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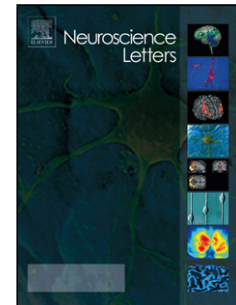
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# Assessment of the H-reflex using two synchronized magnetic stimulators in order to increase stimulus durations: a comparison with electrical stimulation

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## Highlights

- Conventional magnetic stimulation underestimates the H reflex.
- Synchronously coupling 2 magnetic stimulators increases the pulse duration.
- Synchronously coupling 2 magnetic stimulators provides better H-reflex assessment.
- Coupled magnetic stimulation could be used to measure the  $H_{\max}/M_{\max}$  ratio.

## Abstract

Magnetic nerve stimulation (MNS) may be a less painful alternative to electrical nerve stimulation (ENS) for Hoffmann reflex (H-reflex) measurement, however standard MNS (sMNS) techniques utilize a short stimulus duration, thereby limiting its use for H-reflex assessment. This limitation may be partly overcome by coupling two magnetic stimulators to increase the pulse duration (coupled MNS: cMNS). The aim of this study was to test this assumption by comparing the H-reflex characteristics evoked by ENS, sMNS and cMNS.

Thirteen healthy volunteers were tested with ENS and both MNS in the prone position. Maximal *soleus* H-reflex ( $H_{\max}$ ) and M-wave ( $M_{\max}$ ) amplitudes were measured to compute the  $H_{\max}/M_{\max}$  ratio.  $H_{\max}$  was evoked at rest and during both isometric submaximal (10%MVC) and maximal plantar-flexions (MVC).

At rest, MNS techniques underestimated  $H_{\max}$  (ENS:  $8.32 \pm 2.73$  mV; sMNS:  $6.85 \pm 2.29$  mV; cMNS:  $7.48 \pm 2.23$  mV;  $p < 0.05$ ). In contrast, no difference was observed for  $H_{\max}/M_{\max}$  (ENS:  $0.59 \pm 0.17$ ; sMNS:  $0.45 \pm 0.28$ ; cMNS:  $0.47 \pm 0.29$ ;  $p = 0.11$ ). sMNS, cMNS and ENS similarly detected  $H_{\max}$  facilitation during MVC (ENS:  $+120 \pm 248\%$ ; sMNS:  $+228 \pm 350\%$ ; cMNS:  $+162 \pm 180\%$  of the rest value;  $p = 0.344$ ).

Owing to their shorter stimulus duration, both MNS techniques underestimated the  $H_{\max}$  compared to ENS. However, when the gold standard ENS technique cannot be used, coupled MNS may be recommended since it provides better H-reflex characteristic assessment than standard MNS due to its longer stimulus duration.

**Keywords:** Spinal excitability, Recruitment threshold, M-wave, Recruitment curve, Electromyography.

## Introduction

The Hoffmann reflex (H-reflex) can be evoked by electrical stimulation of the peripheral nerve trunk at submaximal intensities [1]. The H-reflex is mediated by Ia sensory afferents and is recorded at the muscle using electromyography (EMG). In the field of neuromuscular function assessment, the H-reflex is frequently used as an indicator of nervous system modulation occurring at the spinal level and is influenced by changes in motoneuron and interneuron excitability as well as the effects of presynaptic inhibition [2].

Electrical nerve stimulation (ENS) has been used to evoke the H-reflex [3] in various experimental settings, such as joint angle manipulation [4], neuromuscular fatigue [5] and exercise training [6]. ENS techniques are associated with a good reproducibility of H-reflex amplitudes and it considered a reliable technique to quantify H-reflex parameters [7,8]. However, a major limitation of this technique is the discomfort and/or pain associated with ENS [9,10]. In sensitive populations such as children [12,13], magnetic nerve stimulation (MNS) may be preferred to ENS. MNS causes less discomfort/pain as the magnetic field induced by the coil tends not to stimulate pain receptors, yet its ability to activate efferent nerves fibers ensures that it provides comparable outcomes to ENS [9,11]. Nevertheless, one limitation of MNS is that the shorter pulse duration will theoretically impact negatively on the H-reflex amplitude [14], thus limiting its use in this regard.

