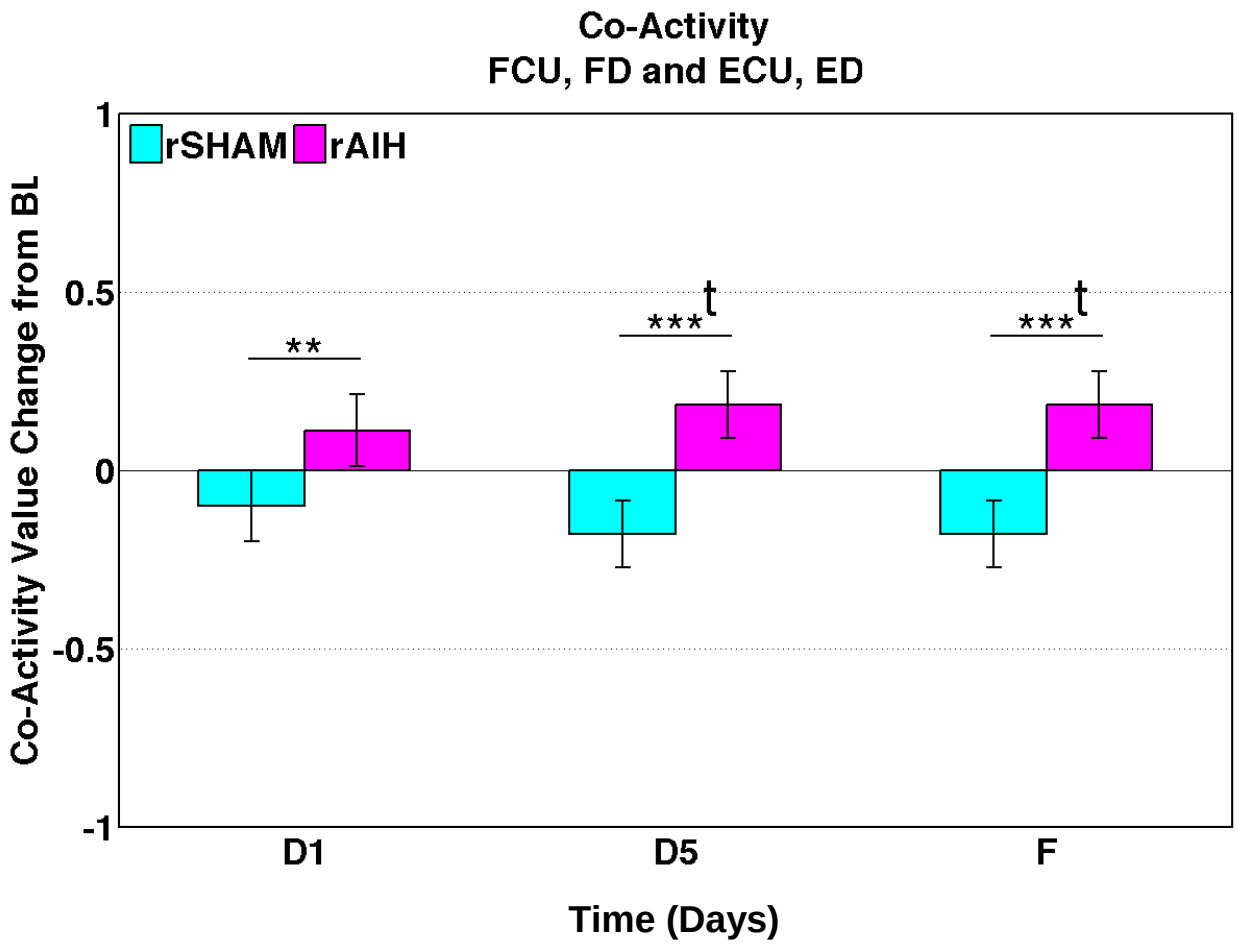
D)



