Effects of acute intermittent hypoxia on corticospinal excitability within the primary motor cortex

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Received: 7 December 2021 / Accepted: 1 June 2022 / Published online: 25 June 2022
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Abstract

Purpose  Acute intermittent hypoxia (AIH) is a safe and non-invasive treatment approach that uses brief, repetitive periods of breathing reduced oxygen air alternated with normoxia. While AIH is known to affect spinal circuit excitability, the effects of AIH on cortical excitability remain largely unknown. We investigated the effects of AIH on cortical excitability within the primary motor cortex.

Methods  Eleven healthy, right-handed participants completed two testing sessions: (1) AIH (comprising 3 min in hypoxia [fraction of inspired oxygen ~ 10%] and 2 min in normoxia repeated over five cycles) and (2) normoxia (NOR) (equivalent duration to AIH). Single- and paired-pulse transcranial magnetic stimulations were delivered to the primary motor cortex, before and 0, 25, and 50 min after AIH and normoxia.

Results  The mean nadir in arterial oxygen saturation was lower (p < 0.001) during the cycles of AIH (82.5 ± 4.9%) than NOR (97.8 ± 0.6%). There was no significant difference in corticospinal excitability, intracortical facilitation, or intracortical inhibition between AIH and normoxia conditions at any time point (all p > 0.05). There was no association between arterial oxygen saturation and changes in corticospinal excitability after AIH (r = 0.05, p = 0.87).

Conclusion  Overall, AIH did not modify either corticospinal excitability or excitability of intracortical facilitatory and inhibitory circuits within the primary motor cortex. Future research should explore whether a more severe or individualised AIH dose would induce consistent, measurable changes in corticospinal excitability.

Keywords  Acute intermittent hypoxia · Corticospinal excitability · Transcranial magnetic stimulation · Primary motor cortex · Intracortical inhibition

Abbreviations

AIH  Acute intermittent hypoxia
CNS  Central nervous system
EMG  Electromyography
FiO2  Fraction of inspired oxygen
ICF  Intracortical facilitation
MEP  Motor-evoked potential
NOR  Normoxia
SICF  Short-interval intracortical facilitation
SICI  Short-interval intracortical inhibition
TMS  Transcranial magnetic stimulation

Introduction

Neurodegenerative disorders represent one of the leading causes of mortality, comprising approximately 12% of deaths globally (Tamburin et al. 2019). Traditional pharmacological
and physical activity-based therapies have limited efficacy in treating neurodegenerative disorders. Neuroplasticity refers to the ability of the central nervous system (CNS) to adapt in response to intrinsic and extrinsic stimuli and forms the basis for functional alterations (Cramer et al. 2011). Neuroplasticity is important for the development and recovery of essential functions, including motor function, following injury or neurodegeneration, e.g., spinal cord injury and stroke (Khan et al. 2017). Recently, conditioning of the CNS using low oxygen exposure has gained popularity as a potential non-pharmacological neurotherapeutic solution to treat or improve symptoms associated with movement disorders, likely via inducing neuroplasticity (Tamburin et al. 2019).

Acute intermittent hypoxia (AIH) refers to brief, repetitive periods of breathing a reduced (e.g., 10%) fraction of normoxia (i.e., FiO2 21%) (Navarrete-Opazo and Mitchell demonstrated chronic benefits for walking speed and endurance in individuals with incomplete lesions (Lovett-Barr et al. 2000). Therefore, this study aimed to examine corticospinal excitability and the excitability of excitatory and inhibitory intracortical circuits (MEP amplitude, input/output curve, SICI, and SICF) using single- and paired-pulse TMS protocols with ICIs after AIH or normoxia.

To our knowledge, only one study has evaluated how AIH for 30 min acutely modulates corticospinal excitability in healthy young adults (Christiansen et al. 2018). Single-pulse MEP amplitude and cervicomедullary MEPs increased by ~40% for at least 75 min following AIH. In the same study, short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF), measured with paired-pulse TMS and F-waves, measured by electrical stimulation, were unchanged. The increase in MEP amplitude elicited by TMS and electrical stimulation (sub-cortical in origin) suggests that AIH can increase corticospinal excitability without modulating cortical processing, indicating that these changes are likely of a sub-cortical origin and primarily related to corticospinal synaptic plasticity (Christiansen et al. 2018). While these results have promising clinical implications, the effects of AIH on corticospinal excitability need to be further investigated by considering additional TMS measures that were not assessed by Christiansen et al. (2018). Specifically, examining the input/output curve, a more comprehensive measure that provides an indication of the states of corticospinal excitability is warranted (Carson et al. 2013). Additionally, measuring short-interval intracortical facilitation (SICF) would provide indications of the neuroplasticity of intracortical facilitatory circuits with AIH (Doeltgen and Ridding 2011).

Therefore, this study aimed to examine corticospinal excitability and the excitability of excitatory and inhibitory intracortical circuits (MEP amplitude, input/output curve, SICI, ICF, and SICF) using single- and paired-pulse TMS protocols, immediately, 25 and 50 min after exposure to AIH and normoxia. We hypothesised that there would be (1) an increase in MEP amplitude and input/output curves will show overall potentiation after AIH but not in normoxia, and (2) no changes in intracortical facilitation or inhibition (i.e., SICI, SICF, and ICF) after AIH or normoxia.
Methods

Participants

Nineteen right-handed participants (Edinburgh Handedness Inventory score > 40; Oldfield 1971) completed the study. All participants were screened with a TMS Safety Screen and excluded if there were any contraindications to TMS based on established international guidelines (Rossi et al. 2011, 2021), if they were taking medications acting on the CNS, or if they had any exposure to terrestrial altitude/intermittent hypoxia in the last 3–6 months. To include data on the participants who demonstrated a considerable response to the entire duration of the hypoxic protocol, a decrease of at least 3% in relative arterial oxygen saturation (SpO2, i.e., mean SpO2 across 25-min hypoxic session relative to that for normoxic session) was required, and only those who met this criterion were included in the analyses (6 participants excluded). All TMS trials were screened for background EMG activity in the 50 ms preceding TMS, and only those participants who did not show EMG activity > 0.01 mV during this time period were included in the analyses (two participants excluded). Following these exclusions, analysis was conducted on results from 11 participants (7 females; age: 29 ± 8 years; age range: 21–54 years). Prior to commencing the study, all participants provided written, informed consent in accordance with the Declaration of Helsinki. This study was approved by the Murdoch University Human Research Ethics Committee (2019/033).

