TIME-DEPENDENT EFFECT OF ACUTE HYPOXIA ON CORTICOSPINAL EXCITABILITY IN HEALTHY HUMANS

Running title: Hypoxia and cortex excitability

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ABSTRACT

Contradictory results regarding the effect of hypoxia on cortex excitability have been reported in healthy subjects, possibly depending on hypoxia exposure duration. We evaluated the effects of 1 and 3 hours hypoxia on motor corticospinal excitability, intracortical inhibition and cortical voluntary activation (VA) using transcranial magnetic stimulation (TMS). TMS to the quadriceps cortex area and femoral nerve electrical stimulations were performed in 14 healthy subjects. Motor-evoked potentials (MEPs at 50-100% maximal voluntary contraction -MVC), recruitment curves (MEPs at 30-100% maximal stimulator power output at 50% MVC), cortical silent periods (CSP) and VA were measured in normoxia and after 1 (n=12) or 3 (n=10) hours of hypoxia (FiO₂=0.12). One-hour hypoxia did not modify any parameters of corticospinal excitability but reduced slightly VA probably due to the repetition of contractions 1-h apart (96±4% vs. 94±4%; P=0.03). Conversely, 3-h hypoxia significantly increased i) MEPs of the quadriceps muscles at all force levels (+26±14%, +24±12% and +27±17% at 50, 75 and 100% MVC, respectively; P=0.01) and stimulator power outputs (e.g. +21±14% at 70% maximal power), and ii) CSP at all force levels (+20±18%, +18±19% and +14±22% at 50, 75 and 100% MVC, respectively; P=0.02) and stimulator power outputs (e.g. +9±8% at 70% maximal power), but did not modify VA (98±1% vs. 97±3%; P=0.42). These data demonstrate a time-dependent hypoxia-induced increase in motor corticospinal excitability and intra-cortical inhibition, without changes in VA. The impact of these cortical changes on physical or psychomotor performances needs to be elucidated to better understand the cerebral effects of hypoxemia.

Key words: Motor cortex, hypoxia, magnetic stimulation, muscle
INTRODUCTION

Integrity of the brain function profoundly depends on oxygen availability and reduced oxygen supply cannot be tolerated for long periods without critical cerebral consequences. Neurons mostly rely on oxidative metabolism for maintenance of ion homeostasis and membrane potential. They quickly sense reduced oxygen availability and consequently decrease their metabolic requirement and activity (Neubauer and Sunderram 2004). This may have functional consequences in healthy humans exposed to hypoxia, such as impaired cognitive performances (Virues-Ortega et al. 2004) or alterations in central motor command during fatiguing exercise (Amann et al. 2007; Goodall et al. 2010). Arguments in favor of hypoxia-induced perturbations of cerebral neuron activity also come from electroencephalographic recordings indicating reduced activity in hypoxia compared to normoxia both at rest (Ozaki et al. 1995) and during mental tasks (Papadelis et al. 2007).

Only a few studies have evaluated in healthy subjects the effect of hypoxia on motor corticospinal excitability as assessed with transcranial magnetic stimulation (TMS). After 10-min wash-in periods with inspiratory oxygen fraction (FiO₂) from 0.16 to 0.10 and pulse oxygen arterial saturation (SpO₂) from 93 to 74%, Goodall et al. (2010) reported no change in MEP amplitudes and CSP compared to normoxia, suggesting that corticospinal excitability and inhibition remained unaffected after some minutes of hypoxic gas inhalation. Conversely, after 20-30 min with FiO₂ = 0.12 (SpO₂ ~75%), Szubski et al. (2006) showed unchanged MEP amplitudes but reduced resting motor threshold (RMT) and shorter CSP compared to normoxia, suggesting that acute hypoxia may increase motor corticospinal excitability and decrease intracortical inhibition. Evaluation of motor corticospinal excitability by TMS after 3-5 days at 4554 m (SpO₂ ~84%) (Miscio et al. 2009) indicated higher RMT and lower short-interval intracortical inhibition as well as tendencies towards lower MEP and intracortical
facilitation compared to normoxia. This suggests that prolonged hypoxic exposure in healthy subject can lead to a hypoexcitability of both the excitatory and inhibitory cortical circuits. These differences between studies regarding the effects of hypoxia at rest on corticospinal excitability suggest that the length of exposure to hypoxia may be critical regarding its impact on cortex excitability and emphasize the need to further investigate the effect of hypoxia on corticospinal excitability. Moreover, the consequences of potential changes in corticospinal excitability and intracortical inhibition due to hypoxia on central motor command remain also to be clarified (Gandevia and Taylor 2006; Kalmar and Cafarelli 2006; Smith et al. 2007).