When electrically stimulating the peripheral nerves, the stimulus duration influences the recruitment of the likelihood of afferent and efferent fibers [15]. The activation threshold for sensory fibers is lower than for motor fibers for long stimulus durations and this relation reverses for short stimulus durations [16]. Therefore, the H-reflex appears at much higher stimulation intensities when short-duration stimuli (e.g.  $\leq 200 \mu\text{s}$ ) are imposed, and consequently the shapes of H-wave-to-M-wave recruitment curves are different when compared to curves evoked with longer stimulus durations (e.g.  $1000 \mu\text{s}$ ). The maximal amplitude of the H-reflex is also reduced when

stimulations are imposed with short durations, but the maximal H-reflex-to-maximal-M-wave ratio ( $H_{\max}/M_{\max}$ ) is not affected by stimulus duration [16].

As compared to ENS (commonly imposed at 1000  $\mu$ s), the stimulus duration delivered by magnetic stimulation is short ( $\sim 80$   $\mu$ s) and not modifiable [14]. In addition, the shape of the triangular waveform delivered by magnetic stimulators reduces the “effective stimulus duration” and may alter the threshold of sensory and motor fibers [14]. Together, these characteristics of standard magnetic nerve stimulation (sMNS) conspire to impair H-reflex assessment [17]. One possible solution to these problems would be to increase stimulus duration by synchronously coupling two or more magnetic stimulators [18]. For example, when two stimulators are coupled, this technical solution leads to the delivery of a single stimulation pulse but of longer duration ( $1.4 \times$  sMNS). While the pulse width (e.g.  $\sim 112$   $\mu$ s) may still be much shorter than with ENS, it should theoretically affect sensory fiber recruitment and improve H-reflex assessment. However, this hypothesis has yet to be tested, so it is unclear if such improvements in stimulus pulse width result in meaningful improvements in H-reflex measurement capacity. For the first time in the present study, we have increased the magnetic pulse width by synchronously coupling two stimulators (cMNS) in order to test this hypothesis.

Therefore, the aim of this study was to compare  $H_{\max}$  and H-M recruitment curves evoked by ENS to those evoked by sMNS and cMNS. H-reflexes were evoked on the resting plantar flexor muscles as well as during submaximal and maximal voluntary contractions, during which H-reflex amplitudes are known to be different. We hypothesized that sMNS would severely underestimate  $H_{\max}$  but not the  $H_{\max}/M_{\max}$  ratio as compared to ENS, but that cMNS would provide better estimates of  $H_{\max}$  than sMNS, owing to its longer stimulus duration.

## Material and Methods

### Population

13 healthy subjects (4 women and 9 men;  $22 \pm 3$  years;  $173 \pm 8$  cm;  $71.3 \pm 11.0$  kg) volunteered to take part in the study. All were tested on their dominant, i.e. right leg. No subjects had any orthopedic or neuromuscular disorders. The local ethic committee approved the study and all procedures were conducted according to the Declaration of Helsinki. Before the experimental session, all subjects gave their written informed consent. When enrolled, the subjects were asked not to participate in any intensive physical activity for 2 days prior to the beginning of the experiment.

## Protocol

Ankle plantar flexor torque measurements were performed using an isokinetic dynamometer (Biodex System 2, Biodex, Shirley, NY). Participants laid prone on the Biodex chair with straps securely fastened at the hip to minimize upper body contribution to force production. The right foot was positioned in a snowboard binding attached to the Biodex foot plate. This set-up was used to minimize movement of the ankle and foot, which is difficult to obtain with the standard (original) Biodex ankle flexion/extension accessory. During all tests, hip and knee angles were maintained at 180° (hip neutral position and knee full extension) and the ankle was set at 90° (neutral position). The subjects were asked to position their head and arm comfortably and to stay in this position during all measurements. ENS, sMNS and cMNS were imposed in a random order within the same experimental session (Fig. 1a).

The experimental procedure commenced with the acquisition of the H-M recruitment curve. Stimuli were delivered every 10 s, which limits post-activation depression during resting H-reflex measurements [19], while ENS intensity was increased and then decreased between 1 and 40 mA (steps of 1 mA between 1 and 20 mA, and 2 mA between 20 and 40 mA). sMNS and cMNS intensities were varied between 30 and 100% of the maximal stimulator output (steps of 2% between 30 and 80%, and 5% between 80 and 100%). EMG responses were recorded twice for all stimulation intensities (i.e. 60 stimulation points per technique).

After the acquisition of recruitment curves in each stimulation condition, a horizontal visual analog scale (10 cm) was presented to the subjects, who were asked to place a vertical mark between “no discomfort” (0 cm) and “worst possible discomfort” (10 cm). This allowed for post-hoc comparisons of the rates of global discomfort associated with the stimulation techniques [9].

