Motor unit pool organization examined via spike-triggered averaging of the surface electromyogram

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Abstract

Voluntary muscle force control is accomplished both by recruitment of motor units (MUs) and by firing rate modulation of active MUs. Typically, MU recruitment and firing rate organization is assessed using piecemeal intramuscular recordings drawn from different experiments, or even from different subjects. As a consequence, it is often difficult to assemble a systematic description of the relations between the different MU properties relevant to the control of muscle force. To address this gap, the objective of our current study was to characterize recruitment and firing rate organization of multiple MUs of differing action potential size, recorded simultaneously from the first dorsal interosseous muscle of intact human subjects, using a recently developed surface electromyogram (EMG) sensor array recording and decomposition system (Delsys). We sought to assess the relation between putative MU size and the recruitment and firing properties for these MUs, recorded at different muscle contraction levels. Spike-triggered averaging (STA) of the surface EMG was performed to estimate the action potential sizes using the firing times of discriminated MUs as the event triggers. The results show that the size principle, which relates MU size to recruitment rank order, was clearly evident during individual force contractions. In addition, the mean firing rate across MUs decreased with increasing size of the MU action potential and was also inversely proportional to the recruitment threshold force. We propose that surface EMG recordings together with advanced decomposition systems, combined with STA methods, may provide an efficient way to systematically examine MU pool organizational properties.

Keywords: surface EMG, size principle, spike-triggered averaging, rate modulation, motor unit decomposition

IN HUMANS, VOLUNTARY MUSCLE force generation is thought to be accomplished by the activation of progressively larger motor units (MUs), as well as by increasing the discharge rate of previously active MUs. On the basis of studies performed on animal models (Henneman 1957; Henneman et al. 1965), it is
widely believed that the recruitment of motoneurons (MNs) is linked to MN size such that, with increasing
excitatory synaptic drive to the MN pool, smaller MNs are recruited first and larger MNs are recruited later.
Similarly, an orderly relation between the recruitment threshold and MU size has also been reported in cat
muscles (Olson et al. 1968) and in human muscles (Goldberg and Derfler 1977; Milner-Brown et al. 1973;

In many intact preparations, MN size cannot be determined by direct measurements, so to validate the size
principle, MN size is often estimated using surrogate measures, such as the estimated MU force-twitch
amplitude (Burke 1967; McPhedran et al. 1965; Milner-Brown et al. 1973) or the peak-to-peak (P-P)
amplitude of the motor unit action potential (MUAP) recorded using intramuscular or surface electrodes
(Olson et al. 1968). Estimates of MU size derived from intramuscular recordings are limited by the
constraints of the intramuscular method in that a relatively small number of MUs are recordable at any
given time. In addition, the intramuscular MUAP size by itself is not a robust marker of MU size, since it is
strongly dependent on the distance of the MU from the recording site, perhaps distorting the estimates of
MU size based on the MUAP size.

In surface electromyogram (sEMG) recordings, it is often difficult to identify and discriminate the
occurrence of action potentials from single MUs consistently, except at very low forces, due to the fact that
the sEMG signals recorded from most electrodes have a lower bandwidth and longer-duration MUAPs than
do intramuscular recordings of MUAPs. This constraint, as well as the larger recording field, leads to a
greater amount of MUAP superposition as the force level increases.

To obtain global estimates of the MUAP amplitude, Milner-Brown and Stein (1975) recorded the sEMG
and intramuscular EMG simultaneously from the first dorsal interosseous (FDI) muscle in intact human
volunteers. The P-P amplitude of the MUAP was extracted from the sEMG using the spike-triggered
averaging (STA) method, with the intramuscular MU spikes used as the “triggers” for averaging the sEMG.
The STA is a type of waveform-averaging method, in which multiple segments of a recorded signal are
time-aligned based on a set of events or triggers (MN firings in this case), and then a time epoch around the
trigger is averaged across all the events in an effort to attenuate the interference of other MUs that are not
time-locked and to reduce the interference of recorded noise. Using the STA method, Milner-Brown and
Stein (1975) reported an association between the computed P-P MUAP amplitude and the threshold force
at which the MU was activated. Although the correlation coefficients of the P-P amplitude vs. threshold
force relation for the five subjects were positive, they were not as strong as the correlations observed for
the twitch tension vs. threshold force relation. It is unclear whether the lack of correlation in this study was
attributable to a signal processing issue or was a function of the basic organization of MU recruitment
during increasing force.

