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Research report

# Changes in cortico-spinal excitability following uphill versus downhill treadmill exercise

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## HIGHLIGHTS

- Locomotor exercise enhance corticospinal excitability in a non-exercised muscle.
- No effect of the mode of muscle contraction in corticospinal excitability changes.
- After $PAS_{25}$, exercise induced changes in corticospinal excitability were different.

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## ABSTRACT

An acute bout of aerobic exercise induces neuroplasticity in the motor cortex. Moreover, paired associative stimulation (PAS) is known to induce neuroplasticity in M1. However, the possible influence of the type of exercise on the neuroplastic changes remains unknown. The present study investigated the effects of two different modes of muscle contraction produced during locomotor exercise on changes in corticospinal (CS) excitability. Subjects performed two 30-min treadmill exercises at an intensity corresponding to 60% of their maximal heart rate with either a +10% (uphill) or −10% (downhill) slope. These exercises were followed or not by paired associative stimulation method ($PAS_{25}$) which consisted of 200 paired stimuli (0.25 Hz, 15 min) of median nerve electrical stimulation followed by transcranial magnetic stimulation of the hand M1 area (ISI 25 ms). Motor evoked potentials (MEP), assessed through abductor pollicis brevis (APB) activity were obtained before exercise, at 5 min, 15 min and 30 min after exercise. A significant ($P < 0.05$) increase of the MEP amplitude was observed 30 min after both exercises but was not different between the two modes of locomotion. On the contrary, MEP amplitude with $PAS_{25}$ increased only 30 min after downhill exercise. We conclude that sub-maximal treadmill exercise increases CS excitability within a period of 30 min. However, the predominant mode of muscle contraction during uphill versus downhill locomotion does not influence CS excitability when assessed using a non-exercised muscle. However, results from $PAS_{25}$ suggest that specific neuroplastic changes occur likely due to homeostatic mechanisms induced by exercise plus a PAS protocol.



## 1. Introduction

It has been shown that physical activity, in particular endurance type training, enhances plasticity of the corticospinal (CS) pathway [1] and improves neurocognitive function [2]. Specifically, individuals who undertake regular physical activity are more responsive to experimentally-induced CS plasticity compared to a sedentary population [3]. The effects of physical activity on CS excitability has previously been studied using transcranial magnetic stimulation (TMS), which elicits a motor evoked potential (MEP) recorded in a relaxed muscle that is not directly implicated in the exercise

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(e.g. abductor polis brevis or first dorsal interosseous). An enhancement of MEP amplitude in non-exercised muscles shows clearly that exercise has an effect upon the neural control of the whole body and not just the specific muscles involved in the activity [4].

Exercise-induced changes in CS excitability in the primary motor cortex (M1) have been demonstrated following an acute bout of aerobic exercise. Additionally, changes in CS excitability were induced using non-invasive brain stimulation techniques such as paired associative stimulation (PAS) [5]. Previous studies have suggested that these changes were mostly mediated at a cortical level [6] and also via indirect *trans*-cerebellar sensory pathways [7]. Using this technique, a greater enhancement of CS excitability was recorded when a facilitating PAS protocol ($PAS_{25}$) was preceded by an acute bout of aerobic exercise than when applied alone [4,8–10]. Interestingly, enhanced sequence specific learning when motor practice was performed after a 20-min submaximal intermittent cycling exercise was demonstrated by Mang et al. (2014). Additionally, this exercise enhanced the long-term potentiation-like (LTP) plasticity induced by a $PAS_{25}$ protocol after the exercise [11]. In the same manner, an inhibitory PAS applied in stroke patients targeting the non-paretic limb during aerobic cycling exercise has been shown to induce a down-regulation of CS excitability [12]. However, Müller et al. [13] demonstrated interestingly that the LTP like-plasticity induced by a $PAS_{25}$ protocol was completely suppressed if neuronal activity was already potentiated. These authors shed light on the homeostatic regulatory mechanism that drives changes in CS excitability in the human M1 as suggested by the Bienenstock-Cooper-Munroe (BCM) theory [14]. The aforementioned studies suggest that there is a potential interest in aerobic exercise and paired associative stimulation as therapeutic tools to drive CS excitability modulation and thus optimize neural repair strategies.

Physical exercise can be characterized by its duration, its intensity and the mode of contraction of the muscles involved. The concentric mode can be defined as muscle shortening during contractions due to the higher muscular torque compared to the external load (e.g., lifting a load, uphill walking/running). In contrast, an eccentric mode occurs when the external load is higher than muscular torque or during action against gravity (e.g. down stairs, jump landing, downhill walking/running) leading to muscle lengthening during the contraction [15]. Eccentric contractions differs from concentric ones in terms of the production of higher torque for both upper and lower limbs [16,17], adding to a lower energy cost for several locomotor exercises, such as walking, running or cycling, performed at the same mechanical workload [18,19]. The exercises involving eccentric contractions may be of interest for patients with disabilities. For example, it has been shown that the higher muscular work achieved during eccentric exercises resulted in more-efficient performance in people with multiple sclerosis [20] and Parkinson's disease [21]. Locomotor exercises with an eccentric component such as downhill walking/running are used for strengthening or rehabilitation programs.