Maximal H-reflex intensity ( $I_{Hmax}$ ; i.e. the stimulus intensity at which H-reflex amplitude was maximal) was determined and used for all subsequent stimulations. After a warm-up, consisting in the completion of 10 sub-maximal contractions of progressive intensity until a maximal level (contraction duration: 5 s; rest: 20 s) and 3 maximal contractions, and a 10-min passive rest, maximal H-reflex responses were evoked at rest (REST) and during submaximal and maximal muscle contractions; these different conditions were used to modulate H-reflex amplitude [20] and assess technique-specific differences in H-reflex measurements. Conditions consisted in evoking the H-reflex during 5-s maximal voluntary isometric contractions (MVC) and submaximal contractions at 10% of MVC. Subjects were strongly encouraged during MVC efforts and torque, feedback was provided on a monitor during

submaximal and maximal contractions. Tests were completed three times in each condition with 30 s between each trial and a resting time of 5 min between conditions.

#### Instrumentation

Skin preparation was performed prior EMG surface electrode placement (Ag-AgCl, Blue Sensor N-00-S, Ambu, Denmark) in order to achieve low impedance ( $Z < 5 \text{ k}\Omega$ ). EMG electrodes were positioned on right *soleus* (SOL) and *tibialis anterior* (TA) muscle bellies, according to the Surface Electromyography for Non-Invasive Assessment of Muscles (SENIAM) recommendations [21], with an inter-electrode distance of 20 mm. EMG signals were amplified (Dual BioAmp, ML 135, ADInstruments, New South Wales, Australia; bandwidth: 10-500 Hz, common mode rejection ratio  $> 85\text{dB}$ , gain = 1000) and simultaneously digitized with torque signal by an external analog-to-digital converter (PowerLab 8/35, ADInstruments, New South Wales, Australia) driven by the LabChart 7.3 Pro software (ADInstruments, New South Wales, Australia). Torque and EMG data were sampled at a frequency of 2 kHz.

ENS was imposed using a constant-current stimulator (Digitimer DS7A, Hertfordshire, UK). Single rectangular-waves pulses (1000  $\mu\text{s}$  duration) were delivered through a cathode electrode (Blue Sensor N-00-S, Ambu, Denmark) placed over the tibial nerve in the popliteal fossa and an anode ( $5 \times 10 \text{ cm}$ , Compex, Ecublens, Switzerland) located 3-4 cm below the inferior part of the right patella.

sMNS and cMNS were delivered with a 70-mm figure-of-eight coil connected to one Magstim 200<sup>2</sup> for standard stimulus duration (peak magnetic field strength 2.2 T, stimulus duration 82  $\mu\text{s}$ ) or two Magstim 200<sup>2</sup> stimulators linked and synchronized by the Bistim<sup>2</sup> module to increase stimulus duration (peak magnetic field strength 2.5 T, stimulus duration 115  $\mu\text{s}$ ; Magstim, Witland, Dyfed, UK). The coil was placed over the posterior tibial nerve in the popliteal fossa. Coil position where the largest peak twitch and M-waves were elicited was marked on the skin.

#### Data analysis

MVC torque ( $\tau_{\text{MVC}}$ ) was considered as the peak torque attained during MVC condition. From the recruitment curves for the three stimulation techniques, peak-to-peak H-reflex and M-wave amplitudes were determined automatically (Fig. 1b) with a Scilab script (Scilab 5.5.2, Scilab Entreprises S.A.S, Orsay, France), after being visually checked individually.  $H_{\text{max}}$  and maximal M-wave ( $M_{\text{max}}$ ) amplitudes were determined, together with

the corresponding stimulation intensities ( $I_{Hmax}$  and  $I_{Mmax}$  respectively). The  $H_{max}/M_{max}$  ratio was calculated to account for spinal excitability. The amplitude of the M-wave associated to  $H_{max}$  (i.e. evoked at  $I_{Hmax}$ ;  $M_{Hmax}$ ) was also measured to characterize motor fiber activation when Ia-fiber activation was maximal. The regression slopes ( $H_{slp}$  and  $M_{slp}$ ) were determined by linear fitting between the thresholds and the maximal amplitudes for individual H and M recruitment curves [4] normalized to  $M_{max}$  intensity. The  $H_{slp}/M_{slp}$  ratio was then computed to further investigate motoneuron excitability since it has been shown to be theoretically independent of the activation threshold [22]. At  $I_{Mmax}$  the amplitude of the TA M-wave ( $M_{TA}$ ) was determined to compare antagonist recruitment between the three techniques of stimulation.