Therefore, the present study aimed to evaluate the effect of 1-h and 3-h hypoxic gas inhalation in healthy subjects on motor corticospinal excitability and inhibition and the effect of potential changes in corticospinal excitability on maximal voluntary activation (VA) assessed by TMS. One-hour hypoxic breathing encompass most of the exposure durations used in previous studies having investigated the neuromuscular effects of hypoxia at rest and during exercise (e.g. (Goodall et al. 2010; Szubski et al. 2006)). Three-hour hypoxic exposure corresponds to the earliest signs of acute mountain sickness including symptoms such as headache and tiredness (Hackett and Roach 2001) that have been previously correlated to changes in cortex excitability (Miscio et al. 2009). We hypothesized that longer hypoxic exposure would induce greater impairment of both excitatory and inhibitory cortical circuits that would be associated with impaired VA level.

**MATERIALS AND METHODS**

**Subjects**

Fourteen healthy physically active male subjects were studied, 12 in the first part of the protocol (age: 35 ± 9 yrs, body mass: 70 ± 9 kg, height: 176 ± 7 cm) and 10 in the second
part (age: 37 ± 7 yrs, body mass: 73 ± 7 kg, height: 180 ± 5 cm; no significant difference compared to part 1, \(P > 0.05\)). Eight subjects performed both parts of the protocol. The study was approved by the local ethics committee and performed according to the Declaration of Helsinki. All subjects gave their written informed consent before participating in the study.

**Experimental setup**

This study was part of a larger project evaluating the effect of hypoxia on exercise-induced neuromuscular fatigue and data measured before exercise only were used in order to assess the effect of hypoxia duration on motor corticospinal excitability and VA. The study protocol included two parts: the first part evaluated the effect of 1-hour hypoxic exposure while the second part evaluated the effect of 3-hour hypoxic exposure (Fig. 1A). In the first part of the protocol, subjects were evaluated on the same day before and after 1 h of hypoxic breathing. In the second part, subjects were evaluated on two occasions (at least 72 h apart) after either 3 hours of normoxic breathing or 3 hours of hypoxic breathing, in a randomized, counterbalanced order. Hence, in both parts of the protocol, normoxic and hypoxic measurements were performed at the same time of the day ±1 h to avoid the effect of diurnal variations in corticospinal excitability (Tamm et al. 2009).

The effect of hypoxia on motor corticospinal excitability, VA, neuromuscular transmission and muscle contractile properties were assessed on the right quadriceps femoris muscle under isometric conditions. TMS was used to assess motor corticospinal excitability and intracortical inhibition from MEP and CSP measurements. Electrical femoral nerve stimulation (ENS) was performed to measure neuromuscular transmission and contractile properties. VA was assessed with both ENS and TMS (see below).

Subjects refrained from physical exercise on the 2 days prior to the tests, refrained from drinking caffeinated beverages on test days, and were required to sleep for at least 7 h
the night before the tests and to have their last meal at least 2 h prior to the tests. Subjects sat on a custom-built quadriceps chair with the right hip angle set at 90° and knee joint angle set at 100° of flexion. A noncompliant strap connected to a strain gauge (Captels, St Mathieu de Treviers, France) was attached around the subject’s shank, 3–5 cm above the tip of the lateral malleoli. The subjects were firmly secured to the chair with noncompliant straps to minimize body movement. Subjects were at rest for at least 20 min before the first measurements, permitting skin preparation and electrode placing. Subjects inhaled the gas mixtures (normoxic with FiO₂ = 0.21 or hypoxic with FiO₂ = 0.12) delivered by an Altitrainer 200® (SMTEC, Nyon, Switzerland) via a face mask and were blinded for the gas mixture composition. This FiO₂ level is equivalent to an altitude of ~4100 m and induced arterial deoxygenation in healthy subjects comparable to values typically observed in hypoxemic respiratory patients at sea level.

Neurophysiological evaluations summarized in Figure 1B included the following stages: i) electromyographic (EMG) electrodes placement, ii) determination of ENS intensity, iii) measurement of maximal voluntary force (~10 submaximal warm-up knee extensions followed by 2 maximal voluntary contractions (MVC), 1-min apart), iv) determination of the optimal TMS site, v) determination of the optimal TMS intensity (recruitment curve), vi) 2 MVCs followed by supramaximal ENS to assess knee extensors contractile properties, and vii) assessment of peripheral (with ENS) and cortical (with TMS) VA. The whole procedure lasted for 1 h approximately. In Part 1 of the protocol, all measurements from stage v) to stage vii) were repeated after 1 h of hypoxic gas inhalation. In Part 2 of the protocol, all neurophysiological evaluations were performed identically after 3 h of normoxic or hypoxic breathing.

