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Extra Forces induced by wide-pulse, high-frequency electrical stimulation: Occurrence, magnitude, variability and underlying mechanisms

Jennifer Wegrzyk [a], Alexandre Fouré [a], Christophe Vilmen [a], Badih Ghattas [b], Nicola A. Maffiuletti [c], Jean-Pierre Mattei [a,d], Nicolas Place [e], David Bendahan [a], Julien Gondin [a,*]

[a] Aix Marseille Université, CNRS, CRMBM UMR 7339, 13385 Marseille, France
[b] Aix Marseille Université, CNRS, Institut de Mathématiques, UMR 7373, 13288 Marseille, France
[c] Neuromuscular Research Laboratory, Schulthess Clinic, Zurich, Switzerland
[d] APHM, Hôpital Sainte Marguerite, Service de Rhumatologie, 13009 Marseille, France
[e] Institute of Sport Sciences and Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland

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HIGHLIGHTS

- The percentage of responders showing “Extra Forces” to wide-pulse, high-frequency (WPHF) neuromuscular electrical stimulation has been previously over-estimated.
- Force output evoked by WPHF shows markedly high inter- and intra-individual variability.
- In the responder group only, H-reflex is depressed immediately after WPHF indicating a significant central contribution to “Extra Forces”.

ABSTRACT

Objective: In contrast to conventional (CONV) neuromuscular electrical stimulation (NMES), the use of “wide-pulse, high-frequencies” (WPHF) can generate higher forces than expected by the direct activation of motor axons alone. We aimed at investigating the occurrence, magnitude, variability and underlying neuromuscular mechanisms of these “Extra Forces” (EF).
Methods: Electrically-evoked isometric plantar flexion force was recorded in 42 healthy subjects. Additionally, twitch potentiation, H-reflex and M-wave responses were assessed in 13 participants. CONV (25 Hz, 0.05 ms) and WPHF (100 Hz, 1 ms) NMES consisted of five stimulation trains (20 s on–90 s off).
Results: K-means clustering analysis disclosed a responder rate of almost 60%. Within this group of responders, force significantly increased from 4% to 16% of the maximal voluntary contraction force and H-reflexes were depressed after WPHF NMES. In contrast, non-responders showed neither EF nor H-reflex depression. Twitch potentiation and resting EMG data were similar between groups. Interestingly, a large inter- and intrasubject variability of EF was observed.
Conclusion: The responder percentage was overestimated in previous studies.
Significance: This study proposes a novel methodological framework for unraveling the neurophysiological mechanisms involved in EF and provides further evidence for a central contribution to EF in responders.

1. Introduction

Neuromuscular electrical stimulation (NMES) is commonly used to enhance muscular performance (Gondin et al., 2011b)

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* Corresponding author at: Aix-Marseille Université, CNRS, CRMBM UMR 7339, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 5, France. Tel.: +33 4 91 32 48 07.
E-mail address: julien.gondin@univ-amu.fr (J. Gondin).

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and to maintain contractile activity in paralyzed or immobilized muscles (Sheffler and Chae, 2007). Electrically-evoked contractions are generated by a combination of peripheral mechanisms (i.e., the direct activation of motor axons under the stimulation electrodes) and central mechanisms (i.e., the depolarization of sensory axons producing a "reflex" response). It has been recently suggested that the relative contribution of peripheral and central factors to force production might be modulated by pulse duration, pulse frequency and stimulation intensity with the aim of generating contractions with a better resistance to fatigue (Collins, 2007; Dean et al., 2008; Bergquist et al., 2011b).

The conventional (CONV) form of NMES typically applied in clinical settings involves the utilization of short stimulus pulses (50–400 μs) delivered at high current intensities and as intermittent low-frequency trains (15–40 Hz) (Hainaut and Duchateau, 1992). These parameters generate contractions predominantly *via* peripheral pathways due to both the preferential activation of motor axons and the large antidromic transmission along them (Bergquist et al., 2011b). Thus, even though being a key component in training and rehabilitation, the major and commonly accepted drawback of CONV is the rapid onset of muscular fatigue due to a non-physiological recruitment of motor units (i.e., random, spatially limited and temporally synchronous) (Vanderthommen et al., 2003; Gregory and Bickel, 2005; Maffiuletti, 2010).

It has recently been suggested that the central contribution to motor unit recruitment could be enhanced when delivering the stimulation at low current intensities and with long pulse duration (Collins, 2007). Low current intensities minimize the antidromic collision in motor axons, thereby allowing orthodromically transmitted signals to descend from spinal circuits. In addition, the use of relatively long pulse durations (0.5–1 ms) favors the recruitment of sensory axons having a longer strength-duration and lower rheobase as compared to motor axons (Veale et al., 1973). Interestingly, the use of wide-pulse (1 ms), high-frequency (>80 Hz) (WPHF) and low-current-intensity NMES has been shown to produce up to three times higher isometric forces than CONV (Collins et al., 2002; Lagerquist et al., 2009). For a given stimulation intensity, this progressively increasing force output that arises in addition to what would be expected from the direct response to motor axon stimulation has been referred to as "Extra Forces" (EF) (Collins et al., 2001, 2002). Based on the fact that a peripheral nerve block abolished the EF phenomenon in some previous studies (Collins et al., 2001; Lagerquist et al., 2009) and that WPHF-induced EF was associated with enhanced H-reflex and/or asynchronous activity (Bergquist et al., 2011a), central mechanisms are likely to be involved in EF generation. Moreover, similar EF patterns have been observed at high frequency tendon vibration. Based on increases in the soleus V/F wave amplitude, vibration-induced EF was attributed to an increased motoneuron excitability (Magalhaes et al., 2013). On that basis, it has been suggested that the central contribution to force production might minimize muscle fatigue due to the preferential recruitment of fatigue-resistant motor units according to the Henneman size principle (Binder-Macleod and Scott, 2001; Gregory et al., 2007), thereby providing a prospective advantage of WPHF over the CONV stimulation pattern for clinical use. However, the central origin hypothesis of EF has been recently challenged by the findings of Frigon et al. suggesting that EF could essentially result from intrinsic muscle properties (Frigon et al., 2011). In the latter study, anesthetic nerve block experiments in human subjects and nerve transection in decerebrate cats failed to abolish EF, instead muscle length changes significantly affected EF. Accordingly, peripheral mechanisms such as length-dependent changes in Ca^{2+} release, sensitivity, and phosphorylation of the myosin light chain have been proposed as underlying mechanisms for EF (Binder-Macleod and Kesar, 2005; Frigon et al., 2011). Another finding that challenges the hypothesis of an enhanced central contribution involved in WPHF is that neuromuscular fatigue was even increased for repeatedly evoked WPHF contractions (Neyroud et al., 2014).

Previous studies reported that WPHF-induced EF occurs in 85–100% of healthy individuals, classified as responders for nerve (Baldwin et al., 2006; Klakowicz et al., 2006) and muscle belly stimulation (Collins et al., 2001; Baldwin et al., 2006; Dean et al., 2007). However, this large proportion has been observed when small sample sizes were tested (i.e., ranging from 5 to 15 subjects). Moreover, the hitherto existing classification approach suffers from methodological limitations given that no comparative analysis has been performed between WPHF and CONV to determine EF occurrence. This is surprising given that force production can also slightly increase in response to CONV, e.g. due to staircase potentiation (Rassier and MacIntosh, 2002). The mechanisms that may account for the differences in force response between subjects and NMES protocols remain to be determined.

In the present study, we investigated, in a first instance, the occurrence of EF in a large cohort of subjects by using a clustering method previously applied for quantifying the inter-individual variability to resistance training (Bamman et al., 2007; Gondin et al., 2011a). In addition to EF occurrence, we studied the magnitude and variability of EF in response to WPHF in order to estimate the protocol's effectiveness and its potential beneficial use. Considering that NMES-induced strength gains are correlated with the electrically-evoked force (i.e., the magnitude of EF) (Maffiuletti, 2010; Gondin et al., 2011b), information regarding both the between- and within-subject variability are of importance in the context of rehabilitation. To account for EF occurrence, magnitude and variability, we investigated, for a subset of responders and non-responders, the potential underlying neuromuscular mechanisms by evaluating twitch potentiation, H-reflex and M-wave. We hypothesized that the responder subjects would exhibit a higher twitch potentiation and a higher resting H-reflex excitability as compared to the non-responders.

2. Methods

The study is divided into two experiment sections (Fig. 1). The first sub-study addresses the EF phenomenon in terms of occurrence and magnitude on a large cohort of subjects. The second sub-study investigates the central and peripheral factors for EF generation by using a smaller sample. The entire study was approved by the Local Human Research Ethics Committee Sud Méditerranée I (n° 2012-A01265–38) and was conducted in conformity with the Declaration of Helsinki.

2.1. Sub-study 1 – EF occurrence and magnitude

2.1.1. Subjects

42 healthy volunteers (20 men, 22 women; age: 28 ± 6 years, weight: 64 ± 10 kg, height: 171 ± 10 cm, mean ± SD) devoid of neurological and musculoskeletal impairment participated in the study after providing written informed consent. All subjects reported to be occasionally but not regularly active in recreational sports. Before testing, subjects were asked to avoid any strenuous exercise 48 h prior to the protocol to minimize possible residual fatigue.