Experimental design

On separate days (at least 3 days apart), participants underwent two laboratory sessions: AIH and normoxic sham (NOR). The order of the session was pseudo-randomised and participants were blinded to the experimental condition. In each session, TMS was used to elicit neurophysiological responses (measured via surface electromyography; EMG) before (pre) and 0, 25, and 50 min after the delivery of the condition (Fig. 1).

Hypoxia was delivered through a mask connected to a hypoxic generator (Altitrainer, SMTEC SA, Nyon, Switzerland). The mask covered the participant’s nose and mouth and sealed around the cheeks and under the chin to prevent leaks through the mask. The AIH protocol consisted of 3 min in hypoxia (FiO2 ~ 10%) followed by 2 min in normoxia (FiO2 ~ 21%), repeated five times (total of 15-min hypoxia and 10-min normoxia). The NOR session consisted of breathing normoxic air (FiO2 ~ 21%) for 25 min. The hypoxic generator was hidden from participants’ view throughout the experimental sessions. For NOR, the hypoxic generator was on and set at a simulated altitude of 100 m, and pre-recorded sounds of the hypoxic generator were played to provide background noise and improve condition blinding. In the AIH session, the mask was removed during periods of normoxia; and mask application was kept the same as the AIH session throughout the NOR session. Safe hypoxic exposure in this body of literature is believed to range between 9 and 16% (Navarrete-Opazo and Mitchell 2014). We used a FiO2 of 10% in this study, because the hypoxic generator could not accommodate FiO2 values less than 10%. While this is slightly less severe than 9.4% FiO2 used by Christiansen et al. (2018), we attempted to counteract this by applying a longer hypoxic duration of 3 min (as opposed to 1 min used by Christiansen et al. 2018). Consequently, participants could reach lower SpO2 levels than they may have done with only 1 min of exposure.

Arterial oxygen saturation levels

The SpO2 was recorded every 20-s during experimental trials using a pulse oximeter positioned on the left index finger (Rossmax SB100, Switzerland; averages data over 4-s epochs). The nadir in SpO2 of each cycle was determined as the minimum of these data. The SpO2 data were also analysed as 1-min mean values for the duration of the 25-min condition, as well as the mean value across the cycles (i.e., mean of the five 3-min cycles).

Electromyographic recordings

Surface EMG activity was recorded from the first dorsal interosseus (FDI) muscle of the right hand through surface electrodes (Ag–AgCl). The skin was cleaned with ethanol and gauze before the active electrode was placed over the muscle belly and the reference electrode was placed on the metacarpophalangeal joint. A grounding electrode was placed on the medial epicondyle. The EMG data were amplified (×1000) and band-pass filtered (20–1000 Hz) using a CED 1902 amplifier (Cambridge Electronic Design, Cambridge, UK), and digitised at a sampling rate of 5000 Hz using a CED 1401 analogue-to-digital converter (Cambridge Electronic Design, Cambridge, UK). All EMG recordings were taken during resting state with participants asked to remain still, quiet and alert.

Transcranial magnetic stimulation

TMS was applied to the left M1 using a 90 mm figure-of-eight coil connected to a BiStim module that connected two MagStim 2002 Bistim magnetic stimulators (Magstim Co., Whitland, UK). The coil was held tangentially, at a 45°
angle (to the sagittal plane) over the scalp to induce a posterior–anterior current flow in the underlying brain tissue.

In each experimental session, the optimal site of stimulation for eliciting an MEP in the right FDI was determined. The optimal site was defined as the scalp site that elicited the largest and most consistent MEPs (Rossini et al. 2015). The optimal site was marked on the scalp at the start of each session and used for all subsequent stimulations in the session. In each session, two TMS intensities were determined: (1) resting motor threshold (RMT), and (2) the 1 mV stimulus intensity (SI1mV). RMT is defined as the lowest stimulation intensity (as a percentage of maximal machine output) that produced MEPs of ≥ 0.05 mV peak-to-peak amplitude at rest, in at least 5 out of 10 consecutive trials (Rothwell 1997; Rossini et al. 2015). SI1mV is defined as the stimulation intensity (as a percentage of maximum machine output) required to evoke a peak-to-peak MEP of ~ 1 mV.

### Transcranial magnetic stimulation outcome measures

During experimental trials, the input/output curve, SICI, ICF, and SICF were obtained before (pre) and at three time-points after (0, 25, and 50 min post) the condition (AIH or NOR). To ensure a stable baseline, two blocks of all TMS measures were conducted. The order of these measures was pseudo-randomised across participants and across sessions.

#### Input–output curve

The excitability of the corticospinal tract was assessed by obtaining input/output curves. Single-pulse TMS at intensities corresponding to 90, 110, 130, and 150% of each individual’s RMT were delivered (Devanne et al. 1997; Rossini et al. 2015). Ten trials were delivered at each stimulus intensity (total of 40 trials per block). The order of stimulus intensities was randomised and the intertrial interval was set at 5 s (± 20% jitter).
Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) Single- and paired-pulse TMS was delivered to measure SICI and ICF. The paired-pulse protocol comprised a subthreshold conditioning stimulus set at 80% of RMT and a test stimulus set at 111mV intensity, separated by an ISI of 3 ms for SICI (Kujirai et al. 1993) and 12 ms for ICF (Ziemann et al. 1998b). Each block comprised 15 paired-pulse trials targeting SICI, 15 paired-pulse trials targeting ICF, and 10 single-pulse trials (total 40 trials per block). The order of trials was pseudo-randomised and the inter-trial interval was set at 5 s (± 20% jitter).