Electrical nerve stimulation
ENS was delivered percutaneously to the femoral nerve by a cathode electrode (20-mm diameter) pressed in the femoral triangle, 3–5 cm below the inguinal ligament. The anode, a 5 × 10 cm gel pad electrode (Compex SA, Ecublens, Switzerland), was located over the gluteal fold. For both single and paired stimulations (see below), square wave pulses (1-ms duration) were produced via a high-voltage (maximal voltage 400 V) constant-current stimulator (Digitimer DS7, Hertfordshire, UK). For all stimulus modalities, stimulation intensity corresponded to 150% of the optimal intensity (range 38–95 mA), i.e. the stimulus intensity at which the maximal amplitude of both twitch force and concomitant quadriceps muscle M-wave were reached. Supramaximal ENS was delivered i) 2 s after the MVCs with paired stimulations at 100 Hz (10-ms interstimulus interval) and ii) during and 2 s after the last MVC of the 4-contraction sets for VA assessment (see below) and M-wave measurement with single stimulations (see Fig. 1B).

Transcranial magnetic stimulation

A magnetic stimulator (Magstim 200, The Magstim Company, Dyfed, UK) was used to stimulate the motor cortex. Single TMS pulses of 1-ms duration were delivered via a concave double-cone coil (110 mm diameter; maximum output 1.4 T) positioned over the vertex of the scalp and held tangentially to the skull. The coil was slightly moved (following sagittal and coronal planes) to preferentially activate the left motor cortex (contralateral to the right leg) until eliciting the largest MEP in the rectus femoris with only a small MEP in the biceps femoris during knee extensions at 20% MVC and with a stimulation intensity of 70% of the maximal stimulator power output. The optimal stimulation site was rigorously marked over the scalp to ensure reproducibility of the stimulation conditions for each subject throughout the entire experimental day. Then, following 3 min of rest and at each measurement time point (i.e. in normoxia and in hypoxia), stimulations during brief (~4 s)
knee extensions at 50% MVC, i.e. the force level inducing the largest MEP on the quadriceps muscles (Sidhu et al. 2009), were performed at 30, 40, 50, 60, 70, 80, 90 and 100% of the maximal stimulator power output, with four trials for each stimulation intensity (10 s separated contractions at the same intensity, 30 s separated series of 4 contractions; stimulation intensities of the series were randomized). The stimulation intensity that elicited the largest *rectus femoris* MEP with small MEP of *biceps femoris* (amplitude < 10% of maximal *rectus femoris* M-wave) was selected for subsequent VA assessment. After another 3 min of rest, VA assessment consisted in 4 sets of 4 brief (~4 s) contractions at 100%, 75%, 50% and 100% MVC, with 10 s of rest between contractions and 30 s between series (Sidhu et al. 2009). TMS was delivered on the first three contractions and ENS (single stimulation) was delivered during and 2 s after the last contraction (Fig. 1). Strong verbal encouragements were given during MVCs and real-time visual feedback of target torque levels were provided via a custom software (Labview 8, National Instrument, Austin, USA) on a computer screen throughout the experiment.

**Electromyographic recordings**

The electromyographic (EMG) signal was recorded from the right *vastus lateralis, rectus femoris, vastus medialis* and *biceps femoris* muscles (as a surrogate for antagonist hamstring muscles) with four pairs of silver chloride surface electrodes of 20 mm diameter (universal ECG electrode, Control Graphic Medical, Brie Comte Robert, France) during ENS and TMS. Low resistance (< 5 kΩ) between the two electrodes was obtained by shaving, light abrasion of the skin and cleaning with alcohol. Recording electrode locations were based on SENIAM recommendations (Hermens and Freriks 1997) with an inter-electrode distance of 20 mm. The reference electrode was fixed over the patella. The positions of the EMG electrodes were marked with indelible ink on the first experimental day to ensure that they
were placed in the same location at subsequent visits. EMG signals were amplified and band-
pass filtered (5 Hz-1 kHz) using BioAmp and PowerLab systems (ADInstruments, Bella
Vista, Australia), and recorded at a sampling rate of 2 kHz and stored on a computer for
subsequent analysis.

Arterial saturation

SpO2 and heart rate (HR) were measured continuously using a pulse oximeter (Pulsox
300, Konica Minolta, Osaka, Japan) placed on the forefinger.

Data analysis

MEP peak-to-peak amplitudes of quadriceps muscles during TMS superimposed on
submaximal and maximal contractions were normalized to peak-to-peak amplitudes of the M
waves during single ENS delivered on relaxed muscle 2 s after the MVC. The duration of the
CSP was determined as the interval from stimulation to the time at which post-stimulus EMG
activity exceeded, for at least 100 ms, ± 2 SD of the pre-stimulus EMG calculated as the
averaged signal over the 500 ms preceding the stimulation (Goodall et al. 2010). Because
similar results were obtained for all quadriceps muscles, the averaged MEP amplitudes and
CSP values from the vastus lateralis, rectus femoris and vastus medialis were calculated in
each condition and used for further analysis.

