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

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Bergquist AJ, Wiest MJ, Collins DF. Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: quadriceps femoris. J Appl Physiol 113: 78–89, 2012. First published May 3, 2012; doi:10.1152/japplphysiol.00074.2011.—Neuromuscular electrical stimulation (NMES) can be delivered over a nerve trunk or muscle belly and both can generate contractions through peripheral and central pathways. Generating contractions through peripheral pathways is associated with a nonphysiological motor unit recruitment order, which may limit the efficacy of NMES rehabilitation. Presently, we compared recruitment through peripheral and central pathways for contractions of the knee extensors evoked by NMES applied over the femoral nerve vs. the quadriceps muscle. NMES was delivered to evoke 10% and 20% of maximum voluntary isometric contraction torque 2–3 s into the NMES (time1) in two patterns: 1) constant frequency (15 Hz for 8 s); and 2) step frequency (15–100–15 Hz and 25–100–25 Hz for 3–2–3 s, respectively). Torque and electromyographic activity recorded from vastus lateralis and medialis were quantified at the beginning (time1) and end (time2; 6–7 s into the NMES) of each pattern. M-waves (peripheral pathway), H-reflexes, and asynchronous activity (central pathways) during NMES were quantified. Torque did not differ regardless of NMES location, pattern, or time. For both muscles, M-waves were ~7–10 times smaller and H-reflexes ~8–9 times larger during NMES over the nerve compared with over the muscle. However, unlike muscles studied previously, neither torque nor activity through central pathways were augmented following 100 Hz NMES, nor was any asynchronous activity evoked during NMES at either location. The coefficient of variation was also quantified at time2 to determine the consistency of each dependent measure between three consecutive contractions. Torque, M-waves, and H-reflexes were most variable during NMES over the nerve. In summary, NMES over the nerve produced contractions with the greatest recruitment through central pathways; however, considering some of the limitations of NMES over the femoral nerve, it may be considered a good complement to, as opposed to a replacement for, NMES over the quadriceps muscle for maintaining muscle quality and reducing contraction fatigue during NMES rehabilitation.

M-wave; H-reflex; electromyography; quadriceps; human

NEUROMUSCULAR ELECTRICAL STIMULATION (NMES) can be delivered using electrodes placed on the skin over either a nerve trunk or a muscle belly. We have recently shown that when NMES is used to generate isometric plantar flexion contractions of the ankle, NMES over the tibial nerve trunk generated contractions through markedly different pathways than NMES over the triceps surae (TS) muscle belly (4). During NMES over the tibial nerve trunk, contractions were generated primarily by the synaptic recruitment of motor neurons in the spinal cord (central pathways), while NMES over the TS muscle generated contractions predominantly through the activation of motor axons beneath the stimulating electrodes (peripheral pathways). However, the ankle plantar flexors are rarely stimulated during NMES rehabilitation programs and whether or not this effect of NMES location can be generalized to muscles more commonly used for NMES has not been tested. Thus, in the present experiments, we extend this line of investigation to the quadriceps femoris. The quadriceps muscle is the most often stimulated muscle for NMES rehabilitation (3) to reduce atrophy (3, 21, 23, 25), and improve cardiovascular function (19, 30), mobility (46, 52) and glucose utilization (32, 42) following damage to the central nervous system (CNS). Whether transmission along central pathways contributes to NMES-evoked contractions of the quadriceps muscle is not known; in the present study we compare the extent to which transmission along central and peripheral pathways contributes to contractions evoked by NMES applied over the femoral nerve trunk vs. the quadriceps muscle belly.

Generating contractions through peripheral pathways by the depolarization of motor axons beneath the stimulating electrodes may limit the efficacy of NMES for maintaining muscle quality and for producing functional movement. The discharge of motor units recruited in this way is synchronous, time locked to each stimulus pulse as represented by successive M-waves in the electromyographic (EMG) signal. The recruitment of motor units through peripheral pathways does not follow Henneman’s size principle (12, 26, 53), and as a result, motor unit recruitment through this pathway during NMES leaves fatigue-resistant muscle fibers less active and consequently more vulnerable to disuse atrophy compared with contractions generated through synaptic recruitment (central pathways). Additionally, the nonphysiological recruitment order and synchronous discharge of motor units contributes to the rapid fatigue associated with NMES-evoked contractions (50). In contrast, activating muscle through central pathways by depolarizing sensory axons and recruiting motor units synaptically follows Henneman’s size principle (28, 29), and motor unit discharge is either time locked to each stimulus pulse as an H-reflex or is temporally unrelated to the NMES and appears as asynchronous activity in the EMG signal (2, 4, 16, 17, 35, 38). Increasing the recruitment of fatigue-resistant muscle fibers by increasing activity through central pathways during NMES may help reduce the atrophy and fiber type transitions associated with prolonged inactivity imposed by damage to the CNS.