For the three explored conditions (REST, MVC and 10% MVC) and all stimulation techniques, H-reflex and associated M-wave amplitudes were automatically determined. The average of the three trials was used for statistical analysis.

## Statistics

Distribution normality and homogeneity of variances were tested using a Shapiro–Wilk normality test and the Bartlett's test, respectively. For recruitment curve data, differences between the three stimulation techniques in extracted parameters ( $H_{max}$ ,  $M_{max}$ ,  $H_{max}/M_{max}$ ,  $H_{slp}$ ,  $M_{slp}$  and  $H_{slp}/M_{slp}$ ) were analyzed separately with one-way Analysis of variance (ANOVAs) with repeated measures. H-reflex and M-wave recruitment curve patterns with stimulation intensities normalized to  $I_{Hmax}$  and  $I_{Mmax}$  were compared with two-way ANOVAs with repeated measures (stimulation technique  $\times$  intensity). Moreover, to discriminate the effect of conditioning on  $H_{max}$  and  $M_{Hmax}$ , two-way ANOVAs with repeated measures (stimulation technique  $\times$  conditions) were used. When ANOVAs revealed significant main or interaction effects, Fisher's LSD post-hoc tests were applied to test the discrimination among pairs of means. Data are reported as mean  $\pm$  SD and the  $\alpha$ -level for statistical significance was set at  $p < 0.05$ . Statistica 8.0 software (Statsoft, Inc, USA) was used for all statistical analysis.

## Results

### Recruitment curves

Maximal intensity ( $I_{Mmax}$ ) was attained for ENS and cMNS (ENS:  $29.5 \pm 8.7$  mA; cMNS:  $86.4 \pm 11.0\%$  of maximal stimulator output), as evidenced by the plateau of the SOL M-wave amplitude (see typical subject data; Fig. 2a & 2b). Conversely, SOL M-wave amplitude did not reach a plateau with sMNS (Fig. 2c). Maximal H-reflex



occurred at  $9.5 \pm 2.9$  mA for ENS,  $65.2 \pm 10.8\%$  and  $55.7 \pm 9.7\%$  of the maximal stimulator output for sMNS and cMNS, respectively.

H-reflex and M-wave recruitment curve parameters are reported in Table 1.  $M_{\max}$  was lower using sMNS compared to ENS and cMNS.  $H_{\max}$  was higher using ENS than both MNS techniques. However, the  $H_{\max}/M_{\max}$  ratio did not differ between the three stimulation techniques.  $M_{\text{slp}}$  was statistically lower using ENS than sMNS and cMNS. Conversely, no difference was observed for  $H_{\text{slp}}$ .  $H_{\text{slp}}/M_{\text{slp}}$  was only found to be different between ENS and sMNS.  $M_{\text{TA}}$  was significantly higher using ENS ( $2.52 \pm 0.79$  mV) than sMNS ( $1.71 \pm 1.04$  mV;  $p < 0.01$ ) and cMNS ( $1.84 \pm 1.07$  mV;  $p < 0.05$ ). No difference was observed between sMNS and cMNS ( $p = 0.854$ ).

Both ANOVAs comparing M-wave and H-reflex amplitudes plotted against relative intensities showed interaction effects (intensity  $\times$  stimulation technique;  $F = 2.23$ ,  $p < 0.01$  and  $F = 7.93$ ,  $p < 0.001$ , respectively). M-wave amplitudes evoked by ENS were significantly higher than M-waves evoked by magnetic stimulation between 32 and 87% of  $I_{M\max}$  (Fig. 3a). Between 95 and 100% of  $I_{M\max}$  M-wave amplitudes evoked by ENS were higher than those evoked by sMNS, while no difference was observed between ENS and cMNS. Moreover, from 63 to 100% of  $I_{M\max}$ , M-wave amplitudes were higher with cMNS than sMNS. The analysis of H-reflex recruitment curves revealed lower H-reflex amplitudes for sMNS and cMNS as compared to ENS between 65 to 158% of  $I_{H\max}$  (Fig. 3b).

#### Discomfort

The ANOVA revealed an effect of the stimulation technique on discomfort scores ( $F = 18.87$ ,  $p < 0.001$ ). Discomfort induced by ENS ( $4.2 \pm 2.5$  cm) was rated to be significantly higher than sMNS ( $1.9 \pm 1.9$  cm) and cMNS ( $1.9 \pm 1.8$  cm). No difference was found between sMNS and cMNS.