Short-interval intracortical facilitation (SICF) Single- and paired-pulse TMS was delivered to measure SICF. The MEP elicited by TMS is the result of a complex descending volley of electrical activity comprising a direct wave (D-wave) and several indirect waves (I-waves): paired-pulse TMS can be used to probe early I-wave and late I-wave circuit excitability by varying the ISI (Tokimura et al. 1996; Ziemann et al. 1998a; Chen and Garg 2000). The paired-pulse protocol for SICF comprised a conditioning stimulus set at 111mV intensity and a subthreshold test stimulus set at 90% of RMT separated by ISIs of 1.5 and 4.5 ms. Each block comprised 15 paired-pulse trials with an ISI of 1.5 ms, 15 paired-pulse trials with an ISI of 4.5 ms, and 10 single-pulse trials (total 40 trials per block). The order of trials was randomised and the inter-trial interval was set at 5 s (± 20% jitter).

Data processing

Arterial oxygen saturation data

For correlation analysis, SpO2 data from the hypoxia session were normalised by presenting as a ratio of the SpO2 values for the normoxia session for each participant.

Transcranial magnetic stimulation data

Single-pulse MEP amplitudes obtained during SICI, ICF, and SICF measurements (total 20 single-pulse trials) were averaged at each time point (pre, 0, 25, and 50 min post-intervention). The mean paired-pulse MEP amplitude (i.e., conditioned MEP) was expressed as a ratio of the mean single-pulse MEP amplitude at each time point. Ratios < 1 reflect inhibition and ratios > 1 reflect facilitation. Neurophysiological measures were also normalised by expressing the post-time point measures (i.e., the average of 0, 25, and 50 min post-intervention) as a percentage of the pre measure.

Statistical analyses

Significance for all statistical analyses was set at p < 0.05. Group data are all presented as the mean (M) ± standard deviation (SD). All analyses were completed using IBM SPSS version 24 (IBM Corp, Armonk, NY).

For SpO2 data, a two-way repeated-measures ANOVA was performed on SpO2 data to determine whether levels differed between cycles (10% and 21%) and sessions (AIH and NOR). Separate ANOVAs were performed on the average and nadir SpO2 data.

For baseline TMS measures, repeated-measures ANOVAs were used to compare the single-pulse MEP amplitudes, SICI, ICF, and SICF between the two Pre-measurement blocks in the two sessions (see Supplementary Table 1). For single-pulse MEP amplitude, SICI and ICF, the ANOVAs had within-subjects factors of Pre-Measurement Block (two levels: Pre-1 and Pre-2) and Session (two levels: AIH and NOR). For SICF, two-way ANOVAs were performed separately for the two ISIs (1.5 ms and 4.5 ms) with the within-subjects factors of Pre-Measurement Block (two levels: Pre-1 and Pre-2) and Session (two levels: AIH and NOR). As there were no significant differences between the two Pre-measurement blocks for any of the measures, the two Pre-measurement blocks for each of the measures were averaged and used for all further analyses. A repeated-measures ANOVA was used to compare the Pre input/output curves between the two sessions, with within-subjects factors of Stimulus Intensity (four levels: 90% RMT, 110% RMT, 130% RMT, and 150% RMT) and Session (two levels: AIH and NOR). There were no significant differences in the Pre input/output curves between the AIH and normoxia sessions (see Supplementary Table 2).

To analyse the effect of AIH on neurophysiological measures, a two-way repeated-measures ANOVA was performed on raw single-pulse MEP amplitude data to test for differences between sessions and over time. A three-way repeated-measures ANOVA was performed on raw input/output curve data to determine whether MEP amplitude at the varying intensities differed between sessions and over time. Separate two-way repeated-measures ANOVAs were performed on the SICI and ICF ratios to determine whether SICI and ICF differed between sessions and over time. A three-way repeated-measures ANOVA was performed on SICF ratios to determine whether SICF at the two peaks differed between sessions and over time.

For all ANOVAs, Mauchley’s test of sphericity was examined, and in the event of a violation of sphericity, the Greenhouse–Geisser correction was applied to adjust degrees of freedom. Effect sizes were described in terms of partial eta-squared ($\eta_p^2$), with $\eta_p^2 < 0.06$ representing a small effect, $\eta_p^2 \geq 0.06$ a moderate effect, and $\eta_p^2 \geq 0.14$ a large effect).
To assess the association between the extent of changes in SpO2 level and changes in TMS measures, Spearman’s correlations were performed to analyse the relationship between change in normalised SpO2 and change in MEP amplitude, SICI, ICF, and SICF (expressed as percentage change from pre) for AIH and NOR sessions separately.

Results

Arterial oxygen saturation (SpO2)

For the participants included in our analyses, there was a significant main effect of Session for the average SpO2 of the five 3-min hypoxic cycles ($F_{1,10} = 98.75$, $p < 0.001$, $\eta_p^2 = 0.91$), with significantly lower SpO2 values in AIH (mean ± SD: 89.8 ± 2.8%, range: 85.4–94.1%) compared with NOR (mean ± SD: 98.3 ± 0.5%, range: 97.6–99.0%). However, for the mean SpO2 for the hypoxic cycles, there was no significant main effect of Cycle ($F_{4,40} = 0.68$, $p = 0.608$, $\eta_p^2 = 0.06$) and no significant Session*Cycle interaction ($F_{4,40} = 0.64$, $p = 0.637$, $\eta_p^2 = 0.06$).