Peripheral VA was assessed using twitch interpolation technique (Merton 1954). Briefly, the force produced during a superimposed single twitch (TwQs) delivered at peak
force during the MVC was compared with the force produced by a single ENS delivered on
relaxed muscle 2 s after the MVC (TwQp) and peripheral VA (%) was calculated using the
equation: \[1 - (\text{TwQs} / \text{TwQp})\] x 100. Cortical VA was quantified by measurement of the
force responses to TMS. Because motor cortex and spinal cord excitability increase during
voluntary contractions, it is necessary to estimate rather than directly measure the amplitude of the resting twitch evoked by motor-cortex TMS (Todd et al. 2003). The mean superimposed twitch (SIT) amplitude evoked during contractions at 100, 75, and 50% MVC was calculated, and the y-intercept of the linear regression between the mean SITs and voluntary force was used to quantify the estimated resting twitch (ERT) (Goodall et al. 2009; Sidhu et al. 2009; Todd et al. 2003). Cortical VA (%) was then calculated using the equation: [1 - (SIT / ERT)] x 100. The reliability of this method for the determination of cortical VA for the knee extensors has been described recently (Goodall et al. 2009; Sidhu et al. 2009).

Peak forces measured during MVC (before the magnetic stimulation) and TwQp, MEPs, CSPs, M-wave and VA were calculated as the averaged values obtained during the four sets of contractions (see Fig. 1) performed at each time point. Peak force during paired ENS at 100 Hz on relaxed muscles was also measured at each time point.

**Statistical analyses**

Normality of distribution and homogeneity of variances of the main variables were confirmed using a Skewness-Kurtosis normality test and the Levene’s test, respectively. In both parts of the protocol, two-way [FiO₂ (normoxic 0.21 or hypoxic 0.12) × force contraction level (50, 75, 100% MVC) or stimulation intensity (30-100% of the maximal stimulator output)] ANOVA with repeated measures was performed for each dependent variable. Post-hoc Fischer tests were applied to determine a difference between two mean values if the ANOVA revealed a significant main effect or interaction effect. Relationships between changes in MEP, CSP, VA and SpO₂ were also determined by Pearson product correlation. For all statistical analyses, an alpha level of 0.05 was used as the cut-off for significance. All descriptive statistics presented are mean values ± SD.
RESULTS

Part 1: Effects of 1-h hypoxic breathing

SpO₂ was 98 ± 1% in normoxia and 83 ± 5% after 1 h of hypoxia (main FiO₂ effect: \( F_{(1,11)} = 127.2, P < 0.001 \)). HR was 65 ± 8 bpm in normoxia and 69 ± 10 bpm after 1 h of hypoxia (\( P = 0.98 \)).

MEP amplitudes during contractions at 50% MVC with TMS intensities from 30 to 100% of the maximal magnetic stimulator output are shown in Figure 2A. No significant difference was observed between normoxia and hypoxia (\( P = 0.93 \)). Similar optimal TMS intensities were determined in normoxia (70 ± 10%) and after 1 h of hypoxic breathing (73 ± 8%; \( P = 0.19 \)). MEP amplitudes during contractions at 50, 75 and 100% MVC with the optimal TMS intensity are shown in Figure 3A. No significant difference was observed between normoxia and hypoxia (\( P = 0.78 \)). Similarly, no significant difference was observed in CSP during contractions at 50% MVC with TMS intensities from 30 to 100% of the maximal magnetic stimulator output (Figure 4A; \( P = 0.49 \)) and during contractions at 50, 75 and 100% MVC with the optimal TMS intensity (Figure 5A; \( P = 0.36 \)).

Peak forces during maximal voluntary and evoked contractions, M-wave amplitudes as well as cortical and peripheral VA are shown in Table 1. Peak forces during MVC (main FiO₂ effect: \( F_{(1,11)} = 7.0, P = 0.02 \)) and paired ENS at 100 Hz (main FiO₂ effect: \( F_{(1,11)} = 11.4, P = 0.006 \)) were significantly reduced after 1 h of hypoxic breathing compared to normoxia while no significant change in peak force during TwQp (\( P = 0.13 \)) was observed. M-wave amplitudes (\( P > 0.20 \) for all quadriceps muscles) and peripheral VA (\( P = 0.48 \)) did not differ significantly between normoxia and hypoxia. Cortical VA however was significantly reduced in hypoxia compared to normoxia (main FiO₂ effect: \( F_{(1,11)} = 5.8, P = 0.03 \)).
Part 2: Effects of 3-h hypoxic breathing

SpO$_2$ was 96 ± 1% in normoxia and 86 ± 5% after 3 h of hypoxia (main FiO$_2$ effect: $F_{(1,9)} = 45.1, P < 0.001$). HR was 75 ± 13 bpm in normoxia and 80 ± 18 bpm after 3 h of hypoxia ($P = 0.41$).

