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Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: triceps surae

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Bergquist AJ, Clair JM, Collins DF. Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: triceps surae. *J Appl Physiol* 110: 627–637, 2011. First published December 23, 2010; doi:10.1152/japplphysiol.01103.2010.—Neuromuscular electrical stimulation (NMES) can be delivered over a nerve trunk or muscle belly and can generate contractions by activating motor (peripheral pathway) and sensory (central pathway) axons. In the present experiments, we compared the peripheral and central contributions to plantar flexion contractions evoked by stimulation over the tibial nerve vs. the triceps surae muscles. Generating contractions through central pathways follows Henneman's size principle, whereby low-threshold motor units are activated first, and this may have advantages for rehabilitation. Statistical analyses were performed on data from trials in which NMES was delivered to evoke 10–30% maximum voluntary torque 2–3 s into the stimulation ($Time_1$). Two patterns of stimulation were delivered: *1*) 20 Hz for 8 s; and *2*) 20–100-20 Hz for 3–2-3 s. Torque and soleus electromyography were quantified at the beginning ($Time_1$) and end ($Time_2$; 6–7 s into the stimulation) of each stimulation train. H reflexes (central pathway) and M waves (peripheral pathway) were quantified. Motor unit activity that was not time-locked to each stimulation pulse as an M wave or H reflex ("asynchronous" activity) was also quantified as a second measure of central recruitment. Torque was not different for stimulation over the nerve or the muscle. In contrast, M waves were approximately five to six times smaller, and H reflexes were approximately two to three times larger during NMES over the nerve vs. the muscle. Asynchronous activity increased by 50% over time, regardless of the stimulation location or pattern, and was largest during NMES over the muscle belly. Compared with NMES over the triceps surae muscles, NMES over the tibial nerve produced contractions with a relatively greater central contribution, and this may help reduce muscle atrophy and fatigue when NMES is used for rehabilitation.

M wave; H reflex; electromyography; human

NEUROMUSCULAR ELECTRICAL STIMULATION (NMES) is commonly used to alleviate muscle atrophy and restore movement following damage to central motor pathways (46). NMES is often applied through electrodes placed on the skin over a peripheral nerve trunk or over a muscle belly. For example, stimulation over the common peroneal nerve has been used for years to restore dorsiflexion during the swing phase of gait (48), and stimulation over the quadriceps muscles is used to produce walking, rowing, and cycling movements (6, 29, 38, 49). In the present experiments, we utilized surface electromyographic (EMG) recordings to establish whether different neural pathways contribute to contractions generated when NMES is applied over a peripheral nerve trunk compared with NMES applied over a muscle belly.

NMES initiates contractions by the excitation of axons under the stimulating electrodes (4) and can recruit motor units in three distinct ways (16). The most direct form of motor unit recruitment utilizes a peripheral pathway via the activation of motor axons and does not involve the central nervous system. Depolarizing motor axons generates an M wave in the EMG and recruits motor units synchronously at a predictable, "time-locked," latency following each stimulation pulse. Generating contractions through this peripheral pathway tends to recruit motor units randomly in relation to motor unit type (13, 24, 50), which may limit the efficacy of NMES for maintaining muscle quality, as fatigue-resistant muscle fibers will be activated less compared with when recruitment is orderly. This relative inactivity leaves fatigue-resistant muscle fibers vulnerable to disuse atrophy. Additionally, the nonphysiological recruitment order and synchronous discharge of motor units contributes to the rapid fatigue that is problematic when NMES is used to restore movement (46).

In addition to activating motor axons, NMES also activates sensory axons, and this can contribute to the evoked contraction by recruiting motor units in two distinct ways (16). One form of this central recruitment is through the H-reflex pathway. Similar to recruitment during the M wave, motor unit recruitment during the H reflex is time-locked to each stimulation pulse, but occurs at a longer latency due to the longer pathway through the spinal cord (43). The other form of central motor unit recruitment results in "asynchronous" motor unit discharge that is not time-locked to each stimulation pulse (17, 41). It has been suggested that this asynchronous activity is brought about by the activation of persistent inward currents in spinal neurons (17). Both forms of central recruitment produce contractions synaptically and, therefore, likely follow Henneman's size principle (28), whereby the lowest threshold and most fatigue-resistant motor units are activated first. Increasing the recruitment of low-threshold motor units may help reduce the atrophy and fiber-type transitions associated with central motor pathway damage and subsequent inactivity (8, 25). Additionally, increasing central motor unit recruitment during NMES may improve the fatigue resistance of electrically evoked contractions (40).

The relative contributions made by central and peripheral pathways to electrically evoked contractions may differ when NMES is applied over a nerve trunk compared with over a muscle belly (5). During NMES over the tibial nerve, H reflexes were prominent in the soleus EMG when contractions were 3–10% of maximum voluntary isometric contraction (MVIC) torque. Conversely, during contractions of similar amplitude evoked by stimulation over the triceps surae (TS)

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muscles, M waves dominated the EMG, and there was little H-reflex activity. From these data, it would seem that NMES over the nerve trunk generates contractions with a greater central contribution than NMES over the muscle belly. However, these data were recorded in only four participants, and no statistical analyses were performed. Despite the apparent lack of an H reflex during NMES over the muscle belly, a contribution from the central nervous system to contractions evoked when NMES is applied over muscle has been established (12, 17, 18, 40). Torque was significantly reduced when NMES was applied during an anesthetic nerve block proximal to the stimulation site, when only activation of motor axons could contribute to the evoked contractions. To reconcile the lack of an H reflex during NMES over the muscle belly with the clearly demonstrated central contribution, we have suggested that asynchronous motor unit activity may provide the majority of the central contribution during NMES over the muscle belly (5). To date, a contribution from asynchronous motor unit activity to contractions evoked by NMES has not been quantified.

The present experiments were designed to compare the contributions made by central and peripheral pathways to motor unit recruitment for contractions of similar amplitude generated by NMES applied over the tibial nerve compared with NMES applied over the TS muscles. We studied the TS muscle group because we have data suggesting that motor units are recruited differently during stimulation over the tibial nerve compared with over the TS muscles (5). Additionally, there is growing interest in stimulating these muscles for rehabilitation of gait for people who have had a stroke or incomplete spinal cord injury (3, 34, 44). Accordingly, we were also interested in characterizing motor unit recruitment during larger, more functionally relevant contractions than have been studied previously (5, 37). Contractions of ~10–40% MVIC torque were examined, as this encompasses the range of plantar flexion torque (20–30% MVIC) estimated for walking (2). We anticipated that stimulation at both locations would generate contractions through peripheral and central pathways, but that the relative contributions would differ. Specifically, we hypothesized that contractions evoked by NMES over the nerve trunk would have smaller M waves and larger H reflexes compared with NMES over the muscle belly. We also hypothesized that NMES over the muscle belly would produce more asynchronous activity than NMES over the nerve trunk, given that we have shown NMES over the muscle belly can produce contractions through central pathways (11, 17, 18, 40) without the presence of H reflexes (5). The results of the present experiments contribute to our understanding of how NMES generates contractions and confirms that NMES applied over the tibial nerve and TS muscles generates contractions with markedly different contributions through central and peripheral pathways.