For the nadir SpO2 data, there was a significant main effect of Session ($F_{1,10} = 97.58$, $p < 0.001$, $\eta_p^2 = 0.91$), with a significantly lower SpO2 in AIH than in NOR. The nadir in SpO2 across each cycle ranged from 76.2 to 90.2% (mean ± SD: 82.5 ± 4.9%). There was no significant main effect of Cycle ($F_{4,40} = 0.86$, $p = 0.495$, $\eta_p^2 = 0.08$) and no significant Session*Cycle interaction ($F_{4,40} = 0.82$, $p = 0.519$, $\eta_p^2 = 0.08$) for the nadir. There was a cyclical reduction in SpO2 levels during AIH but no significant change in SpO2 levels during NOR (Fig. 2). Mean SpO2 across the final 60 s of each hypoxic cycle ranged from 77.1% to 91.2% (mean ± SD: 83.9 ± 4.8%), which was similar to the results observed for the nadir.

Single-pulse TMS measures

The baseline single-pulse MEP amplitude was 2.31 ± 1.46 mV for the AIH session and 1.94 ± 0.84 mV for the NOR session. A repeated-measures ANOVA performed on single-pulse MEP amplitude showed no significant main effect of Session ($F_{1,10} = 0.67$, $p = 0.433$, $\eta_p^2 = 0.06$) or

![Fig. 2](image-url)
Time \( (F_{3,30} = 0.14, p = 0.938, \eta_p^2 = 0.01) \), and no significant Session*Time interaction \( (F_{3,30} = 0.15, p = 0.932, \eta_p^2 = 0.01) \) (Fig. 3A, B).

**Input/output curve**

For the AIH condition, the baseline MEP amplitude for the I/O curve was 0.03 ± 0.03 mV for 90%RMT, 0.79 ± 1.03 mV for 110%RMT, 2.31 ± 1.45 mV for 130%RMT, and 3.76 ± 1.73 mV for 150%RMT. For the NOR condition, the baseline MEP amplitude for the I/O curve was 0.19 ± 0.55 mV for 90%RMT, 0.99 ± 1.04 mV for 110%RMT, 2.46 ± 1.65 mV for 130%RMT, and 3.39 ± 1.81 mV for 150%RMT. As expected, the ANOVA showed a significant main effect of Intensity \( (F_{1,11,12} = 31.42, p < 0.000, \eta_p^2 = 0.76) \) but no significant main effect of Session \( (F_{1,10} = 0.26, p = 0.875, \eta_p^2 < 0.01) \) or Time \( (F_{3,30} = 2.42, p = 0.086, \eta_p^2 = 0.20) \). There was a significant Session*Intensity interaction \( (F_{2.2,21.7} = 4.23, p = 0.026, \eta_p^2 = 0.30) \). Post hoc analyses revealed that, for both AIH and NOR, MEP amplitude enlarged as TMS intensity increased from 90 to 150% of rMT, but remained unchanged across all time-points in AIH and NOR sessions (Fig. 3C, D). The significant Session*Intensity interaction was driven by the higher MEP amplitude in AIH relative to NOR at 150% MSO, which failed to reach the conventional significance level \( (p = 0.095) \). There were no other significant interactions: Session * Time \( (F_{3,30} = 0.09, p = 0.931, \eta_p^2 = 0.01) \); Time * Intensity \( (F_{3.0,30.0} = 1.26, p = 0.307, \eta_p^2 = 0.11) \); Session * Time * Intensity \( (F_{4.7,46.9} = 0.49, p = 0.774, \eta_p^2 = 0.05) \).

**Fig. 3** No change in Motor-Evoked Potentials (MEPs) by single-pulse TMS and in Input/Output curves after exposure to hypoxia compared to normoxia. Normalised MEP amplitude data observed before and after hypoxia (filled symbols) and normoxia (open symbols) exposure; each symbol reflects data from one individual (A). Column scatterplots of normalised MEP amplitude (post-time-points averaged and presented as a percentage of pre) data \( (n = 18) \) at baseline (dotted line) and after exposure to hypoxia and normoxia (B). Group MEP amplitude as a function of stimulation intensity hypoxia and normoxia sessions, respectively (C, D). Data points are offset horizontally for clarity in representation of data points.
Paired-pulse TMS measures

Short-interval intracortical inhibition (SICI)

The baseline SICI ratio was 0.29±0.17 for the AIH session and 0.33±0.24 for the NOR session. The repeated-measures ANOVA showed no significant main effect of Session (F1,10 = 2.34, p = 0.157, η2 = 0.19), no significant main effect of Time (F3,30 = 2.29, p = 0.098, η2 = 0.19), and no significant Session*Time interaction (F3,30 = 0.35, p = 0.789, η2 = 0.03). SICI elicited by paired-pulse TMS did not differ after exposure to either AIH or NOR (Fig. 4).

Intracortical Facilitation (ICF)

The baseline ICF ratio was 1.52±0.39 for the AIH session and 1.37±0.32 for the NOR session. There was no significant main effect of Session (F1,10 = 0.17, p = 0.685, η2 = 0.02) or Time (F3,30 = 0.30, p = 0.823, η2 = 0.03), and there was no significant Session*Time interaction (F3,30 = 0.90, p = 0.452, η2 = 0.08). As shown in Fig. 4, ICF elicited by paired-pulse TMS did not significantly differ across session or time-points.

Short-interval intracortical facilitation (SICF)

The baseline SICF ratio for the 1.5 ms ISI was 1.41±0.52 for the hypoxia session and 1.37±0.38 for the normoxia session. The baseline SICF ratio for the 4.5 ms ISI was 1.15±0.30 for the hypoxia session and 1.11±0.29 for the normoxia session. There was a significant main effect of Peak (F1,10 = 11.58, p = 0.007, η2 = 0.54) but no significant main effect of Session (F1,10 = 0.96, p = 0.351, η2 = 0.09) or Time (F3,30 = 2.24, p = 0.105, η2 = 0.18). There were also no significant Session*Peak (F1,10 = 0.12, p = 0.734, η2 = 0.01), Session*Time (F3,30 = 0.85, p = 0.479, η2 = 0.08), Peak*Time (F3,30 = 0.26, p = 0.853, η2 = 0.03), and Session*Peak*Time (F3,30 = 0.35, p = 0.793, η2 = 0.03) interactions. SICF at both peaks (1.5 and 4.5 ms ISIs) did not differ significantly across sessions or time-points (Fig. 4).

