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Julien Gondin, Julien Duclay, Alain Martin. Soleus- and Gastrocnemii-Evoked V-Wave Responses Increase After Neuromuscular Electrical Stimulation Training. *Journal of Neurophysiology*, 2006, 95, pp.3328 - 3335. <10.1152/jn.01002.2005>. <hal-04645751>

**HAL Id: hal-04645751**

**<https://hal.science/hal-04645751v1>**

Submitted on 12 Jul 2024

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*J Neurophysiol* 95:3328-3335, 2006. First published Feb 15, 2006; doi:10.1152/jn.01002.2005

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This information is current as of September 29, 2006 .

## Soleus- and Gastrocnemii-Evoked V-Wave Responses Increase After Neuromuscular Electrical Stimulation Training

Julien Gondin, Julien Duclay, and Alain Martin

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Submitted 23 September 2005; accepted in final form 11 February 2006

**Gondin, Julien, Julien Duclay, and Alain Martin.** Soleus- and gastrocnemii-evoked V-wave responses increase after neuromuscular electrical stimulation training. *J Neurophysiol* 95: 3328–3335, 2006. First published February 15, 2006; doi:10.1152/jn.01002.2005. The aim of the study was to use combined longitudinal measurements of soleus (SOL) and gastrocnemii evoked V-wave and H-reflex responses to determine the site of adaptations within the central nervous system induced by 5 wk of neuromuscular electrical stimulation (NMES) training of the plantar flexor muscles. Nineteen healthy males subjects were divided into a neuromuscular electrostimulated group ( $n = 12$ ) and a control group ( $n = 7$ ). The training program consisted of 15 sessions of isometric NMES over a 5-wk period. All subjects were tested before and after the 5-wk period. SOL, lateral gastrocnemius (LG), and medial gastrocnemius (MG) maximal H-reflex and M-wave potentials were evoked at rest (i.e.,  $H_{\max}$  and  $M_{\max}$ , respectively) and during maximal voluntary contraction (MVC) (i.e.,  $H_{\sup}$  and  $M_{\sup}$ , respectively). During MVC, a supramaximal stimulus was delivered that allowed us to record the V-wave peak-to-peak amplitudes from all three muscles. The SOL, LG, and MG electromyographic (EMG) activity as well as muscle activation (twitch interpolation technique) were also quantified during MVC. After training, plantar flexor MVC increased significantly by 22% ( $P < 0.001$ ). Torque gains were accompanied by an increase in muscle activation (+11%,  $P < 0.05$ ), SOL, LG, and MG normalized EMG activity (+51, +54, and +60%, respectively,  $P < 0.05$ ) and  $V/M_{\sup}$  ratios (+81, +76, and +97%, respectively,  $P < 0.05$ ).  $H_{\max}/M_{\max}$  and  $H_{\sup}/M_{\sup}$  ratios for all three muscles were unchanged after training. In conclusion, the increase in voluntary torque after 5 wk of NMES training could be ascribed to an increased volitional drive from the supraspinal centers and/or adaptations occurring at the spinal level.

### INTRODUCTION

Neuromuscular electrical stimulation (NMES) is commonly used as a means of strength training in healthy humans (Colson et al. 2000; Gondin et al. 2005; Maffiuletti et al. 2002; Parker et al. 2003). Many studies have also reported that the application of electrical stimulation to a muscle is effective in improving maximal voluntary strength and functional capacity in hypoactive patients with spinal cord injury (Belanger et al. 2000; Kern et al. 2005) or stroke (Glanz et al. 1996; Kimberley et al. 2004; Powell et al. 1999). Several authors have suggested that neural factors largely account for NMES training-induced strength gains, especially in the case of programs lasting <4–5 wk (Enoka 1988; Gondin et al. 2005; Maffiuletti et al. 2002). Indeed, increases in surface electromyographic (EMG) activity

and muscle activation (Colson et al. 2000; Gondin et al. 2005; Maffiuletti et al. 2002) as well as significant cross-education effect (Hortobagyi et al. 1999; Zhou 2000) have been reported after multiple sessions of NMES. Several arguments can be advanced to explain the occurrence of neural adaptations. Indeed, NMES evokes action potential in both intramuscular nerve branches and cutaneous receptors, thus generating force directly by activation of motor axons (Hultman et al. 1983) and, indirectly, by reflex recruitment of spinal motoneurons (Collins et al. 2001). Moreover, Smith et al. (2003) recently reported that NMES applied over the quadriceps femoris muscle activated specific supraspinal regions (e.g., primary motor cortex, primary sensory cortex, cingulate gyrus) in a dose-response manner. Therefore NMES does not actually bypass the nervous system, at least in an intact system. Nevertheless, the sites of adaptations within the central nervous system (i.e., spinal and/or supraspinal) after multiple sessions of NMES remain unclear.

In an attempt to investigate neural adaptations to NMES training, only one longitudinal study has examined spinal reflex pathways on the basis of the recording of evoked H-reflex responses in healthy humans (Maffiuletti et al. 2003). Indeed, training-induced modifications in H-reflex amplitude are usually ascribed to changes in alpha motoneuron excitability and/or changes in presynaptic inhibition of Ia afferents (Zehr 2002). Maffiuletti et al. (2003) reported no change in the resting soleus (SOL) and gastrocnemius maximal H-reflex ( $H_{\max}$ ) to maximal M-wave ( $M_{\max}$ ) ratios after 4 wk of NMES training and concluded that the mechanism accounting for the strength gains could be an increased volitional drive from the supraspinal centers. However, several investigations have indicated that H-reflexes evoked during actual voluntary contractions (Aagaard et al. 2002) and/or during the performance of the training exercise (Voigt et al. 1998; Zehr 2002) likely represent a more functional estimate of the training-induced adaptations at the spinal cord level. By recording H-reflex responses evoked both at rest and during maximal voluntary contraction (MVC), it would therefore be possible to examine neural adaptations occurring within the spinal locus of the motor system.