2.1.2. Experimental design

The testing session included: (1) a warm-up period consisting of 5–7 submaximal plantar flexion contractions of 3–5 s, (2) assessment of isometric maximal voluntary contraction (MVC) force; (3) adjustment of NMES intensity by using 2-s testing trains and

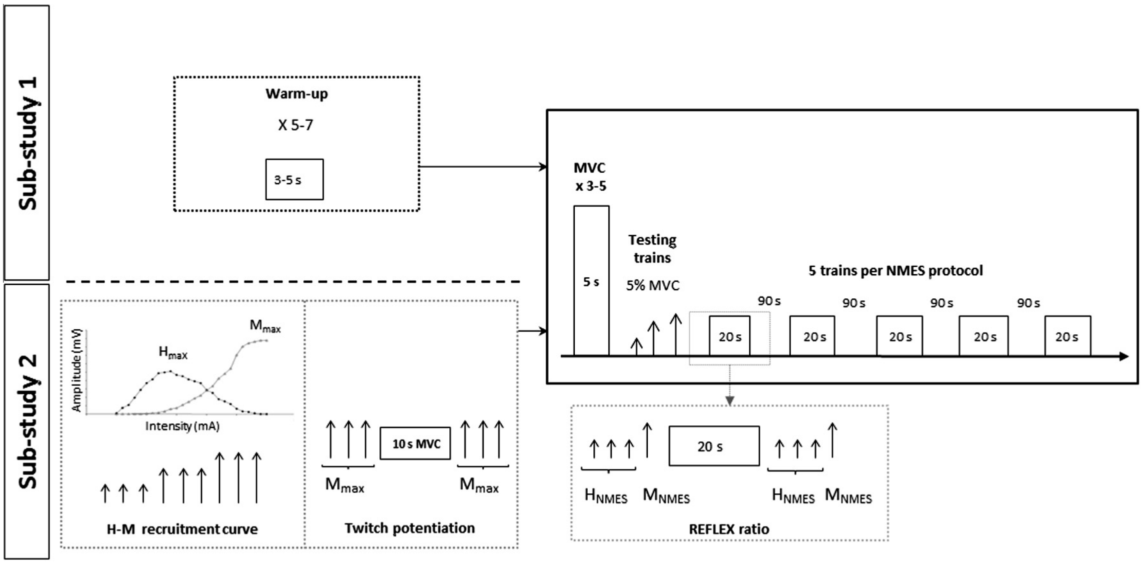

Fig. 1. Schematic over view of sub-study 1 and sub-study 2. The "warm-up" of sub-study 1 was replaced by the recording of the H–M recruitment curve and by the testing of twitch potentiation in sub-study 2. In addition to sub-study 1, the reflex ratio was tested before and after each of the five NMES trains.

(4) two NMES protocols (i.e., CONV vs. WPHF) consisting of 5 × 20 s trains applied in a single-blinded, randomized order across subjects. The experiment lasted $\sim$1 h and the participants were not informed about the purpose of the study until the end of the testing session. The *triceps surae* muscles were examined because large EF has been previously reported for this muscle group (Collins et al., 2001, 2002; Baldwin et al., 2006; Klakowicz et al., 2006; Baldwin et al., 2006; Bergquist et al., 2011a).

2.1.3. Experimental setup

All experimental procedures were performed on the right calf muscles while subjects were lying supine on a table. Two flexible surface electrodes of 5 × 13 cm and 5 × 9 cm (STIMEX schwa-medico GmbH, Ehringshausen, Germany) were placed on the *triceps surae* muscles. The proximal (and largest) electrode was placed over the *gastrocnemius* at approximately the point of the largest circumference. The distal electrode was placed over the *soleus* muscle below the bottom of the *gastrocnemius* muscle belly (Collins et al., 2001). As previously performed (Frigon et al., 2011), the knee angle was fixed at $\sim$170° (180° = full extension) and the right heel and forefoot were firmly strapped to a custom-made ergometer consisting of a foot pedal coupled to a force transducer (U9B, 1 kN; sensitivity: 1 mV/V, HBM, Darmstadt, Germany) and amplifier (Meggitt, Sensorex, Archamps, France). The foot was securely held in position with an ankle angle of 90° while the knee and hips were securely fixed on the table in order to avoid force generation by other muscle groups than the calf muscles.

2.1.4. Assessment of MVC Force

After a warm-up period, subjects performed three to five MVCs of 5 s until the highest value could not be further increased. Each contraction was separated by 2 min of rest. Participants were asked to fold their arms over their chest and to concentrate on contracting exclusively the calf muscles. During the testing session, they were not given any visual feedback of the generated force. MVC force was quantified as the maximal value achieved across the different trials. The force signal was recorded at a sampling frequency of 1 kHz using Powerlab 16/36 data acquisition system and software (LabChart 7, ADInstruments, Sydney, Australia).

2.1.5. NMES protocols

Monophasic rectangular pulses were delivered using a constant-current stimulator (Digitimer DS7A, Hertfordshire, UK; maximal voltage: 400 V) at 100 Hz (1-ms pulse duration) and 25 Hz (0.05 ms pulse duration) for the WPHF and the CONV protocols, respectively. At the beginning of each protocol, testing trains of 2 s using the stimulation parameters of each NMES protocol were applied to adjust stimulation intensity in order to reach $\sim$5% of the MVC force. This intensity level has been previously reported to be low enough to limit antidromic collision, thereby leading to large EF (Collins et al., 2002; Dean et al., 2007). The optimal stimulation intensity was considered to be reached when the evoked peak force was 5.0 ± 0.3% of MVC. Each NMES protocol (CONV and WPHF) included 5 stimulation trains delivered at a constant frequency, each lasting 20 s and separated by a 90-s resting period. The two protocols were separated by 5–10 min of recovery to minimize the effect of fatigue. Throughout the experiment, subjects were instructed to relax as much as possible and to focus on one freely-chosen point on the ceiling.

2.2. Sub-study 2 – Underlying mechanisms and variability of EF

2.2.1. Subjects

Surface electromyographic (EMG) activity and contractile properties were additionally assessed for 13 healthy individuals (8 men, 5 women; age: 26 ± 4 years, weight: 67 ± 10 kg, height: 172 ± 10 cm, mean ± SD) that participated in the second sub-study. Four of them (3 responders/1 non-responder) were retested on the basis of the first sub-study and re-tested. Muscle electrode placement and the subjects' positioning were strictly identical to sub-study 1.

2.2.2. Experimental design

H-reflex responses were quantified to explore potential central contributions to EF production whereas twitch potentiation and the associated M wave provided information on peripheral mechanisms potentially involved in EF generation. The testing session comprised (1) H–M recruitment curves at rest to determine maximal H reflex (H_{max}) and M wave (M_{max}) peak-to-peak amplitudes, (2) assessment of twitch potentiation with supramaximal single stimuli delivered before and after a MVC, (3) two to three additional MVC measurements, (4) adjustment of NMES intensity by using 2-s testing trains, and (5) NMES protocols (i.e., 5 × 20 s CONV vs. WPHF, see experimental protocol of study 1) with H-reflex and M-wave measurements before and immediately after each train.

2.2.3. Stimulation

For evoking H and M responses the posterior tibial nerve was stimulated with a single rectangular pulse (1 ms) delivered by a second Digitimer stimulator (Digitimer DS7A, Hertfordshire, UK; maximal voltage: 400 V). The self-adhesive cathode (10-mm diameter, Asept Inmed, Quint Fonsegrives, France) was located in the popliteal fossa. Once the stimulation site was determined, the electrode was firmly fixed with taping. The anode, which was a large rectangular electrode (5 × 13 cm, STIMEX schwa-medico GmbH, Ehringshausen, Germany), was placed on the anterior surface of the knee.

2.2.4. EMG recordings

Surface EMG activity of the *soleus* (SOL), the *gastrocnemius lateralis* (GL) and the *tibialis anterior* (TA) muscles was recorded by pairs of silver chloride (Ag/AgCl) circular (10-mm diameter, Asept Inmed, Quint Fonsegrives, France) electrodes. For the GL, EMG electrodes were placed in between the NMES electrodes (Bergquist et al., 2011a; Neyroud et al., 2014). For the SOL, recording electrodes were placed along the middorsal line of the leg, just below the (smaller) distal NMES electrode. To exclude the possibility that the electrophysiological responses of the plantar flexors to tibial nerve stimulation were contaminated by concomitant activation of the TA, electrodes were also positioned on the line of the fibula at 1/3 of the distance from the tip of the medial malleolus. One reference electrode was positioned over the contralateral patella. Low resistance between the two electrodes ($<5\ \mathrm{k}\Omega$) was obtained by careful skin shaving, abrasion and cleaning with alcohol. The EMG signals were amplified with a bandwidth frequency ranging from 0.3 Hz to 1 kHz and simultaneously recorded at a sampling frequency of 2 kHz (Powerlab) on a connecting computer running Labchart software (see sub-study 1).