MEP amplitudes during contractions at 50% MVC with TMS intensities from 30 to 100% of the maximal magnetic stimulator output are shown in Figure 2B. MEP amplitudes were significantly higher in hypoxia compared to normoxia (main FiO$_2$ effect: $F_{(1,63)} = 7.1, P = 0.03$). Similar optimal TMS intensities were determined in normoxia (60 ± 10%) and after 3 h of hypoxic breathing (63 ± 9%; $P = 0.19$). MEP amplitudes during contractions at 50, 75 and 100% MVC with the optimal TMS intensity are shown in Figure 3B. MEP amplitudes were significantly higher in hypoxia compared to normoxia (main FiO$_2$ effect: $F_{(1,36)} = 10.3, P = 0.01$). Also, CSP were significantly longer in hypoxia compared to normoxia during contractions at 50% MVC with TMS intensities from 30 to 100% of the maximal magnetic stimulator output (Figure 4B; main FiO$_2$ effect: $F_{(1,63)} = 6.3, P = 0.04$) and during contractions at 50, 75 and 100% MVC with the optimal TMS intensity (Figure 5B; main FiO$_2$ effect: $F_{(1,36)} = 8.1, P = 0.02$).

Peak forces during voluntary and evoked contractions, M-wave amplitudes as well as cortical and peripheral VA are shown in Table 1. No difference was observed between normoxia and hypoxia (all $P > 0.05$).

Hypoxia-induced changes in MEP or CSP did not correlate with changes in VA or SpO$_2$ (all $r^2 < 0.15$ and $P > 0.05$).

When comparing the effect of 1 h and 3 h of hypoxic breathing in the 8 subjects having performed both parts of the protocol, 3 h hypoxia induced significantly larger increase in MEP amplitudes during contractions at 50% MVC with TMS intensities from 30 to 100%
of the maximal magnetic stimulator output (main FiO₂ effect: $F_{(1,49)} = 6.9, P = 0.03$) and
during contractions at 50, 75 and 100% MVC with the optimal TMS intensity (main FiO₂
effect: $F_{(1,14)} = 8.2, P = 0.02$) compared to 1 h hypoxia. Changes in CSP were also larger after
3 h compared to 1 h hypoxic breathing at 50% MVC with TMS intensities from 30 to 100%
of the maximal magnetic stimulator output (main FiO₂ effect: $F_{(1,49)} = 6.5, P = 0.04$) while a
tendency toward larger increase during contractions at 50, 75 and 100% MVC with the
optimal TMS intensity was observed (main FiO₂ effect: $F_{(1,14)} = 2.7, P = 0.10$). No other
difference was observed between responses to 1 h and 3 h hypoxic breathing.

**DISCUSSION**

In the present study, we used the recently described methods based on quadriceps (*i.e.*
a major locomotor muscle) responses during TMS (Goodall et al. 2009; Sidhu et al. 2009) to
evaluate the effects of 1 and 3 h hypoxic exposure on motor corticospinal excitability and
cortical VA. MEP amplitudes as well as CSP were significantly increased after 3 h of hypoxic
exposure only, indicating a time-dependent effect of hypoxia on corticospinal excitability.
These changes in corticospinal excitability after 3 h of hypoxia were not accompanied by any
change in cortical VA and MVC.