During MVC, the study of the so-called V-wave, which is an electrophysiological variant of the H-reflex (Hultborn and Pierrot-Deseilligny 1979; Upton et al. 1971), may be also used to reflect the level of efferent neural drive from spinal  $\alpha$ -motoneurons (Aagaard et al. 2002; Duclay and Martin 2005;

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Pensini and Martin 2004; Sale et al. 1983). The V-wave consists of a volley of H-reflex impulses that are allowed to reach the muscle as a result of the removal of antidromic action potential impulses generated by  $\alpha$ -motor axon stimulation by collision with descending neural drive generated by the voluntary motor effort (Upton et al. 1971). Moreover, the supra-maximal level of nerve stimulation used during the recording of V-wave also causes massive excitation of all afferent axons in the peripheral nerve. As a result, the evoked V-wave response will recruit both large and small motoneurons, whereas H-reflex primarily relies on the pool of smaller motoneurons (Aagaard et al. 2002; Duclay and Martin 2005). An increase in V-wave amplitude may result from an enhanced neural drive in descending corticospinal pathways, elevated motoneuron excitability, and/or alterations in presynaptic inhibition (Aagaard et al. 2002), i.e., from changes occurring at both spinal and supraspinal levels. Because modulations in H-reflex responses could be ascribed to spinal mechanisms, a concomitant analysis of these two types of evoked responses would therefore allow us to determine the sites mediating neural adaptations in response to NMES training.

The purpose of the present study was therefore to use combined longitudinal measurements of SOL but also gastrocnemii-evoked V-wave and H-reflex responses to examine the neural adaptations induced by a 5-wk NMES training program on the plantar flexor muscles. Because NMES activates both motor and sensory fibers (Collins et al. 2001; Hultman et al. 1983) as well as supraspinal centers (Han et al. 2003; Smith et al. 2003), it can be hypothesized that the strength gains observed after short-term NMES training might be associated with adaptations at both spinal and supraspinal levels.

## METHODS

### Subjects

Nineteen healthy male students gave written informed consent to participate in this study. They were randomly assigned to a neuromuscular electrostimulated group (EG), composed of 12 subjects (age  $21.7 \pm 3.4$  yr, height  $176.6 \pm 7.4$  cm, weight  $70.5 \pm 4.7$  kg, means  $\pm$  SD) or to a control group (CG) ( $n = 7$ , age  $26.6 \pm 5.5$  yr, height  $178.3 \pm 8.4$  cm, weight  $71.9 \pm 6.6$  kg, means  $\pm$  SD). None of them had engaged in systematic strength training or NMES in the 12 mo before the experiments began but some were active in recreational sports. CG subjects did not engage in any form of resistance training exercise and were asked not to begin a new exercise program for the duration of the study. Approval for the project was obtained from the University of Burgundy Committee on Human Research. All procedures used in this study were in conformity with the Declaration of Helsinki.

### NMES training

**TRAINING SESSION** One week before the stimulation period began, the EG subjects participated in one practice session to familiarize themselves with stimulation parameters. The training program consisted of fifteen 18-min sessions of isometric (bilateral) NMES over a 5-wk period, with three sessions per week. Forty isometric contractions were carried out during each training session. The subjects were trained on custom-made equipment especially created for this investigation. During the stimulation, they were placed in a supine position on a comfortable mattress, with the ankle, knee, and hip joints fixed at a  $90^\circ$  angle and the head resting on a pillow. Each foot was placed in a shoe, which was firmly attached to a footplate, fixed parallel to the

wall. To stabilize and to secure the feet in the shoes, straps were firmly fastened. A strap also secured the feet to the footplate and another strap was fastened to avoid any movement of the ankle. Finally, a strap fixed between the knees and the footplate, surrounding the legs, maintained the  $90^\circ$  knee joint angle during the evoked contractions. Three 2-mm-thick, self-adhesive electrodes were placed over each leg. The cathodes, measuring  $25 \text{ cm}^2$  ( $5 \times 5 \text{ cm}$ ) and exhibiting membrane-depolarizing properties, were placed over the superficial aspect of the SOL muscle, about 5 cm distal from where the two heads of the gastrocnemius join the Achilles tendon. The anode measuring  $50 \text{ cm}^2$  ( $10 \times 5 \text{ cm}$ ) was placed along the middorsal line of the leg, over both medial and lateral gastrocnemii. A portable battery-powered stimulator (Sport P, Compex Medical SA, Ecublens, Switzerland) was used. Rectangular-wave pulsed currents (75 Hz) lasting 400  $\mu\text{s}$  were delivered with a rise time of 1.5 s, a steady tetanic stimulation time of 4 s, and a fall time of 0.75 s (total duration of the contraction: 6.25 s). Each stimulation was followed by a pause lasting 20 s. Stimulation intensity was monitored on-line and was gradually increased throughout the training session to the level of maximally tolerated intensity, which varied between 32 and 120 mA (mean  $74 \pm 32 \text{ mA}$ ), according to individual pain threshold. No subject reported serious discomfort from the current. Each session was preceded by a standardized warm-up, consisting of 5 min of submaximal NMES at a freely chosen intensity (5 Hz, pulses lasting 200  $\mu\text{s}$ ). The average torque produced during the NMES session was  $61 \pm 11\%$  of the MVC.

### Measurements

**STUDY DESIGN** The EG subjects were tested at baseline and again 3–4 days after the last NMES training session. CG subjects received no exercise training and were tested before and after a 5-wk period. All subjects were asked not to perform any strenuous exercise for  $\geq 48$  h before the two testing sessions.