METHODS

Participants

Fourteen human participants with no known neurological or musculoskeletal impairments (20 to 48 yr of age; 10 men and 4 women) volunteered after providing informed, written consent. Four of these participants (2 men and 2 women) did not complete the experiments, and their data were not included in the analysis. One of these participants withdrew because she found the NMES uncomfortable before an adequate contraction could be evoked. Two participants were excluded because we could not activate the TS without strong coactivation of the tibialis anterior (TA) muscle during stimulation over the tibial nerve in the popliteal fossa. Another participant was excluded because the latency of his H reflex was such that accurate peak-to-peak measurements were not possible due to contamination by the subsequent stimulus artifacts during 20-Hz stimulation. Two of the ten participants whose data were grouped for the statistical analyses were completely naive to NMES. These experiments were conducted in accordance with the Declaration of Helsinki and were approved by the Health Research Ethics Board at the University of Alberta.

Protocol

All participants took part in one experimental session, which lasted between 1.5 and 2.5 h. All procedures were performed on the right leg. Participants were seated in the chair of a Biodex Dynamometer (System 3, Biodex Medical Systems, Shirley, NY) with the hip at 110°, the knee at 120°, and the ankle at 90° with the lateral malleolus aligned with the axis of the dynamometer. The foot was secured to the Biodex footplate to measure isometric plantar flexion torque.

EMG. Surface EMG was recorded from the right soleus and TA muscles using adhesive gel electrodes (2.25 cm^2; Vermed Medical, Bellows Falls, VT) in a bipolar configuration. The electrodes were placed parallel to the predicted path of the muscle fibers with ~1 cm interelectrode distance (Fig. 1). A common reference electrode (not shown) was placed over the tibia or patella of the right leg. EMG signals were amplified 1,000 times and band-pass filtered at 30–3,000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

MVICs. Before the trials involving NMES, participants performed MVICs of the TS by plantar-flexing the ankle against the footplate to increase torque to a maximum and held this contraction for 3–5 s. Participants were provided with visual feedback of their torque production on a computer monitor and received verbal encouragement to promote maximal performance during each MVIC. Each participant completed two to three MVICs until peak plantar flexion torque differed by <10% between trials. Each MVIC was separated by at least 3 min of rest to minimize fatigue. After collecting MVICs, participants were no longer provided any feedback of their torque production for the remainder of the experiment.

NMES. NMES was delivered either over the tibial nerve or over the TS (Fig. 1) muscle group using a constant-current stimulator and 1-ms rectangular pulses (DS7A Digitimer, Welwyn Garden City, UK). A 1-ms pulse duration was used as long-pulse durations generate contractions with a larger central contribution than short-pulse durations (17, 18, 39, 39a). Stimulation current was measured using a current probe (mA 2000 Non-contact Milliammeter; Bell Technologies, Orlando, FL). The tibial nerve stimulation was delivered through two adhesive gel electrodes (2 × 3 cm; Vermed Medical, Bellows Falls, VT) placed on the skin of the popliteal fossa with an interelectrode

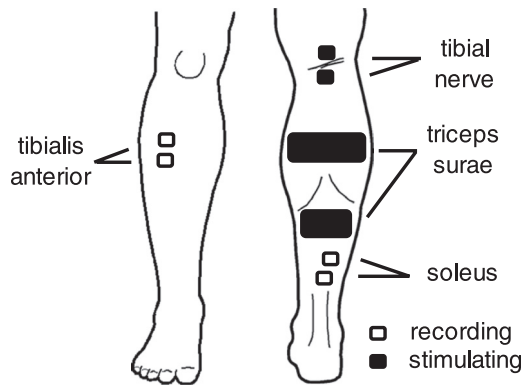

Fig. 1. Schematic of the stimulating and recording locations on the right leg.

distance of 1 cm. Electrodes were placed on the site at which a single pulse evoked a soleus EMG response (M wave or H reflex) at the lowest intensity. Stimulation over the TS was delivered through two flexible adhesive electrodes (4 × 16 cm; Electrosurgical Patient Plate 1180: Split, 3M Health Care, St. Paul, MN), trimmed to fit over the TS muscles of each participant. The anode was placed over the lateral and medial gastrocnemii at the point of approximately the largest circumference. The cathode was placed over the soleus, just distal to the gastrocnemii. If contractions of the peroneus muscles were observed through visual inspection and palpation during stimulation, the electrodes were repositioned medially and/or were cut smaller to more selectively activate the TS muscles.

M-wave-H-reflex recruitment curve. Separate M-wave-H-reflex (M-H) recruitment curves were constructed for stimulation over the tibial nerve and the TS muscles from soleus EMG responses to 50 stimulation pulses. Stimuli were delivered randomly every 3–5 s at current levels ranging from below M-wave and H-reflex threshold to 1.5 times the minimum current required to evoke the largest M wave (M_{max}). To maintain similar levels of motoneuron excitability during collection of the recruitment curve data (14), participants held a background contraction of ~10% of the maximal rectified soleus EMG using visual feedback displayed on a computer monitor. However, the 3- to 5-s interstimulus interval may be too short to completely avoid the effects of postactivation depression on H-reflex amplitude, even while holding a background contraction (47), and thus H_{max} (largest H reflex)-to-M_{max} ratios in the present study may be slightly underestimated.

Stimulation patterns. NMES was delivered in two patterns as illustrated by the dotted lines in Fig. 2: *1*) a constant frequency pattern of 20 Hz for 8 s; and *2*) a step-frequency pattern of 20–100-20 Hz for 3–2-3 s for each phase, respectively [adapted from Collins et al. (17)]. The 20-Hz frequency was chosen because it was the highest frequency that allowed for H-reflex analysis between stimulation artifacts (50-ms interstimulus interval). This frequency is also within a recommended frequency range (18–25 Hz) for NMES of the lower limb (46). The step-frequency pattern was chosen because it allowed us to examine contractions evoked by NMES at 20 Hz before and after a period of 100-Hz stimulation, which has been shown to enhance the central contribution to the evoked contractions (18, 37). The constant frequency pattern then also acted as a control, allowing us to determine the effects of the 100-Hz step on torque and motor unit recruitment.