Associations between oxygen saturation and neurophysiological measures

Spearman’s bivariate correlations showed that there was no significant relationship between normalised SpO2 and changes in MEP amplitude, SICI, ICF, SICF Peak 1 (ISI 1.5 ms), or SICF Peak 3 (ISI 4.5 ms) (all r<0.32, all p>0.339).

Discussion

This study aimed to characterise the effects of AIH on changes in corticospinal excitability, as well as the excitability of intracortical facilitatory and inhibitory circuits within M1. Results show that MEP amplitude and input/output curves did not change with AIH or NOR. Contrary to our hypothesis, these results suggest that exposure to hypoxia did not affect corticospinal excitability. In addition, neither SICI, ICF, nor SICF changed with AIH or NOR. This suggests, in agreement with our hypothesis, that exposure to hypoxia did not alter the excitability of intracortical inhibitory or facilitatory circuits. Our results do not align with those from Christiansen et al. (2018), as AIH failed to induce short-term neuroplastic adjustments in the corticospinal system. These findings, however, remain specific to the experimental parameters used (FiO2 = 10% for 3-min on and 2-min off, over 5 cycles in healthy young adults).

Variable oxygen saturation levels during acute intermittent hypoxia exposure

As expected, AIH induced cyclical reductions in SpO2 levels. However, there was large inter-individual variability with minimum SpO2 values ranging from 76.2 to 90.2% (mean±SD: 82.5±4.9%). The fact that the same external stimulus (i.e., induced FiO2 levels in this study) initiates a different internal response (i.e., SpO2), to a fixed FiO2 (i.e., as used in this study), variability in the SpO2 response for a given FiO2 is known as AIH. Indeed, FiO2 levels below 9% are generally avoided as this is associated with greater risk for side effects such as hypoxic brain injury and cardiac arrhythmias (Navarrete-Opazo and Mitchell 2014). A large inter-individual variation in the degree of ventilatory drive in response to hypoxia may have increased variability in arterial hypoxemia (i.e., indirectly assessed here from SpO2 values) across participants. In the absence of direct minute ventilator measurement in our study, however, this remains speculative. Using a clamped SpO2, as opposed to a fixed FiO2 (i.e., as used in this study), variability in the response would have been decreased (Soo et al. 2020). Future research could also consider measuring an index of hyperventilation (e.g., partial pressure of end tidal carbon
Fig. 4 No change in intracortical inhibitory and facilitatory circuits after exposure to hypoxia compared to normoxia. The left column shows Normalised average SICI (A), ICF (C), SICF Peak 1 (E), and SICF Peak 3 (G) ratio (mean ± SD) data observed before and after hypoxia (filled symbol) and normoxia (open symbol) exposure. Data points are off-set horizontally for clarity in representation of data points. The right column shows scatterplots of normalised group SICI (B), ICF (D), SICF Peak 1 (F), and SICF Peak 3 (H) ratio (pre- as a percentage of post-average) data after exposure to hypoxia and normoxia.
dioxide) to determine whether the ventilatory response is associated with changes in corticospinal excitability.

Compared to the present study, Christiansen et al. (2018) delivered slightly lower oxygen levels (FiO2 ~ 9.4%) during their AIH protocol composed of 15 cycles of 1-min on and 1-min off pattern for 30-min, ultimately totaling 15-min of hypoxic exposure (identical to the current study). While Christiansen et al. (2018) also showed large inter-individual variability in the reduction of SpO2 levels with hypoxic exposure, their SpO2 levels were lower across the 15-min of hypoxia (range: ~ 80–85%) than those observed for the 15-min exposure in our study (89.8 ± 2.8%, range: 85.4–94.1%). While we did observe lower SpO2 values in the final minute of each 3-min cycle (83.9 ± 4.8%), it is possible that the different cycle times we implemented (3-min hypoxia followed by 2-min normoxia) compared to that used by Christiansen et al. (2018) underpins these differences. Indeed, data presented by Christiansen et al. (2018) appear to show a trend for SpO2 to decrease across the first six 1-min cycles (between cycle analyses not reported), which may represent an accumulative effect of the hypoxic exposure with more frequent but shorter normoxic recovery periods, which we did not observe.

Although there was no association between change in SpO2 levels and change in MEP amplitude from baseline to post-exposure, it is possible that there is some interplay between SpO2 and plastic changes in the corticospinal system that might be observed with a larger sample. The relationship between changes in SpO2 levels and changes in MEP was not directly reported by Christiansen et al. (2018). However, the difference in results between the two studies suggests that a slightly more severe FiO2 level of 9.4% (used by Christiansen et al. 2018) compared to 10% used in the current study, as well as the more frequent yet briefer hypoxic exposures, might have resulted in larger changes in SpO2 levels which might have consequently evoked changes in TMS measures. This explanation is speculative and remains to be tested.

Corticospinal excitability

There were no significant changes in single-pulse MEP amplitude or input/output curve following exposure to AIH, compared to normoxia, across all time-points. This result is inconsistent with the study by Christiansen et al. (2018), who did report increases in MEP amplitude following exposure to a 30-min AIH protocol. The lack of change in MEP amplitude at any of the tested TMS intensities suggests that neuronal recruitment patterns in M1 were not affected by AIH for the participants tested in our study.