The present experiments were designed to compare the contributions made by peripheral (M-wave) and central (H-reflex and asynchronous activity) pathways to motor unit recruitment for isometric knee extension contractions of the quadriceps muscle. Based on our experiments conducted on the TS muscles (4), we hypothesized that: 1) contractions evoked by NMES over the femoral nerve trunk would have smaller...
M-waves, larger H-reflexes, and less asynchronous activity compared with contractions of equal amplitude evoked by NMES over the quadriceps muscle belly; and 2) both locations of NMES would generate equivalent increases in torque following a brief period of high-frequency NMES (100 Hz), delivered during NMES at a lower frequency (15 or 25 Hz), which would be accompanied by enhanced H-reflexes during NMES over the nerve trunk and enhanced asynchronous activity during NMES over the muscle belly. The results of the present study contribute to our understanding of how NMES generates contractions in the muscle most commonly used for NMES rehabilitation programs. We show for the first time that contractions of the quadriceps muscle can be generated through central pathways and that the effect of NMES location on the balance between motor unit recruitment through peripheral and central pathways is not unique to the TS muscle, but that NMES location also affects motor unit recruitment of the quadriceps.

METHODS

Participants

Thirteen human participants with no known neurological or musculoskeletal impairments volunteered for this study after providing informed written consent. Eleven participants (8 males and 3 females; age between 21 and 48 yr; 29.3 ± 8.33 (SD) yr) volunteered for the initial experiments (see Initial Experiments). Each initial experiment lasted ~2.5 h. Seven participants (4 males and 3 females; age range: 25 to 48 yr; 32.0 ± 8.05 (SD) yr) volunteered for the additional experiments (see Additional Experiments), 5 of whom had participated in the initial experiments. Each additional experiment lasted ~1 h. All experiments were conducted in accordance with the Declaration of Helsinki and were approved by the Health Research Ethics Board at the University of Alberta.

Initial Experiments

All procedures were performed on the right thigh. To measure isometric knee extension torque, participants were seated in the chair of a Biodex dynamometer (System 3, Biodex Medical Systems, Shirley, NY) with the hip at 120° and the knee at 90°. The axis of the dynamometer was aligned with the axis of rotation of the participant’s knee joint. The arm of the dynamometer was parallel to the anterior aspect of the tibia, with the lower edge of the pad positioned proximal to the lateral malleolus. The trunk, waist, and thigh were stabilized using straps on the Biodex dynamometer chair.

Electromyography. Surface electromyography (EMG) was recorded from the vastus lateralis (VL) and vastus medialis (VM) using adhesive gel electrodes (2.25 cm²; Vermed Medical, Bellows Falls, VT) in a bipolar configuration (Fig. 1A). The electrodes were placed parallel to the predicted path of the muscle fibers with ~1-cm interelectrode distance. For VL, the distal electrode was positioned 8–12 cm from the patella while for VM the distal electrode was placed between 2 and 3 cm from the lateral border of the patella. A common reference electrode was placed over the patella. EMG signals were amplified 500 times and band-pass filtered at 10–1,000 Hz (NeuroLog System; Digitimer, Welwyn Garden City, UK).

Maximum voluntary isometric contractions. Prior to trials involving NMES, participants performed maximum voluntary isometric contractions (MVCs) of the quadriceps, extending against the arm of the dynamometer for 3–5 s as forcefully as possible. Participants were provided with visual feedback of their torque production on a computer monitor and received verbal encouragement to promote maximal performance during each MVC. Each participant completed two to three MVCs until peak isometric knee extension torque differed by less than 10% between trials. Each MVC was separated by at least 3 min of rest to minimize fatigue.

Neuromuscular electrical stimulation. NMES was delivered either over the femoral nerve trunk or over the quadriceps muscle belly (Fig. 1A) using 1-ms square-wave pulses from a single-channel constant-current stimulator (DS7A Digitimer, Welwyn Garden City, UK). Stimulation current was measured using a current probe (mA 2000 Noncontact Milliammeter; Bell Technologies, Orlando, FL). Stimulation over the femoral nerve trunk was delivered through two adhesive gel electrodes in a monopolar arrangement. The anode (7.5 × 13 cm; model CF7515, Axelgaard Manufacturing, Lystrup, Denmark) was positioned on the skin at the gluteal fold. The cathode (3.2 cm round; model CF3200, Axelgaard Manufacturing) was placed on the skin of the femoral triangle at a position where a single pulse evoked a response (M-wave or H-reflex) in VL at the lowest stimulation amplitude. When a suitable position was identified, a small foam ball was placed over the cathode and was wrapped with a tensor bandage to apply pressure over the stimulation site. Stimulation over the quadriceps muscle belly was delivered in a bipolar configuration. The output of the single-channel stimulator was divided between two pairs of flexible adhesive electrodes (7.5 × 13 cm; model CF7515, Axelgaard Manufacturing). This configuration was found to maximize the activation of the quadriceps and reduce stimulation discomfort in pilot experiments. The anodes were placed proximally over the muscle belly, while the
cathodes were placed over the motor point of VL and VM. Motor points were identified by the location on the surface of the skin in which an electrical pulse evoked a visible muscle twitch with the least current. If contractions of the adductors were observed, through visual inspection and palpation during stimulation, the electrodes were repositioned laterally and/or were cut smaller to more selectively activate the quadriceps muscle.