#### Rest and contraction condition

During the MVC condition, subjects produced comparable  $\tau_{\text{MVC}}$  levels across trials (ENS:  $128 \pm 27$  N.m; sMNS:  $127 \pm 21$  N.m; cMNS:  $125 \pm 24$  N.m). ANOVA revealed both condition and stimulation effects for M-wave amplitude ( $F = 4.446$ ,  $p < 0.01$ ; and  $F = 3.556$ ,  $p < 0.05$ ) but no interaction effect ( $F = 1.570$ ,  $p = 0.168$ ). With the three techniques, no difference in  $M_{H\max}$  was observed between REST and 10%MVC conditions (Fig. 4a). Under these three conditions,  $M_{H\max}$  was significantly lower with ENS than with sMNS and cMNS ( $1.17 \pm 1.58$  mV,  $2.57 \pm 1.49$  mV and  $2.36 \pm 1.61$  mV, respectively). In the MVC condition,  $M_{H\max}$  was increased in comparison to REST for the three stimulation techniques (Fig 4a; ENS:  $3.44 \pm 3.76$  mV; sMNS:  $3.97 \pm 3.08$  mV; cMNS:  $3.31 \pm 2.62$  mV).

Absolute M-wave values in the MVC condition ( $p = 0.105$ ) and relative values compared to REST ( $\approx +250\%$ ;  $p = 0.068$ ) did not differ between stimulation techniques.

For  $H_{\max}$  ANOVA showed both condition and stimulation effects ( $F = 23.407$ ,  $p < 0.001$ ; and  $F = 8.517$ ,  $p < 0.01$ ) but no interaction effect ( $F = 1.309$ ,  $p = 0.263$ ). Similar to that observed for the recruitment curves,  $H_{\max}$  evoked by ENS was higher than sMNS and cMNS under the different conditions ( $p < 0.05$ ). Among these conditioning modalities,  $H_{\max}$  was greater in the MVC condition, with no difference between stimulation techniques (Fig 4b; ENS:  $+120 \pm 248\%$ ; sMNS:  $+228 \pm 350\%$ ; cMNS:  $+162 \pm 180\%$ ;  $F = 1.12$ ,  $p = 0.344$ ).

## Discussion

The aim of this study was to compare the characteristics of the H-reflex and recruitment curves evoked by ENS and both sMNS and cMNS. Our hypothesis was that both MNS techniques would result in underestimation of the  $H_{\max}$  but not the  $H_{\max}/M_{\max}$  ratio when compared to the traditional ENS technique, and that cMNS would provide better outcomes than sMNS owing to its longer stimulus duration. The results of this study support these hypotheses.

### H-reflex characteristics

Both cMNS and sMNS underestimated the  $H_{\max}$  when compared to ENS. This can be ascribed to the shorter stimulus duration of MNS techniques as compared to ENS [14,17]. Furthermore, it is noteworthy that the  $M_{H\max}$  was lower with ENS than with both MNS. This result is consistent with previous works reporting that a short stimulus duration preferentially activates efferent fibers [14]. Consequently, the H-reflex appears after the M-wave occurrence during the recruitment curve (Fig. 2c). In addition, the amplitude of the H-reflex is reduced because there is a major effect of the antidromic collision in motor axons, as illustrated by the greater  $M_{H\max}$  with MNS. Conversely, with a 1000- $\mu$ s, i.e. optimal, stimulus duration [16,23] the threshold for Ia-afferent fibers is lower than for motor fibers and the H reflex appears before the M wave (Fig. 2a). The magnitude of antidromic collision is reduced because relatively fewer efferent fibers are depolarized by the stimulation. Beyond duration, the shape of stimulus waveform may also have affected nerve fiber recruitment. Indeed, the triangular waveforms typically used for MNS can easily activate efferent motor fibers [14]. However, to conclude on the effect of the stimulus waveform, we should have compared ENS and MNS with similar stimulus duration. In addition, to conclude about the effect of the stimulation nature (ENS vs. MNS), there should be a comparison between ENS and MNS applied with similar pulse duration

and waveform shape. Nevertheless, the main aim of this study was to compare ENS and MNS under ecologic conditions, i.e. applied with different pulse duration and waveform.