**Methodological aspects**

We defined the optimal TMS site and intensity during submaximal contractions that
are known to radically increase cortical excitability compared to muscles in a relaxed state,
rather than setting the TMS intensity based on the resting motor threshold, because all
subsequent MEP, CSP and VA measurements were performed during voluntary contractions.
This has nevertheless the inconvenience to require numerous submaximal voluntary
contractions (about 50) that may be demanding for the subjects and even lead to some degree
of fatigue despite the rest periods set between contractions and stages of the
neurophysiological evaluations. In the first part of the protocol, when neuromuscular
evaluations were repeated twice before and after 1 h of hypoxic breathing, we observed a
slight but significant force reduction for MVC and paired ENS at 100 Hz (Table 1),
suggesting some neuromuscular fatigue. Because acute hypoxia (from minutes to hours) has
been clearly shown to have no significant effect on MVC (Perrey and Rupp 2009), we believe
that the force reduction observed after 1 h hypoxia only is likely the consequence of the
numerous contractions performed at 50% MVC during the neuromuscular evaluations. Such a
high percentage of MVC (50%) was chosen to elicit large MEPs as previously reported
(Sidhu et al. 2009) and to minimize the effects of instructions and individual reaction time on
CSP (Mathis et al. 1998). This result underlines the drawback of TMS assessment during
voluntary contractions to measure cortex excitability and voluntary activation that can induce
per se some amount of muscle fatigue. To avoid this problem, submaximal contractions at
lower intensity (e.g. 20% MVC) may be used, although it remains to be evaluated whether it
would lead to similar results. Nevertheless, because in the minutes following fatiguing muscle
contractions MEP amplitude and CSP during voluntary contractions are back to pre-fatigue
values (Taylor et al. 1996), we believe that the fatigue induced in Part 1 of the protocol did
not preclude our ability to assess the effect of hypoxia per se on corticospinal excitability. At
last, the reduction in cortical VA without significant reduction in peripheral VA after 1 h
hypoxia suggests that cortical VA is more sensitive to central fatigue occurrence and therefore
emphasizes its significance to evaluate impaired neuromuscular function.

Hypoxia and corticospinal excitability
After 3 h of hypoxic breathing, the present results showed significantly greater MEP amplitudes and CSP durations, suggesting hyperexcitability of both excitatory and inhibitory corticospinal circuits. Interestingly, these effects were observed independently of force contraction levels (at least between 50 and 100% MVC) and TMS intensities (from 30 to 100% of the maximal stimulator output). Comparing the effect of 1 h and 3 h hypoxia in the 8 subjects having performed both parts of the protocol confirmed the time-dependent effect of hypoxia on MEP and CSP, although the effect on CSP at 50, 75 and 100% MVC with the optimal TMS intensity did not reach significance ($P = 0.10$) probably due to a type II error. Of note, no change in M-wave amplitude was observed (Table 1), suggesting that these changes were the consequences of adaptation mechanisms at the spinal and/or supraspinal levels. Measurement of the Hofmann-reflex response and calculation of the $H_{\text{max}}/M_{\text{max}}$ ratio provides an index of net $\alpha$-motoneuron excitability and/or modulation of the presynaptic inhibition of Ia afferents that has been evaluated in hypoxia. No significant change in $H_{\text{max}}/M_{\text{max}}$ ratio was observed after 20-min hypoxic exposure ($\text{FiO}_2 \sim 11%$; (Willer et al. 1987)) or after 1 week at 5050 m of altitude (Kayser et al. 1993). Also, F-wave considered as another index of spinal excitability, were reported to be unchanged after 20-30 min hypoxic breathing ($\text{FiO}_2 = 12%$; (Szubski et al. 2006)) or after 3-5 days at 4554 m of altitude (Miscio et al. 2009). Hence, although no data are available regarding spinal excitability following 1-3 h of hypoxic exposure, measurements performed after short and prolonged exposure indicate that net spinal excitability (i.e. the sum of all inhibitory and excitatory influences on the $\alpha$-motoneuron pool) is likely unchanged in hypoxia at rest. Therefore, we are confident that our results are likely to illustrate changes occurring at the motor cortex level. Further investigations (with measurements of cervicomedullary MEP for instance) are however needed to explore the changes at the spinal level during prolonged hypoxic exposure.
The MEP and CSP increases following 3 h hypoxic exposure are in contrast to previous results in healthy humans where no change in MEP amplitude (Goodall et al. 2010; Miscio et al. 2009; Szubski et al. 2006; 2007) and reduced (Szubski et al. 2006) or unchanged (Goodall et al. 2010; Miscio et al. 2009; Szubski et al. 2007) CSP in hypoxia were observed. Since we found no changes in MEP and CSP after 1 h of hypoxic exposure, we propose that the effect of hypoxic exposure on corticospinal excitability in healthy humans is time-dependent. However, in addition to hypoxic exposure duration, differences between the present results and previous observations in healthy subjects may also arise from methodological aspects such as MEP normalization (e.g. not normalized in (Miscio et al. 2009)), different muscle evaluated (e.g. first dorsal interosseus (Szubski et al. 2006)) and different procedures to determine CSP (Mathis et al. 1998). We did not observe any correlation between changes in SpO₂ and corticospinal excitability as previously reported after 20-30 min of hypoxic exposure in healthy humans (Szubski et al. 2006), while other observations in healthy subjects after more prolonged hypoxic exposure (3-5 days at high altitude (Miscio et al. 2009)) or in hypoxemic patients (Mohamed-Hussein et al. 2007) indicated significant correlation between the level of hypoxemia and changes in cortex excitability. Hence, the severity of hypoxemia may influence changes in corticospinal excitability at least during chronic hypoxic exposure. This remains however to be further investigated as well as the potential relationships between these changes and symptoms of acute mountain sickness (Miscio et al. 2009). Potential mechanisms underlying a time-dependent effect of hypoxic exposure in healthy subjects may relate to ventilatory and acid-base balance adaptations over the first hours and days in hypoxia (Dempsey et al. 1975; Forster et al. 1975). Such changes may be able to influence neuron excitability that is sensitive not only to hypoxia but also to changes in arterial CO₂ and circulating ions such as bicarbonates (Bonnet et al. 1998; Bruehl and Witte 2003; Gu et al. 2007; Gu et al. 2000).
Some previous results (Ainslie et al. 2007; Goodall et al. 2010) as well as personal observations (unpublished data) regarding cortex oxygenation assessed with near-infrared spectroscopy in healthy subjects inhaling an hypoxic gas mixture at rest indicated that the kinetics of cortex deoxygenation response is noticeably longer than arterial blood deoxygenation, requiring > 20-30 min to reach a steady state. Also, recent animal studies regarding brain oxygenation under hypoxemic conditions emphasize the specificity of brain tissue oxygenation response to hypoxemia, with for instance some cerebral reoxygenation being observed over the course of a 1-h stable periodic pattern of hypoxemia (Almendros et al. 2010). Therefore, the specific pattern of cerebral tissue deoxygenation under hypoxemic conditions could contribute to a time-dependent effect of hypoxic breathing on cortex excitability.