**M-wave-H-reflex (M-H) recruitment curve.** Separate M-H recruitment curves were constructed for stimulation over the femoral nerve trunk and the quadriceps muscle from responses to 50 stimulation pulses delivered randomly every 8–10 s. For stimulation over the femoral nerve trunk, current was delivered from below M-wave and H-reflex threshold to 1.2 times the minimum current required to evoke a maximal M-wave (M_{max}) in VL. This amplitude was also sufficient to evoke M_{max} in VM for all participants. To maintain similar levels of motor pool excitability during collection of the recruitment curve data (12), participants held a background contraction to produce ~5% MVIC torque using visual feedback displayed on a computer monitor. After collecting the data for the M-H recruitment curves, participants did not receive feedback of their torque production for the remainder of the experiment.

**NMES patterns.** In initial experiments, NMES was delivered in two patterns: 1) a constant-frequency pattern of 15 Hz for 8 s; and 2) a step-frequency pattern of 15–100-15 Hz for 3–2.3 s for each phase, respectively (adapted from Refs. 16, 17). The 15-Hz frequency was chosen because in pilot experiments it was determined that 15 Hz was the highest frequency that allowed for quantifying asynchronous activity (see **Data Acquisition and Analysis**) and is just below a recommended frequency range (18–25 Hz) for NMES of the lower limb (50). The step-frequency pattern was chosen because it allowed us to examine contractions evoked by NMES at 15 Hz before and after a period of 100-Hz NMES, which has been shown to enhance the central motor unit recruitment during NMES-evoked contractions (4, 35). The constant-frequency pattern then also acted as a control, allowing us to determine the effects of the 100-Hz step on motor unit recruitment.

A single trial of NMES consisted of three repetitions of a NMES pattern, with 60 s separating each pattern. For each NMES location, trials were collected using both NMES patterns and amplitudes. 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 NMES-evoked contractions.

**NMES amplitude.** To set the NMES amplitude, 2 s of NMES was delivered every 5–10 s while the amplitude was increased by ~2-mA increments during NMES over the nerve trunk and ~5-mA increments during NMES over the muscle belly until the desired torque was achieved. If more than ~10 contractions were required to achieve the desired torque, participants were provided ~3 min of rest before continuing. NMES was delivered to evoke contractions of 10, 20, or 30% MVIC torque during the interval 2–3 s into the NMES (time; see Fig. 3A). For all trials, if the NMES was uncomfortable, the experimental session was concluded or trials at lower NMES amplitudes were collected. As a result, data were obtained for both NMES over the nerve trunk and over the muscle belly from 11 participants at 10% MVIC torque, 8 participants at 20% MVIC torque, and 1 participant at 30% MVIC torque. For all participants, increases in NMES amplitude were limited by discomfort during NMES over the muscle belly.

**Additional Experiments.**

During the initial experiments, the lower frequency of NMES was delivered at 15 Hz to enable the recording of asynchronous activity. However, this frequency is lower than the 20- to 25-Hz frequencies that we have used to stimulate the ankle musculature in previous experiments that have shown that torque, H-reflexes, and asynchronous activity are augmented after a period of 100 Hz NMES (2, 4, 16, 17, 35). Thus we conducted additional experiments in which the lower frequency in the NMES pattern was 25 Hz to provide a more valid comparison with the results of our previous studies.

These additional experiments followed the same protocol as the initial experiments except that the NMES was delivered at 25 Hz for 8 s (constant-frequency pattern) or 25–100-25 Hz for 3–2.3 s for each phase, respectively (step-frequency pattern) and was only delivered at one amplitude, which evoked 10% MVIC torque at time1. This contraction amplitude was chosen to maximize the chances of generating augmented torque and H-reflexes, as lower contraction amplitudes have produced the greatest levels of additional torque following 100-Hz NMES (2, 4). In these additional experiments, we collected M_{max} for each participant, but data for M-H recruitment curves were not collected and EMG data were not analyzed during NMES patterns because H-reflex peak-to-peak measurements were contaminated by stimulation artefacts during 25-Hz NMES.

**Data Acquisition and Analysis.**

Data were sampled at 5 kHz using custom-written Labview software (National Instruments, Austin, TX) and stored on a computer for subsequent analysis that was 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 isometric knee extensor torque during the MVIC. Torque generated during NMES was normalized to each participant’s MVIC. The amplitudes of each M-wave and H-reflex recorded for the M-H recruitment curves and during 15-Hz NMES were measured peak-to-peak. Recruitment curves were generated by plotting M-wave and H-reflex amplitudes as a function of stimulus amplitude. For stimulation over the quadriceps muscle belly, we sometimes failed to observe a clear plateau in M-wave amplitude in the recruitment curves, even at maximal stimulator output (100 mA). Thus, for each participant, all M-waves and H-reflexes were normalized to the single largest M-wave (M_{max}) from the recruitment curve for stimulation over the femoral nerve trunk. The single largest H-reflex (H_{max}) from the recruitment curves and M_{max} from the recruitment curve for stimulation over the femoral nerve trunk were used to calculate H_{max}-to-M_{max} ratios. EMG during 25-Hz and 100-Hz NMES was not quantified due to contamination by stimulation artefacts.