Stimulation intensity. NMES was delivered at two intensities. Low-intensity stimulation was delivered to evoke a peak torque of ~10% MVIC during the interval 2–3 s into the stimulation in 10 participants ($Time_1$; see Fig. 2). The mean current for this low-stimulation intensity was 7.8 ± 0.9 mA for NMES over the nerve trunk and 28.3 ± 1.9 mA for NMES over the muscle belly. Higher intensity stimulation was delivered to generate between 20 and 40% MVIC torque at $Time_1$. The mean current for this higher intensity stimulation was 8.4 ± 0.8 mA for NMES over the nerve trunk and 34.2 ± 2.7 mA for NMES over the muscle belly. For all trials, if the stimulation was uncomfortable, the experimental session was concluded. Four participants found the stimulation uncomfortable before a contraction of 20% MVIC torque could be achieved. Data from one participant who received stimulation to evoke a contraction of ~40% MVIC were excluded from the group statistical analyses as there was strong coactivation of the TA muscle during NMES over the tibial

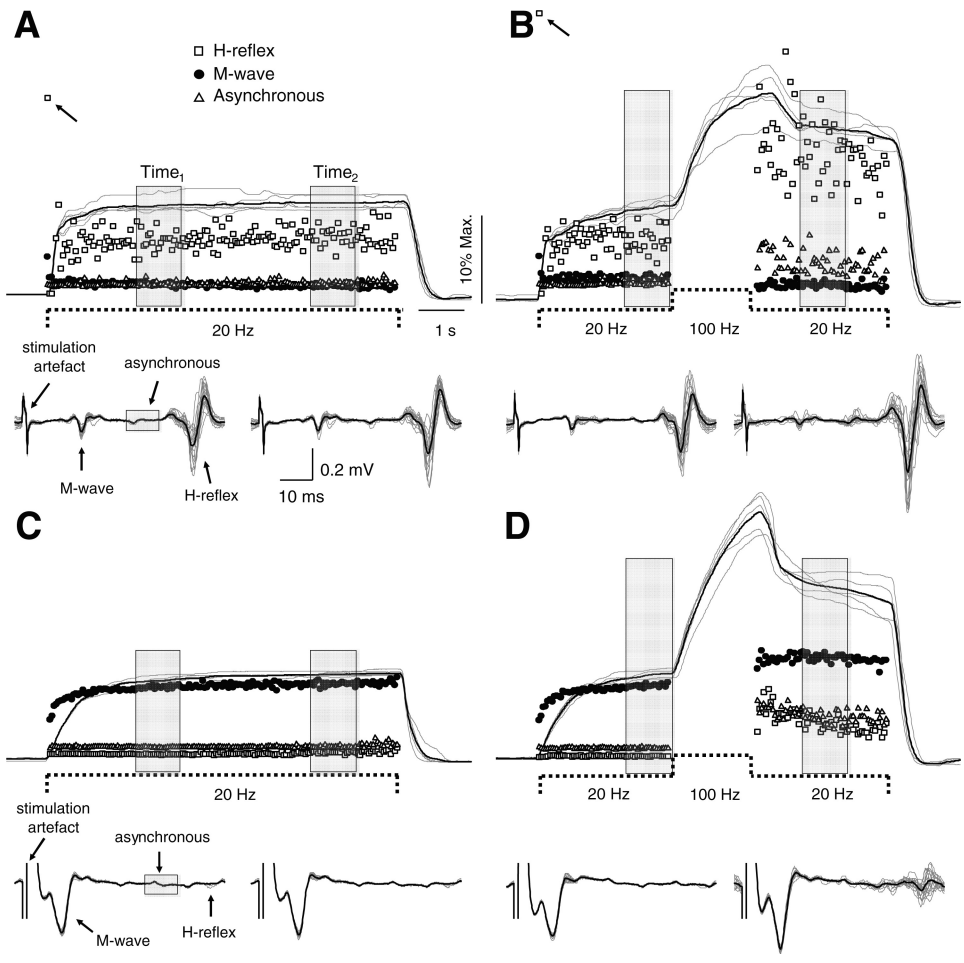

Fig. 2. Torque and electromyographic (EMG) responses evoked by stimulation over the tibial nerve (*A* and *B*) and the triceps surae (TS) muscles (*C* and *D*) to evoke ~10% maximum voluntary isometric contraction (MVIC) torque at $Time_1$ (2–3 s into the stimulation) in a single participant. *A* and *C*: responses to the 20-Hz constant-frequency pattern are displayed. *B* and *D*: responses to the 20–100-20-Hz pattern are displayed. In the *top* of each panel, torque profiles represented by the solid black lines are averages of 5 shaded lines in response to 5 trains of neuromuscular electrical stimulation (NMES), and the symbols represent the average EMG data over 5 repetitions during a single trial. Vertical calibration represents 10% of the largest M wave (M_{max}) for EMG and 10% MVIC for torque. The *bottom* of each panel shows EMG recorded at $Time_1$ (*left* trace) and $Time_2$ (6–7 s into the stimulation; *right* trace) during a single train of NMES. Solid black lines represent the average of 20 single responses (shaded lines) to NMES. Stimulation artifacts for data recorded during NMES over the TS muscles have been truncated (*C* and *D*). All data are shown on the same scale, as indicated by the calibration bars in *A*. EMG during 100-Hz stimulation was not quantified due to contamination by stimulation artifacts.

nerve (Fig. 5). Therefore, data that were grouped for analyses were obtained from five participants with higher intensity stimulation. Of these five participants, four received stimulation to evoke ~20% MVIC torque, and one received stimulation to evoke ~30% MVIC torque.

Data Acquisition and Analysis

A single trial of NMES consisted of five repetitions of a stimulation pattern with 45 s between each repetition. For each stimulation location, trials were collected using both patterns at both intensities. The order of trials was randomized for each participant. Throughout the NMES trials, participants were asked to remain relaxed and refrain from contributing voluntarily to the evoked contractions.

Data were sampled at 5 kHz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for subsequent analyses that were conducted using custom-written Matlab software (The Mathworks, Natick, MA). MVIC torque was calculated by averaging data over a 500-ms window centered on the peak plantar flexion torque recorded during the largest MVIC. Recruitment curves were generated by plotting peak-to-peak M-wave and H-reflex amplitudes as a function of stimulus intensity. The single H_{max} and M_{max} from each recruitment curve were used to calculate the H_{max}-to-M_{max} ratio. To determine whether the gastrocnemii were equally well activated during stimulation over the nerve trunk and over the muscle belly, peak twitch torques from the recruitment curve data evoked by similar sized M waves were compared. Data were compared between locations for stimulation intensities from 60–100% M_{max} when no H reflex was present during stimulation over the nerve trunk. Torque during M-H recruitment curves and NMES was normalized to that recorded during each participant's MVIC. The amplitude of each M wave and H reflex during 20-Hz stimulation was measured peak to peak and normalized to each participant's M_{max}. EMG during 100-Hz stimulation was not quantified due to contamination by stimulation artifacts.