Interestingly, compared to concentric contractions, eccentric contractions are known to induce specific neural adaptations [22]. Specifically, EMG activity is decreased during eccentric muscle contraction compared to concentric contraction for the same submaximal torque achieved [23,24]. This results in a lower number or a weaker discharging rate of motor units [25,26] explained by inhibitory spinal mechanisms that occur during eccentric contraction [27]. These mechanisms are suggested to prevent muscle damage by reducing the motoneuronal excitability [25,28] which leads to a reduced activation level [29]. However, a greater cortical excitability is associated with eccentric contractions [30], explained by a larger amount of sensory information to the brain from muscle lengthening, and the improved planning of eccentric actions [31]. Furthermore, this greater excitability was also suggested to counteract the inhibitory spinal mechanisms, during eccentric contraction [24,28]. Thereafter, when assessed using TMS, CS excitability was shown to be reduced during eccentric contractions [32,33].

Within this context, the aim of the present study was to (i) determine whether the mode of locomotor exercise influences CS excitability and (ii) if it could differentially modulate changes in CS excitability changes induced using a PAS protocol. We hypothesized that locomotor exercise with dominant eccentric contractions (i.e. downhill treadmill exercise) would lead to greater CS changes compared to exercise with dominant concentric contractions (i.e. uphill treadmill exercise) due to specific neural adaptations that occur during the former, compared to the latter. In addition, it has been suggested that brain activation during movement preparation and execution increases more for eccentric than concentric exercise, a finding that may have implications for CS excitability following specific physical training in either contraction modes [24,31,34]. Thus, the application of a $PAS_{25}$ protocol after exercise should lead to a different modulation of CS excitability in a non-exercised muscle with an up-regulation of CS excitability after uphill treadmill exercise, and a down-regulation after downhill treadmill exercise according to the BCM theory [13,14]. To test our hypothesis, we asked individuals who undertook regular physical activity to execute uphill and downhill treadmill exercises at the same intensity with and without the application of a $PAS_{25}$ protocol immediately after exercise. We then examined changes in the MEP evoked in a muscle of the dominant hand that was not involved in the exercise, using TMS. Thus, we were able to assess changes in CS excitability occurring in the whole system and not specifically-induced changes in the exercised muscles.

## 2. Materials and methods

### 2.1. Participants

Twelve (age: $25 \pm 4$ years) healthy active volunteers ($7.5 \pm 3.3$ h of physical activity per week) were recruited for this study, which included two paradigms. All were right handed and gave their written consent prior to experimentation. The study conformed to the standards set by the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (2008) and was approved by the Institutional Research Ethics Board.

### 2.2. Experimental protocol

This study was divided into two paradigms; 1) exercise alone (EX) and 2) exercise following by $PAS_{25}$ (EX + $PAS_{25}$). First, subjects were familiarized with TMS and $PAS_{25}$ protocols in order to determine whether they were responsive to $PAS_{25}$ or not. Subjects were included in the study if the MEP amplitude assessed after the $PAS_{25}$ protocol alone was enhanced to at least 120% of pre-stimulation values. For all included subjects, we then determined the maximal aerobic running velocity and the maximal heart rate ($HR_{max}$) during an incremental treadmill running test.

Both paradigms included 2 sessions each consisting of a 30-min treadmill uphill (UP) or downhill (DOWN) exercise at a submaximal intensity with assessment of CS excitability before and following the intervention (EX and EX + $PAS_{25}$) (see Fig. 1). The four sessions were completed over a period of 4 weeks with a minimum of 48 h recovery between visits. All participants were given instructions not to undertake vigorous physical activity the day before each session. Participants were also instructed not to consume caffeine and nicotine at least 3 h before testing, and were asked to declare if they had taken any medication or had any acute illness, injury or

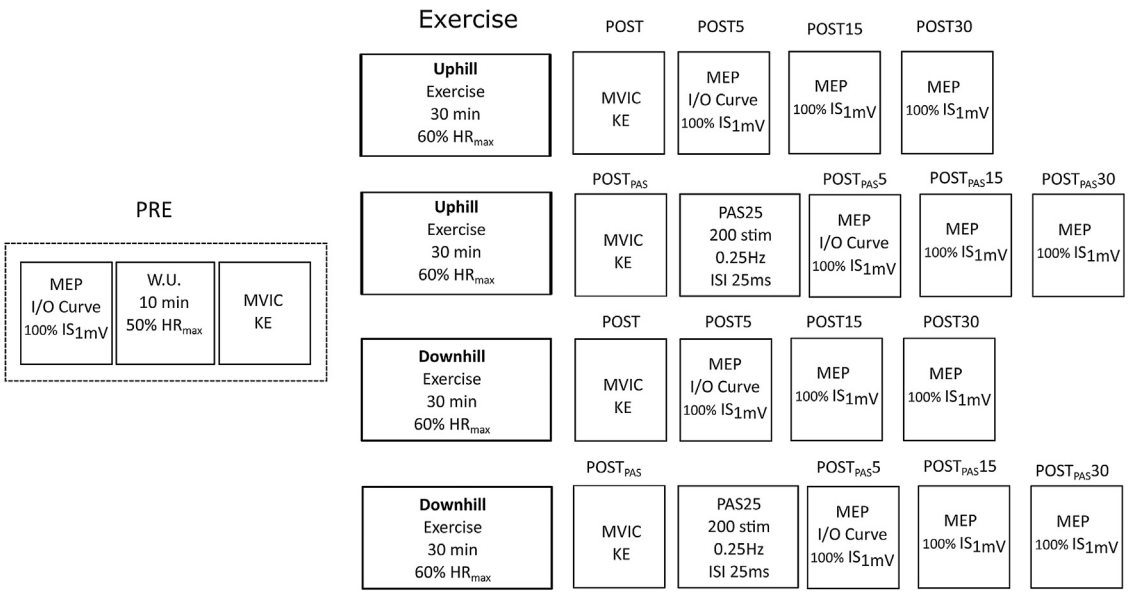


**Fig. 1.** Time-line of the experimental protocol for both exercises (uphill and downhill), and paradigms (exercise alone and exercise + $PAS_{25}$). MEP: Motor Evoked Potential; I/O Curve: Input-Output curve; W.U.: Warm-up; MVIC KE: Maximal Voluntary Isometric Contraction of the Knee Extensor muscles; $IS_{1mV}$: Stimulation intensity inducing a 1 mV MEP in the Abductor Pollicis Brevis; HRmax: maximal heart rate.