During NMES over the muscle belly, M-wave amplitude can be contaminated by the preceding stimulation artefact due to the close proximity of the stimulating and recording electrodes. Thus, to prevent overestimation of the M-wave amplitude, we analyzed the data post hoc using a two-step software-based signal processing procedure (48). The algorithm removes the complete stimulation artefact including both positive and negative spikes as well as any exponentially decaying tail. Likewise, during NMES over the nerve trunk, H-reflex amplitude can be contaminated by the preceding M-wave due to the proximity of the NMES location to the spinal cord. In other words, the H-reflex may begin on the tail of the M-wave. To calculate the H-reflex amplitude, we adopted a four-step software-based signal processing procedure (39). This process isolated the tail of the H_{max} signal from the M-H recruitment curve, when no reflex was present, and scaled the tail using a template according to the amplitude of the M-wave to be removed. The scaled M-wave tail was then subtracted, leaving the uncontaminated H-reflex for peak-to-peak analysis. All data were analyzed using both signal-processing algorithms, regardless of NMES location.

To quantify asynchronous activity during 15-Hz NMES, we calculated the root mean square (RMS) of the EMG activity over a 10-ms interval between 55 and 65 ms after each stimulation artefact (see Fig. 1B). As in a previous study (4), a duration of 10 ms was chosen as this time window was not contaminated by the stimulus artefact, M-wave, or H-reflex, and for the present study it minimized the effect of the H-reflex silent period on the asynchronous measurement. To 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 or H-reflex. The second-order polynomial was subtracted from the raw data before the RMS was calculated, leaving the detrended data with a mean of zero. RMS values were normalized to the maximum RMS (RMS$_{max}$) calculated separately over a 500-ms period centered on the peak VL and VM EMG during each participant’s MVIC. Pilot experiments, in which we delivered 8 s of 15-Hz NMES to evoke 10% MVIC torque of the quadriceps while participants were at rest or held voluntary isometric contractions to generate 5–20% MVIC, additional to the NMES-evoked contraction, confirmed that we could measure asynchronous activity in the quadriceps and that our measure of RMS activity increased during increasing levels of voluntary contraction. Further, at a given background contraction amplitude, asynchronous activity was not different between NMES locations and could be measured in every participant. However, the pilot experiments showed that 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 20% MVIC torque was measured as 2, 4, and 7% RMS$_{max}$, respectively. As such, RMS is reported here to provide a relative measure of the involuntary asynchronous activity generated by the sensory volley during NMES over the nerve trunk and muscle belly and between time$_1$ and time$_2$. Fifteen M-wave, H-reflex, and asynchronous activity measurements were averaged over each of two time periods (time$_1$: 2–3 s into the NMES; time$_2$: 6–7 s into the NMES) during a single NMES pattern. For each participant, isometric knee extension torque, M-waves, H-reflexes, and asynchronous activity measured at time$_1$ and time$_2$ were averaged separately over the three repetitions of a NMES pattern in a single trial. Group means were calculated by pooling these mean data from each participant. The consistency of isometric knee extension torque, M-waves, and H-reflexes between successive contractions was measured by calculating the coefficient of variation (CV = (SD/mean) × 100) between the mean values calculated for the three consecutive contractions at time$_2$.

Statistical analyses were performed using Statistica software (StatSoft, Tulsa, OK). Kolmogorov-Smirnoff and Lilliefs tests for normality showed that group data were normally distributed. For the initial experiments, analyses were performed on group torque, VL, and VM data from trials in which NMES was delivered to evoke 10% and 20% MVIC torque. Paired t-tests were used to test for differences in M$_{max}$ and H$_{max}$-to-M$_{max}$ ratios, obtained from the M-H recruitment curves produced at each stimulation location. Paired t-tests were also used to compare stimulation current between the two locations separately for each amplitude. For data from trials with NMES, separate three-factor repeated-measures analyses of variance (rmANOVA) tests were run on each dependent variable (torque, H-reflex, M-wave, and asynchronous activity) at both NMES amplitudes (10% and 20% MVIC) to determine the influence of NMES location, NMES pattern (constant frequency vs. step frequency), and time (time$_1$ vs. time$_2$) on the amplitude of the evoked response. A two-factor rmANOVA test was run on torque CV data to determine the influence of NMES location and NMES pattern on the consistency of the evoked torque at time$_2$. An α-level of 0.05 was used to evaluate statistical significance. All data are reported as means ± SD.