Despite this underestimation of the  $H_{\max}$ , no difference between techniques was found for the  $H_{\max}/M_{\max}$  ratio. This is consistent with a previous study showing that the stimulus duration does not affect the  $H_{\max}/M_{\max}$  ratio [23] and indicates that any technique may be used when the  $H_{\max}/M_{\max}$  ratio is the primary variable of interest. However, in the current study  $M_{\max}$  was not reached with sMNS, as previously reported [24], whereas it was with cMNS. Although not observed in the present study, the  $M_{\max}$  underestimation could potentially induce an  $H_{\max}/M_{\max}$  overestimation with sMNS. Therefore, we suggest that the use of cMNS should be preferred to sMNS for the assessment of the  $H_{\max}/M_{\max}$  ratio, when the use of ENS is not possible. A greater recruitment of the antagonist muscle was also observed with ENS than with sMNS and cMNS, as evidenced by the significantly higher  $M_{TA}$  with ENS. It has been previously reported that a supra-maximal electrical stimulus can activate antagonist muscles and severely affect twitch responses [25]. The reduced recruitment of the fibular nerve with MNS techniques, also observed by Neyroud et al. [9], may ensure a better assessment of evoked responses (*i.e.* M-wave and H-reflex).

It has been reported that the  $H_{slp}/M_{slp}$  assesses the excitability of the motoneuron pool with a high degree of significance, and is theoretically independent from the activation threshold of the H-reflex and M-wave [22]. Our results show that  $H_{slp}/M_{slp}$  can be reliably evaluated with cMNS but not with sMNS. The underestimation of  $H_{slp}/M_{slp}$  cannot be attributed to  $H_{slp}$  changes because no difference was found between techniques. Rather, it is more likely related to the inability to achieve a maximal intensity with sMNS; consequently,  $M_{\max}$  was underestimated and  $M_{slp}$  could have been overestimated. These results suggest that  $H_{slp}/M_{slp}$  can be reliably assessed with cMNS, but not with sMNS.

#### H-reflex facilitation

In the current study, different contractions were used to modulate the H-reflex [20] and consequently compare the ability of the three stimulation techniques to evaluate H-reflex facilitation. The submaximal contractions had no effect on the H-reflex amplitude, even though peak-to-peak H-reflex amplitude is commonly observed to be influenced by the contraction level [7,26]. Voluntary muscular contraction increases the excitability of efferent fibers and, consequently, H-reflex amplitude increases (*i.e.* facilitation occurs). In the present study, it is possible that the subject position, i.e. prone position, could explain the discrepancy between our results and previous studies, which have been conducted in a sitting position [7,26]. Indeed, it has been demonstrated that the pre-synaptic inhibition is

reduced in the prone position [27]. Therefore, any manoeuvre, such as a voluntary contraction, aimed at modulating the H-reflex amplitude would have less effect in a prone as compared to a sitting position, especially at low force levels [7,26]. This certainly explains why a 10%-MVC contraction was not sufficient to increase motoneuron excitability and significantly increase H-reflex amplitude from the resting condition. Conversely, all the stimulation techniques consistently detected the increase of H-reflex amplitude during the MVC. However, M-wave increment was also observed during MVC with the three techniques, although we cannot exclude the possibility that any electrode movement relative to the nerve during MVC may have led to an underestimation of the effect of facilitation. Given that facilitation was similar for the three techniques, we nevertheless suggest that both sMNS and cMNS ~~can~~ could be confidently used to assess H-reflex variation during MVC.

In conclusion, the results of this study confirm that H-reflex assessment is not accurate with a standard magnetic simulator, owing to its short stimulus duration. However, synchronizing two magnetic stimulators in order to increase the stimulus duration can reduce these limitations. cMNS used to measure the  $H_{\max}/M_{\max}$  ratio. Furthermore, cMNS induces less discomfort than ENS, which may be of benefit when testing is conducted in non-adult, elderly or clinical populations. Based on the current findings, it is reasonable to recommend the use of cMNS over sMNS for the assessment of the H-reflex characteristics, when the use of the gold standard technique, i.e. ENS, is not possible.