infection. Moreover, the different sessions were performed at the same hour of the day for each subject in order to minimize variability of M1 neuroplasticity due to changes in neurotransmitter levels between sessions and particularly from cortisol levels, well known to impair neuroplasticity [8,35].

### 2.3. Maximal aerobic running velocity test

During the familiarisation session, the participants performed an incremental treadmill level running exercise with a velocity beginning at 2.2 $ms^{-1}$ (8 $kmh^{-1}$) for 2 min and then increasing by 0.13 $ms^{-1}$ (0.5 $kmh^{-1}$) each min until exhaustion (as per methods described in Assadi & Lepers [36]). Heart rate was recorded continuously during the test and $HR_{max}$ was determined as the highest HR value reached by each subject at the end of the test.

### 2.4. Treadmill exercise

After the pre-exercise measures were taken, participants exercised on a treadmill either uphill or downhill (Fig. 1). We randomly assigned either uphill or downhill exercise with or without PAS as the first treadmill exercise session for each participant so that the participant cohort was counter-balanced between those that experienced uphill or downhill exercise followed by $PAS_{25}$ or not in their first session. For each session, participants started with a warm-up period consisting of 10-min treadmill level running at 50% of $HR_{max}$ followed by a knee extensor maximal torque test (MVIC KE in Fig. 1, see details in the paragraph 2.7). Following this, the 30-min treadmill exercise was carried out with either a +10% slope or −10% slope as previously used to induce mostly CON (uphill) or ECC (downhill) contractions of the knee extensor muscles [37,38]. For all sessions, the experimenter adjusted the speed of the treadmill during the exercise in order to maintain a stable level of intensity corresponding to 60% of $HR_{max}$ for each participant. Two minutes were allowed to reach the targeting speed at the start of the exercise for all subjects. The moderate intensity has previously been shown to limit a systemic increase in neurochemical neuroplasticity inhibitors such as cortisol [35,39]. Heart rate, running velocity and rate of perceived exertion (RPE) of effort using the 15 point RPE 6-20 scale [40] were recorded every 5 min during the exercises.

### 2.5. Corticospinal excitability

Corticospinal excitability was assessed by means of single-pulse TMS applied to the abductor pollicis brevis (APB) area of the left motor cortex. TMS was delivered using a figure of eight magnetic coil connected to a magnetic stimulator Magstim 200[2] (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with an angle of 45° from the anterior-posterior axis. The location of stimulation was firstly defined with reference to the cortical map and then precisely measured by assessing the hotspot of the APB. Hotspot was assessed by stimulating several positions using the same intensity and observing which gave the highest peak-to-peak MEP amplitude over 5 consecutive MEPs. MEP was recorded using two EMG electrodes placed on the APB and a ground electrode placed on the elbow of the same arm. Recording and direct observations were performed with the Biopac software (Biopac MP150, Biopac System Inc, USA).

To quantify changes in CS excitability in this study, the same protocol was applied. We used an intensity sufficient enough to evoke a peak-to-peak MEP amplitude of 1 mV ($IS_{1mV}$) in the resting APB at the beginning of the each experiment. The intensity was validated if 5 consecutive MEPs of the same amplitude were obtained [5]. An input-output curve (I/O curve) for 7 intensities varying from 70% to 130% of the $IS_{1mV}$ by step of 10% was performed before (PRE) and 5 min after (POST5, $POST_{PAS}$ 5) each intervention protocol (EX and EX + $PAS_{25}$) (see Fig. 1). Corticospinal excitability was also assessed 15 min (POST15, $POST_{PAS}$15) and 30 min (POST30, $POST_{PAS}$ 30) after the intervention by means of 15 single TMS pulses at $IS_{1mV}$ intensity. This procedure permitted the assessment of the strength and integrity of corticomotor network and the analysis of exercise-induced effects [41]. The order of intensities for the I/O curve was randomized across participants and sessions, but remained fixed during each session. For each intensity, 15 single magnetic pulses were delivered with an interval of 4–5 s and averaged for statistical analyses. During the entire assessment and measurement, we asked to participants to remain in a rested state. All procedures were performed with the APB relaxed, and the participant seated in a chair, with their elbows and forearms resting on the table.

### 2.6. $PAS_{25}$ protocol

A facilitating $PAS_{25}$ stimulation paradigm was used as it was expected to increase corticospinal excitability [5]. Square wave pulses of 1 ms were electrically applied to the right median nerve by use of a single bipolar surface electrode placed on the wrist (Digitimer DS7AH, Digitimer LTD, UK). Stimulus intensity was set at 300% of each subjects' perceptual threshold, i.e. the lowest stimulation intensity a subject could perceive in the relaxed APB. The TMS pulse was applied 25 ms after the electrical stimuli at 100% of $IS_{1mV}$. This protocol was repeated 200 times at 0.25 Hz for a total duration of 15 min. Because PAS effectiveness has been shown to be influenced by attention, we asked subjects to count the number of stimuli during $PAS_{25}$ and to report it at different moments. This protocol was applied 5 min after the end of exercise, which was the time required to complete the POST MVIC and to ready the subject for testing.

### 2.7. Knee extensor maximal voluntary contraction torque

Maximal voluntary isometric contraction (MVIC) torque of the knee extensor muscles was measured before and immediately after the exercise (POST) to quantify any induced muscle fatigue. This test was performed on the right knee extensors with an isokinetic system (System pro, Biodex Medical System, New-York). MVIC was defined as the best performance obtained on two consecutive trials (1-min rest in between). Participants were seated comfortably and attached with a noncompliant strap on the trunk and ankle above the malleoli with knee angle of 70° (0° = leg extension) and hip angle of 90°. They performed a specific warm up prior to the PRE test to become accustomed to the movement and torques developed. The warm-up included several leg extensions at different angular velocities and two 5-s submaximal isometric contractions. Participants were verbally encouraged during each trial to perform the best performance possible.

### 2.8. Statistical analysis

For all variables, the Shapiro-Wilk test was first applied to determine the nature of the data distribution. Because variables were not normally distributed, the Friedman's' ANOVA was used to highlight statistical differences which were then assessed by the Wilcoxon T-test with a significance level set at $p < 0.05$ and confirmed by the Cohen's effect size (ES). If the Friedman's' ANOVA was not statistically different, a paired *t*-test was used with a significance level of $p < 0.05$. For each participant, MEP amplitudes out of the defined range (*Mean ± 2SD)* were excluded from the measures. All values were expressed as *Mean ± SD* and *Mean ± SE* for figures.

## 3. Results

### 3.1. Locomotor velocity, HR and RPE

Despite different velocities of locomotion, HR was similar during all exercises (see Fig. 2a). However, average locomotor velocity was greater during downhill ($2.4 \pm 0.86\ ms^{-1}$) than during uphill ($1.25 \pm 0.25\ ms^{-1}$) exercises ($Z = 2.22$, $p = 0.01$; $ES = 1.9$) (see Fig. 2b). Perceived exhaustion (RPE) increased significantly for both conditions between the fifth minute and the end of the exercise until it reached $10.4 \pm 3.9$ for uphill ($Z = 2.19$, $p = 0.02$; $ES = 0.5$) and $10.3 \pm 4.4$ for downhill exercises ($Z = 2.7$, $p = 0.007$; $ES = 0.6$) (see Fig. 2c). No differences were found for RPE between conditions for each time point of the exercises ($Z < 1.8$, $p = 0.07$).

### 3.2. MVIC torque of knee extensors

No significant difference in knee extensor MVIC torque was found, neither between conditions, nor for time ($X^2 = 0.48$, $p = 0.92$), suggesting that no muscle fatigue occurred in knee extensor muscles following exercises (see Fig. 3).

### 3.3. MEP amplitude changes

No difference was found for the average stimulator intensity used to assess the resting motor threshold between all conditions before exercise ($X^2 = 4.22$, $p = 0.24$). Likewise, $IS_{1mV}$ used during the experiment was the same for all conditions in these experiments (uphill $50.3 \pm 5.9\%$, downhill $49.4 \pm 5.3\%$) ($X^2 = 3.98$, $p = 0.27$).

Typical MEP traces for one subject are shown in Fig. 4. When exercise alone was undertaken, no MEP amplitude differences were seen between PRE and POST5 and POST15 for uphill and downhill locomotion. However, a significant increase ($X^2 = 10$, $p = 0.02$) of MEP amplitude at $IS_{1mV}$ was observed at POST 30 compared to PRE for uphill and downhill locomotion (see Fig. 4). Furthermore, no significant differences were found for the slope of the I/O-curve at POST5 compared to PRE for both conditions (uphill: $Z = 0.53$, $p = 0.6$, $ES = 0.16$; downhill: $Z = 1.06$, $p = 0.28$, $ES = 0.25$) (see Fig. 5). Fig. 6 shows that compared to PRE values, MEP amplitude at POST30 was significantly greater than PRE, POST5 and POST15 values for both uphill ($Z < 2.35$, $p < 0.03$; *ES = 1.07*) and downhill ($Z < 2.6$, $p < 0.012$; *ES = 0.76*) exercises. However, MEP amplitude at POST30 was not significantly different between uphill and downhill exercises.

Similarly to exercise alone, $EX + PAS_{25}$ did not change the I/O curve slope between PRE and $POST_{PAS}$ 5 for both conditions (uphill: $Z = 0.7$, $p = 0.47$, $ES = 0.16$; downhill: $Z = 0.08$, $p = 0.92$, $ES = 0.04$). Likewise, MEP amplitudes at $POST_{PAS}$ 5, $POST_{PAS}$ 15 and $POST_{PAS}$ 30 were not different from PRE values after uphill running ($Z < 1.8$, $p < 0.48$, $ES < 1.04$). On the contrary, the MEP amplitude after downhill running at $POST_{PAS}$ 30 was significantly greater than PRE values ($Z = 2.35$, $p = 0.02$, $ES = 1.4$) and $POST_{PAS}$ 5 and $POST_{PAS}$ 15 values ($Z < 2.9$, $p < 0.03$, $ES > 0.83$). Furthermore, the MEP amplitude at $POST_{PAS}$ 5 was significantly greater after downhill than uphill running ($Z = 2.11$, $p = 0.03$, $ES = 1.4$).

## 4. Discussion

The aim of this study was to examine the changes in CS excitability following uphill versus downhill treadmill locomotor exercises performed at the same sub-maximal intensity without induced muscle fatigue. Moreover, using a $PAS_{25}$ protocol, we aimed to highlight if a subsequent PAS protocol modulate differently CS excitability changes compared to exercise alone. The main results were that there was an increase in CS excitability occurring 30-min after uphill and downhill exercise alone, without any difference between conditions. When exercise was following by $PAS_{25}$, only the downhill condition induced an increase in CS excitability 30-min after the end of the exercise.

### 4.1. Similar exercise intensity without muscle fatigue

To avoid any confounding effects related to exercise intensity and muscle fatigue, we fixed a submaximal intensity of exercise for both locomotor exercises. Indeed, although the nature of the exercises differed in terms of the knee extensor contraction mode (i.e. mostly concentric for uphill versus eccentric for downhill locomotion), both exercises were performed at the same intensity (60% of $HR_{max}$) with the same average rate of perceived exertion. Greater speed during downhill compared to uphill locomotor exercise despite the same heart rate is in agreement with previous findings that have documented lower metabolic cost associated

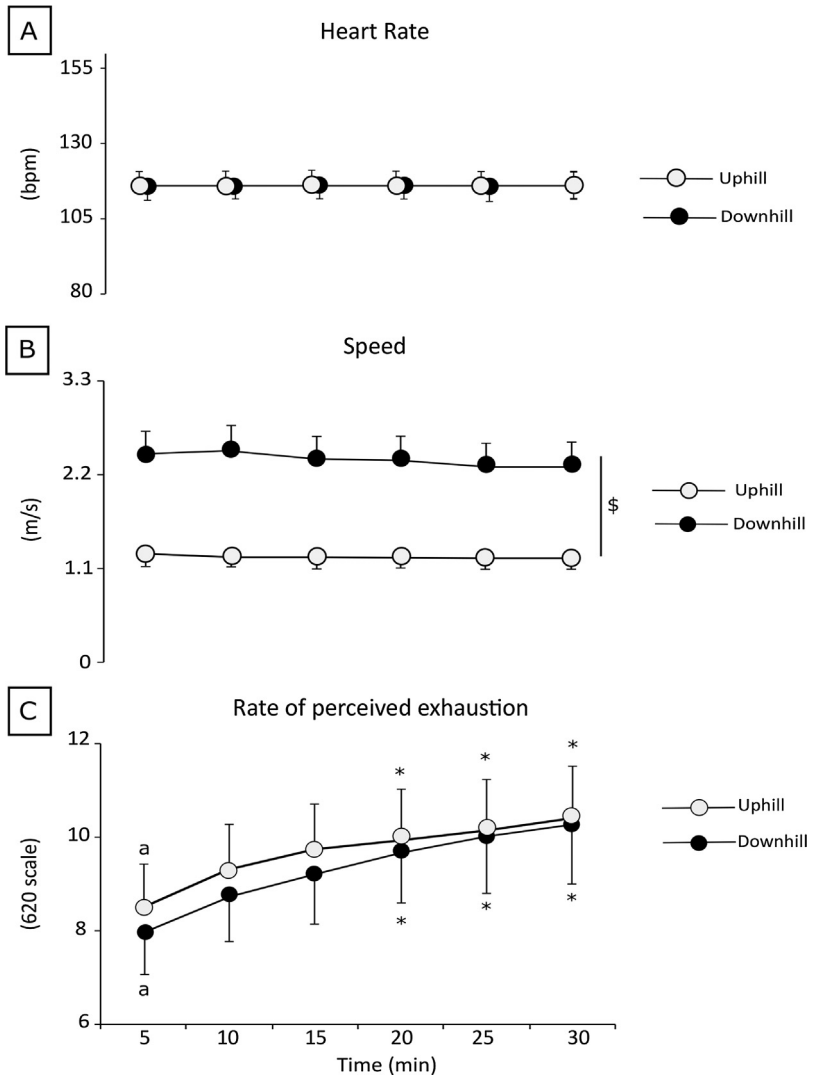


**Fig. 2.** Average heart rate (Panel A), treadmill speed (Panel B) and rate of perceived exertion (Panel C) (Borg scale) during exercises for both paradigms (Mean ± SE). * = Significantly different from previous values ($p < 0.05$). a = Significantly different from all other values ($p < 0.05$); $ = Significantly different between conditions ($p < 0.05$).