RESULTS

Recruitment Curve

M-H recruitment curve data recorded from VL for one participant during stimulation over the nerve trunk (A) and over the muscle belly (B) are shown in Fig. 2. The right side of each panel shows single EMG traces from the corresponding numerical location in the recruitment curve. In this participant, the H$_{max}$-to-M$_{max}$ ratio was 0.22 for stimulation over the nerve trunk and 0.04 for stimulation over the muscle belly. For the group (n = 11), there was no significant difference between M$_{max}$ evoked by stimulation at both locations (t$_{10}$ = 1.05, P = 0.32). M$_{max}$ was 10.4 ± 3.8 mV for stimulation over the nerve trunk and 9.7 ± 2.7 mV for stimulation over the muscle belly. H$_{max}$-to-M$_{max}$ ratios were significantly larger (t$_{10}$ = 3.8, P < 0.01) for NMES over the nerve trunk (0.21 ± 0.10; range 0.09–0.37) compared with NMES over the muscle belly (0.02 ± 0.01; range 0.01–0.03). Robust H-reflexes could be evoked in all 11 participants during stimulation over the nerve trunk. Conversely, H-reflexes were rare and very small when present during stimulation over the muscle belly.

NMES: Single Participant Data

Data recorded from one participant during NMES over the nerve trunk (A, C, and E) and over the muscle belly (B, D, and F) during the constant-frequency and step-frequency pattern are shown in Figs. 3 and 4, respectively. In the top half of each panel, the solid lines show torque and the symbols represent the amplitude of the EMG measures from VL during NMES at 15 Hz. There was no asynchronous activity present during NMES at either location or amplitude and thus these data are not shown in Figs. 3 or 4. During constant-frequency NMES at both locations (Fig. 3), mean torque remained stable throughout the NMES (i.e., was similar at time$_1$ and time$_2$). However, there were periods of time when torque oscillated rapidly (~7 to 8 Hz) during NMES over the nerve trunk, as can be seen in the individual traces (gray lines) in Fig. 3, A, C, and E. During these periods, and throughout the NMES, H-reflexes alternated between large and small (see the open squares, which are an average of three H-reflex measurements across three consecutive contractions, and the gray lines in the EMG traces in Fig. 3, A, C, and E) while M-waves were relatively consistent. During NMES over the nerve trunk, H-reflexes dominated the EMG at all three contraction amplitudes (10, 20, and 30% MVIC torque) while M-waves were small and relatively stable. In contrast, during NMES over the muscle belly, M-waves dominated the EMG. When the step-frequency pattern was delivered in this participant (Fig. 4), torque, M-waves, and H-reflexes were not augmented after 100-Hz NMES.

NMES: Group Data

Initial experiments. Statistical analyses were performed on data recorded when 15-Hz NMES was delivered to evoke 10% and 20% MVIC torque at time$_1$ for the group. There was no...
asynchronous activity, significantly greater than measured at baseline, during NMES at either location or amplitude and thus these data are not shown in Figs. 5 or 6. For both contraction amplitudes, significantly less current was required for NMES over the nerve trunk than over the muscle belly. The mean current required to produce 10% MVIC torque at time1 was 12.6 ± 4.7 mA for NMES over the nerve trunk and 46.0 ± 13.1 mA for NMES over the muscle belly (t_{10} = 8.1, P < 0.01). The mean current to produce 20% MVIC torque was 14.4 ± 6.5 mA for NMES over the nerve trunk and 65.3 ± 22.8 mA for NMES over the muscle belly (t_{10} = 7.1, P < 0.01). The current required for one participant who received NMES to produce 30% MVIC torque was 12 mA for NMES over the nerve trunk and 54 mA for NMES over the muscle belly.

Figure 5 shows group (n = 11) torque and VL EMG data for trials in which the NMES amplitude was adjusted to evoke 10% MVIC torque at time1. There were no significant differences in torque across all factors (Fig. 5A). However, the CV for torque (Fig. 5B) showed a significant main effect of NMES location (F_{1,10} = 9.79, P = 0.01). Torque was more consistent between contractions during NMES over the muscle belly compared with NMES over the nerve trunk, regardless of NMES pattern. For M-wave amplitude (Fig. 5C), there was a significant main effect of NMES location (F_{1,10} = 17.19, P < 0.01). M-waves were ~10 times larger during NMES over the muscle belly compared with NMES over the nerve trunk, regardless of NMES pattern or time. Additionally, the CV for M-waves (Fig. 5D) showed a significant main effect of NMES location (F_{1,10} = 17.29, P = 0.02). M-waves were more consistent between contractions during NMES over the muscle belly compared with NMES over the nerve trunk, regardless of NMES pattern. For H-reflex amplitude (Fig. 5E), there was a significant main effect of NMES location (F_{1,10} = 19.55, P < 0.01). H-reflexes were ~9 times larger during NMES over the nerve trunk compared with NMES over the muscle belly, regardless of NMES pattern or time. Additionally, the CV for H-reflexes (Fig. 5F) showed a significant main effect of NMES location (F_{1,10} = 19.55, P < 0.01). H-reflexes were more consistent between contractions during NMES over the muscle belly compared with NMES over the nerve trunk, regardless of NMES pattern.
consistent between contractions during NMES over the muscle belly compared with NMES over the nerve trunk, regardless of NMES pattern. However, we acknowledge that since H-reflexes were very small, when present, during NMES over the muscle belly, this may not be a relevant comparison.