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Table

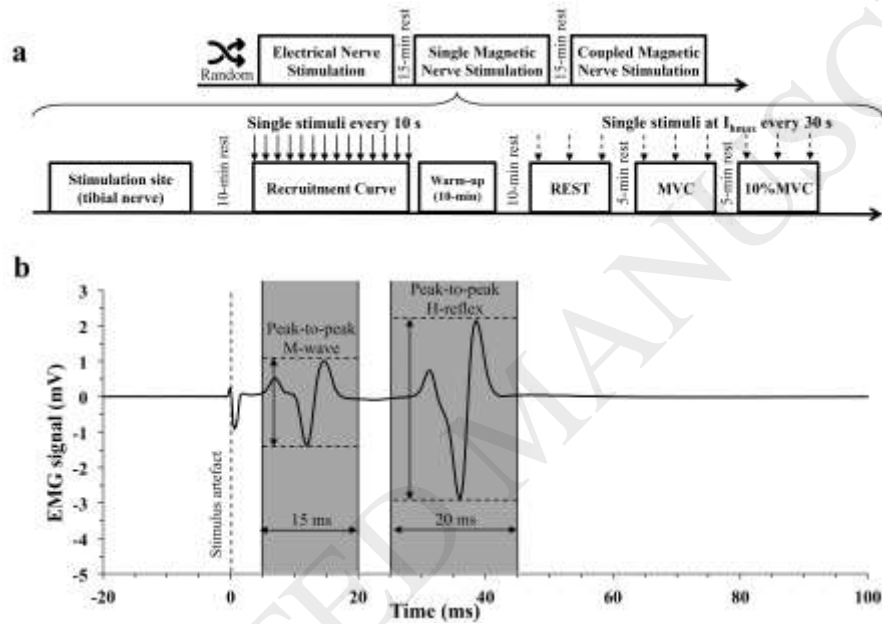
Parameters	Stimulation methods	Mean $\pm$ SD	ANOVA		Paired comparisons	
			F-values	p-values		p-values
<b>M<sub>max</sub> (mV)</b>	sMNS	6.85 $\pm$ 2.29	11.412	< 0.01	sMNS vs. cMNS	< 0.05
	cMNS	7.49 $\pm$ 2.23			sMNS vs. ENS	< 0.01
	ENS	8.32 $\pm$ 2.73			cMNS vs. ENS	0.221
<b>H<sub>max</sub> (mV)</b>	sMNS	2.72 $\pm$ 1.47	12.745	< 0.01	sMNS vs. cMNS	0.182
	cMNS	3.17 $\pm$ 1.71			sMNS vs. ENS	< 0.01
	ENS	4.75 $\pm$ 1.63			cMNS vs. ENS	< 0.05
<b>M<sub>Hmax</sub> (mV)</b>	sMNS	2.67 $\pm$ 1.58	9.099	< 0.01	sMNS vs. cMNS	0.850
	cMNS	2.61 $\pm$ 1.61			sMNS vs. ENS	< 0.01
	ENS	0.97 $\pm$ 0.74			cMNS vs. ENS	< 0.01
<b>H<sub>max</sub>/M<sub>max</sub></b>	sMNS	0.45 $\pm$ 0.28	2.407	0.111		
	cMNS	0.47 $\pm$ 0.29				
	ENS	0.59 $\pm$ 0.17				
<b>M<sub>slp</sub> (a.u.)</b>	sMNS	0.97 $\pm$ 0.02	10.533	< 0.001	sMNS vs. cMNS	0.173
	cMNS	0.91 $\pm$ 0.07			sMNS vs. ENS	< 0.001
	ENS	0.78 $\pm$ 0.13			cMNS vs. ENS	< 0.01
<b>H<sub>slp</sub> (a.u.)</b>	sMNS	0.89 $\pm$ 0.08	1.193	0.324		
	cMNS	0.92 $\pm$ 0.06				
	ENS	0.87 $\pm$ 0.09				
<b>H<sub>slp</sub>/M<sub>slp</sub></b>	sMNS	0.92 $\pm$ 0.08	4.392	< 0.05	sMNS vs. cMNS	0.355
	cMNS	1.02 $\pm$ 0.13			sMNS vs. ENS	< 0.01
	ENS	1.15 $\pm$ 0.26			cMNS vs. ENS	0.064

Table 1: Characteristics of the H-reflexes and M-waves evoked by (sMNS) standard and coupled (cMNS) magnetic nerve stimulation and electrical nerve stimulation (ENS). M<sub>max</sub>: maximal M-wave amplitude; H<sub>max</sub>: maximal H-reflex amplitude; M<sub>Hmax</sub>: M-wave associated with H<sub>max</sub>, M<sub>slp</sub>: M-wave regression slope, H<sub>slp</sub>: H-reflex regression slope.