To quantify asynchronous motor unit activity, we calculated the root mean square (RMS) of the EMG immediately before each H reflex during 20-Hz NMES (see Fig. 2*A*, *bottom left* trace). From this value, we subtracted the baseline RMS of the EMG with each participant at rest before each NMES trial. The intervals over which asynchronous activity was quantified were determined on an individual basis by the onset latency of the H_{max} recorded during the recruitment curve for stimulation over the nerve trunk. An interval duration of 10–12 ms was chosen because it was the only period of time when asynchronous activity was not contaminated by the stimulus artifact, M wave, or H reflex. In some instances during NMES over the muscle belly, large M-wave amplitudes prevented the EMG from returning to baseline by the H-reflex onset. To address this and prevent overestimation of the RMS calculation, all data in the intervals over which asynchronous activity was quantified were fit to a second-order polynomial using the least squares procedure to remove any trend in the baseline associated with the preceding M wave. The second-order polynomial was subtracted from the raw data, leaving the detrended data with a mean of zero. RMS values were normalized to the maximum RMS (RMS_{max}) calculated over a 500-ms period centered on the peak soleus EMG during each participant's MVIC. Pilot work indicated that RMS calculations increased during increasing levels of voluntary plantar flexion contraction, were stable across stimulation intensities, were not different between stimulation locations, and could be measured in every participant across stimulation pattern and intensity. However, the asynchronous activity measure did not accurately reflect the voluntary contraction amplitude as a percentage of RMS_{max}. For example, a voluntary contraction of 5, 10, and 15% MVIC torque during the pilot work was measured as 4, 6, and 9% RMS_{max}, respectively, not 5, 10, and 15% RMS_{max} as one might expect. As such, RMS is reported here to provide a relative measure of the asynchronous activity during NMES over the nerve trunk and muscle belly and between $Time_1$ and $Time_2$ (6–7 s into the stimulation).

Twenty M-wave, H reflex, and asynchronous activity measurements were averaged at each $Time_1$ and $Time_2$ during a single stimulation pattern. For each participant, plantar flexion torque, M waves, H reflexes, and asynchronous activity measured at $Time_1$ and $Time_2$ were averaged separately over the five repetitions of a stimulation pattern in a single trial. Group means were calculated by pooling these mean data from each participant.

Statistical analyses were performed on group data using Statistica software (StatSoft, Tulsa, OK). Paired *t*-tests were used to test for differences in M_{max}, H_{max}-to-M_{max} ratios, and peak twitch torques obtained from the M-H recruitment curves, produced at each stimulation location. For data from trials with NMES, separate three-factor repeated-measures ANOVA were run on each dependent variable (torque, H reflex, M wave, and asynchronous activity) at both intensities (low and higher) to determine the influence of "stimulation location" (nerve trunk vs. muscle belly), "stimulation pattern" (20-Hz constant frequency vs. 20–100-20-Hz step frequency) and "time" ($Time_1$ vs. $Time_2$) on the evoked response. Significant main effects and interactions were tested post hoc using Tukey's honestly significant difference tests when appropriate. An α level of $P < 0.05$ was used to evaluate statistical significance. All data are reported as means ± SE.

RESULTS

M-H Recruitment Curve

There were no significant differences between M_{max} evoked by stimulation at both locations [$t_{(9)} = 1.2$, $P = 0.3$]. M_{max} was 6.9 ± 0.5 mV for stimulation over the nerve trunk, and 6.4 ± 0.5 mV for stimulation over the muscle belly. H_{max}-to-M_{max} ratios were significantly larger [$t_{(9)} = 6.7$, $P < 0.001$] for NMES over the nerve trunk (0.6 ± 0.1) compared with NMES over the muscle belly (0.1 ± 0.01). There were no significant differences between peak twitch torques evoked by stimulation at both locations [$t_{(9)} = 0.3$, $P = 0.79$] when M-wave amplitudes were not different [$t_{(9)} = 0.5$, $P = 0.61$]. Twitch torques were 12.3 ± 1.6% MVIC for stimulation over the nerve trunk and 12.2 ± 1.8% MVIC for stimulation over the muscle belly when M waves were 80.1 ± 15.2% M_{max} and 79.7 ± 15.3% M_{max}, respectively.

Low-intensity Stimulation

Figure 2 shows data recorded from one participant during NMES over the nerve trunk (*A* and *B*) and over the muscle belly (*C* and *D*). In this participant, during NMES over the nerve trunk and over the muscle belly, torque was stable during constant frequency stimulation, but was augmented after the 100-Hz stimulation during the step-frequency pattern. During NMES over the nerve trunk using the constant-frequency pattern, H reflexes were attenuated after the first response (see arrow; Fig. 2*A*) and remained small, but relatively stable, throughout the stimulation. When the step-frequency pattern was delivered over the nerve trunk (Fig. 2*B*), a similar reflex depression was observed initially; however, H reflexes and asynchronous activity were augmented following the 100-Hz stimulation. M waves were also depressed after the first response, but then remained small and stable for both patterns. During NMES over the muscle belly (Fig. 2, *C* and *D*), M waves dominated the EMG for both patterns of stimulation; however, during the step-frequency pattern, M waves, H re-

flexes, and asynchronous activity were larger after the 100-Hz stimulation.

Figure 3 shows group data ($n = 10$) for all dependent variables (torque, M waves, H reflexes, and asynchronous activity) during NMES over the tibial nerve and TS muscles using constant and step-frequency patterns. For torque (Fig. 3*A*), there was a significant interaction between stimulation pattern and time [$F_{(1,9)} = 10.2$, $P = 0.01$]. There was no main effect of stimulation location [$F_{(1,9)} = 0.009$, $P = 0.9$]; hence there was no difference in the torque generated by stimulation over the nerve trunk vs. over the muscle belly at either $Time_1$ or $Time_2$. As shown in the inset in Fig. 3*A*, torque recorded at $Time_2$ was larger than torque at $Time_1$, only during the step pattern. For M-wave amplitude (Fig. 3*B*), there was a significant interaction between stimulation location and time [$F_{(1,9)} = 5.5$, $P = 0.04$], and there was no significant main effect of stimulation pattern [$F_{(1,9)} = 1.1$, $P = 0.3$]. Thus, although M-wave amplitude was independent of stimulation pattern, M waves were significantly larger (5–6 times) during NMES over the muscle belly at both $Time_1$ and $Time_2$ compared with NMES over the nerve trunk and were larger at $Time_2$ compared with $Time_1$ during NMES over the muscle. H-reflex amplitude (Fig. 3*C*) also showed a significant interaction between stimulation location and time [$F_{(1,9)} = 6.88$, $P = 0.02$] and no main effect of stimulation pattern [$F_{(1,9)} = 3.4$, $P = 0.1$]. H-reflex amplitude was also independent of stimulation pattern; however, H reflexes were larger (2–3 times) during NMES over the nerve trunk at $Time_1$ and $Time_2$ compared with NMES over the muscle belly. For asynchronous activity (Fig. 3*D*), there was a significant interaction between stimulation location and time [$F_{(1,9)} = 5.1$, $P = 0.04$], and there was no significant main effect of stimulation pattern [$F_{(1,9)} = 4.6$, $P = 0.09$]. Asynchronous activity during NMES over the muscle belly at $Time_2$ was significantly greater than it was during NMES over the muscle belly at $Time_1$, as well as at both time points during NMES over the nerve trunk.

Higher Intensity Stimulation

Figure 4 shows data recorded from the same participant as in Fig. 2 during NMES over the nerve trunk (*A* and *B*) and over the muscle belly (*C* and *D*) at a stimulation intensity to evoke ~20% MVIC torque at $Time_1$. During stimulation at both locations, torque remained relatively stable during constant-frequency stimulation, but was augmented after a period of 100-Hz stimulation during the step-frequency pattern. During NMES over the nerve trunk using the constant-frequency pattern, H reflexes were attenuated compared with the first response and remained depressed throughout the stimulation, while M waves were small and stable throughout. During the step-frequency pattern, a similar reflex depression was observed during the initial 20-Hz stimulation; however, H reflexes and asynchronous activity were augmented after 100-Hz stimulation, whereas M waves were depressed. During NMES over the muscle belly, M waves dominated the EMG for both patterns of stimulation; however, during the step-frequency pattern, M waves, H reflexes, and asynchronous activity were larger after 100-Hz stimulation.