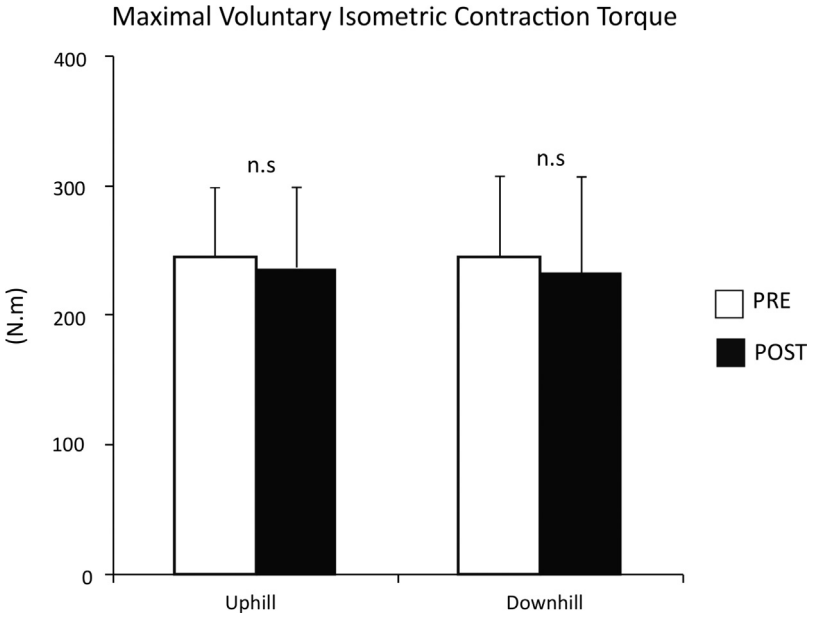


**Fig. 3.** MVIC torque of the knee extensor muscles before (PRE) and immediately after (POST) treadmill exercises (Mean ± SE). n.s.: not statistically different.

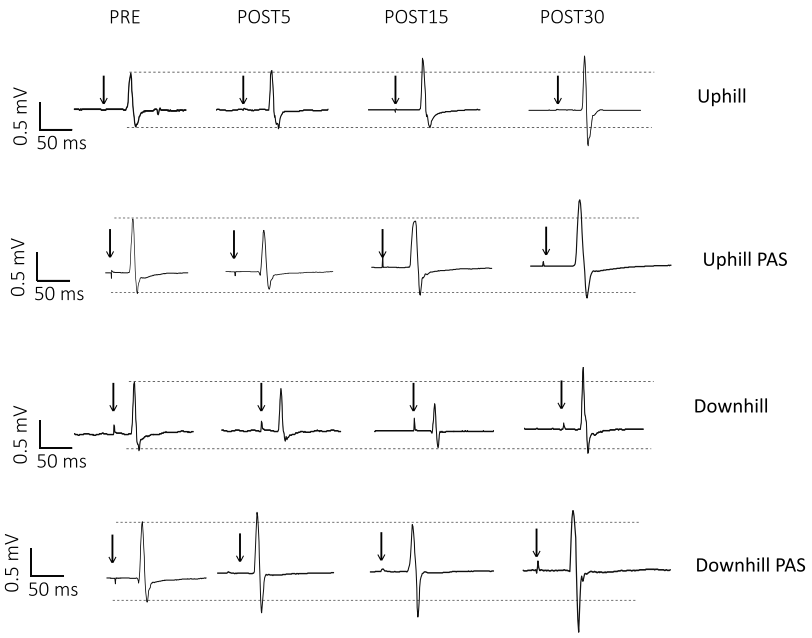


**Fig. 4.** EMG responses of the Abductor Pollicis Brevis in one representative subject to transcranial magnetic stimulation (100% $IS_{1mV}$) for both paradigms before and following uphill and downhill treadmill exercise.