Group data \((n = \text{8})\) for torque and VL EMG are shown in Fig. 6 for trials in which the NMES amplitude was adjusted to evoke 20% MVIC torque. There were no significant differences in the amplitude of torque across all factors (Fig. 6A). Additionally, there were no significant differences in the CV for torque (Fig. 6B). Thus, at this higher level of NMES, there were no differences in the consistency of torque between contractions across both factors. For M-wave amplitude (Fig. 6C), there was a significant main effect of NMES location \((F_{1,7} = 22.94, P < 0.01)\). M-waves were \(\sim 7\) times larger for NMES over the muscle belly compared with NMES over the nerve trunk, regardless of the NMES pattern or time. The CV for M-waves (Fig. 6D) showed a significant main effect of NMES location \((F_{1,7} = 6.17, P = 0.04)\). M-waves were more consistent between contractions during NMES over the muscle belly compared with NMES over the nerve trunk, regardless of NMES pattern. For H-reflex amplitude (Fig. 6E), there was a significant main effect of NMES location \((F_{1,7} = 13.79, P < 0.01)\). H-reflexes were \(\sim 8\) times larger for NMES over the nerve trunk compared with NMES over the muscle belly, regardless of NMES pattern or time. However, there were no significant differences in the CV for H-reflexes (Fig. 6F). Thus, at this higher level of NMES, there were no differences in the consistency of H-reflexes between contractions across both factors.

Additional experiments. Statistical analyses were performed on group torque data \((n = \text{7})\) recorded when 25-Hz NMES was
delivered to evoke 10% MVIC torque at time1. There were no significant differences in torque across all factors (Fig. 7A). However, the CV for torque (Fig. 7B) showed a significant main effect of NMES location ($F_{1,10} = 12.13, P = 0.01$). Torque was more consistent between contractions during NMES over the muscle belly compared with NMES over the nerve trunk, regardless of NMES pattern.

**DISCUSSION**

In this study, we compared the contributions made by peripheral (M-wave) and central (H-reflex, asynchronous activity) pathways to motor unit recruitment during isometric contractions of similar amplitude generated by NMES applied over the femoral nerve trunk and the quadriceps muscle. We found that, similar to the results obtained from experiments on the TS (4), NMES location (nerve trunk vs. muscle belly) largely determined the pathways by which motor units were recruited when NMES was delivered to activate the quadriceps muscle and generate knee extension torque. However, unlike the TS and other muscles studied previously, neither torque nor activity through central pathways was augmented following 100-Hz NMES, nor was any asynchronous activity evoked during NMES at either location.

**Torque**

*Contraction amplitude.* NMES amplitude was adjusted to generate similar torque at time1 for both locations. Accordingly, torque was not significantly different during NMES over the nerve trunk compared with NMES over the muscle belly for any of the relevant comparisons in the present study.
Additionally, torque at time2 was not different from that at time1 for all relevant comparisons. Thus torque did not increase over time during constant-frequency NMES or, contrary to our hypothesis, following the brief periods of 100-Hz NMES during the step-frequency pattern. In a recent study, Thompson et al. (51) delivered stimulation over the quadriceps muscle belly using a step-frequency pattern, similar to that used presently, in nine neurologically intact participants and reported an increase in torque of 21% from before to after a period of 100-Hz NMES; whether this increase was statistically significant was not tested. This apparent increase in torque is in marked contrast to the present results in which there were no differences in torque for the same comparison. Regardless, the results of Thompson et al. (51) and those reported presently are unclear; however, we do not believe that the lack of an increase in torque in the present study was the result of a sampling bias related to the recruitment of “nonresponders.” Of the 13 participants in the present study, 6 participated in our previous study investigating similar effects in the TS (4). In the previous study, these 6 participants generated on average a 48% increase in plantar flexion torque, and presently these same participants generated on average only a 9% increase in knee extension torque. The present study was not designed to distinguish between “responders” and “nonresponders,” although such a study may shed light on neural mechanisms that distinguish these two groups, some of which are discussed below (see Pathways During NMES Over the Nerve Trunk vs. Over the Muscle Belly).

Contraction consistency. Although torque did not differ between NMES locations, the amplitude of consecutive contractions was more consistent during NMES over the muscle belly (CV ~10%) compared with NMES over the nerve trunk.
Fig. 6. Group torque and EMG data (n = 8) during NMES (15 Hz for 8 s and 15–100–15 Hz for 3–2–3 s, respectively) over the femoral nerve trunk (nerve stimulation) and quadriceps muscle belly (muscle stimulation) at an amplitude to evoke 20% MVIC torque at time1. Normalized data averaged at time1 and time2 are shown in A, C, and E. Coefficient of variation data averaged only at time1 are shown in B, D, and F. Significant main effects identified by the rmANOVA are displayed within insets.

(CV ~20%), regardless of NMES pattern or frequency (15 vs. 25 Hz) when NMES was delivered to evoke 10% MVIC torque at time1. When 15-Hz NMES was delivered to evoke 20% MVIC torque, no significant difference in contraction consistency was found. These differences in contraction consistency at lower contraction amplitudes are consistent with data from the ankle plantar flexors (2). When NMES was delivered to evoke ~5% MVIC torque in the plantar flexors, Baldwin et al. (2) found that NMES over the TS muscle belly was more consistent between consecutive contractions (CV ~10%) com-

Fig. 7. Group torque data (n = 7) during NMES (25 Hz for 8 s and 25–100–25 Hz for 3–2–3 s, respectively) over the femoral nerve trunk (nerve stimulation) and quadriceps muscle belly (muscle stimulation) at an amplitude to evoke 10% MVIC torque at time1. Normalized data averaged at time1 and time2 are displayed in A. Coefficient of variation data averaged at time2 are displayed in B. A significant main effect identified by the rmANOVA is displayed within inset.
pared with NMES over the tibial nerve trunk (CV ~20%). The variability in torque in the present study during NMES over the nerve trunk at time$_2$ may be due to the variability in H-reflex amplitude observed during this same period of time, as described in the following section.

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

The relative contributions made by peripheral and central pathways to motor unit recruitment were markedly different between NMES locations. As hypothesized, NMES over the femoral nerve trunk generated contractions with smaller M-waves (7–10 times) and larger H-reflexes (8–9 times) compared with contractions of equal amplitude generated by NMES over the quadriceps muscle belly, regardless of NMES pattern or amplitude. Thus NMES over the nerve trunk generated contractions predominantly through central pathways, while NMES over the muscle belly generated contractions predominantly through peripheral pathways. This effect of NMES location is consistent with the larger $H_{max}$-to-$M_{max}$ ratios obtained with stimulation over the femoral nerve trunk compared with stimulation over the quadriceps muscle belly. Similar to NMES over the tibial nerve (4), much of the motor unit recruitment during NMES over the femoral nerve trunk was via central pathways in the form of H-reflexes; for both muscle groups, contractions of up to 30% MVIC torque could be produced almost exclusively through this pathway in some participants. For both the TS (4) and quadriceps muscle (present study), NMES over the nerve trunk, where all the sensory and motor axons are located in close proximity to the stimulating electrodes, likely recruited a relatively greater proportion of sensory axons compared with NMES delivered over the muscle belly near the motor points, where sensory axons are more widely dispersed throughout the muscle.

Our hypothesis that there would be more asynchronous activity during NMES over the quadriceps muscle belly compared with NMES over the femoral nerve trunk was not supported. Unlike the TS (4), we recorded no asynchronous activity during NMES at either location. This is despite the fact that in pilot experiments, we were able to measure asynchronous activity during NMES that was generated voluntarily (see METHODS) and measured increases in this activity when voluntary contraction amplitude increased. Thus we do not believe that the lack of asynchronous activity recorded in the present study was due to an inability to measure it. Rather, we believe that there was no asynchronous activity generated during NMES of the quadriceps muscle. We have previously proposed that asynchronous activity is due, at least in part, to the activation of persistent inward currents in spinal neurons (16, 17). The lack of asynchronous activity in the quadriceps EMG may indicate that neurons in circuits controlling the quadriceps are less likely to exhibit this behavior.

Our second hypothesis was not supported by the present data, as neither torque, H-reflex, nor asynchronous activity increased following 100-Hz NMES during the step-frequency pattern. Increases in torque, H-reflexes, and asynchronous activity following 100-Hz NMES have been attributed to mechanisms in central circuits (4, 35), such as increased probability of neurotransmitter release from presynaptic terminals, associated with posttetanic potentiation, and/or increased motor neuron excitability, due to the activation of persistent inward currents in spinal neurons. Thus the lack of such increases in the present study indicates that there may be differences in the frequency-dependent changes in sensorimotor integration in central circuits controlling the quadriceps muscle, compared with muscles studied previously. However, small increases in torque, which are not accompanied by increases in EMG activity, may also be due to an intrinsic muscle property (8) that is dependent upon muscle length (22).

**Implications for NMES**

An interesting feature of NMES is the unique pattern of motor unit recruitment underlying the evoked contractions (5, 6, 41). Unlike voluntary contractions, when motor unit recruitment is temporally asynchronous, spatially diffuse (1), and orderly from slow-fatigue-resistant to fast-fatigable with increasing contraction amplitude (28, 29, 33), it is generally accepted that motor unit recruitment during NMES, at least when applied over the muscle belly, is temporally synchronous (1, 41), mainly, but not exclusively (1), superficial (41, 45, 49, 54), and occurs randomly without obvious sequencing related to motor unit type (1, 26, 33, 41). As a consequence, the capacity to produce repeated contractions that do not fatigue rapidly with NMES over the muscle belly is compromised compared with voluntary exercise (6, 24, 34, 41). This may be particularly relevant for the quadriceps where fatigue-resistant motor units are mainly in deeper compartments of the muscle (36, 40) and thus are more difficult to activate during NMES over the muscle belly compared with voluntary contractions, even at rather high NMES amplitudes (41, 49). Despite this, hypertrophy of fatigue-resistant muscle fibers in the quadriceps has been reported, but such adaptation requires a high volume (4 h/day, 7 days/wk; Ref. 47) or amplitude of training (~60% MVIC; Ref. 24), the former of which may not be practical to achieve as part of a long-term exercise program and the latter of which can be problematic for individuals with residual sensation (50) or compromised bone density (21).