**TORQUE MEASUREMENTS** All measurements were taken on the left leg muscles. Subjects wore a shoe that was firmly secured to the footplate of the isokinetic dynamometer (Biodex, Shirley, NY). Subjects were examined in the supine position with the hip, knee, and ankle joints at  $90^\circ$  flexion. To minimize hip and thigh motion during the contractions, and therefore to avoid the contribution of muscles other than the plantar flexors (e.g., knee extensors, hip flexors), straps were fastened across the chest and pelvis. The foot was also secured to the footplate by two straps. One strap was placed around the ankle and the second strap was placed around the foot, 1–2 cm proximal to the metatarsophalangeal joint of the toes. The alignment between the center of rotation of the dynamometer shaft and the axis of the ankle joint was checked at the beginning of each session. Particular care was taken in monitoring the posture of the subjects and in avoiding head rotations during the test to maintain constant corticovestibular influences on the excitability of the motor pool and limit afferent feedback from other peripheral receptors, i.e., Golgi tendon organs, cutaneous and joint afferents (Schieppati 1987; Zehr 2002).

**STIMULATION** The posterior tibial nerve was stimulated with a single rectangular pulse (1 ms) delivered by a Digitimer stimulator (model DS7, Hertfordshire, UK). The self-adhesive cathode (8-mm diameter, Ag–AgCL) was located in the popliteal fossa and the anode, which was a large rectangular electrode ( $5 \times 10 \text{ cm}$ , Compex Medical SA), was placed on the anterior surface of the knee. Each subject was initially familiarized with several submaximal (range 1–20 mA) electrical stimuli over a period of nearly 10 min. The stimulation site resulting in the greatest M-wave amplitude was first located by a handheld cathode ball electrode (0.5-cm diameter). Once the stimulation site was determined, the stimulation electrode was firmly fixed to this site with rigid straps and taping.

**ELECTROMYOGRAPHY** Surface EMG activity of the SOL, lateral gastrocnemius (LG), and medial gastrocnemius (MG) muscles was



recorded bipolarly, during voluntary and electrically evoked contractions, by silver chloride circular electrodes with a diameter of 20 mm and a recording diameter of 10 mm. For the SOL, recording electrodes were placed along the middorsal line of the leg, about 5 cm below the insertion of the gastrocnemii on the Achilles tendon. MG and LG electrodes were fixed lengthwise over the middle of the muscle belly. These sites were determined in pilot testing by eliciting at a given intensity the greatest M-wave amplitude for each muscle by tibial nerve stimulation. This procedure was performed to avoid the innervation zone and therefore to obtain the optimal amplitude of EMG response (Merletti et al. 2001). Because the electrophysiological responses induced by tibial nerve stimulation are generated by the plantar flexors and possibly contaminated by concomitant activation of the tibialis anterior, the EMG activity of the tibialis anterior was also recorded. For this muscle, the electrodes were positioned on the line of the fibula at 1/3 of the distance from the tip of the medial malleolus (Hermens et al. 2000). The reference electrode was placed in a central position on the same leg. The placement of the electrodes was marked on the skin with indelible ink, so that they could be exactly repositioned for the posttests. Low resistance ( $<5$  k $\Omega$ ) between the two electrodes was obtained by abrading the skin with emery paper and cleaning with alcohol. EMG signals were amplified with a bandwidth frequency ranging from 15 Hz to 5.0 kHz (common mode rejection ratio = 90 dB; impedance = 100 M $\Omega$ ; gain = 1,000). The EMG and mechanical signals were digitized on-line (sampling frequency 5 kHz) and stored for analysis with commercially available software (Tida, Heka Elektronik, Lambrecht/Pfalz, Germany).

### Experimental procedure

The duration of each testing session was between 90 and 120 min with each subject performing between 12 and 18 maximal efforts.

Testing procedure and recordings started by progressively increasing the current intensity by 2-mA increments from 0 until there was no further increase in peak twitch torque (i.e., the highest value of the plantar flexor twitch torque was reached) nor in concomitant peak-to-peak M-wave amplitudes. Four stimuli were delivered at rest (i.e., without ongoing EMG activity) at each intensity level, at 10-s intervals. The average EMG and mechanical signals obtained at each intensity were stored to obtain later H-M recruitment curves for SOL, LG, and MG muscles. The stimulus intensity needed to obtain the maximal SOL H reflex ( $H_{\max}$ ) was carefully searched. SOL  $H_{\max}$  intensity ranged from 11 to 52 mA. After the H-M calibration procedure, four single pulses at supramaximal intensity (150% of the stimulus intensity) each separated by 8 s were delivered and the recorded M-wave was considered as  $M_{\max}$  for the respective muscles. Three paired stimuli (10-ms interspike interval), each separated by 6 s were also evoked at rest. Individual supramaximal intensities were constituted between 36 and 130 mA. Subjects were then instructed to perform two MVCs of the plantar flexor muscles with paired stimuli delivered over the isometric plateau (superimposed doublet) and 3 s after the contraction (potentiated doublet) to assess muscle activation according to the twitch interpolation technique (Allen et al. 1995). To ensure reproducible trials, subjects were also provided with visual feedback of the plantar flexor muscle torque on a monitor that was placed 1 m in front of them. After a 5-min resting period, subjects were then asked to perform five MVCs of the plantar flexor muscles for 3 s separated by 1-min rest periods. Single stimulation at SOL  $H_{\max}$  intensity was superimposed about 1.5 s after the beginning of each MVC to record superimposed H-reflex ( $H_{\sup}$ ) and M-wave at  $H_{\sup}$  ( $M_{H\sup}$ ) (H superimposed tests). After a rest period of 5 min, subjects were instructed to perform five more MVCs with the same contraction protocol. During these MVCs, a supramaximal stimulus allowed us to record the superimposed M-wave ( $M_{\sup}$ ) and the V-wave from the three plantar flexor muscles (V superimposed tests). For each subject, the coefficient of variation across trials was calculated. If the V-wave or  $H_{\sup}$  amplitudes varied excessively (coefficient

of variation  $>5\%$ ), three additional MVCs were performed. H and V recordings during MVC were performed in a randomized order within and across the testing sessions. Finally, two MVCs of the dorsiflexor muscles separated by  $\geq 60$  s were performed.