2.2.5. Experimental protocol

For the H–M recruitment curve, single pulses were delivered at rest with an inter-stimulus interval of 10 s. After three stimuli delivered at identical stimulation intensity, the current was increased by 2-mA increments until there was no further increase in peak twitch torque and in concomitant peak-to-peak M-wave amplitude. This intensity was further increased in intensity by 20% to achieve a supramaximal level. The average EMG and mechanical signals obtained at each intensity were stored to obtain later H–M recruitment curves for SOL and GL muscles. Particular care was taken in determining the stimulus intensity needed to obtain SOL H_{max} and in monitoring the posture of the subjects. The stimulus intensity needed to obtain H_{max} in SOL was comprised between 11 and 36 mA (average: 23 ± 7 mA). Single stimuli at individual H_{max} intensity (for the SOL) were subsequently delivered before and after each NMES train of both protocols (see below). Individual supramaximal intensities ranged between 28 and 96 mA (average 58 ± 20 mA). To assess twitch potentiation, subjects were then instructed to perform one single MVC contraction of 10 s duration (without any prior warm-up) with supramaximal twitches delivered over the tibial nerve 7 and 10 s before the contraction and 2 and 5 s after the end of the contraction. After a resting period of 5 min, subjects were instructed to perform 2–3 additional MVCs separated by 2 min rest periods to ensure that the highest force value was obtained. On the basis of the highest MVC value, the threshold intensity corresponding to 5% of the MVC was calculated for NMES and the current intensity was set by means of a second stimulator (cf. sub-study 1, testing trains). Given that the artifact resulting from NMES trains inevitably contaminated the EMG signals, H-reflex and M-wave measurements were performed before and immediately after each NMES train in order to assess the central contribution to EF. Stimulations consisted of three single pulses delivered at SOL H_{max} intensity, hereafter referred to as (H_{NMES}) intensity (5-s interval), followed by one supramaximal stimulation, i.e., at SOL M_{max}, hereafter referred to (M_{NMES}) delivered 5 s apart. The pulses were delivered at the time points 20, 15, 10 (H_{NMES}) and 5 s (M_{NMES}) before and 5, 10, 15, (H_{NMES}) and 20 s (M_{NMES}) after each contraction.

2.3. Data analysis (sub-study 1 and sub-study 2)

2.3.1. Force traces

For both study 1 and 2, the NMES-evoked plantar flexion force (N) was consistently expressed relative to the highest plantar flexor MVC force. According to the method proposed by Dean and colleagues (Dean et al., 2007), the NMES-evoked force was quantified in a 0.5-s time-window within the initial 2 s (START) and the last 2 s of the stimulation period (END). Both time windows were chosen on the basis of the smallest coefficient of variation (CV). According to previous reports (Collins et al., 2001, 2002; Baldwin et al., 2006), we calculated EF, first of all, as the difference between END and START of each stimulation train ($\mathrm{Delta}_{END-START}$) not only for WPHF (Fig. 2A) but also for CONV NMES for which force increases could also be observed in some subjects (Fig. 2B). To avoid the inclusion of those subjects showing a progressive force increase for both protocols and to provide one single data set required for the clustering analysis, we calculated the difference between the delta values of each train for the WPHF and the CONV protocol so as to obtain a relative DELTA EF (i.e., $\mathrm{Delta}_{WPHF(END-START)} - \mathrm{Delta}_{CONV(END-START)}$). Additionally, force time integral (FTI) was used as an index of continuous measures of EF (Fig. 2C). This second EF calculation referred to the integrated EF that was calculated by the difference between the delta FTI values of each train for WPHF and CONV NMES as follows: DELTA FTI = $\mathrm{Delta}_{WPHF(FTI)} - \mathrm{Delta}_{CONV(FTI)}$. In doing so, we accounted for differences in individual force generation profiles while considering those subjects as potential responders whose peak force values were attained within the initial phase during the stimulation train and dropped towards the end.

Peak twitch (Pt) was analyzed from the averaged two first unpotentiated (pre) and the averaged two first potentiated (post) twitches. Twitch potentiation was calculated as a ratio: [(Pt post − Pt pre)/Pt pre] × 100. Since sustained force after the cessation of NMES has previously been considered as an indirect evidence of central recruitment (Lagerquist et al., 2009), we measured the time between the end of the stimulation (marked by the end of the stimulation trigger signal) and the return of the force signal to baseline values after each NMES trains.

2.3.2. Evoked potentials

For H–M curves of respective muscles (i.e., SOL and GL) obtained at rest, the average EMG signal of the three stimuli per intensity was used to determine peak-to-peak amplitude of (1) H_{max}, (2) M-waves at H_{max} (M_{Hmax}) and (3) M_{max}. The following ratios were then calculated for respective muscles: H_{max}/M_{max} and M_{Hmax}/M_{max}.

For H and M responses evoked before and after NMES, the stimulation intensity was optimized for the SOL muscle, so that H-reflexes for the GL muscle were probably submaximal for some subjects. Hereafter, we will refer to H_{NMES} and M_{NMES} for the corresponding evoked potentials. Changes in peak-to-peak amplitude of H_{NMES} as well as peak-to-peak amplitude and total area of M_{NMES} wave were compared for each of the 5 trains of both CONV and WPHF NMES. The first stimulus after each train was not considered to eliminate the effects of sustained force production after the stim-

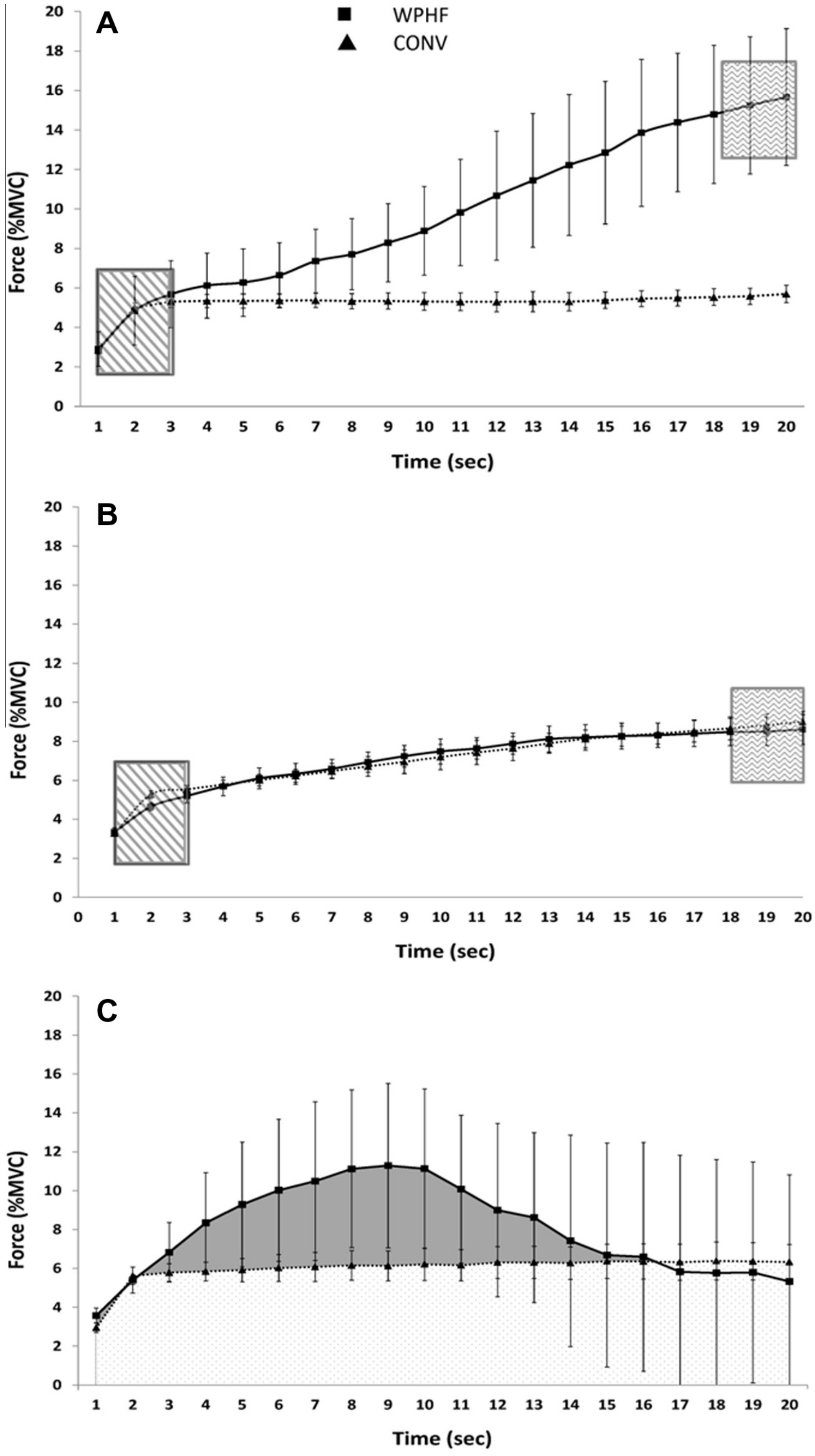

Fig. 2. Extra Forces (EF) calculation of WPHF (■) and CONV (▲) illustrated for the force generation profiles of three representative subjects. (A) EF calculation model used in former approaches considers EF during WPHF only, (B) EF DELTA calculation taking into account potential EF generation for both WPHF and CONV. The shaded areas represent second 1–3 (START) ▨ and second 18–20 (END) ▒ of the stimulation. (C) DELTA force time integral (FTI) calculation taking into account continuous force recordings ░ and peak values ■ occurring before END for both CONV and WPHF. Results are presented as means ± SE.