**Functional consequences of changes in corticospinal excitability**

Increased motor corticospinal excitability after 3 h of hypoxia did not induce changes in MVC or VA. This is in accordance with previous results showing changes in motor corticospinal excitability in healthy humans exposed to hypoxia for ~30 min without concomitant changes in force production and VA during brief MVCs (Szubski et al. 2006). These results as well as the reduction in cortical VA observed after 1 h of hypoxic exposure without changes in MEP amplitudes and CSP durations underline that cortex excitability and suboptimal output from the motor cortex during voluntary contractions reflect, at least in part, distinct mechanisms (Taylor and Gandevia 2001). It remains to be evaluated whether the effect of 3 h of hypoxia on corticospinal excitability may have functional consequences during intense sustained cerebral activation such as during physical exercise or demanding psychomotor tasks.
In conclusion, the present results demonstrate that acute hypoxia has a time-dependent effect on corticospinal excitability, as evidenced by significant increases in MEP and CSP after 3 hours but not after 1 hour of hypoxic breathing. These changes suggest an increase in motor cortex excitability and intra-cortical inhibition after 3 hours of hypoxic exposure. However, these changes had no impact on maximal force production and voluntary activation. Further investigations are needed to clarify the impact of these motor corticospinal changes on physical or psychomotor performances - in particular during intense sustained cerebral activation such as during physical exercise or demanding psychomotor tasks - to better understand the cerebral effects of hypoxemia.

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Author contributions: TR, MJ, SP, GM and SV designed the study and performed the measurements. TR, MJ and SV analyzed the data. TR, MJ, BW, SP, PL, GM and SV discussed the data and approved the final version of the manuscript.


FIGURE LEGENDS

Figure 1. Description of the study protocol (A) and the neurophysiological evaluations (B). A. Normoxic/hypoxic exposure and neurophysiological evaluations (hatched square) in parts 1 and 2 of the protocol. B. After setting electromyographic recording and electrical femoral nerve stimulation (ENS), maximal voluntary and evoked quadriceps responses were measured, the optimal transcranial magnetic stimulation (TMS) site was determined (in part 1, the optimal TMS site was defined in normoxia only and kept the same in hypoxia), TMS at different stimulator intensities were performed to establish the optimal intensity, and peripheral and cortical maximal voluntary activation levels were assessed (see Materials and Methods). MVC, maximal voluntary contraction.

Figure 2. Motor evoked potentials (MEP, normalized to maximum M-wave (Mmax)) measured during transcranial magnetic stimulation at different stimulator power outputs in normoxia and after 1 h (panel A) or 3 h (panel B) of hypoxic breathing. * significant main effect for hypoxia vs. normoxia ($P < 0.05$)

Figure 3. Motor evoked potentials (MEP, normalized to maximum M-wave (Mmax)) measured during transcranial magnetic stimulation at 50, 75 and 100% of the maximal voluntary force (MVC) in normoxia and after 1 h (panel A) or 3 h (panel B) of hypoxic breathing. * significant main effect for hypoxia vs. normoxia ($P < 0.05$)

Figure 4. Cortical silent periods (CSP) measured during transcranial magnetic stimulation at different stimulator power outputs in normoxia and after 1 h (panel A) or 3 h (panel B) of hypoxic breathing. * significant main effect for hypoxia vs. normoxia ($P < 0.05$)
Figure 5. Cortical silent periods (CSP) measured during transcranial magnetic stimulation at 50, 75 and 100% of the maximal voluntary force (MVC) in normoxia and after 1 h (panel A) or 3 h (panel B) of hypoxic breathing. * significant main effect for hypoxia vs. normoxia ($P < 0.05$)
Table 1. Mechanical and EMG quadriceps responses during maximal voluntary or evoked contractions as well as maximal peripheral and cortical voluntary activation levels in normoxia and after 1 h (Part 1) or 3 h (Part 2) of hypoxic breathing.