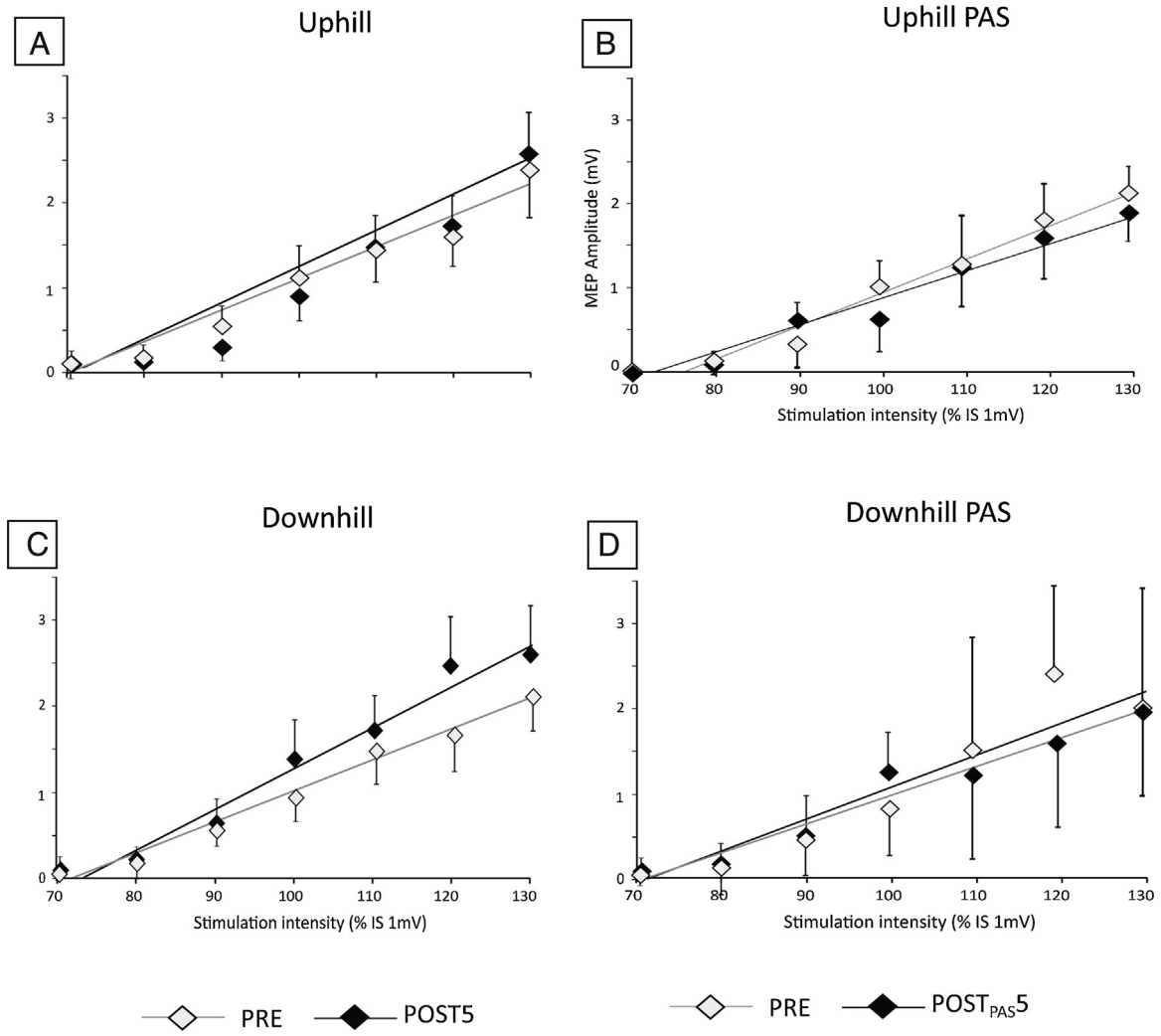


**Fig. 5.** Input-output curve before (PRE) and 5 min after (POST5) uphill (panel A) and downhill (panel C) treadmill exercises and after ($POST_{PAS}5$) uphill + PAS (panel B and downhill + PAS (panel D) (Mean ± SE). The slopes were not significantly different between PRE and POST5 for both conditions.

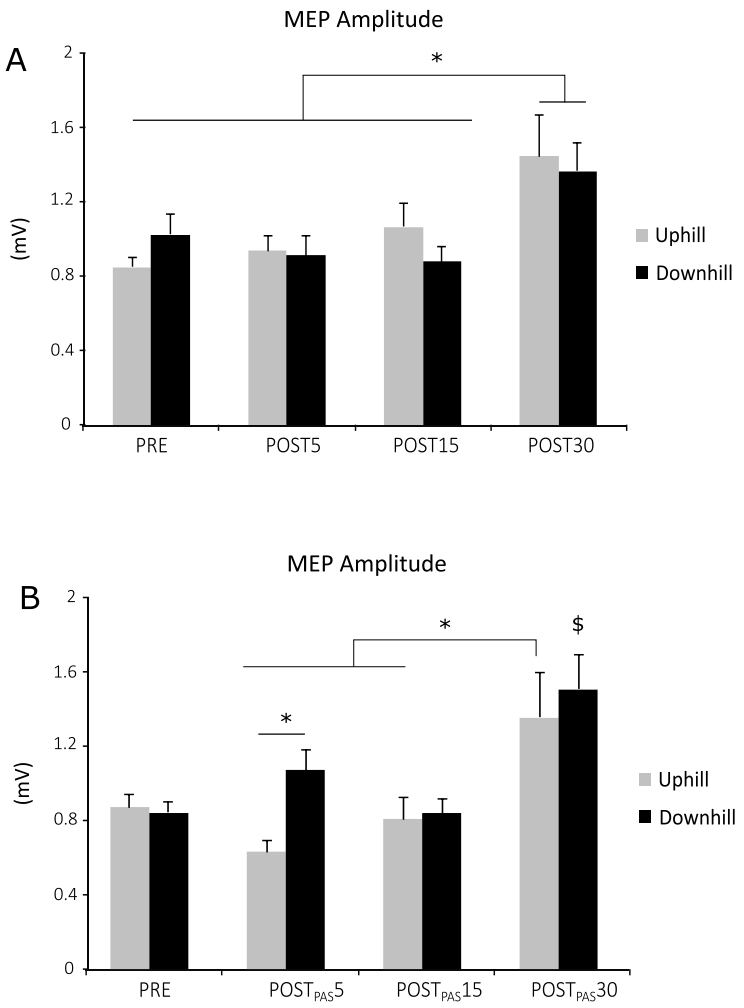


**Fig. 6.** MEP amplitude changes for exercise alone (Panel A) and exercise + PAS (Panel B) before (PRE) and following (POST5, POST15 and POST30) uphill (CON) and downhill (ECC) exercises. * = Significantly different for both conditions ($p < 0.05$, $Z < 2.8$). (Mean ± SE).

with eccentric modes of contraction [19,42,43]. Moreover, maximal strength capacity was not altered following both exercises, suggesting that muscle fatigue did not occur in the knee extensor muscles. The absence of exercise-induced muscle fatigue may be explained by the low intensity of the exercise. Our data suggest therefore, that we successfully reproduced similar non-fatiguing conditions between both locomotor exercises that solicited the knee extensors with different modes of muscle contraction.

### 4.2. Effect of exercise on corticospinal excitability

Our findings also showed that an acute bout of locomotor aerobic exercise promotes CS excitability when tested in a non-exercise muscle; here the Abductor Pollicis Brevis, (APB). This result is in accordance with previous studies [4,10]. Enhancement of CS excitability in the APB likely results from an increase of M1 activity due to motor outputs acting on a facilitator system at the cortical level [44]. Also, increasing cardiovascular and metabolic demands during locomotor exercises [45] could in turn increase the effects of exercise upon neuroplasticity [39,46,47]. However, contrary to changes assessed in the exercised muscles, which have been observed immediately after exercise [48,49], our data suggest that a delayed onset (30 mins post exercise) is necessary to achieve CS excitability changes in a non-exercised muscle. This delayed effect could explain the lack of significant differences between I/O values between PRE and POST5 and POST$_{PAS}$5.

Our findings are of interest when considering physical activity as a therapeutic tool to improve brain function, the cardiovascular system and muscle strength. Specifically, the interest of eccentric non-fatiguing exercises to increase corticospinal excitability in a non-exercised-muscle (e.g. in the upper limbs) similarly to concentric is wide-ranging and manifold [50,51]. Indeed, as showed previously [18,19] and confirmed by the present results, the lower metabolic cost of eccentric exercises compared to concentric exercise allowed the attainment of higher muscular work for the same intensity (e.g.% maximal heart rate). This result in higher total work achieved at the end of eccentric exercises compared to concentric ones, and potentially to greater enhancement of neuromuscular system [52,53].

### 4.3. Effects of the mode of muscle contraction

Contrary to our original hypothesis, the present results suggest that changes in CS excitability induced by an aerobic locomotor exercise are not dependent upon the mode of muscle contraction, at least when tested in a non-exercised muscle. To our knowledge, all of the previous studies that have investigated the effects of the muscle mode of contraction on CS excitability have focused upon the exercised-muscle during isolated single-joint exercises. The findings of these studies highlighted an increase of CS excitability during concentric and eccentric contractions relative to rest [54], but with a lower amplitude for the eccentric than the con-

centric exercises [33,55]. The reduced CS excitability during ECC contraction was also obtained at rest, assessed by a decreases MEP amplitude during muscle lengthening compared to isometric contraction [56]. Likewise the slope of I/O curve was reduced adding to longer MEP latencies during lengthening compared to shortening [57]. However, only one study has provided findings concerning the duration of these effects after contractions, without showing differences in MEP area under the curve after fatiguing concentric and eccentric contraction in elbow flexors relative to rest [54].

It seems therefore that whole-body sub-maximal locomotor exercise with eccentric contractions of lower limb muscles leads to a delayed increase in CS excitability of upper-limb muscle. Thus, it appears that inhibitory mechanisms acting at the spinal level, which depress corticospinal excitability in the exercising muscle did not influence the CS excitability of the upper limb in the non-exercised muscle. However, due to lack of measurement of spinal excitability (e.g. H reflex), we cannot identify the precise origin of the observed changes. We hypothesize that with respect to the different medullary level of upper limb and lower limb muscles, inhibitory spinal mechanisms acting on the lower limb muscles would not have influenced the upper limb muscle. Moreover, we can also hypothesize that M-wave changes in the APB were the same for both exercises as, except for the slope, exercise characteristics were the same (intensity, duration, and hour of day).

### 4.4. Homeostatic regulation of corticospinal plasticity

Contrary to previous findings that applied a facilitating PAS protocol after an aerobic bout of exercise [8,10], CS excitability was significantly enhanced at $POST_{PAS}30$ only after downhill running. Furthermore, when exercise was following by $PAS_{25}$, CS excitability was not changed at $POST_{PAS}5$ compared to PRE values for both exercises, whereas it was significantly greater after downhill than uphill running. These results suggest that $PAS_{25}$ enables us to highlight specific modulations of CS excitability depending on the mode of muscle contraction during exercise, likely related to indirect *trans*-cerebellar sensory pathways [7]. Indeed, it is known that an acute bout of aerobic exercise increases CS excitability (see 4.2) and can prime cortical changes [7]. Moreover, according to Carson and Kennedy [6], CS changes induced by PAS are mostly mediated at cortical level. In this study, exercise intensity was defined at 60% of the maximal HR for each subject. Knowing the specificities of the eccentric muscle mode of contraction, it could be suggested that for the same%HR, downhill running induced a greater cortical demand [24] also increased the cerebral blood flow [34] thus promoting exercise-induced CS changes. However, we can not explain precisely why this specific exercise-induced effect was not shown with the exercise only paradigm. This result could probably be explained by the Bienenstock-Cooper-Munro (BCM) theory of bidirectional synaptic plasticity whereby the threshold leading to a potentiation or a depression of the synaptic excitability could vary according to the history of synaptic activity [14].

## 5. Conclusion

The present study shows that sub-maximal non-fatiguing locomotor exercise on a treadmill affects CS excitability within a delayed period of 30-min when it is tested in a muscle not directly implicated in the exercise. The predominant mode of muscle contraction of the knee extensors during uphill versus downhill locomotor activities does not influence the CS excitability changes. However, it appears that specific neural changes exist in uphill compared to downhill running exercises as highlighted by different responses when exercises were followed by a facilitating $PAS_{25}$ protocol. The main limitation of this study was that we did not record the M-wave compound in both the exercised (quadriceps) and non-exercised (APB) muscles. Hence, we cannot speculate as to the neuromuscular changes which could have potentially affected changes in MEP. Further studies should focus on the cortical systems targeting by PAS to explain the different CS changes between these two paradigms: exercise alone and exercise + PAS.

## Conflicts of interest

All authors declare no conflicts of interest. The results of the present study do not constitute endorsement by ACSM. All results are presented honestly without fabrication or falsification data.

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