### Data analysis

**MVC, EMG ACTIVITY, AND MUSCLE ACTIVATION** Only the highest plantar flexor MVC was considered for analysis. EMG was analyzed over a 500-ms period before stimulation. The root mean square (RMS) EMG values of SOL, LG, and MG muscles were calculated and then normalized: 1) to the corresponding amplitude of the  $M_{\max}$  during resting conditions to obtain the background level of EMG and 2) to the corresponding amplitude of the  $M_{\sup}$  during MVC to obtain the normalized EMG. Tibialis anterior background EMG values were calculated by normalizing the RMS values obtained during resting conditions to the RMS values obtained during dorsiflexor MVC, and were expressed as a percentage. The level of coactivation was calculated by normalizing the RMS values of the tibialis anterior when this muscle was acting as an antagonist to the RMS obtained when this muscle was acting as an agonist (i.e., during dorsiflexion) and was expressed as a percentage. Muscle activation was estimated according to the following formula: % activation =  $(1 - \text{superimposed doublet/potentiated doublet}) \times 100$  (Allen et al. 1995).

**EVOKED POTENTIALS** The average EMG signal for all trials in respective muscles (i.e., SOL, LG, and MG) was used to calculate peak-to-peak amplitude of 1) H-reflexes ( $H_{\max}$  and  $H_{\sup}$ ), 2) small M-waves preceding  $H_{\max}$  and  $H_{\sup}$  (i.e.,  $M_{H\max}$  and  $M_{H\sup}$ , respectively), maximal M-waves ( $M_{\max}$  and  $M_{\sup}$ ), and V-wave. Because the electrical stimulation was optimized for the SOL muscle, LG and MG H-reflexes were not maximal for all subjects at SOL  $H_{\max}$  intensity. At this intensity and for the EG subjects, LG and MG H-reflexes were found to be maximal in 22 (about 92%) and 20 (about 83%) of 24 sessions and were obtained in the ascending part of the recruitment curve in two (about 8%) of the 24 sessions, respectively. The following ratios were then calculated for respective muscles:  $M_{H\max}/M_{\max}$ ,  $M_{H\sup}/M_{\sup}$ ,  $H_{\max}/M_{\max}$ ,  $H_{\sup}/M_{\sup}$ , and  $V/M_{\sup}$  (Ducloy and Martin 2005).

**CONTRACTILE PROPERTIES** The torque signals associated with the electrically evoked contractions under resting conditions were averaged. The following contractile properties for both single and paired stimuli were then analyzed as previously described (Gondin et al. 2005): 1) peak twitch, 2) time-to-peak twitch, and 3) half-relaxation time.

**STATISTICAL ANALYSIS** Normality of the data was checked and subsequently confirmed using the Kolmogorov-Smirnov test. A separate two-factor [group (EG vs. CG)  $\times$  test (muscle activation vs. H superimposed test vs. V superimposed test)] ANOVA with repeated measures was used to compare EMG activity obtained during MVC. Separate two-factor [group (EG vs. CG)  $\times$  session (PRE vs. POST)] ANOVAs with repeated measures on session were used to compare the MVC, background level of EMG recorded at SOL  $H_{\max}$  intensity, normalized EMG activity and muscle activation obtained during MVC, twitch contractile properties, V-wave amplitudes, and  $V/M_{\sup}$  ratio. Separate three-factor [group  $\times$  session  $\times$  condition (rest vs. MVC)] ANOVAs with repeated measures on session and on condition were performed to compare both the H-reflex ( $H_{\max}$  and  $H_{\sup}$ ) and M-wave ( $M_{\max}$  and  $M_{\sup}$ ) amplitudes, the  $M_{H\max}/M_{\max}$  and  $M_{H\sup}/M_{\sup}$  ratios, and the  $H_{\max}/M_{\max}$  and  $H_{\sup}/M_{\sup}$  ratios before and after the 5-wk period. When a main effect or a significant interaction was found, Tukey post hoc analysis was performed. Significance was accepted when  $P < 0.05$ . The statistical analyses were performed using Statistica software for Microsoft Windows (StatSoft, version 6.1, Tulsa, OK). All data are expressed as means  $\pm$  SD within the text and the table and means  $\pm$  SE in the figures.