Intracortical circuit excitability

There were no significant changes in SICI and ICF following exposure to AIH across all time periods. SICI provides a measure of intracortical inhibition and likely reflects activation of GABA inhibitory circuits (Kujirai et al. 1993; Di Lazzaro et al. 2006), while ICF is suggested to be a facilitatory circuit modulated by glutamatergic mediated processes (Di Lazzaro and Ziemann, 2013). The lack of changes in SICI and ICF may suggest that AIH exposure did not cause changes in GABAergic-mediated intracortical inhibitory and glutamatergic intracortical facilitatory pathways within M1. Our study was the first to measure SICF in response to AIH. The results also showed that there were no differences in SICF between the AIH and NOR sessions, suggesting that the excitability of intracortical facilitatory circuits mediated by 1-wave generating processes within M1 was not likely affected by AIH exposure (Stefan et al. 2004; Sale et al. 2007; Kamke et al. 2012, 2014).

Given the absence of any changes in intracortical processes in the current study, it is interesting to speculate on potential mechanisms of AIH-induced neuroplasticity. Studies examining the physiological mechanisms of hypoxia-induced neuroplasticity have largely focussed on SCI injury and on respiratory and spinal motoneurons (Fuller et al. 2000, 2003; Dale-Nagle et al. 2010; Gonzalez-Rothi et al. 2015a; Prosser-Loose et al. 2015). In SCI patients, the specific mechanisms of neuroplasticity are sensitive to the hypoxic dose. AIH activates serotonin-dependent mechanisms, known as respiratory long-term facilitation (Hayashi et al. 1993; Mitchell et al. 2001), which enhance ventilation or respiratory motor output through the release of serotonin that, in turn, strengthens synaptic pathways to phrenic motor neurons (Hayashi et al. 1993; Wilkerson and Mitchell 2009). Sustained or ‘severe’ doses of induced hypoxia activate adenosine-mediated pathways of long-term facilitation (Nichols et al. 2012; Devinney et al. 2016). Serotonin and adenosine, while both inducing long-term facilitation, are opposing mechanisms that could result in no net effect of inducing hypoxia. Given the severity of the hypoxia protocol affects these pathways, AIH dose is a key consideration for hypoxia research (Nichols et al. 2012).

Limitations and future considerations

The current study recruited a larger proportion of female than male participants, whereas the Christiansen et al.’s (2018) study only recruited male participants. The inclusion of female subjects in our study, while critical for ensuring results that are generalizable within the population, requires further consideration. Menstrual cycle and menopause are known to affect corticospinal excitability (Zoghi et al. 2015), implying that the stage of the menstrual cycle should be...
controlled for or recorded in research studies. In the current investigation, female participants could have been at different stages of their menstrual cycle in the two experimental sessions. Furthermore, the sample size of the study was further reduced based on participants not demonstrating a substantial response to hypoxic exposure or based on pre-stimulus EMG which overall likely decreased the power of the study. The effect sizes associated with the Session * Time interaction for all measures are < 0.08 (small-to-medium effect size). Accordingly, the study would have required a larger sample size to detect significant differences.

MEP amplitude elicited by single-pulse TMS and I/O curves reflect excitability in both cortical and sub-cortical pathways including the spinal cord (Rothwell 1997, 2011; Ridding and Rothwell 2007; Rossini et al. 2015). While we did not observe changes in single-pulse TMS and I/O curve measures, these TMS measures may not have been as sensitive for identification of changes occurring specifically in the spinal cord. As shown by Christiansen et al. (2021), AIH can induce changes at the spinal level; future research should include targeted examination of spinal circuit excitability using techniques such as the Hoffmann–Reflex and cervicomедullary motor-evoked potential (McNeil et al. 2013) to further characterise changes at the spinal level following AIH.

For future studies, a clamp-based approach to inducing hypoxia could be used whereby the external FiO2 levels are manipulated to evoke a desired SpO2 level in each participant. A desired level of hypoxia FiO2 may be considered at values less than 10% as prior literature on continuous or intermittent hypoxia exposure suggests that levels above 10% may not affect corticospinal excitability (Szubski et al. 2006; Goodall et al. 2010; Rupp et al. 2012; Christiansen et al. 2018). The pattern of exposure (i.e., the time of exposure to hypoxia and normoxia in each cycle and the number of cycles) must also be factored in when determining the optimal AIH dose. Currently, technological limitations prevent studies from being able to rapidly and accurately adjust the FiO2 levels in response to an individual’s SpO2 when inducing intermittent hypoxia; therefore, with future advances in technology, such manipulations and clamp-based studies may be warranted. Finally, in the current study, we did not ask participants to state which condition they thought they had in the two sessions to verify the effectiveness of our condition blinding strategy.

Conclusions

Overall, AIH did not modify corticospinal excitability and excitability of GABAergic, glutamatergic, and I-wave generating processes acting within M1 when measured up to 50-min after the intervention. Our findings do not support using AIH to facilitate neuroplasticity, at least under present circumstances (FiO2 10%). Future research systematically examining the effects of varying doses of AIH on cortical excitability is warranted given that AIH has been shown to improve function in patients with spinal cord injury.

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s00421-022-04982-8.

Author contributions  All authors contributed to the study conception and design. Data collection was performed by RF, and material preparation and data analysis were performed by SR, AMV, and HF. The first draft of the manuscript was written by SR and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding  Open Access funding enabled and organized by CAUL and its Member Institutions. The research leading to these results received funding from an institutional Small Research Grant scheme. Author BRS is supported by an NHMRC Investigator Grant (APP1196462). Author AMV is supported by an Australian Research Council Discovery Early Career Researcher Award (DE190100694).

Declarations

Competing interests  The authors have no relevant financial or non-financial interests to disclose.

Ethics approval (research involving human participants)  All procedures performed involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Murdoch University Human Research Ethics Committee (2019/033).