Generating contractions by stimulating over the femoral nerve trunk may alleviate some of these issues. First, NMES over the nerve trunk required significantly less current than NMES over the muscle belly. Second, increases in NMES amplitude in the present study were limited, in every case, by discomfort during NMES over the muscle belly. Thus NMES over the femoral nerve trunk produces contractions that require less battery power and generate less discomfort for the participant; however, there is evidence that NMES over the femoral nerve trunk generates more discomfort for the participant (44, 49), and thus this line of inquiry requires further investigation. Third, both in vivo (20) and computational modeling (43) data support the idea that NMES recruits motor units randomly in relation to axon diameter, in which case motor units recruited as M-waves during NMES over the nerve trunk would be expected to be randomly distributed throughout the muscle. Finally, motor unit recruitment through central pathways follows Henneman’s size principle (28, 29) and thus recruits fatigue-resistant motor units first. These fatigue-resistant muscle fibers are located deep in the quadriceps muscle (36, 40) and may therefore be less accessible during NMES over the muscle belly (45, 49, 51). Thus contractions mediated through central pathways should minimize the nonphysiological recruit-
off the quadriceps femoris, one of the most utilized muscle through central pathways during NMES-evoked contractions.

Conclusion

would require less precise control of evoked contraction am-

apeutic purposes (TES), such as muscle conditioning which

nerve trunk would be most immediately beneficial for thera-

central pathways. Thus, overall, it may be that NMES over the

injury (7, 14, 21, 27). Consequently, the greater recruitment

from atrophy and transformation to fast-fatigable units, a common

occurance after periods of inactivity as the result of spinal cord

muscle belly, in the present study, suggests that NMES over the

femoral nerve trunk holds promise for maintaining muscle quality

(therapeutic electrical stimulation; TES) and possibly for produc-

functioning movements (functional electrical stimulation; FES)

following damage to the CNS compared with NMES over the

Despite these promising theoretical advantages of delivering

over a nerve trunk, there are potential practical limita-

stimulating the femoral nerve trunk for FES. First, the position

of the cathode in the femoral triangle is highly sus-

ceptible to movement as a result of the contraction itself, due

the nearby tendon, and as a result of limb movements,

making it difficult to deliver consistent current. Second, even

movement of the NMES electrode can be minimized, contrac-

tions generated through central pathways are less consistent

between successive contractions compared with contractions

generated through peripheral pathways (2); however, this may

only be the case at lower NMES amplitudes. Although con-

traction stability within a contraction was not quantified in the

present study, we did observe instances in which torque oscil-

lated during NMES over the femoral nerve trunk. These

oscillations in torque occurred simultaneously with oscillations

in H-reflex amplitude, similar to that to which we have observed

for soleus H-reflexes (15). Third, it is unclear whether contrac-

tions with a significant contribution through central pathways

will be of sufficient amplitude for FES applications, although

presently we show such contractions up to 30% MVIC torque.

Fourth, during FES-assisted movements, it may be that motor

unit recruitment through central pathways would diminish, as it

is well known that H-reflexes reduce in size during passive and

voluntary movement (10, 11, 18, 31); however, such H-reflex

modulation is reduced or absent in people with spinal cord

injury (37). Finally, as NMES amplitude is increased beyond

what was tested in the present study, for example, in response

to fatigue during FES-assisted exercise, increased levels of

antidromic transmission in motor axons will develop (55),

which will progressively block motor unit recruitment through

central pathways. Thus, overall, it may be that NMES over the

nerve trunk would be most immediately beneficial for thera-
pie purposes (TES), such as muscle conditioning which

would require less precise control of evoked contraction am-

plitudes, until some of the anticipated limitations associated

with NMES over the femoral nerve trunk for functional move-

ment applications (FES) can be addressed.

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

Author Contributions

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the study; A.J.B. data collection; A.J.B., M.J.W., and D.F.C. data analysis;
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References

2. Baldwin ER, Klakowicz PM, Collins DF. Wide-pulse-width, high-

frequency neuromuscular stimulation: implications for functional electri-

9. Blouin JS, Walsh LD, Nickolls P, Gandevia SC. High-frequency sub-

11. Brooke JD, McIlroy WE, Collins DF, Misiaszek JE. Mechanisms within the human spinal cord suppress fast reflexes to control the move-


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