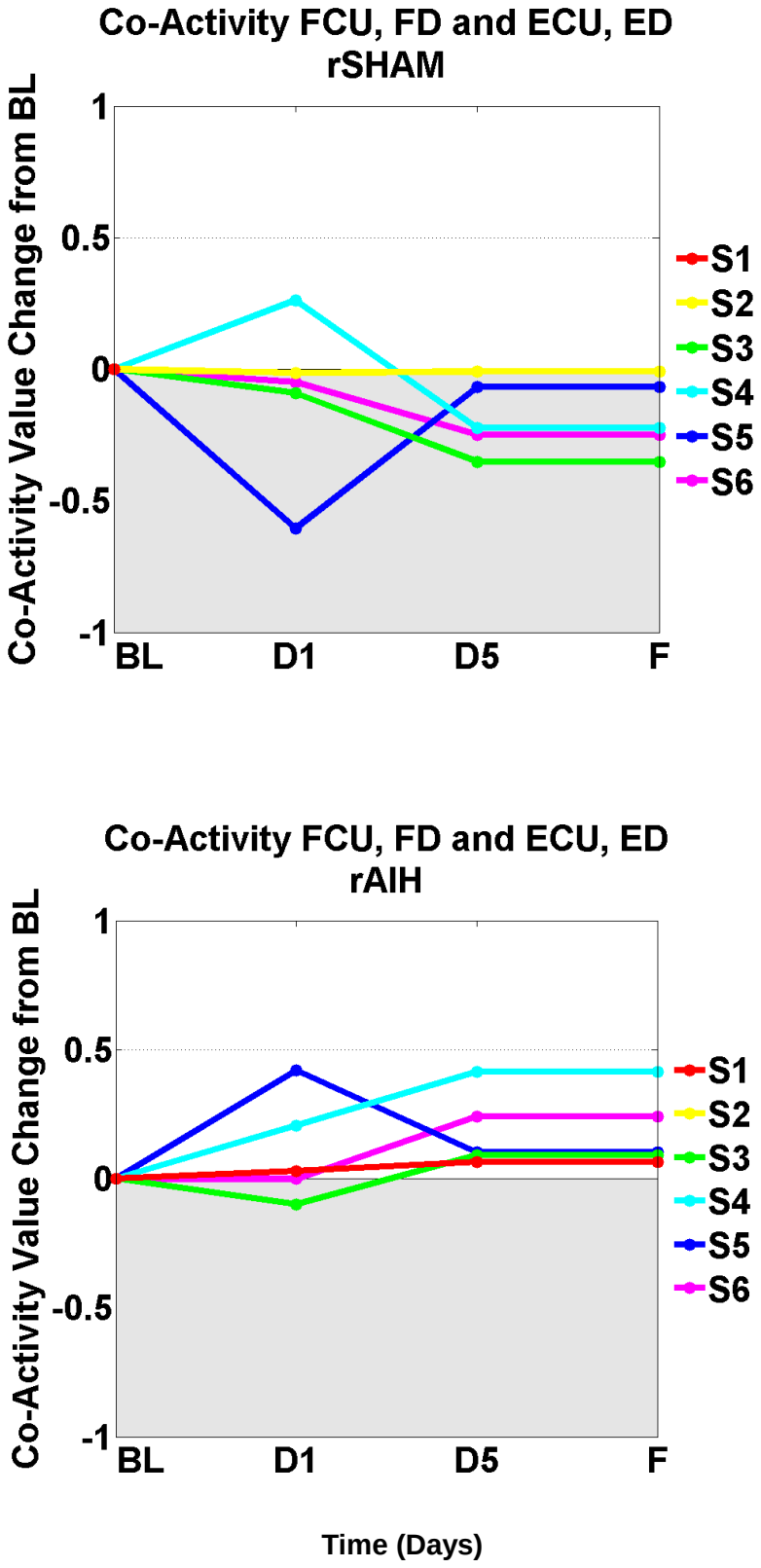
Datum omitted from figure: S5, D1, 1.3



E)


Co-Activity
FCU, FD and ECU, ED
rSHAM
rAIH
Co-Activity Value Change from BL
1
0.5
0
-0.5
-1
**
***t
***t
D1
D5
F
Time (Days)

F)


Co-Activity FCU, FD and ECU, ED
rSHAM
Co-Activity Value Change from BL
1
0.5
0
-0.5
-1
BL
D1
D5
F
S1
S2
S3
S4
S5
S6
Co-Activity FCU, FD and ECU, ED
rAIH
Co-Activity Value Change from BL
1
0.5
0
-0.5
-1
BL
D1
D5
F
S1
S2
S3
S4
S5
S6
Time (Days)