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References

Bailleul S, Chacaroun S, Doutreleau S, Detante O, Pepin JL, Verges S (2017) Hypoxic conditioning and the central nervous system: a new therapeutic opportunity for brain and spinal cord injuries? Exp Biol Med (maywood) 242:1198–1206
Barker AT, Jalinous R, Freeston IL (1985) Non-invasive magnetic stimulation of human motor cortex. Lancet 1:1106–1107
Chen R, Garg R (2000) Facilitatory I wave interaction in proximal arm and lower limb muscle representations of the human motor cortex. J Neurophysiol 83:1426–1434
Christiansen L, Urbin MA, Mitchell GS, Perez MA (2018) Acute intermittent hypoxia enhances corticospinal synaptic plasticity in humans. Elife 7:e34304

Christiansen L, Chen B, Lei Y, Urbin MA, Richardson MSA, Oudega M, Sandhu M, Rymer WZ, Trumbower RD, Mitchell GS, Perez MA (2021) Acute intermittent hypoxia boosts spinal plasticity in humans with tetraplegia. Exp Neurol 335:113483

Costello JT, Bhogal AS, Williams TB, Bekoe R, Sabir A, Tipton MJ, Corbett J, Mani AR (2020) Effects of normobaric hypoxia on oxygen saturation variability. High Alt Med Biol 21:76–83

Cramer SC, Sur M, Dobkin BH, O’Brien C, Sanger TD, Trojanowski JQ, Rumsey JM, Hicks R, Cameron J, Chen D, Chen WG, Cohen LG, deCharms C, Duffy CJ, Eden GF, Fetz EE, Filart R, Freund M, Grant SJ, Haber S, Kalivas PW, Kolb B, Kramer AF, Lynch M, Mayberg HS, McQuillen PS, Ntikin R, Pascual-Leone A, Reuter-Lorenz P, Schiff N, Sharma A, Shekim L, Stryker M, Sullivan EV, Vinogradov S (2011) Harnessing neuroplasticity for clinical applications. Brain 134:1591–1609

Dale EA, Ben Mabrouk F, Mitchell GS (2014) Unexpected benefits of intermittent hypoxia: enhanced respiratory and nonrespiratory motor function. Physiology (bethesda) 29:39–48

Dale-Nagle EA, Hoffman MS, MacFarlane PM, Satriotomo I, Lovett-Barr MR, Vinit S, Mitchell GS (2010) Spinal plasticity following intermittent hypoxia: implications for spinal injury. Ann N Y Acad Sci 1198:252–259

Deevane H, Lavoie BA, Capaday C (1997) Input-output properties and gain changes in the human corticospinal pathway. Exp Brain Res 114:329–338

Deviney MJ, Nichols NL, Mitchell GS (2016) Sustained hypoxia elicits competitive spinal mechanisms of phrenic motor facilitation. J Neurosci 36:7877–7885

Di Lazzaro V, Ziemann U (2013) The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. Front Neural Circuits 7:18

Di Lazzaro V, Pilato F, DiLeone M, Ranieri F, Ricci V, Prolice P, Bria P, Tonali PA, Ziemann U (2006) GABAAB receptor subtype specific enhancement of inhibition in human motor cortex. J Physiol 575:721–726

Di Lazzaro V, Prolice P, Ranieri F, Capone F, DiLeone M, Oliviero A, Pilato F (2012) I-wave origin and modulation. Brain Stimul 5:512–525

Doelgen SH, Ridding MC (2011) Modulation of cortical motor networks following primed theta burst transcranial magnetic stimulation. Exp Brain Res 215:199–206

Fuller DD, Bach KB, Baker TL, Kinkead R, Mitchell GS (2000) Long term facilitation of phrenic motor output. Respir Physiol 121:135–146

Fuller DD, Johnson SM, Olson EB Jr, Mitchell GS (2003) Synaptic pathways to phrenic motoneurons are enhanced by chronic intermittent hypoxia after cervical spinal cord injury. J Neurosci 23:2993–3000

Gangwar A, Paul S, Ahmad Y, Bhargava K (2020) Intermittent hypoxia modulates redox homeostasis, lipid metabolism associated inflammatory processes and redox post-translational modifications: benefits at high altitude. Sci Rep 10:7899

Gonzalez-Rothi EJ, Lee K-Z, Dale EA, Reier PJ, Mitchell GS, Fuller DD (2015a) Intermittent hypoxia and neurorehabilitation. J Appl Physiol 119:1455–1465

Gonzalez-Rothi EJ, Lee KZ, Dale EA, Reier PJ, Mitchell GS, Fuller DD (2015b) Intermittent hypoxia and neurorehabilitation. J Appl Physiol (1985) 119:1455–1465

Goodall S, Ross EZ, Romer LM (2010) Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. J Appl Physiol (1985) 109:1842–1851

Hallett M (2000) Transcranial magnetic stimulation and the human brain. Nature 406:147–150

Hallett M (2007) Transcranial magnetic stimulation: a primer. Neuron 55:187–199

Hayashi F, Coles SK, Bach KB, Mitchell GS, McEchron DM (1991) Time-dependent phrenic nerve responses to carotid afferent activation: intact vs. decerebellate rats. Am J Physiol 265:R811-819

Hurtado A (1960) Some clinical aspects of life at high altitudes. Ann Intern Med 53:247–258

Kamke MR, Hall MG, Lyce HF, Sale MV, Fenlon LR, Carroll TJ, Riek S, Mattingley JB (2012) Visual attentional load influences plasticity in the human motor cortex. J Neurosci 32:7001

Kamke MR, Ryan AE, Sale MV, Campbell MEJ, Riek S, Carroll TJ, Mattingley JB (2014) Visual spatial attention has opposite effects on bidirectional plasticity in the human motor cortex. J Neurosci 34:1475

Khan F, Amaya B, Galea MP, Gonzenbach R, Kesselring J (2017) Neurorehabilitation: applied neuroplasticity. J Neurol 264:603–615

Kuijira T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroo S, Asselman P, Marsden CD (1993) Corticocortical inhibition in human motor cortex. J Physiol 471:501–519

Lovett-Barr MR, Satriotomo I, Muir GD, Wilkerson JE, Hoffman MS, Vinit S, Mitchell GS (2012) Repetitive intermittent hypoxia induces respiratory and somatic motor recovery after chronic spinal spinal injury. J Neurosci 32:3591–3600