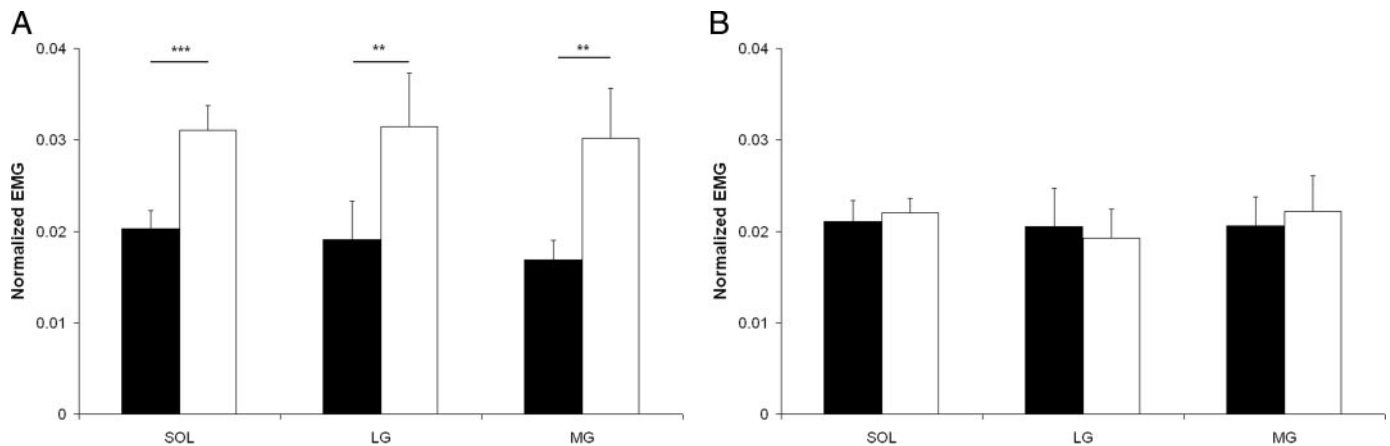


FIG. 1. Normalized EMG activity in soleus (SOL), lateral gastrocnemius (LG), and medial gastrocnemius (MG) muscles obtained during maximal voluntary plantar flexion before (black bars) and after (white bars) the 5-wk period: *A*: neuromuscular electrostimulated group ( $n = 12$ ). *B*: control group ( $n = 7$ ). All values are means  $\pm$  SE. Significantly higher than values before the 5-wk period: \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

## RESULTS

No significant baseline differences were observed between the two groups for the set of the dependent variables.

### MVC, EMG activity, and activation level

The plantar flexor MVC torque increased significantly by  $22 \pm 19\%$  after training in the EG (from  $106 \pm 14$  to  $128 \pm 15$  Nm,  $P < 0.001$ ), whereas no significant change occurred in the CG ( $118 \pm 18$  vs.  $114 \pm 10$  Nm).

In the EG, muscle activation increased significantly by  $11 \pm 15\%$  after training (from  $90 \pm 11$  to  $99 \pm 4\%$ ,  $P < 0.05$ ), whereas no significant change was found in the CG between the two testing sessions ( $96 \pm 4$  vs.  $94 \pm 11\%$ ).

No main effect or significant interaction was found for SOL, LG, MG, or tibialis anterior background EMG level obtained at SOL  $H_{\max}$  intensity.

There was no main effect or significant interaction for SOL, LG, or MG EMG values recorded during muscle activation assessment, H and V superimposed tests.

SOL, LG, and MG normalized EMG increased significantly after training in the EG by  $51 \pm 44\%$  ( $P < 0.001$ ),  $54 \pm 41\%$  ( $P < 0.01$ ), and  $60 \pm 59\%$  ( $P < 0.01$ ), respectively (Fig. 1*A*). No significant changes were observed in the CG before and after the 5-wk period (Fig. 1*B*).

There was no main effect or significant interaction for the level of coactivation ( $0.29 < P < 0.49$ ). The level of coactivation before and after the 5-wk period was  $8.9 \pm 4.9$  and  $8.5 \pm 2.4\%$  in the EG and  $7.0 \pm 1.6$  and  $8.3 \pm 3.6\%$  in the CG, respectively.

### Evoked potentials

There was a significant condition effect ( $P < 0.05$ ) for both H-reflex and M-wave amplitudes in all three muscles (data not shown).  $H_{\text{sup}}$  and  $M_{\text{sup}}$  during MVC were significantly higher than  $H_{\max}$  and  $M_{\max}$  at rest for the SOL ( $P < 0.05$  and  $P < 0.001$ , respectively), LG ( $P < 0.001$  and  $P < 0.05$ , respectively), and MG ( $P < 0.05$  and  $P < 0.01$ , respectively). There was no session or group effect and no significant interaction for H-reflexes and M-waves obtained at rest and during MVC in any of the three muscles (data not shown).

Examples of SOL, LG, and MG V-waves and M-waves evoked during MVC before and after the 5-wk training period are shown in Fig. 2. SOL, LG, and MG V-wave amplitude increased significantly in the EG after training by  $75 \pm 75\%$  ( $P < 0.05$ ),  $80 \pm 56\%$  ( $P < 0.05$ ), and  $84 \pm 88\%$  ( $P < 0.01$ ),

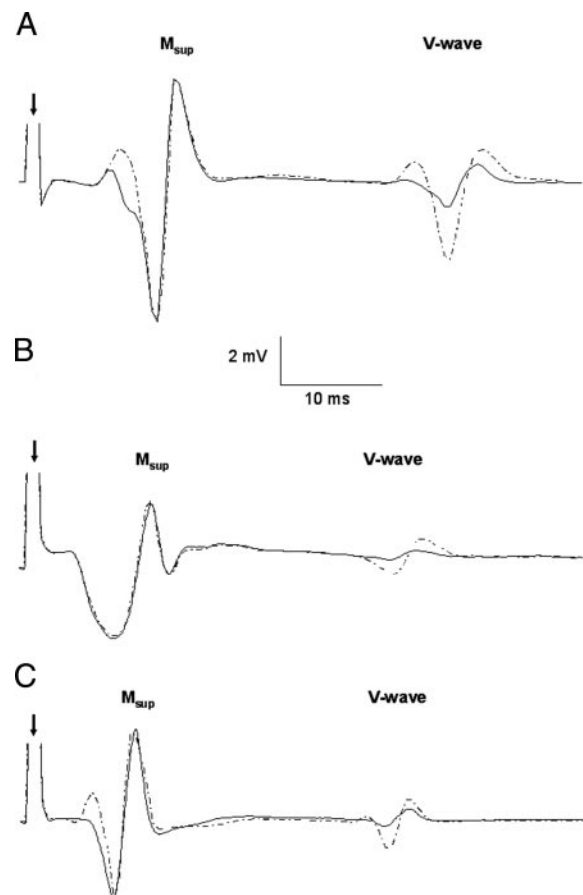


FIG. 2. Recordings from one representative subject of the evoked V-wave responses and the associated superimposed M-wave ( $M_{\text{sup}}$ ) before (solid line) and after the 5-wk neuromuscular electrical stimulation (NMES) training program (dotted line) in the soleus (*A*), the gastrocnemius lateralis (*B*), and the gastrocnemius medialis (*C*) muscles. Initial spikes represent the stimulus artifact. Note that the superimposed M-wave did not change, whereas the V response was higher after training in all 3 muscles.

respectively (Fig. 2). V-wave amplitude showed no changes in the CG in any of the three muscles between the two testing sessions (data not shown).

### Reflex ratios

No main effect or significant interaction was found for  $M_{Hmax}/M_{max}$  and  $M_{Hsup}/M_{sup}$  ratios (Table 1), suggesting that stimulus conditions were stable. No main effect or significant interaction was observed between  $H_{max}/M_{max}$  and  $H_{sup}/M_{sup}$  ratios (Fig. 3) in any of the three muscles. On the other hand, SOL, LG, and MG  $V/M_{sup}$  ratios increased significantly in the EG after training by  $81 \pm 89\%$  ( $P < 0.01$ ),  $76 \pm 52\%$  ( $P < 0.05$ ), and  $97 \pm 67\%$  ( $P < 0.01$ ), respectively (Fig. 3, A–C), whereas no difference was noted in the CG before and after the 5-wk period (Fig. 3, D–F).

### Contractile properties

No significant interaction ( $0.24 < P < 0.85$ ) or main effect ( $0.21 < P < 0.95$ ) was noted for the contractile properties associated with single and paired stimuli (data not shown).

### DISCUSSION

This study demonstrated that the significant increase in plantar flexor muscles MVC observed after 5 wk of NMES training was accompanied by a significant increase in 1) muscle activation and 2) normalized EMG activity and V-wave response (both raw and normalized to the maximal M wave) in all three muscles. The data also indicated that H-reflex responses obtained both at rest and during MVC were not significantly affected by the NMES training program.

### Methodological considerations

According to Zehr (2002), certain methodological requirements must be respected to ensure accurate interpretation of H-reflex in exercise study. Usually, H-reflexes should be evoked at a stimulation level sufficient to provide a corresponding small M-wave, to help ensure stimulus constancy (Schiepatti 1987). Moreover, H-reflexes should be obtained in the ascending part of the recruitment curve to be sensitive to excitation and/or inhibition mechanisms (Crone et al. 1990; Pierrot-Deseilligny and Mazevet 2000). For these reasons, the

electrical stimulation intensity used in the present investigation was optimized to record the SOL maximal H-reflex. In the SOL muscle, we observed that this small M-wave represented nearly 10–15% of the maximal M-wave during both resting and active conditions, which is in agreement with the values reported in the literature (Duclay and Martin 2005; Maffiuletti et al. 2001; Scaglioni et al. 2003). Despite the optimization of stimulus intensity for the SOL muscle, gastrocnemii H-reflexes were found to be maximal or obtained in the ascending part of the recruitment curve in about 90% of the trials. Our results also indicated that the ratio between the small M-wave accompanying H-reflex and the corresponding maximal M-wave either at rest or during MVC was almost identical after the 5-wk period. We can thus suppose that the same proportion of  $\alpha$ -motoneurons was activated by the stimulation during both testing sessions in all three muscles. Moreover, background EMG activity in all three muscles, which could also induce changes in H-reflex amplitude (Schiepatti 1987), was no different before and after the 5-wk period. All these findings clearly indicated that H-reflex recording conditions were similar over the two testing sessions in all three muscles.

### Effects of NMES training on MVC, EMG activity, and muscle activation

An average increase of 22% in plantar flexion MVC was observed after 5 wk of NMES training. This finding is in agreement with several studies using similar training programs on the same muscular group (Maffiuletti et al. 2002; Martin et al. 1993).

In the present study, both muscle activation and normalized EMG of three plantar flexor muscles increased significantly after the treatment, therefore confirming the occurrence of neural adaptations as a result of a short-term NMES training (Gondin et al. 2005; Maffiuletti et al. 2002). Similarly, Maffiuletti et al. (2002) reported a higher EMG activity of the agonist muscles and an enhancement of the voluntary activation of the plantar flexor muscles after 4 wk of NMES. We recently demonstrated that neural adaptations accounted for the larger proportion of initial strength increment after multiple sessions of NMES (Gondin et al. 2005). Such adaptations within the nervous system could thus be ascribed to changes occurring at the spinal and/or supraspinal level. Electrically evoked reflexes (H and V) represent a good tool to investigate whether adaptation occurs at the spinal level.