<table>
<thead>
<tr>
<th></th>
<th>MVC (Kg)</th>
<th>Db100 (Kg)</th>
<th>TwQp (Kg)</th>
<th>Mmax (mV)</th>
<th>VA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RF</td>
<td>VL</td>
<td>VM</td>
<td>Periph.</td>
<td>Cortical</td>
</tr>
<tr>
<td>Normoxia</td>
<td>74.0</td>
<td>28.5</td>
<td>16.8</td>
<td>8.6</td>
<td>14.6</td>
</tr>
<tr>
<td>(8.9)</td>
<td>(2.5)</td>
<td>(2.2)</td>
<td>(2.9)</td>
<td>(4.1)</td>
<td>(3.2)</td>
</tr>
<tr>
<td>1-h Hypoxia</td>
<td>68.5</td>
<td>26.8</td>
<td>16.4</td>
<td>8.1</td>
<td>15.1</td>
</tr>
<tr>
<td>(12.0)*</td>
<td>(3.1)*</td>
<td>(2.9)</td>
<td>(2.6)</td>
<td>(4.0)</td>
<td>(3.1)</td>
</tr>
<tr>
<td>Part 2</td>
<td>76.0</td>
<td>18.8</td>
<td>17.1</td>
<td>9.0</td>
<td>16.4</td>
</tr>
<tr>
<td>(10.4)</td>
<td>(1.9)</td>
<td>(2.6)</td>
<td>(2.2)</td>
<td>(3.0)</td>
<td>(3.5)</td>
</tr>
<tr>
<td>3-h Hypoxia</td>
<td>72.2</td>
<td>17.9</td>
<td>15.7</td>
<td>8.5</td>
<td>15.0</td>
</tr>
<tr>
<td>(8.8)</td>
<td>(1.3)</td>
<td>(1.8)</td>
<td>(2.3)</td>
<td>(2.6)</td>
<td>(4.0)</td>
</tr>
</tbody>
</table>

Values are Means (SD). MVC, maximal voluntary contraction; Db100, paired stimulation at 100 Hz; TwQp, potentiated single twitch; Mmax, M-wave amplitude; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; VA, maximum voluntary activation; Periph., VA obtained from femoral nerve stimulation; Cortical, VA obtained from cortical stimulation. * significantly different from Normoxia (P < 0.05)
**Figure 1.**

### Part 1:
- **Normoxia**
- 1 h Hypoxia

### Part 2:
- Randomized order
- 3 h Normoxia
- 3 h Hypoxia

### A

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>2 MVCs followed by paired ENS at 100 Hz</td>
</tr>
<tr>
<td>10 min</td>
<td>Contractions at 20% MVC superimposed with TMS at 70% maximal power output</td>
</tr>
<tr>
<td>15 min</td>
<td>Contractions at 50% MVC superimposed with TMS at 30-100% maximal power output (4 contractions at each of the 8 intensities)</td>
</tr>
<tr>
<td>20 min</td>
<td>Contractions at 100, 75, 50 and 100% MVC superimposed with TMS or ENS (4 sets of 4 contractions)</td>
</tr>
</tbody>
</table>

### B

<table>
<thead>
<tr>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal voluntary contractions and electrically evoked quadriceps responses</td>
</tr>
<tr>
<td>Definition of the optimal TMS site</td>
</tr>
<tr>
<td>Definition of the optimal TMS intensity</td>
</tr>
<tr>
<td>Maximal voluntary activation with TMS</td>
</tr>
</tbody>
</table>

- **Electrical femoral nerve stimulation (ENS)**
- **Transcranial magnetic stimulation (TMS)**
Figure 2.

A

B

Stimulation intensity (% maximal stimulator power output)

MEP amplitude (% Mmax)

□ Normoxia
■ 1h Hypoxia

□ Normoxia
■ 3h Hypoxia

*
Figure 3.
Figure 4.

A

B

CSP duration (ms)

Stimulation intensity (% maximal stimulator power output)

CSP duration (ms)

Stimulation intensity (% maximal stimulator power output)

*
Figure 5.