McNeil CJ, Butler JE, Taylor JL, Gandevia SC (2013) Testing the excitability of human motoneurons. Front Hum Neurosci 7:152

Merton PA, Morton HB (1980) Stimulation of the cerebral cortex in the intact human subject. Nature 285:227

Mitchell GS, Baker TL, Nanda SA, Fuller DD, Zakha AG, Hodgeman BA, Bavis RW, Mack KJ, Olson EB Jr (1985) (2001) Invited review: Intermittent hypoxia and respiratory plasticity. J Appl Physiol 90:2466–2475

Morton JP, Cable NT (2005) Effects of intermittent hypoxic training on aerobic and anaerobic performance. Ergonomics 48:1535–1546

Navarrete-Opazo A, Mitchell GS (2014) Therapeutic potential of intermittent hypoxia: a matter of dose. Am J Physiol Regul Integr Comp Physiol 307:R1181-1197

Nichols NL, Dale EA, Mitchell GS (2012) Severe acute intermittent hypoxia elicits phrenic long-term facilitation by a novel adenosine-dependent mechanism. J Appl Physiol 112:1678–1688

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113

Oudega M, Perez MA (2012) Corticospinal reorganization after spinal cord injury. J Physiol 590:3647–3663

Prabhakar NR (2001) Oxygen sensing during intermittent hypoxia: cellular and molecular mechanisms. J Appl Physiol (1985) 90:1986–1994

Prosser-Loose EJ, Hassan A, Mitchell GS, Muir GD (2015) Delayed intervention with intermittent hypoxia and task training improves forelimb function in a rat model of cervical spinal injury. J Neurotrauma 32:1403–1412

Ridding MC, Rothwell JC (2007) Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 8:559–567

Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2011) Screening questionnaire before TMS: an update. Clin Neurophysiol 122:1686

Rossi S, Antal A, Bestmann S, Bikson M, Brewer C, Brockmöller J, Carpenter LL, Cincotta M, Chen R, Daskalakis JD, Di Lazzaro V, Fox MD, George MS, Gilbert D, Kimiskidis V, Koch G, Ilmoniemi RJ, Lefaucheur JP, Leocani L, Lisanby SH, Minussi C, Padberg F, Pascual-Leone A, Wroe S, Asselman P, Marsden CD (2001) Invited review: Intermittent hypoxia and respiratory plasticity. J Appl Physiol 90:2466–2475

Shafi MM, Siebner HR, Ugawa Y, Wassermann EM, Zangen A, Carpenter LL, Cincotta M, Chen R, Daskalakis JD, Di Lazzaro V, TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: expert Guidelines. Clin Neurophysiol 132:269–306
Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, Di Lazzaro V, Ferreri F, Fitzgerald PB, George MS, Hallett M, Lefaucheur JP, Langguth B, Matsumoto H, Miniussi C, Nitsche MA, Pascual-Leone A, Paulus W, Rossi S, Rothwell JC, Siebner HR, Ugawa Y, Walsh V, Ziemann U (2015) Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 126:1071–1107
Rothwell JC (1997) Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. J Neurosci Methods 74:113–122
Rupp T, Jubeau M, Wuyam B, Perrey S, Levy P, Millet GY, Verges S (2012) Time-dependent effect of acute hypoxia on corticospinal excitability in healthy humans. J Neurophysiol 108:1270–1277
Sale MV, Ridding MC, Nordstrom MA (2007) Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 181:615–626
Sandhu MS, Perez MA, Oudega M, Mitchell GS, Rymer WZ (2021) Efficacy and time course of acute intermittent hypoxia effects in the upper extremities of people with cervical spinal cord injury. Exp Neurol 342:113722
Stefan K, Wycislo M, Classen J (2004) Modulation of associative human motor cortical plasticity by attention. J Neurophysiol 92:66–72
Sutor T, Cavka K, Vose AK, Welch JF, Davenport P, Fuller DD, Mitch-ell GS, Fox EJ (2021) Single-session effects of acute intermittent hypoxia on breathing function after human spinal cord injury. Exp Neurol 342:113735
Szukski C, Burtscher M, Loscher WN (1985) (2006) The effects of short-term hypoxia on motor cortex excitability and neuromuscular activation. J Appl Physiol 101:1673–1677
Tamburin S, Smania N, Saltuari L, Hoemberg V, Sandrini G (2019) Editorial: New advances in neurorehabilitation. Front Neurol 10:1090
Tan AQ, Sohn WJ, Naidu A, Trumbower RD (2021) Daily acute intermittent hypoxia combined with walking practice enhances walking performance but not intralimb motor coordination in persons with chronic incomplete spinal cord injury. Exp Neurol 340:113669
Tokimura H, Ridding MC, Tokimura Y, Amassian VE, Rothwell JC (1996) Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex. Electromyogr Control-Electroencephalo-Graph Clin Neurophysiol 101:263–272
Welch JF, Perim RR, Argento PJ, Sutor TW, Vose AK, Nair J, Mitch-ell GS, Fox EJ (2021) Effect of acute intermittent hypoxia on cortico-diaphragmatic conduction in healthy humans. Exp Neurol 339:113651
Wilkerson JE, Mitchell GS (2009) Daily intermittent hypoxia augments spinal BDNF levels, ERK phosphorylation and respiratory long-term facilitation. Exp Neurol 217:116–123
Ziemann U, Tergau F, Wassermann EM, Wischer S, Hildebrandt J, Paulus W (1998a) Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation. J Physiol (lond) 511:181–190
Ziemann U, Tergau F, Wassermann EM, Wischer S, Hildebrandt J, Paulus W (1998b) Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation. J Physiol 511(Pt 1):181–190
Zoghi M, Vaseghi B, Bastani A, Jaberzadeh S, Galea MP (2015) The effects of sex hormonal fluctuations during menstrual cycle on cortical excitability and manual dexterity (a Pilot study). PLoS ONE 10:e0136081

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