ulation was turned off (Collins, 2007; Bergquist et al., 2011b). The following ratios were then calculated for respective muscles: H_{NMES}/M_{NMES} and M_{H_NMES}/M_{NMES}. In order to assess the modulation of H-reflex responses by the NMES protocols, we calculated the following ratio: $[(H_{NMES}/M_{NMES}\,(post) - H_{NMES}/M_{NMES}\,(pre))/H_{NMES}/M_{NMES}\,(pre)] \times 100$, hereafter referred to as REFLEX ratio. For the responder group, 3 reflex ratios had to be discarded because evoked potentials were affected by continuous forces production after the contraction. The root mean square (RMS) EMG values of the SOL and GL were calculated over a 500-ms resting period before each single pulse and then normalized to the corresponding amplitude of the M-wave to obtain the background level of EMG. *Tibialis anterior* background EMG values were also obtained by measuring the RMS values during the same resting period.

2.4. Statistical analysis

Statistical analyses were performed using Statistica software (Stat Soft 9, Tulsa, OK, USA). We used k-means cluster analysis to classify the subjects according to the magnitude of DELTA EF/DELTA FTI calculated on the basis of the stimulation protocols. Clustering on EF was performed for all 5 trains per individual for both sub-studies separately (i.e. for the 42 subjects of the first study and for the 13 subjects of the second study). For both indexes of EF (i.e., DELTA EF and DELTA FTI), responders were clustered into three groups consisting of (1) non-responders, (2) moderate responders and (3) high responders. We have chosen a three clusters approach due to the high inter-individual variability of EF within the responder group. The split of the responder group into "moderate and high responders" allowed us to demarcate outliers with an extreme EF response, thereby increasing the sensitivity to responder detection and avoiding the underestimation of EF occurrence.

For sub-study 1, a two-way ANOVA (protocol × time (s)) with repeated measures on time was used to detect the onset of EF for WPHF and the point from which force values were significantly different between the two protocols. For sub-study 2, EMG data were analyzed separately for SOL and GL muscles. Normality was checked before each analysis using Kolmogorov–Smirnov test. A four-factor ANOVA (group × protocol × time (pre/post) × train) with repeated measures were used to compare background level of EMG. To assess inter-train variability of force, a one way ANOVA with repeated measures on train illustrated variations in EF magnitude and FTI for the responders. Unpaired *t*-tests were used to assess differences in H_{max}/M_{max}, M_{Hmax}/M_{max} and M_{max} at rest and twitch potentiation between responders and non-responders. Values of H_{NMES}/M_{NMES} ratio recorded before the NMES session were compared for all protocols by a three-way repeated measures ANOVA (group × protocol × train). Subsequently, a three-way repeated measures ANOVA (group × protocol × train) was performed to compare REFLEX ratio. Changes in M_{HNMES}/M_{NMES} ratio and both peak-to-peak amplitude and area of M_{NMES} were assessed by using a four-way repeated measures ANOVA (group × protocol × time (pre/post) × train). A two-way repeated measures ANOVA (train × protocol) was used to assess differences in sustained forces. In order to compare the degree of association between variables, Pearson's correlation was performed.

When a main effect or a significant interaction was found for ANOVA, Newman's Keuls post hoc analyses were performed. Data are presented as mean ± SD in text and tables and as mean ± SE in figures. Significance was accepted when $P < 0.05$.

3. Results

3.1. Sub-study 1 – EF occurrence and magnitude

According to the k-means clustering analysis based on the data set DELTA EF, 17 out of 42 subjects were identified as responders (i.e., 40% composed of 16 moderate responders and 1 high responder) and 25 subjects as non-responders. The clustering analysis based on the data set DELTA FTI resulted in 21 responders (i.e., 50% composed of 17 moderate responders and 4 high responders) (Fig. 3). Interestingly, a significant positive correlation was found between DELTA FTI and Delta EF ($r^2 = 0.82$, $P < 0.05$). Whereas two individuals were classified as responders for the DELTA EF but not for the DELTA FTI analysis (*force traces of one representative subject shown in* Fig. 4, *Responder A*), six subjects showed significant EF for the DELTA FTI calculation but not for the DELTA EF analysis (*force traces of one representative subject shown in* Fig. 4, *Responder B*). All responder subjects, resulting from either DELTA EF or DELTA FTI, were taken together so that our clustering analysis identified 23 responders (i.e., 57%; 10 men/13 women) and 19 non-responders.

In the responder group, the mean force increased from 4.2 ± 1.9% to 15.5 ± 7.7% MVC (i.e., more than threefold) throughout the WPHF protocol (Fig. 5A). The resulting EF was 11.2 ± 7.8% MVC. No significant EF was observed for the CONV protocol (from 4.6 ± 0.9% to 5.4 ± 2.0% MVC, $P > 0.05$). A significant onset of EF occurred from the 4th second for WPHF and from the 8th second the force production was significantly different between the two protocols. In the non-responder group, force values did not significantly change for both the WPHF (from 4.6 ± 1.2% to 4.6 ± 2.4% MVC) and the CONV protocol (from 4.9 ± 0.9% to 5.6 ± 1.7% MVC) (Fig. 5B).

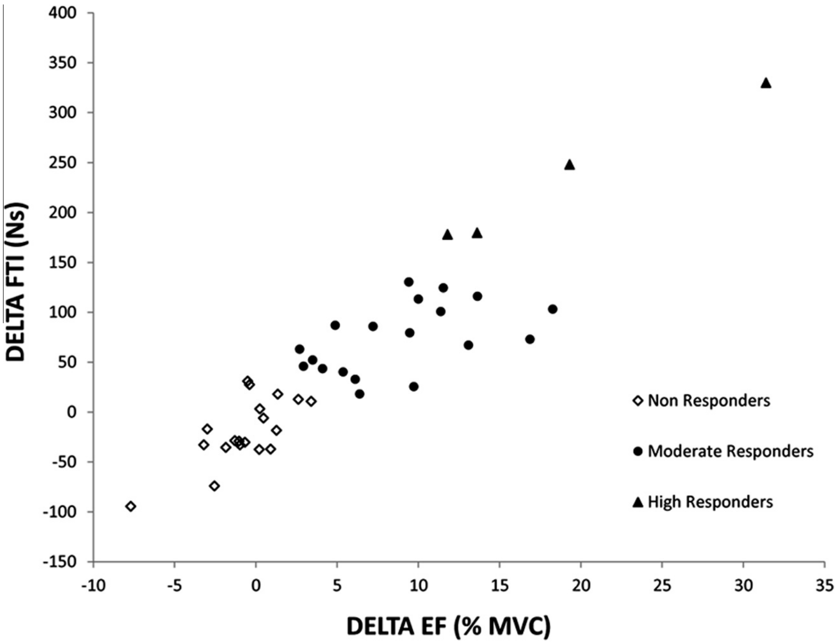

Fig. 3. K-means clustering analyses: Scatter plot illustrates subject classification into "Non Responders" ◇ for both DELTA EF and DELTA FTI, "Moderate Responders" ● for either DELTA EF or DELTA FTI or both and "High responders" ▲ for either DELTA EF or DELTA FTI or both. Negative values indicate that for some non-responders WPHF evoked lower forces than CONV for either FTI or EF or both.