Figure 5 shows data recorded from a single participant during NMES over the nerve trunk (*A* and *B*) and over the muscle belly (*C* and *D*) at a stimulation intensity that evoked ~40% MVIC torque at $Time_1$. During stimulation at both locations, torque remained stable during constant-frequency stimulation. Torque was also not augmented following 100-Hz stimulation at either location. Interestingly, during the 100-Hz period of NMES over the nerve trunk, torque decreased due to the activation of the common peroneal nerve in this participant, as indicated by TA EMG activity (not shown). As such, these data were not included in the statistical analysis of group data. During NMES over the nerve trunk using the constant-frequency pattern, H reflexes were attenuated after the first response, but recovered to an amplitude equal to the first response by the end of the stimulation. During the step-

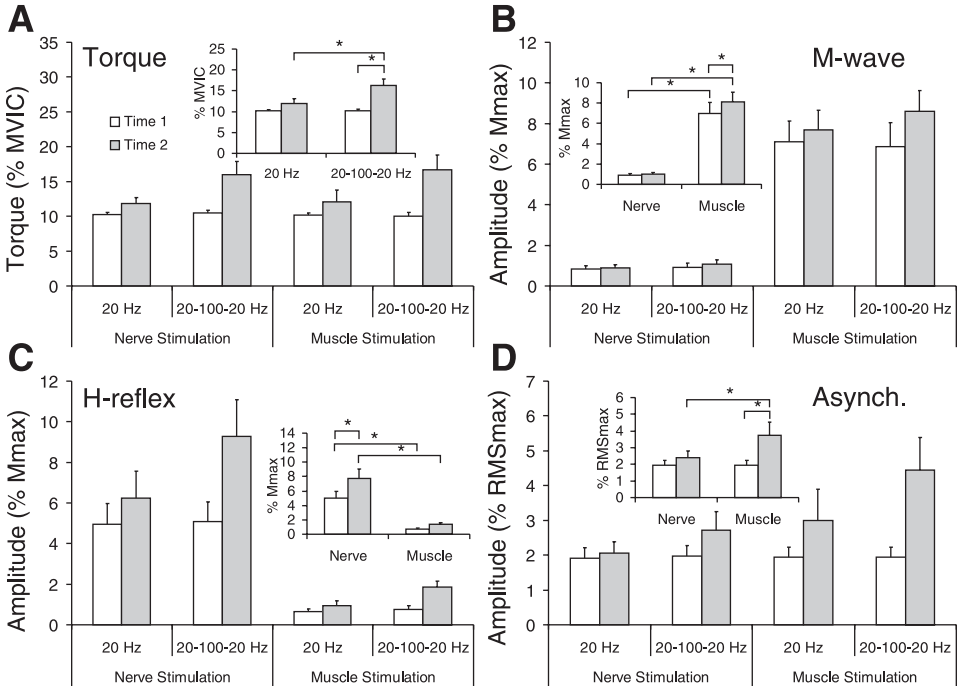

Fig. 3. Normalized group data ($n = 10$) averaged at two time points ($Time_1$ and $Time_2$) during NMES over the tibial nerve (Nerve Stimulation) and the TS muscles (Muscle Stimulation) at an intensity to evoke ~10% MVIC torque at $Time_1$. *A*: torque. *B*: M waves. *C*: H reflexes. *D*: asynchronous activity. Significant two-way interactions identified by statistical analyses are displayed within the *insets*. *Significant difference at a level $P < 0.05$.

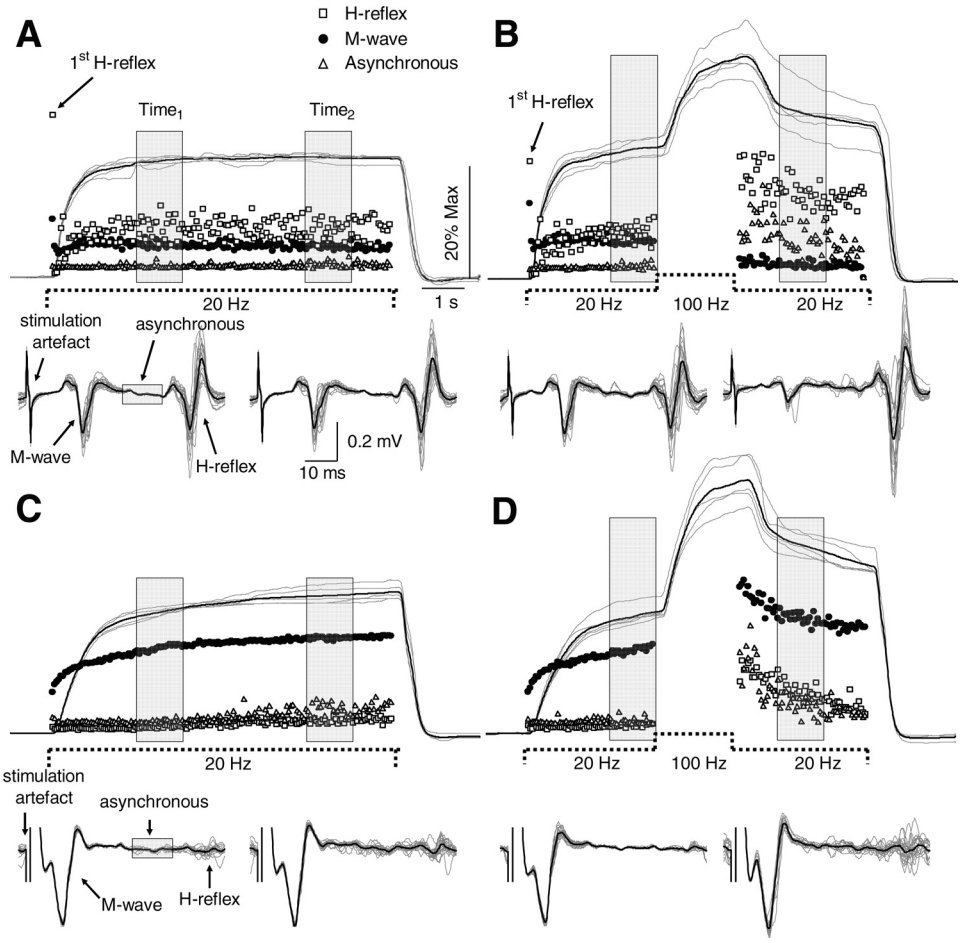

Fig. 4. Torque and EMG responses evoked by stimulation over the tibial nerve (*A* and *B*) and the TS muscles (*C* and *D*) to evoke ~20% MVIC torque at $Time_1$ in a single participant. *A* and *C*: responses to the 20-Hz constant-frequency pattern are displayed. *B* and *D*: responses to the 20–100-20-Hz pattern are displayed. In the *top* of each panel, torque profiles represented by the solid black lines are averages of 5 shaded lines in response to 5 trains of NMES, and the symbols represent the average EMG data over 5 repetitions during a single trial. Vertical calibration represents 20% M_{max} for EMG and 20% MVIC for torque. The *bottom* of each panel shows EMG recorded at $Time_1$ (*left* trace) and $Time_2$ (*right* trace) during a single train of NMES. Solid black lines represent the average of 20 single responses (shaded lines) to NMES. *C* and *D*: stimulation artifacts for data recorded during NMES over the TS muscles have been truncated. All data are shown on the same scale, as indicated by the calibration bars in *A*.