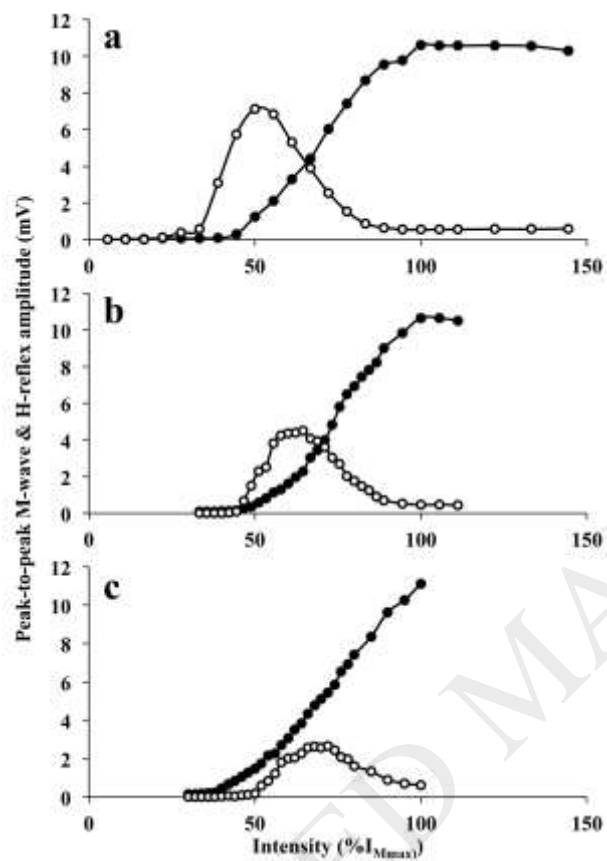


# Figure legends

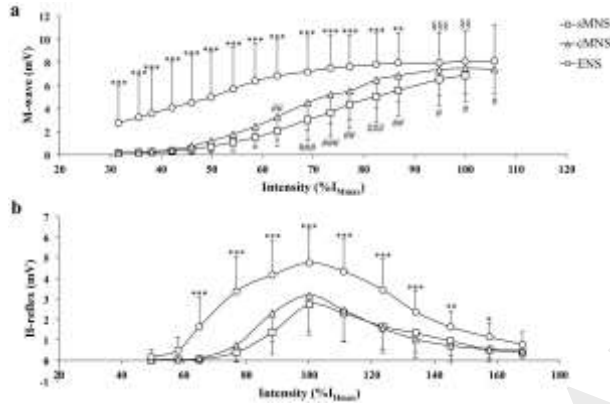
**Fig. 1** Panel a: Overview of the experimental protocol. The three stimulation techniques were randomly tested. After completing the recruitment curve, subjects were stimulated at maximal H-reflex intensity ( $I_{Hmax}$ ; dashed arrows) during the rest period (REST) and two conditions aimed at modulating the H-reflex amplitude (MVC: maximal voluntary contraction; 10%MVC: 10% of MVC). Panel b: Typical EMG recording (solid line) after a single stimulation (vertical dashed line; time = 0 ms). Peak-to-peak M-wave and H-reflex amplitudes were directly calculated from these signals.



**Fig. 2** Typical M-wave (close circle) and H-reflex (open circle) recruitment curves obtained with electrical nerve stimulation (panel a) and coupled (panel b) and standard (panel c) magnetic nerve stimulation. Intensities were normalized to the maximal M-wave intensity ( $I_{Mmax}$ ).



**Fig. 3** M-wave (panel a) and H-reflex amplitudes (panel b) evoked by standard (sMNS) and coupled (cMNS) magnetic stimulation and electrical nerve stimulation (ENS). Intensities were normalized to the maximal M-wave intensity ( $I_{Mmax}$ ) and the maximal H-reflex intensity ( $I_{Hmax}$ ) for M-wave and H-reflex recruitment curves, respectively. Data are expressed as mean  $\pm$  SD. Significant difference between ENS and both MNS techniques: \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ . Significant difference between ENS and sMNS: §§:  $p < 0.01$ ; §§§:  $p < 0.001$ . Significant difference between sMNS and cMNS: #:  $p < 0.05$ ; ##:  $p < 0.01$ ; ###:  $p < 0.001$ .



**Fig. 4** M-wave associated to H-reflex (panel a) and H-reflex amplitudes (panel b) evoked by standard (sMNS) and coupled (cMNS) magnetic stimulation and electrical nerve stimulation (ENS) at rest (REST) and during active contractions (MVC: Maximal Voluntary Contraction; 10%MVC: 10% of MVC). \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ .