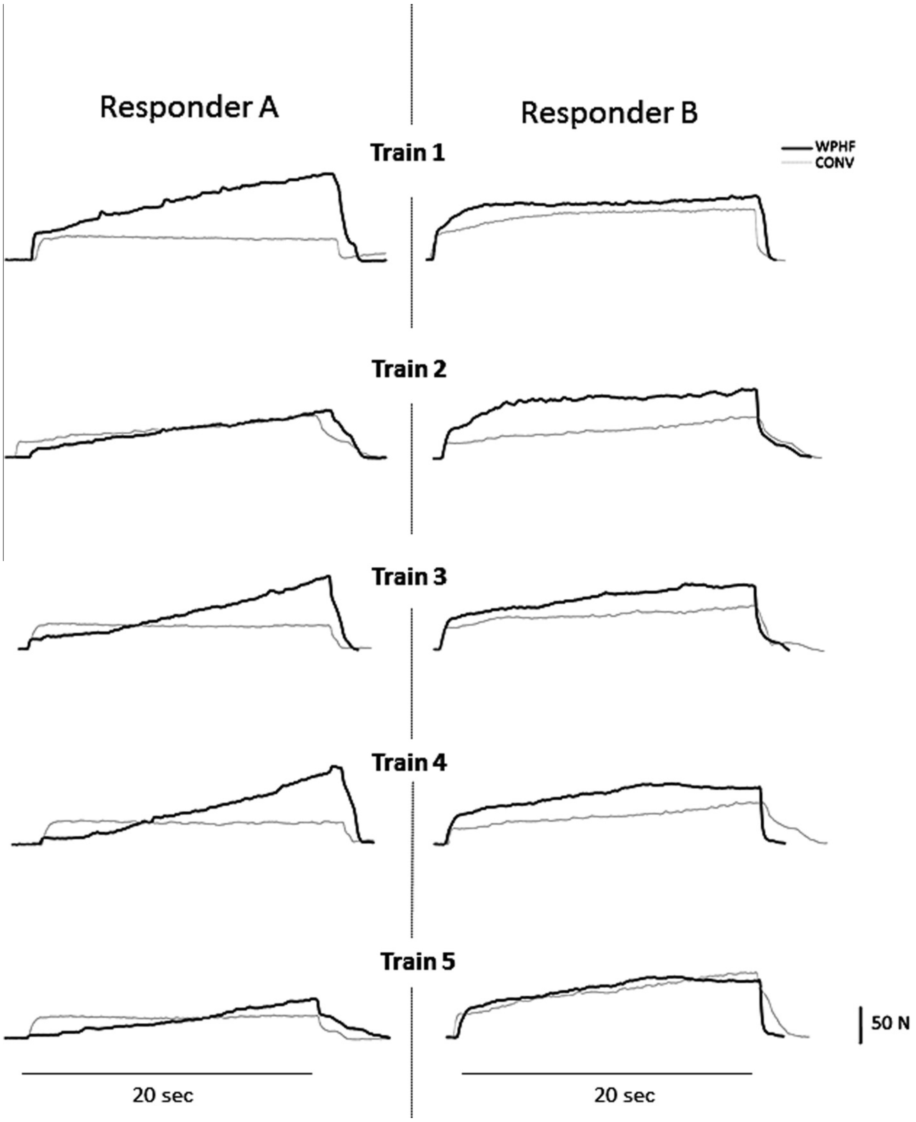

Fig. 4. Representative force generation profiles during the five trains evoked by WPHF (black lines) and CONV (gray lines) for Responder (A): one responder subject according to DELTA EF analysis but not DELTA FTI analysis, and for Responder (B): one responder subject according to DELTA FTI analysis but not DELTA EF analysis.

FTI was significantly higher (almost twofold) for the WPHF (1980 ± 1003 Ns) than for the CONV protocol (1038 ± 449 Ns) in the responder group. No difference was found in the non-responder group (951 ± 490 Ns vs. 1086 ± 378 Ns for WPHF and CONV, respectively). Age, body weight, height, MVC force, current intensity, and sex distribution did not differ between the responder and non-responder groups (Table 1).

3.2. Sub-study 2 – Variability of EF and underlying mechanisms

On the basis of k-means cluster analysis, six subjects were identified as responders and seven subjects as non-responders, respectively. For the responder group, WPHF-induced EF was 20 ± 7% MVC (mean force increased from 6 ± 3% to 26 ± 11% MVC) and FTI was 4734 ± 2445 Ns. The intersubject variability of EF was high as illustrated by a CV of 43% (ranging from 9% to 44% MVC) and 52% (ranging from mean 1603 to 8016 Ns) for EF and FTI, respectively. Moreover, a high intrasubject variability of CV was observed. The corresponding mean CVs were 24% and 17% for EF and FTI, respectively. The values of the EF and FTI magnitude were consistent and not statistically different within the 5 trains ($P > 0.05$).

3.2.1. Neuromuscular variables at rest

Resting H_{max}/M_{max} and M_{Hmax}/M_{max} ratios were not different between the responders vs. non-responders for the two investigated muscles. In the same way, M_{max} amplitudes and areas were similar between the two groups for SOL and GL muscles (Table 2).

No significant difference in twitch potentiation was observed between groups ($P > 0.05$). Force increased from 101 ± 25 N to 145 ± 31 N and from 111 ± 16 N to 150 ± 16 N for the responders and non-responders, respectively. This corresponds to a ratio of 44 ± 13% for the responders and 43 ± 26% for the non-responders, respectively.

3.2.2. Neuromuscular variables in response to NMES

H_{NMES}/M_{NMES} ratio recorded before the NMES trains were not different between groups, trains and protocols ($P > 0.05$). Furthermore, it is noteworthy that M_{HNMES}/M_{NMES} ratios and background EMG level for SOL, GL and the *tibialis anterior* were not affected by groups, trains and protocols for the three muscles, thereby indicating that the conditions of H-reflex recordings were stable (Table 3). The similar M_{HNMES}/M_{NMES} ratios pre- and post-intervention indicate that the same proportion of alpha motoneurons has

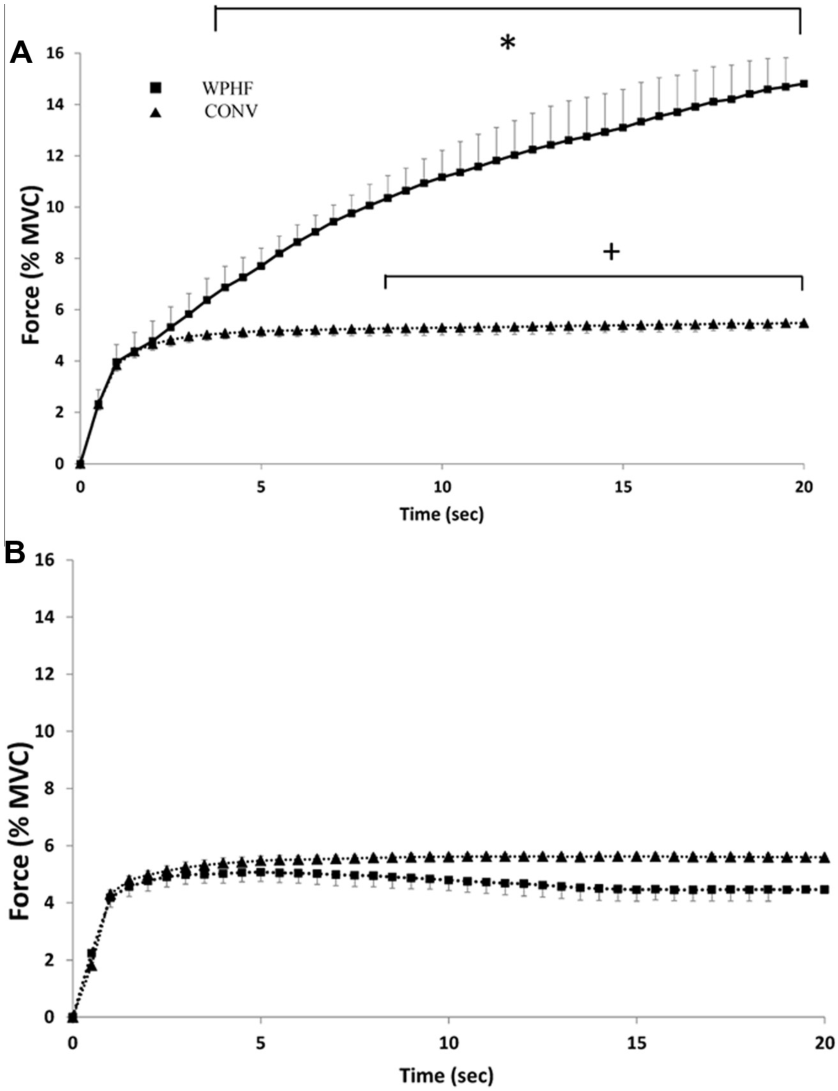

Fig. 5. Mean force generation patterns of WPHF (■) and CONV (▲) and for the responder (A) and non-responder group (B). * significantly different from the 4th sec for WPHF: $P < 0.05$. + significantly different from WPHF from the 8th sec: $P < 0.05$.

Table 1
Comparison of demographic and anthropometric variables, MVC force, absolute and relative stimulation intensity between responders and non-responders.

	Responders	Non-responders
Numbers	23	19
Age (yrs)	27.4 ± 5.7	29.4 ± 5.0
Sex (%) (w/m)	53/47	50/50
Weight (kg)	63.3 ± 10.4	65.0 ± 10.7
Height (cm)	170.6 ± 9.6	172.7 ± 10.4
MVC force (N)	994 ± 353	962 ± 237
Absolute intensity (mA)	WPHF: 12.9 ± 5.4 CONV: 124.0 ± 29.8	WPHF: 15.4 ± 20.9 CONV: 112.3 ± 25.1
Relative intensity (% MVC)	WPHF: 4.3 ± 1.3 CONV: 4.7 ± 0.8	WPHF: 4.7 ± 1.0 CONV: 4.9 ± 0.8

Table 2
Ratio, amplitude and area of the evoked potentials obtained at rest in the responders and non-responders.

	Responders	Non responders
Soleus		
H_{max}/M_{max}	0.46 ± 0.17	0.51 ± 0.23
M_{Hmax}/M_{max}	0.22 ± 0.19	0.32 ± 0.24
M_{max} amplitude (mV)	9.4 ± 2.8	9.9 ± 2.7
M_{max} area (mV·ms)	0.11 ± 0.05	0.07 ± 0.04
Gastrocnemius lateralis		
H_{max}/M_{max}	0.14 ± 0.07	0.20 ± 0.11
M_{Hmax}/M_{max}	0.61 ± 0.36	0.48 ± 0.39
M_{max} amplitude (mV)	10.0 ± 3.3	9.1 ± 3.1
M_{max} area (mV·ms)	0.18 ± 0.14	0.15 ± 0.09

been activated and that no obvious change in axonal excitability has occurred.

For SOL, a significant effect of protocol was found for M_{NMES} amplitude ($P < 0.05$) and area ($P < 0.05$) indicating higher values for CONV than for WPHF NMES. For the GL, no significant effect of group, protocol, time and train was observed.

Fig. 6 illustrates SOL H_{NMES} and M_{NMES} amplitudes recorded before (pre) and after (post) one exemplary WPHF train for one representative responder (showing considerable EF) and one representative non-responder (showing no EF). While the two subjects had a similar H_{max}/M_{max} ratio before WPHF, SOL H-reflex amplitude was considerably reduced by WPHF for the responder while no change occurred for the non-responder. For both subjects, neither M_{HNMES}/M_{NMES} nor M_{NMES} were affected by the WPHF train.

No significant train effect was observed for the REFLEX ratios. Since both force output and reflex ratios did not change in the

Table 3
Ratio, amplitude and area of the evoked potentials obtained and background EMG activity before (pre) and after (post) WPHF and CONV in the responders and non-responders.

	WPHF				CONV			
	Responders		Non Responders		Responders		Non Responders	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Soleus								
M_{HNMES}/M_{NMES}	0.20 ± 0.16	0.21 ± 0.17	0.25 ± 0.22	0.24 ± 0.20	0.24 ± 0.18	0.22 ± 0.25	0.29 ± 0.27	0.30 ± 3.01
M_{NMES} area (mV·ms)*	0.018 ± 0.029	0.018 ± 0.030	0.022 ± 0.035	0.020 ± 0.030	0.025 ± 0.030	0.022 ± 0.029	0.041 ± 0.072	0.040 ± 0.068
M_{NMES} amplitude (mV)*	8.4 ± 2.3	8.4 ± 2.2	9.3 ± 3.2	9.1 ± 3.2	9.2 ± 2.4	9.3 ± 2.4	10.2 ± 3.0	10.0 ± 3.1
Background activity	0.0031 ± 0.0013	0.0029 ± 0.0010	0.0023 ± 0.0010	0.0024 ± 0.0011	0.0031 ± 0.0019	0.0031 ± 0.0018	0.0021 ± 0.0007	0.0022 ± 0.0007
Gastrocnemius lateralis								
M_{HNMES}/M_{NMES}	0.63 ± 0.32	0.68 ± 0.31	0.48 ± 0.37	0.47 ± 0.37	0.70 ± 0.17	0.76 ± 0.18	0.46 ± 0.36	0.45 ± 0.36
M_{NMES} area (mV·ms)	0.048 ± 0.035	0.065 ± 0.051	0.071 ± 0.090	0.073 ± 0.092	0.077 ± 0.044	0.084 ± 0.051	0.060 ± 0.073	0.059 ± 0.072
M_{NMES} amplitude (mV)	9.3 ± 2.9	9.6 ± 3.1	9.1 ± 3.3	9.1 ± 3.3	9.2 ± 2.8	9.4 ± 2.9	9.1 ± 3.2	9.1 ± 3.2
Background activity	0.0036 ± 0.0024	0.0035 ± 0.0024	0.0026 ± 0.0014	0.0031 ± 0.0020	0.0033 ± 0.0023	0.0032 ± 0.0021	0.0027 ± 0.0015	0.0028 ± 0.0016

* Significantly different between protocols: $P < 0.05$.

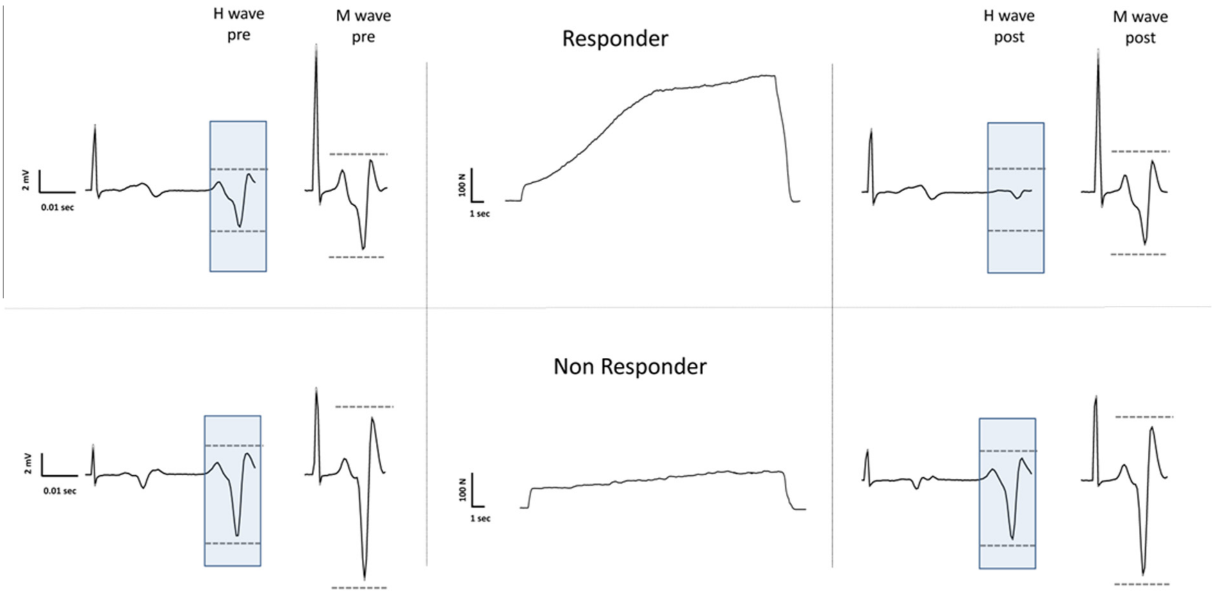

Fig. 6. Maximal soleus H and M waves obtained before (pre) and after (post) WPHF and the resulting force traces from one responder and one non-responder subject. Note that, although the two subjects displayed a similar H_{max}/M_{max} ratio before NMES, the H reflex amplitude was only reduced for the responder subject (top traces, right panel). For both subjects, both the amplitude of the small M-waves preceding the maximal H wave and the maximal M wave remained constant, thereby indicating that the stimulus conditions were stable.

course of the five trains we assumed that fatigue did not occur during the protocol and averaged the values of the five trains for the subsequent analyses. For the responders, the SOL REFLEX ratio was significantly depressed after WPHF (i.e., H_{NMES}/M_{NMES} decreased from 0.38 ± 0.17 to 0.27 ± 0.14) but not after CONV (ratio from 0.36 ± 0.17 to 0.34 ± 0.20, $P < 0.05$) (Fig. 7A). Interestingly, SOL REFLEX ratio was lower after WPHF for the responders as compared with the values obtained for the non-responder group for both WPHF and CONV (Fig. 7A). For the non-responders H_{NMES}/M_{NMES} values ranged from 0.55 ± 0.20 (pre) to 0.54 ± 0.21 (post) for WPHF and from 0.56 ± 0.24 to 0.53 ± 0.24 for CONV.

For the GL muscle, REFLEX ratio was significantly lower after WPHF (from 0.09 ± 0.04 to 0.07 ± 0.03) as compared to CONV (from 0.09 ± 0.05 to 0.09 ± 0.06) for the responders (Fig. 7B). On the contrary, no significant difference was found between the GL REFLEX ratio obtained from the responders and those measured in the non-responder group for both WPHF and CONV (Fig. 7B). For the non-responders H_{NMES}/M_{NMES} values were 0.17 ± 0.10 (pre) vs. 0.17 ± 0.10 (post) for WPHF and 0.17 ± 0.12 (pre) vs. 0.15 ± 0.10 (post) for CONV, respectively.

Moreover, the responder group showed a trend ($P = 0.087$) toward a longer duration of sustained forces after WPHF as compared to CONV (2.04 ± 1.02 vs. 0.81 ± 0.19 s). For the non-responders, sustained forces were 1.40 ± 0.60 s for WPHF and 0.75 ± 0.16 s for CONV. A significant negative correlation was found between sustained forces after WPHF and SOL REFLEX ratio ($r = -0.32$, $P < 0.05$) indicating that higher sustained forces go along with a higher depression of the SOL H reflex.