frequency pattern, similar reflex depression and recovery were observed during the initial 20-Hz stimulation, and H reflexes were large, but variable, following 100-Hz stimulation. Regardless of the stimulation pattern, M waves were initially large, but decreased in size over the first 1 s of NMES and remained small and stable throughout the remaining stimulation. Asynchronous activity was small and stable throughout and was unaffected by the 100-Hz stimulation. During NMES over the muscle belly, only M waves were evident in the EMG for both patterns of stimulation.

Figure 6 shows group ($n = 5$) torque and EMG data for the higher intensity stimulation trials. For torque amplitude (Fig. 6*A*), there was a significant main effect of time [$F_{(1,4)} = 18.5$, $P = 0.01$] and no significant main effect of stimulation location [$F_{(1,4)} = 0.3$, $P = 0.63$] or stimulation pattern [$F_{(1,4)} = 3.4$, $P = 0.14$]. Torque was significantly larger at $Time_2$ compared with $Time_1$, regardless of the stimulation location or pattern. For M-wave amplitude (Fig. 6*B*), there was a significant interaction between stimulation location and time [$F_{(1,4)} = 26.3$, $P < 0.01$] and no significant main effect of stimulation pattern [$F_{(1,4)} = 0.04$, $P = 0.8$]. M waves were larger (5–6 times) for NMES over the muscle belly at both time points compared with NMES over the nerve trunk and were larger at $Time_2$ compared with $Time_1$ during NMES over the muscle belly. For H-reflex amplitude (Fig. 6*C*), there was a significant two-way interaction between stimulation location and time [$F_{(1,4)} = 10.9$, $P = 0.03$], and no significant main effect of stimulation pattern [$F_{(1,4)} = 4.7$, $P = 0.1$]. H reflexes were larger (2–3 times) during NMES over the nerve trunk at $Time_1$ and $Time_2$ compared with NMES over the muscle belly at $Time_1$ and $Time_2$, respectively. Furthermore, following a period of 100-Hz stimulation, H reflexes were larger at $Time_2$ compared with $Time_1$ only during stimulation over the nerve. For asynchronous activity (Fig. 6*D*), there was a significant main effect of stimulation location [$F_{(1,4)} = 12.9$, $P = 0.02$] and time [$F_{(1,4)} = 12.6$, $P = 0.02$] and no significant main effect of stimulation pattern [$F_{(1,4)} = 5.0$, $P = 0.09$]. Asynchronous activity during NMES at both locations increased over time, regardless of stimulation location or pattern. Furthermore, asynchronous activity was larger for NMES over the muscle belly compared with NMES over the nerve, regardless of the stimulation pattern or time.

DISCUSSION

In this study, we compared the contributions made by central and peripheral pathways to motor unit recruitment for contractions of similar amplitude generated by NMES applied over the tibial nerve and the TS muscles. As we anticipated, NMES at both locations recruited motor units through peripheral and central pathways, but the contributions made by these pathways for the two locations of stimulation differed markedly. Specifically, during NMES over the nerve trunk, contractions were generated primarily through a central pathway (H re-

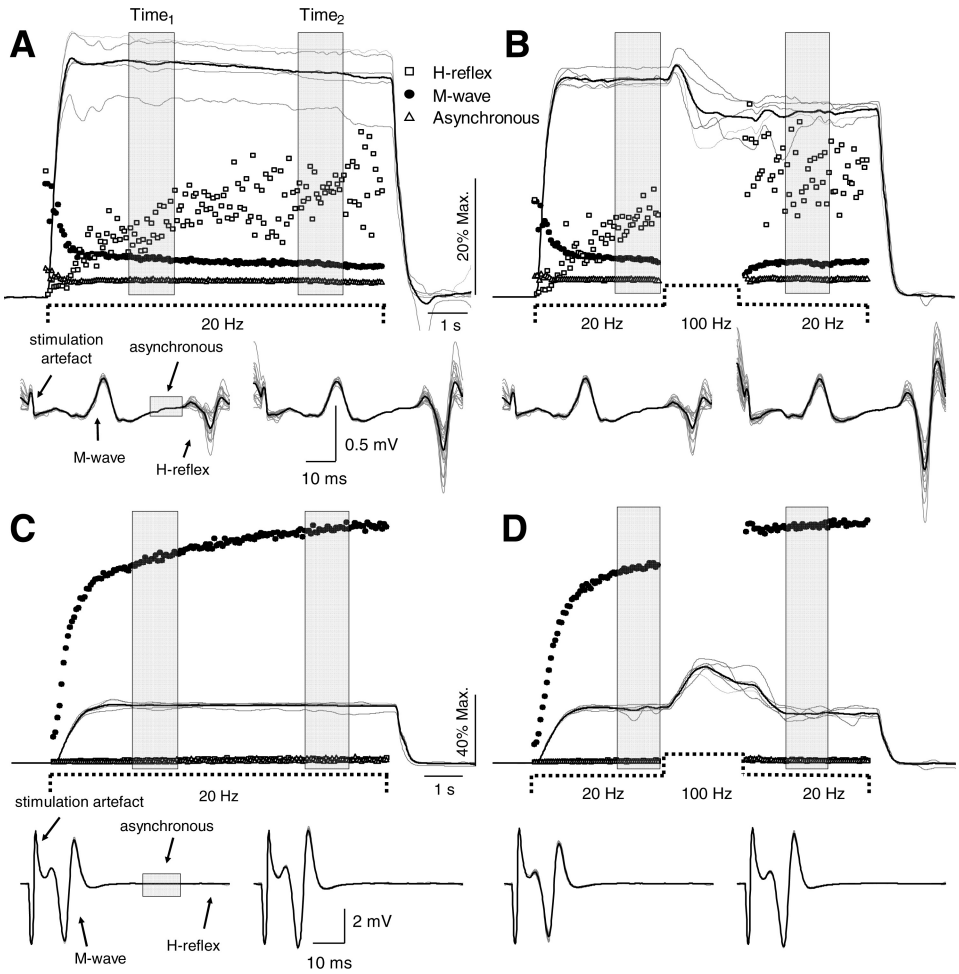

Fig. 5. Torque and EMG responses evoked by stimulation over the tibial nerve (*A* and *B*) and the TS muscles (*C* and *D*) to evoke ~40% MVIC torque at $Time_1$ in a single participant. *A* and *C*: responses to the 20-Hz constant-frequency pattern are displayed. *B* and *D*: responses to the 20–100-20-Hz pattern are displayed. In the *top* of each panel, torque profiles represented by the solid black lines are averages of 5 shaded lines in response to 5 trains of NMES, and the symbols represent the average EMG data over 5 repetitions during a single trial. The *bottom* of each panel shows EMG recorded at $Time_1$ (*left* trace) and $Time_2$ (*right* trace) during a single train of NMES. Solid black lines represent the average of 20 single responses (shaded lines) to NMES. *A* and *B* are shown on the same scale, as indicated by the calibration bars in *A*. *C* and *D* are shown on the same scale, as indicated by the calibration bars in *C*.

flexes), while NMES over the muscle belly generated contractions primarily through a peripheral pathway (M waves). For stimulation at both locations, the central contribution increased over time and could be augmented following a brief period of NMES at 100 Hz.

Torque

Torque was not significantly different during NMES over the nerve trunk compared with NMES over the muscle belly for both stimulation patterns and intensities. During low-intensity, constant-frequency stimulation, torque did not change from the beginning ($Time_1$) to the end ($Time_2$) of the stimulation. The "extra torque" we did observe after brief periods of 100-Hz stimulation during low-intensity stimulation and over time during the high-intensity stimulation has been attributed to multiple central mechanisms (see *Central Mechanisms* below).

Pathways During NMES Over the Nerve Trunk vs. Over the Muscle Belly

Although torque did not differ between stimulation locations, different neural pathways contributed to contractions generated when NMES was applied over the tibial nerve compared with over the TS muscles. Consistent with our first hypothesis and previous work in our laboratory (5), contractions evoked by NMES over the tibial nerve had significantly smaller M waves and significantly larger H reflexes compared with NMES over the TS muscles. M waves were five to six times larger during NMES over the TS muscles compared with NMES over the tibial nerve. H reflexes were evident in the EMG during NMES at both locations, but were two to three times larger during NMES over the nerve trunk compared with NMES over the muscle. In line with our second hypothesis, NMES over the muscle produced more asynchronous activity than NMES over the nerve trunk, regardless of the stimulation pattern. Asynchronous activity was low at the beginning and increased over several seconds for NMES at both locations. Together, these results support previous assertions that NMES over the muscle belly can produce contractions with a significant central contribution (5, 17, 18, 40) and shows that this contribution is in the form of H reflexes and asynchronous activity. The contribution of asynchronous activity to the evoked torque, however, may be less than that of the H reflex. The extra torque generated by NMES over the nerve trunk was accompanied by enhanced H reflexes, whereas equal levels of extra torque generated by NMES over the muscle belly were generated by enhanced asynchronous activity and enhanced M waves. Thus a portion of the extra torque during NMES over the muscle belly may originate from a peripheral mechanism. In general, NMES over the nerve trunk generated contractions with a greater contribution through central pathways, whereas NMES over the muscle belly generated contractions with a greater peripheral contribution.

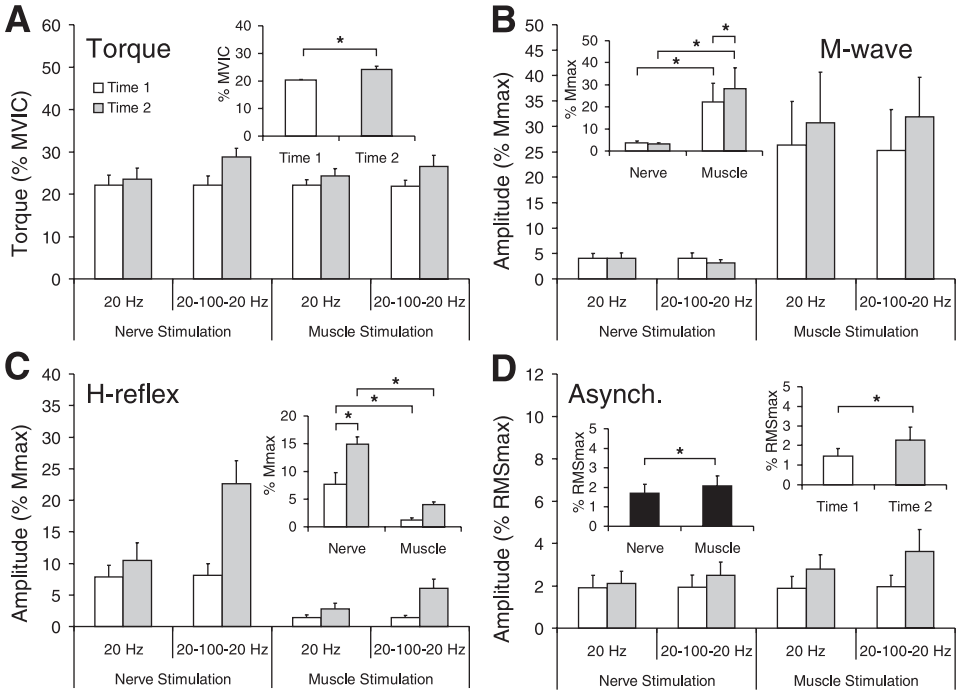

Fig. 6. Normalized group data ($n = 5$) averaged at two time points ($Time_1$ and $Time_2$) during stimulation over the tibial nerve (Nerve Stimulation) and the TS muscles (Muscle Stimulation) at an intensity to evoke between 20 and 30% MVIC torque at $Time_1$. *A*: torque. *B*: M waves. *C*: H reflexes. *D*: asynchronous activity. Significant main effects and two-way interactions identified by statistical analyses are displayed within the *insets*. *Significant difference at a level $P < 0.05$.

When stimulation intensity was increased to produce contraction amplitudes of ~20–30% MVIC torque, H reflexes and asynchronous activity were present during constant-frequency stimulation at both locations. During the step-frequency pattern, H-reflex amplitudes increased after stimulation at 100 Hz and reached ~24% M_{max} during NMES over the nerve trunk and 5% M_{max} during NMES over the muscle belly. Although H reflexes are initially depressed during repetitive stimulation due to postactivation depression of neurotransmitter release from Ia afferents (31), our laboratory has previously reported large H reflexes during NMES over the nerve trunk (5, 37). In the present study, even at the higher stimulation intensity, when antidromic transmission in motor axons (23) would be more pronounced, H reflexes were present during NMES at both locations. In the individual who received stimulation to generate ~40% MVIC torque (see Fig. 5), H reflexes were present only during stimulation over the nerve trunk, whereas only M waves were evident in the EMG during stimulation over the muscle belly; although these data were not included in the group due to coactivation of TA. Thus, at this highest stimulation intensity studied, a central contribution was only present during stimulation over the nerve trunk, but further study at these higher intensities is required to substantiate this finding.