Outcome Measure	MCID	rSHAM Subjects Improved at F	rAIH Subjects Improved at F
Box and Blocks Test	6 blocks (Lang et al., 2013)	0% (0/5)	40% (2/5)
Grip Strength Test	5 kg (Lang et al., 2008)	0% (0/4)	20% (1/5)
Pinch Strength Test	0.14 kg (Smaby et al., 2004)	0% (0/4)	60% (3/5)
Jebsen Taylor Test	30 sec (Beekhuizen and Field-Fote, 2005)	**Dom** 20% (1/5)	20% (1/5)
		Non-Dom 20% (1/5)	40% (2/5)
Maximal Hand Aperture	5 mm (DeJong et al., 2012)	25% (1/4)	50% (2/4)
Magnitude of Muscle Activity	20% extensor increase 25% flexor decrease (Hodgson, 2008)	**ECU & ED** 25% (1/4)	25% (1/4)
		FCU & FD 50% (2/4)	75% (3/4)
Overall Flexor/Extensor Co-Activity	20% decrease (Carolan and Cafarelli, 1992)	40% (2/5)	60% (3/5)

More subjects attained MCID with rAIH than rSHAM.

Table 4. Clinical Significance Summary. The number of subjects who maintained a MCID in hand function at follow-up is listed for each outcome measure and for both interventions (rAIH and rSHAM). Abbreviations: MCID, minimal clinically important difference; F, follow up day; Dom, dominant hand; Non-Dom, non-dominant hand; ECU, extensor carpi ulnaris; ED, extensor digitorum; FCU, flexor carpi ulnaris; FD, flexor digitorum; rSHAM, repetitive SHAM; rAIH, repetitive acute intermittent hypoxia.

Discussion

This study found that rAIH increases hand function in persons with chronic, cervical iSCI. Particularly, we observed statistically significant improvement in hand dexterity (higher BBT score), pinch strength (greater force production), and hand speed (reduced JTHFT time), as compared to rSHAM. These improvements remained evident at follow-up, several days after cessation of rAIH, suggesting long-lasting intervention effects. Maximal aperture increased significantly following 5 days of rAIH (Figure 9), along with hand and wrist extensor activity contributing to maximal aperture (Figure 10). Hand extensor activity remained elevated at follow-up (Figure 10B). Interestingly, flexor/extensor co-activity in the hand and wrist reached markedly more positive values across all assessed days following rAIH, as compared to rSHAM (Figure 11). This change suggests greater extensor than flexor activity during hand opening in tested subjects, a muscle activation strategy reminiscent of that used by able-bodied individuals (Stahl et al., 2012). Although no significant rAIH effect was observed on grip strength or non-dominant hand speed versus rSHAM, existing effects may have been obscured by the relatively low sample size (n=6). Indeed, non-dominant hand speed increased markedly above baseline at rAIH follow-up (Figure 8C). Combined, these findings confirm the hypothesis that rAIH elicits enduring enhancement of hand function in SCI.

Kinematic and EMG data provide much insight about how rAIH may affect spinal plasticity. The increased maximal aperture observed after 5 days of rAIH, along with significantly greater wrist and hand extensor activity, are consistent with the LTF hypothesis, whereby rAIH increases activation of extensors by augmenting somatic motor output. However, we did not observe a significant increase in hand or wrist flexor activity after rAIH versus rSHAM (Figure 10 D, E). This finding may signify that rAIH does not activate flexors during

maximal aperture production to the same extent as extensors. At the same time, flexor/extensor co-activity in the fingers and wrist decreased significantly at rAIH follow-up (Figure 11). Since values were more positive following rAIH, extensors were activated more and flexors inhibited more during hand opening. This finding suggests that rAIH may inhibit motorneurons via GABAergic pathways, in addition to its excitatory glutamategic effect. Also, since muscle co-activity is concurrent activity of agonist and antagonist muscles surrounding a joint (Hortobagyi and DeVita, 2000) and has been linked to joint stiffness (Baratta et al., 1988; Solomonow et al., 1988; Humphrey and Reed, 1983), our findings suggest that a possible effect of rAIH is to decrease joint stiffness.