4. Discussion

The first objective of the present study was to determine EF occurrence (i.e., the percentage of subjects susceptible to WPHF), magnitude and variability. Within a group of 42 subjects, we identified 57% of responders with an average EF magnitude amounting to three times the initial force production. Secondly, we assessed several neuromuscular variables to explore the underlying mechanisms that account for the difference between responders and non-responders. While twitch potentiation, muscle membrane properties and resting H-reflex excitability were not different between the two groups, H-reflex in response to WPHF was depressed only for the responder group. This suggests a potential involvement of central mechanisms in EF generation.

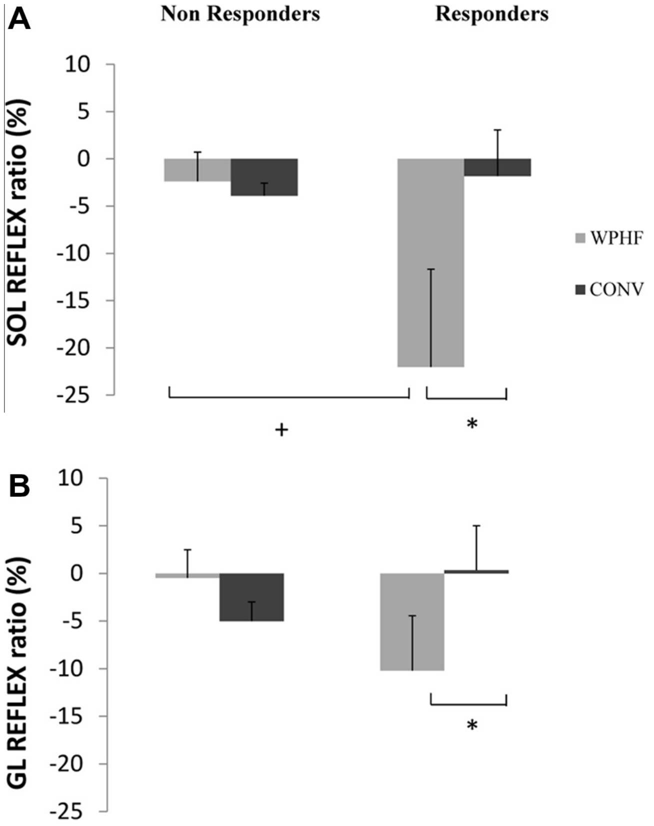

Fig. 7. *Soleus* (SOL; A) and *gastrocnemius lateralis* (GL; B) REFLEX ratio obtained after WPHF and CONV for the responders and non-responders. * significantly different from CONV (within responder group) and + significantly different between WPHF and CONV (between groups), $P < 0.05$

4.1. EF occurrence and magnitude

K-means clustering analyses resulted in 57% of responders which is a considerably lower percentage than the range of 85–100% previously reported in much smaller groups of subjects ($n = 5–15$) for nerve (Baldwin et al., 2006; Klakowicz et al., 2006) and muscle belly stimulation (Collins et al., 2001, 2002; Baldwin et al., 2006; Collins, 2007; Dean et al., 2007). A lower percentage was found in one recent study that compared CONV and WPHF for 13 subjects out of which only 5 subjects developed EF over 40% (da Silva et al., 2014). The high discrepancies in responder percentage in between the latter studies point out the need to develop a standardized classification method of responders. The original and additional feature of our clustering classification model consisted in (1) providing a statistical approach of greater selectivity and sensitivity, (2) excluding those responders that showed significant EF not only for WPHF but also for CONV and (3) including those responders showing EF for DELTA FTI but not for DELTA EF calculation. Our findings reveal that EF is not exclusively attributed to WPHF and that EF occurrence has been overestimated in some previous studies in which no CONV control trials were conducted (Baldwin et al., 2006). Another limitation of previous studies could be seen in the fact that only two single time points (i.e., "END" vs. "START") were considered for force calculation instead of continuous measures of force generation (i.e., FTI), thus disregarding an early development of EF that we observed for several subjects.

It should be pointed out that the difference in EF occurrence between our current investigation and previous studies cannot be explained by either the stimulation parameters (pulse duration and stimulation frequency), the stimulation intensity or by the different stimulation locations (Collins et al., 2001, 2002; Lagerquist and Collins, 2010; Bergquist et al., 2011a). In line with former literature, we used repetitive constant frequency stimulation trains with an identical or similar resting period in between the trains (Dean et al., 2007; Lagerquist et al., 2009; Dean et al., 2007; Lagerquist and Collins, 2010). The main differences between our study and previously-conducted WPHF studies are related to the chosen knee angle and the body position. The knee angle ranged from 90° (Klakowicz et al., 2006) to 110–120° (Baldwin et al., 2006; Dean et al., 2007; Bergquist et al., 2011a) in previous studies whereas the subjects involved in the present study had the knee almost extended at ~170° (Frigon et al., 2011). It has been recently reported that the magnitude of EF was inversely related to muscle length, indicating that the shorter the muscle length, the larger the EF (Frigon et al., 2011). However, previous studies investigating the *gastrocnemius* at short muscle length observed either exactly the same or even lower EF magnitudes (being not higher than twofold) (Collins et al., 2002; Magalhaes and Kohn, 2010) thereby ruling out knee angle position as a potential accounting factor of the reduced responder rate in our study. This is actually further supported by a recent study conducted with a knee angle of 90° and reporting an even smaller responder rate of 50% in a group of 6 subjects showing no EF for the majority of WPHF bursts they have been exposed to (Magalhaes and Kohn, 2010). The other posture-related difference is due to the fact that the subjects of our study were lying supine whereas previous studies performed NMES in sitting position. Despite the differences in body tilt, postural head and body stability was ensured for both set-ups, i.e., subjects were either securely fixed on a chair or a bed so that it is rather unlikely that EF occurrence could have been affected by any change in the excitability of the H-reflex circuit between sitting and lying positions. Previous studies that compared reclined sitting with lying positions reported no significant differences in SOL H-reflex amplitude and latency (Al-Jawayed et al., 1999; Goulart et al., 2000). The higher responder rate previously reported might be possibly related to a selection bias, i.e. the re-recruitment of subjects pre-identified as responders in former studies (Baldwin et al., 2006; Lagerquist et al., 2009).

With regard to EF magnitude in responders, mean plantar flexion force increased more than threefold, leading to an EF magnitude of at least 10% MVC, a finding consistent with those previously reported in the literature (Baldwin et al., 2006; Dean et al., 2007; Lagerquist and Collins, 2010). We observed that the FTI was almost twofold for the WPHF as compared to the CONV NMES protocol. Considering that NMES-induced strength gains are correlated with the level of electrically-evoked force (Maffiuletti, 2010; Gondin et al., 2011b) and the related FTI, the use of WPHF might be particularly effective in the context of rehabilitation for preserving or improving muscle strength. However, it is noteworthy that the stimulation intensity was adjusted to reach only 5% MVC force. Considering that higher stimulation intensities promote antidromic collision in motor axons and might alleviate the central contribution to force production, a comparative analysis of both protocols would be of interest using higher stimulation intensities.

4.2. Underlying mechanisms of EF

Based on a large sample we can state that age, body weight, height, MVC force, current intensity, and sex distribution did not differ between the responder and non-responder group. In the literature, potential peripheral (Sandercock, 2006; Frigon et al., 2011) and/or central (Collins et al., 2001, 2002; Nickolls et al., 2004; Thompson et al., 2011) contributions to EF generation are currently subject of debate.

4.2.1. Peripheral mechanisms

We found that resting M_{max} amplitudes and areas, as rough indicators of muscle membrane excitability, were not different between groups suggesting that the responders and non-responders might have similar muscle membrane excitability. Additionally, the extent of twitch potentiation after a single MVC was similar between the two groups. These findings indicate that the peripheral mechanisms, essentially phosphorylation of myosin light chains (Zhi et al., 2005), involved in twitch potentiation resulting from a MVC do not directly determine the classification into responders and non-responders. M-wave amplitudes were generally higher for CONV as compared to WPHF indicating that the direct activation of motor axons is more prevalent for CONV. No between group differences could be found. Therefore, the peripheral properties measured by force and M-wave amplitude do not present an accounting factor of EF occurrence in our study. Within the scope of our EMG recordings, however, we cannot address the issue of intrinsic muscle properties (i.e., Ca^{2+} release and sensitivity and/or muscle stiffness) contributing to EF (Mela et al., 2002; Rassier and MacIntosh, 2002; Binder-Macleod and Kesar, 2005; Frigon et al., 2011; Fortuna et al., 2012). EF has been previously shown for asynchronous muscle stimulation at short muscle length and for a subtetanic frequency of 5 Hz but has already been abolished for 10 Hz at neutral muscle length when the common-elastic elements of cat soleus muscle was stretched (Sandercock, 2006). Given that we applied tetanic frequencies of 100 Hz to a muscle at long length (i.e. higher than slack length), we exclude that EF observed in our study was due to changes in the series elastic component. Further studies are needed to investigate whether the above mentioned intrinsic muscle properties differ between responders and non-responders.