The significantly greater H_{max}-to-M_{max} ratio and predominance of H reflexes during NMES over the nerve trunk compared with NMES over the muscle belly are likely explained in part by the neuronal architecture beneath the stimulating electrodes. NMES over the nerve trunk, where sensory and motor axons are bundled close together beneath the stimulating electrodes, likely recruited a relatively greater proportion of sensory axons than NMES delivered over the muscle belly near the TS motor points. At the level of the TS muscles, axons of the tibial nerve branch diffusely (36). This branching, in combination with the increased interelectrode distance and use of larger electrodes during stimulation over the muscle, may have activated axons over a broader spatial distribution, resulting in a less synchronous afferent volley arriving at the motoneuron during NMES over the muscle belly compared with NMES over the nerve trunk. Thus, during stimulation over the muscle belly, the sensory volleys may not depolarize motoneurons synchronously and generate an H reflex; rather, they may be more temporally dispersed and contribute to enhanced asynchronous activity. This effect of stimulation location would be less for the M wave, as the pathway to the muscle is shorter and circumvents central synapses compared with the pathway for the H reflex.

During stimulation over the muscle belly, M waves were significantly enhanced over time during low- and high-intensity stimulation. Some change in the amplitude of the M wave can be expected due to changes in muscle architecture beneath the recording electrodes (20), but M-wave amplitude did not change over time during NMES over the nerve trunk. Since the recording site and contraction intensities were not different between stimulation locations, a change in muscle architecture beneath the recording electrode does not explain the larger M waves evoked during stimulation over the muscle belly. However, muscle conformational changes beneath the stimulating electrodes may explain larger M waves during stimulation over the muscle belly. In isometric muscle contractions, the muscle fibers shorten and develop tension as the tendon stretches (26). This shortening would alter the position of muscle fibers beneath the stimulating electrodes in such a way that more axons and possibly more motor points converge beneath the stimulating electrodes, resulting in greater numbers of activated axons, further enhancing the muscle contraction. Support for this rationale lies in the slow rise of M-wave amplitude in concert with the slow rise in torque during the first second of stimulation when the muscle is shortening during NMES over the muscle belly.

Central Mechanisms

Several central mechanisms may account for the enhanced H reflexes and asynchronous activity that develop over time during NMES. Such mechanisms include the following: inadvertent or voluntary descending drive, posttetanic potentiation at the Ia synapse, and activation of persistent inward currents in spinal neurons. Inadvertent voluntary activation of motoneurons could account for the increase in H-reflex amplitude (51) and asynchronous activity; however, evidence suggests that this is not what occurred. Similar levels of extra torque generated through central pathways, as occurred during the low-intensity stimulation in this study, can develop in people who are sleeping (18) or who have complete spinal cord injury (45). Furthermore, participants in this study did not find the stimulation uncomfortable and remained relaxed throughout the NMES and did not voluntarily contract the muscles of the ankle. Posttetanic potentiation may also add to the enhanced central motor unit recruitment observed. Following repetitive stimulation of Ia afferents, posttetanic potentiation at the Ia synapse enhances excitatory postsynaptic potentials (27, 30). The development of persistent inward currents in spinal neurons have also been suggested as a mechanism underlying enhanced central motor unit recruitment (5, 17, 18, 37). Persistent inward currents have been demonstrated directly in spinal neurons in animals initiated by high-frequency synaptic drive (7) and indirectly in humans during periods of electrical stimulation (17, 18) or vibration (21, 35).

Implications for NMES

NMES is used to generate contractions for maintaining muscle quality [therapeutic electrical stimulation (TES)] and producing functional movements [functional electrical stimulation (FES)] following damage to central motor pathways (22, 32, 33, 46). However, the nonphysiological recruitment order of motor units during NMES limits the activation of low-threshold motor units during TES, and that, combined with synchronous motor unit activation, contributes to accelerated muscle fatigue during FES (46). The random recruitment order and synchronous discharge associated with recruitment through peripheral pathways (M waves) is in sharp contrast to the asynchronous and orderly motor unit recruitment that occurs during a voluntary contraction. The synchronous discharge of motor units during NMES means that nonphysiologically high firing rates are required to produce smooth contractions, and these high firing rates increase the energy demand from each active motor unit, resulting in premature fatigue (1). Additionally, the random recruitment order enhances the susceptibility of low-threshold motor units to disuse atrophy and fiber-type transitions, leaving the muscle with a smaller proportion of fatigue-resistant motor units (46). The limited recruitment of low-threshold motor units could be overcome by increasing the stimulation intensity to depolarize all of the motor axons, but the disadvantage of synchronous motor unit recruitment would remain, and such high intensities can be problematic for individuals with residual sensation (15) or compromised bone density (19). For this reason, developing methods that recruit low-threshold motor units at relatively low-stimulation intensities may have advantages for both TES and FES. Enhancing the extent to which NMES activates sensory axons and contributes to the evoked contractions through a central pathway in the form of H reflexes or asynchronous activity may be one such method.

The data from the present experiments confirm previous indications that the contribution made by central and peripheral pathways to electrically evoked contractions differs when stimulation is applied over a nerve trunk compared with over a muscle belly (5). Contractions produced by NMES over the nerve trunk generated a larger central contribution (H reflexes). NMES over the muscle belly evoked contractions with a greater contribution from direct motor axon activation (M waves). Thus NMES over the nerve trunk may hold greater promise for maintaining muscle quality following central motor pathway damage, as well as in the prevention of muscle fatigue during FES. Although there may be issues around control for FES using NMES over the nerve trunk, as contractions evoked by stimulation over the tibial nerve have been shown to be less stable within a single contraction and less consistent between successive contractions compared with stimulation over the TS muscles (5). The potential to reflexively activate a sufficiently large proportion of motor units to be useful for TES and FES may require a muscle with particularly strong reflex inputs, such as the TS muscles. Whether recruitment during NMES over the nerve trunk and over the muscle belly differs for other muscle groups has not yet been tested. However, a central contribution to electrically evoked contractions has been demonstrated for the TS (5, 18, 37), TA (37), quadriceps (A. J. Bergquist, unpublished observation), wrist extensors (5), biceps brachii (10, 42), and flexor pollicis longus (9).

Additionally, as stimulation intensity is increased beyond what was tested in this study, for example, in response to fatigue during FES exercise, increased levels of anti-dromic collision will develop (51). This will progressively block H-reflex and asynchronous contributions to evoked contractions. Although it has been estimated that 20–30% MVIC plantar flexion torque is required for walking (2), the present results indicate that a central contribution to evoked contractions occurs over this range during stimulation over the tibial nerve and, to a lesser extent, the TS muscles. However, considerably greater levels of plantar flexion torque, as a percent of MVIC, may be required for walking in individuals with severely atrophied muscle, and whether this can be achieved through central recruitment remains to be determined.

Summary

The contributions made by central and peripheral pathways to motor unit recruitment during NMES differed markedly for plantar flexion contractions of equal amplitude generated by NMES applied over the tibial nerve compared with the TS muscles. During NMES over the nerve trunk, contractions were generated primarily through a central pathway, while NMES over the muscle belly generated contractions predominantly through a peripheral pathway. Thus NMES over the tibial nerve may be more advantageous for maintaining muscle quality and reducing muscle contraction fatigue for rehabilitation compared with NMES over the TS muscles.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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