TABLE 1. Ratios between small M-wave accompanying H-reflex and corresponding maximal M-wave for soleus, lateral gastrocnemius, and medial gastrocnemius muscles at rest ( $M_{Hmax}/M_{max}$ ) and during MVC ( $M_{Hsup}/M_{sup}$ ) before (PRE) and after 5-wk period (POST) for neuromuscular electrostimulated group (EG) and control group (CG)

	EG		CG	
	PRE	POST	PRE	POST
Soleus				
$M_{Hmax}/M_{max}$	$0.12 \pm 0.11$	$0.10 \pm 0.05$	$0.12 \pm 0.06$	$0.11 \pm 0.04$
$M_{Hsup}/M_{sup}$	$0.14 \pm 0.12$	$0.12 \pm 0.15$	$0.10 \pm 0.06$	$0.12 \pm 0.13$
Lateralis gastrocnemius				
$M_{Hmax}/M_{max}$	$0.40 \pm 0.34$	$0.40 \pm 0.32$	$0.37 \pm 0.33$	$0.36 \pm 0.26$
$M_{Hsup}/M_{sup}$	$0.40 \pm 0.40$	$0.36 \pm 0.31$	$0.37 \pm 0.41$	$0.36 \pm 0.43$
Medialis gastrocnemius				
$M_{Hmax}/M_{max}$	$0.26 \pm 0.32$	$0.27 \pm 0.26$	$0.27 \pm 0.35$	$0.30 \pm 0.37$
$M_{Hsup}/M_{sup}$	$0.30 \pm 0.23$	$0.28 \pm 0.32$	$0.28 \pm 0.26$	$0.25 \pm 0.33$

Values are means  $\pm$  SD. EG,  $n = 12$ ; CG,  $n = 7$ .

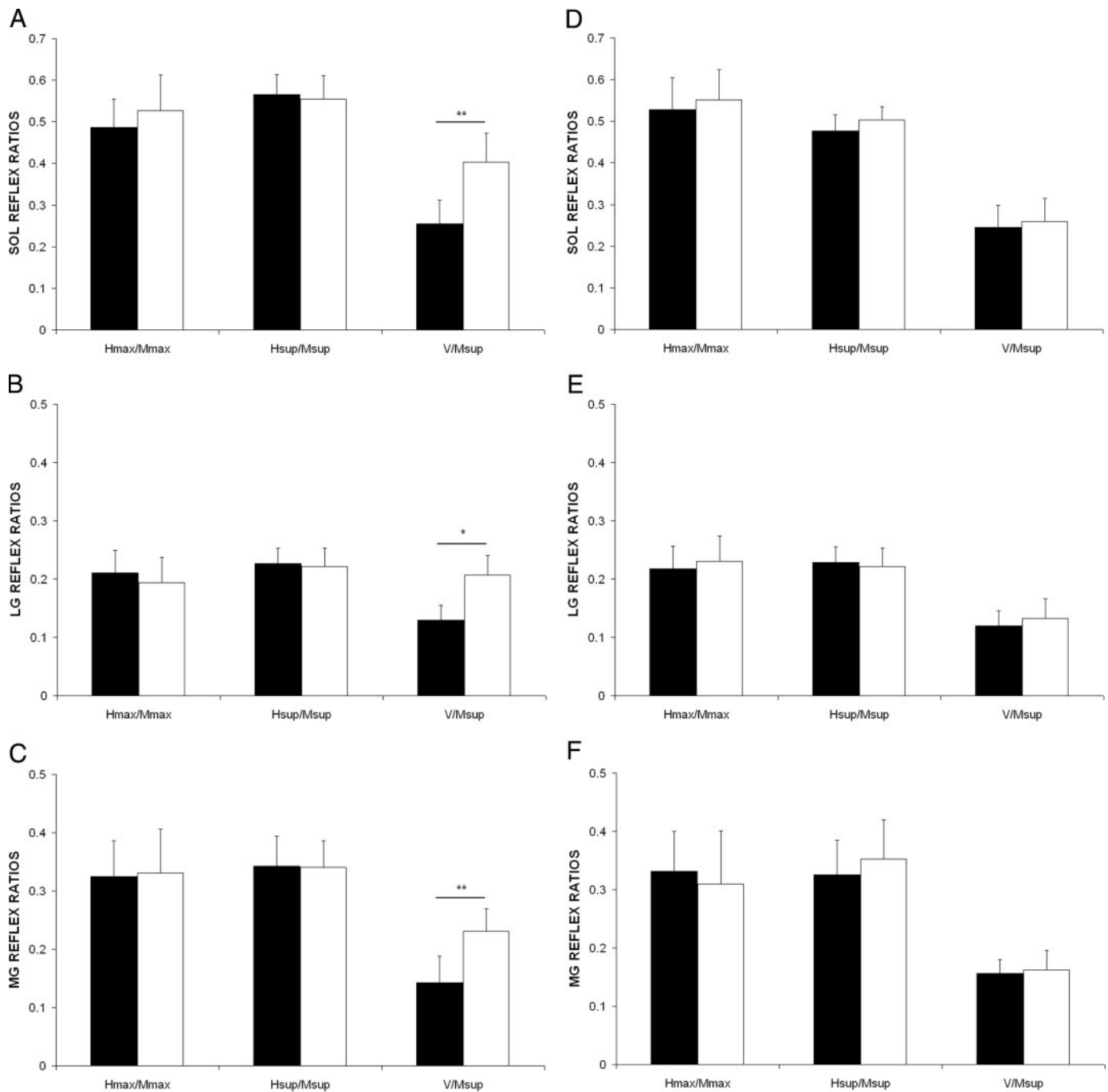


FIG. 3. Soleus (SOL), lateral gastrocnemius (LG), and medial gastrocnemius (MG)  $H_{\max}/M_{\max}$ ,  $H_{\sup}/M_{\sup}$ , and  $V/M_{\sup}$  ratios obtained at rest ( $H_{\max}/M_{\max}$ ) and during maximal voluntary plantar flexion ( $H_{\sup}/M_{\sup}$  and  $V/M_{\sup}$ ) before (black bars) and after the 5-wk period (white bars). A–C: for the neuromuscular electrostimulated group ( $n = 12$ ). D–F: for the control group ( $n = 7$ ). All values are means  $\pm$  SE. Significantly higher than values before the 5-wk period: \* $P < 0.05$ , \*\* $P < 0.01$ .

#### Effects of NMES training on H-reflex responses

In the present study, SOL and gastrocnemii resting  $H_{\max}/M_{\max}$  ratios did not change significantly in response to multiple sessions of NMES. These results are in accordance with those recently reported (Maffiuletti et al. 2003) after 4 wk of NMES training of the plantar flexor muscles. Nevertheless, several authors have suggested that evoked reflex responses obtained during actual voluntary contraction (Aagaard et al. 2002) and/or during the performance of the training exercise (Voigt et

al. 1998) likely represent a more functional estimate of the training-induced adaptations at the spinal cord level. For example, Aagaard et al. (2002) showed no changes in resting SOL H-reflex amplitude after 14 wk of heavy weight-lifting exercises, whereas the H-reflex recorded during maximal isometric ramp contractions significantly increased with training. However, these authors evoked SOL H-reflexes at rest and during MVC with stimulation intensity that produced an M-wave corresponding to 10 and 20% of the maximal M-wave,



respectively. Thus the proportion of motor axons directly recruited was probably different between active and resting conditions, thus indicating that H-reflex modulations observed in this study may be partially attributed to recording methodologies. Moreover, Lagerquist et al. (2006) recently demonstrated no change in the soleus  $H_{\max}/M_{\max}$  ratio obtained at a 10% EMG background contraction after a 5-wk isometric plantar flexor voluntary strength training program (i.e., with a training duration similar to our NMES program).

In the current study, not only SOL but also gastrocnemii H-reflexes recorded during actual MVC were used for the first time to evaluate the modulation of the spinal loop after NMES resistance training. Although NMES applied over the triceps surae muscle evokes action potentials in both intramuscular nerve branches (Hultman et al. 1983) and cutaneous receptors, thus resulting in reflex recruitment of spinal motoneurons (Collins et al. 2001), the  $H_{\text{sup}}/M_{\text{sup}}$  ratio of all three muscles was unaffected by the NMES training program. It can therefore be suggested that the elements of the H-reflex pathway are not directly involved in the chronic adjustments in response to NMES training.

#### *Effects of NMES training on V-waves*

This study is the first to demonstrate a significant increase in SOL and gastrocnemii  $V/M_{\text{sup}}$  ratios with NMES training. Although there are discrepancies in the literature concerning the specific response to NMES versus voluntary actions (Bax et al. 2005), similar findings have been reported after voluntary resistance training programs (Aagaard et al. 2002; Sale et al. 1983). For example, a 55% increase in SOL V-wave amplitude was reported after 14 wk of heavy-resistance strength training (Aagaard et al. 2002). An increase in both V-wave amplitude and normalized EMG such as those obtained in our study for both SOL and gastrocnemii may result from an enhanced neural drive in descending corticospinal and/or extrapyramidal pathways, elevated motoneuron excitability, and/or alterations in presynaptic inhibition (Aagaard et al. 2002).

Thus the observed increase in SOL and gastrocnemii V-wave amplitude could be attributable to an increase in motoneuron firing frequency and/or recruitment. Both mechanisms result indeed in a direct proportional increase in the probability of antidromic collision arising from an enhanced volitional drive from higher motor centers. In support of this hypothesis, recent functional neuroimaging studies have demonstrated significant cerebral cortex activation during electrically evoked contractions of human wrist extensor (Han et al. 2003) and knee extensor muscles (Smith et al. 2003). Such activation of selected brain regions during NMES might be involved in the increased volitional drive from the supraspinal centers. In the same way, several studies (Khaslavskaja and Sinkjaer 2005; Khaslavskaja et al. 2002; Thompson et al. 2005), using transcranial magnetic stimulation, found increased amplitude of motor evoked potentials in the tibialis anterior after a single session of NMES. Nevertheless, further research is warranted to determine the relative contribution of motor unit recruitment versus discharge rate in the NMES training-induced V-wave enhancement.

Although the lack of change in  $H_{\text{sup}}/M_{\text{sup}}$  ratio indicates that the spinal reflex was unchanged after the present NMES training program, the V-wave increment could be explained, at

least partially, by neural mechanisms at the spinal level. To compare H-reflex and V-wave modulations, we have to take into account that these two types of evoked responses could recruit different portions of the spinal motoneuron pool because of the large difference in stimulation intensities (i.e., submaximal vs. supramaximal, respectively). Indeed, V-wave response will recruit both large and small motoneurons, whereas H-reflex primarily relies on the pool of smaller motoneurons (Aagaard et al. 2002; Ducloy and Martin 2005). The training effect could therefore be attributed to spinal elements that affect motoneuron firing without affecting the efficacy of the elements of the H-reflex pathways.

#### *Functional implications*

In the current work, the voluntary strength gains induced by our NMES training were associated with neurological changes occurring within the spinal and/or supraspinal locus of the motor system. Thus the benefits of NMES might be useful in the design of rehabilitation programs to improve recovery of upper-limb function (Wade 1989) and to increase cortical activation in subjects with cerebral damage. However, the short-term NMES effects we observed in healthy subjects may not be the same in a patient population and further studies are needed to examine the effects of multiple sessions of NMES in patients with CNS disorders. Moreover, it is crucial to investigate whether such neural drive improvement is maintained after the interruption of NMES training.

In conclusion, the present study demonstrated that the significant increase in both plantar flexor MVC and associated normalized EMG activity observed after 5 wk of NMES training could be attributed to an enhanced neural drive in descending corticospinal and/or extrapyramidal pathways from supraspinal centers and/or neural mechanisms occurring at the spinal level.

#### ACKNOWLEDGMENTS

The authors thank the subjects for participation in this study. We also express our gratitude to Dr. Nicola Maffiuletti for critical reading of the manuscript, to L. Rochette for helpful contribution to data analyses, and to M. Bouley for the English revision of the manuscript.

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