4.2.2. Central mechanisms

Resting H_{max}/M_{max} ratios were not different between groups, thereby indicating the same excitability of the H-reflex circuit for comparable stimulation conditions (illustrated by similar M_{Hmax}/M_{max} ratios) between the responders and non-responders. Our results showed that both SOL and GL H reflex in response to WPHF were significantly depressed for the responders (-22% and -13%, respectively) but not for the non-responders. This group difference in REFLEX ratio suggests that spinal mechanisms were differently involved between responders and non-responders during WPHF in such a way that the force might have been provoked by a higher proportion of activated sensory axons as compared to the non-responders. A possible preferential activation of sensory axons in the responder group as compared to the non-responder group cannot be explained by electrode position that was carefully controlled for all subjects and performed by the same examiner. However, the difference in the relative activation of sensory and motor axons between groups could be explained by the individual's muscle-nerve architecture (Frigon et al., 2007) given that axonal activation depends on both the distance from the stimulating electrodes to the axons as well as on axon diameter (Burke and Schiller, 1976). Further studies are needed to investigate whether a greater proportion of sensory axons might have been localized closer to the NMES electrodes in the responder group as compared to the non-responders. Maffiuletti et al. (2008) observed a large inter-individual variability in sensory threshold activation and reported a higher sensory excitability in women having larger skinfold thicknesses and smaller cross-sectional areas than men (Maffiuletti et al., 2008). However, it seems unlikely that these two latter parameters could have affected EF given that we observed no sex differences between responders and non-responders.

Solid evidence of spinal involvement in motor unit recruitment during WPHF is given by previous studies (Collins et al., 2001, 2002; Blouin et al., 2009; Lagerquist et al., 2009). The significant reduction of H-reflex amplitude after WPHF in the responder group can be mainly related to three factors including: (1) changes in the excitability of the motoneuron pool, (2) postactivation depression and/or (3) presynaptic inhibition of the Ia afferent terminals (Zehr, 2002; Misiaszek, 2003). For instance, Magalhaes et al. reported that vibration-induced EF might be related to increased motoneuron excitability as illustrated by the enhanced V/F wave amplitudes (Magalhaes et al., 2013). However, the latter findings have been observed in response to high-frequency vibration of the Achilles tendon and have not been directly confirmed in WPHF NMES studies. Collins et al. suggested that WPHF may induce persistent inward current activation in spinal motoneurons or interneurons being possibly related to EF occurrence (Collins et al., 2001). Leading to continuous depolarization through intrinsic membrane properties known as plateau potentials (Hounsgaard et al., 1988; Hultborn et al., 2004), sustained forces that occur after the end of the stimulation train might be an indicator of a prolonged motoneuron discharge. In accordance with previous studies (Gorassini et al., 1998; Collins et al., 2001; Magalhaes et al., 2013), our results indicated that forces tended to outlast WPHF. However, the influence of persistent inward current on EF is still debated (Frigon et al., 2011) and it remains to be determined if and how this phenomenon could explain the reduction of H-reflex amplitude.

Postactivation depression could explain the decreased REFLEX ratio in the responder group as a consequence of a pronounced reduction in neurotransmitter release from synaptic afferents that have been repeatedly activated during the stimulation (Crone and Nielsen, 1989; Hultborn et al., 1996). Previous studies have shown increased H-reflex and asynchronous activity during a "top hat" stimulation pattern consisting of a frequency sequence of 20–100–20 Hz (Klakowicz et al., 2006; Bergquist et al., 2011a; Clair-Auger et al., 2012). When stimulating the tibial nerve of healthy subjects, Klakowicz et al. (2006) found that H reflex was depressed by 85% during the first 20-Hz bout and recovered back to approximately 40% of the initial reflex amplitude during the second 20-Hz bout in consequence to high-frequency burst. These findings illustrated a significant contribution of Ia pathway to EF generation. Our EMG data recorded after constant-frequency stimulation suggest that the repeated activation of predominantly Ia afferents via WPHF in the responder group resulted in a reduction of H reflex amplitude. Likewise, previous studies investigating postactivation depression for electrical and mechanical reflex stimuli showed an inhibition of reflex transmission (Van Boxtel, 1986; Aymard et al., 2000).

Presynaptic inhibition might have also modulated the REFLEX ratio in the responder group by afferent feedback from other peripheral receptors such as muscle spindles and Golgi tendon organs (Burke et al., 1984; Pierrot-Deseilligny, 1997) and descending supraspinal commands (Zehr, 2002). Indeed, WPHF may also elicit a response in cutaneous afferents and other proximal muscle nerve fibers, given their similar activation thresholds (Burke et al., 1983). Even though none of our subjects reported any pain during the stimulation, nociceptors and other sensory receptors could have been activated differently between groups and within the responder group which led to a modulation in monosynaptic transmission and EF variability accordingly. A possible activation of different sensory fibers might have modulated the excitation within the subliminal fringe and brought subliminally excited motoneurons to firing threshold. These changes may have been induced by the addition of small excitatory inputs (not only from proprioceptors but also voluntary drive) and could account for the inconsistency in EF among responder subjects despite a given low stimulus current intensity (Burke et al., 1989; Funase and Miles, 1999; Oya and Cresswell, 2008).

One of the most conclusive results of the present study is the high variability in force output resulting from WPHF in contrast to the high consistency of CONV NMES. This variability was not only found within the responder group showing EF but also within the non-responders. For the responders, the high intersubject variability we found for Delta EF (CV of 43%) is consistent with previous results (Dean et al., 2007). From a neurophysiological point of view, not only the predisposition (i.e. the nerve-muscle architecture which is determined by the orientation and dispersion of nerve axons relative to the electrodes) but also the neuromodulatory state within neural circuits might have differently affected the individual's response to WPHF. A high inter-individual variability has already been shown for recruitment patterns and/or cutaneous reflexes under resting conditions and during anisometric voluntary contractions (Zehr, 2002; Frigon, 2011). Since modulators act on single neurons and synapses (Marder et al., 2014) they might also account for differences in monoamine tone that was previously suggested to influence persistent inward currents associated with EF (Hounsgaard et al., 1988; Rank et al., 2007; Dean et al., 2007). For the first time, we additionally reported a large intrasubject (i.e. train-to-train) variability illustrated by a CV of 24%. The latter findings do not confirm the previous results from Collins et al. (2002) indicating that during a 7-s stimulation period, EF was "relatively consistent in individual subjects both within and between experimental trials." and those from Baldwin et al. (2006) indicating a 10% CV between consecutive WPHF burst trains. The high intrasubject variability observed in our study occurred rather randomly given that the duty cycle of the stimulation was strictly standardized and that no signs of fatigue, i.e., no significant decrease in EF magnitude has been observed in the course of the 5 trains. This unsteadiness in intrasubject EF magnitudes could have been linked to certain cognitive processes such as concentration and distraction during the testing despite the fact that all participants were given the instructions to maintain their visual attention to one point at the ceiling and to relax completely. We experienced WPHF stimulation at low current intensities (favoring orthodromic responses) to be more susceptible to cognitive processes as compared to CONV. Accordingly, the fact that sustained forces could be rapidly terminated by brief voluntary contractions of the antagonist muscle or a focused effort to "relax completely" as shown in previous studies suggests a supraspinal contribution in response to the evoked contraction (Collins et al., 2001) but could not be quantified in our study.

4.3. Perspectives

The responder percentage observed in our study was lower as compared to previous findings which must be considered as a constraint when integrating WPHF in practice. Even though WPHF-induced high FTI values may certainly prove beneficial in order to counterbalance muscle atrophy, EF occurrence is highly individual and does only occur in a part of the healthy population (∼60% based on our sample). Thus, it would be worthwhile if future studies could assign typical characteristics and/or predispositions to responder profiles in order to foresee EF occurrence. Also, the high inter- and intrasubjects variability of EF could be a limitation for some NMES applications in rehabilitation such as functional electrical stimulation (FES) that aims at producing (and reproducing) precise dynamic movement and controlled force exertion following neurological insults such as stroke (Westerveld et al., 2013). The exploration of EF under different exercise conditions (e.g., anisometric movement) would help in this regard.

5. Conclusion

In a large cohort of subjects and by using a robust classification approach, we identified almost 60% of responders to WPHF with an average EF magnitude amounting to three times the initial force production. For future investigations, our methodological considerations (taking into account EF occurrence for CONV and continuous measures of EF) provide a framework to build upon. Twitch potentiation, M wave and H-reflex properties at rest did not discriminate between responders and non-responders. However, for the first time, we observed that both SOL and GL H-reflex amplitudes were depressed only for the responder group in response to WPHF, indicating a central contribution to EF generation. The high inter- and intrasubject variability of EF could be partly attributed to a voluntary and/or involuntary supraspinal descending drive that inevitably affected the balance of inhibition and facilitation of the central circuit involved in WPHF.

Conflict of interest

None of the authors have potential conflicts of interest to be disclosed.

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