Most, but not all, of the clinical findings in this study may be explained by previously suggested rAIH mechanisms. Particularly, manual dexterity, pinch strength, and dominant hand speed improvements are consistent with the hypothesis that rAIH increases somatic motor output in humans (Mahamed and Mitchell, 2007; Trumbower et al., 2012). Yet the insignificant effect of rAIH on grip strength and non-dominant hand speed is puzzling. Negligible grip strength increases may be explained by considering compensatory mechanisms SCI subjects commonly use to achieve grip strength. While able-bodied individuals grip by activating finger flexors, SCI persons experience tight flexors, which they cannot activate to produce grip. They compensate for this loss of function by tenodesis, a cocking of the wrist using preserved extensors to generate passive finger closing (Frank et al., 1984). Since we found that rAIH may inhibit flexors, it is plausible that this inhibition may have made cocking the wrist more challenging, which lead to lowered grip strength performance. Pinch strength may not have been similarly affected, because there are no compensatory extensor strategies to produce this movement. Insignificant change in non-dominant hand speed due to rAIH may be explained by considering task-dependent

plasticity. Since subjects used the dominant hand to a greater extent in daily activities while receiving the 5-day intervention, the effect of rAIH may have been greater in that hand. The relatively lesser motor training of the non-dominant hand may also explain the higher variability in non-dominant hand JTHFT performance (Figure 8C, D).

Aside from statistically significant hand function changes following rAIH, clinically significant improvements were observed in a majority of the quantified outcome measures (Table 4). Clinically significant improvements were defined as the minimal change in outcome measure necessary for subjects to experience improved quality of life (Jaeschke et al., 1989). A pre-specified minimal clinically important difference (MCID) was obtained for each outcome measure from previous studies (references and values in Table 4). These studies determined the MCID by collecting regular quality of life questionnaires from subjects receiving a course of treatment thought to improve daily function (Jaeschke et al., 1989). We quantified the number of subjects who attained an MCID over all outcome measures in rAIH and rSHAM. In the majority of assessed outcome measures, more subjects attained a MCID at rAIH follow-up than at rSHAM follow-up (Table 4). In the remaining outcome measures, the number of subjects attaining MCID was the same following rAIH and rSHAM.

Despite these exciting findings, this study has limitations. The exact mechanism by which rAIH elicits functional changes in humans cannot be determined conclusively from the present data. We suggest that rAIH increases hand function in cervical iSCI by recruiting LTF-dependent mechanisms similar to those observed in rodent respiratory and somatic motor systems. However, possible alternative or auxiliary rAIH mechanisms include altered sympathetic responses, regional changes in blood flow, and action of serotonin on central downstream targets in addition to its effect on spinal motor neurons. Further experimental work is required to

elucidate conclusively the mechanism of action of rAIH in humans, particularly at the molecular level. This knowledge would make it possible to manipulate downstream targets in the signaling cascade initiated by rAIH, potentially augmenting functional gains. We also considered inter-subject variations, including differing medications, assistive devices, level of cervical injury, amount of physical activity, diet, age, and sex, as potential study confounds. Relative homogeneity of the sample was attained by including male subjects only, consistent with the fact that over 80 percent of spinal cord injuries occur among males (NSCISC, 2013). Subjects were also instructed to maintain consistency in their medication, physical activity regimen, daily routine, and diet for the duration of data collection. Statistical analyses accounted for large inter-subject variations in motor performance by comparing changes in functional outcomes relative to baseline.

Repetitive AIH has intriguing possibilities as a safe and effective therapeutic tool. Current and past data suggest that mild rAIH breathing protocols are safe in human (Hayes et al., 2013; Serebrovskaya et al., 2008; Trumbower et al., 2012). A distinctive rAIH property is that it elicits functional improvements preferentially in longstanding injuries (Golder and Mitchell, 2005), a time when few SCI therapies are available. This preferential effect is highly correlated with loss and subsequent recovery of serotonergic innervation in spinal injury. Notably, the intervention also elicits an effect without concurrent exercise training, making it suitable for individuals with severe mobility impairments. Thus, rAIH breathing offers a promising new direction in rehabilitation to facilitate functional gains in people with longstanding SCI injuries.

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