To explain further, the STA-based estimate (Milner-Brown and Stein 1975) of the MU size recorded on the
surface electrode may have been modified due to one or more barriers, such as diminished amplitude of the
corresponding sEMG signal or small offsets in timing (between the intramuscular and surface recorded
potentials). As mentioned above, with the use of this STA method, only a small number of MUs can be
detected during each testing session with each intramuscular electrode; therefore, the size principle was
examined, necessarily, by combining recorded MUs from multiple contractions of different force levels.
The method is further limited by the time-consuming process of MU extraction and by the fact that the
force is constrained to be held at relatively low levels to enable MU decomposition from the intramuscular
recordings and to avoid excessive MUAP superposition on the sEMG.

To address these limitations, our first objective was to determine whether the size principle can be
discerned in a relatively large MU sample of simultaneously activated MUs recorded during a single force
production task (in which force is rising progressively during the ramp phase of the contraction) in muscles
of neurologically intact humans. To achieve this, we used a recently developed sEMG sensor array
combined with a MU decomposition method (De Luca et al. 2006; Nawab et al. 2010) that together yield a large number of MUs, recorded simultaneously over a relatively large force range. This system is described further in METHODS. The use of this surface sensor array with the decomposition method combines the advantage of a larger and more uniform recording field of sEMG recording with the ability to identify individual MUAP waveforms.

Another mechanism to increase muscle force output is to modulate the discharge rate of active MUs (rate modulation). There are two competing observations regarding the firing rate patterns recorded in a MU pool. The first is that earlier-recruited (and presumably smaller) MUs tend to fire at a higher rate than the later-recruited larger MUs, giving rise to the so-called “onion-skin” property, as reported in both cat (Hoffer et al. 1987) and human muscles (De Luca et al. 1982a; Freund et al. 1975; Tanji and Kato 1973) during voluntary contractions. In contrast, other researchers have reported that the later-recruited MUs tend to fire at a higher rate during isometric force generation, such as during reflex activation of cat muscles (Burke 1968; Kernell 1965) and during voluntary activation of human limb muscles (Grimby et al. 1979; Moritz et al. 2005; Oya et al. 2009).

In these referenced studies, investigators examined the relationship between MU firing rates and MU recruitment thresholds. To our knowledge, there has been no systematic examination of the relationship between MU firing rate and MU size (as approximated by the MUAP amplitude) in human subjects. Accordingly, a second focus of our current study was to investigate the relationship between MU firing rates, during steady-state isometric force tasks, as a function of MU size (MUAP P-P amplitude).

To verify and quantify the surface representation of the MUAPs, we developed an analytical method whereby a waveform average of the MUAP was constructed utilizing the MU spike times derived from the decomposition system, i.e., an STA-derived MUAP estimate; we describe our analytical techniques in detail in METHODS.

Using these methods, and assuming an orderly relation between MUAP size and MU size, we are able to confirm the size principle consistently in all tested subjects and at each tested force level. In addition, we consistently observed an inverse relation between MU firing rates and MU size during steady-state force generation.

METHODS

Participants

Eight right-dominant neurologically intact individuals (4 men, 4 women) volunteered to participate in this study. The EMG activity of the FDI muscle was examined during isometric abduction of the right index finger about the second metacarpophalangeal (MCP) joint. All participants gave informed consent via protocols approved by the Institutional Review Board under the Office for the Protection of Human Subjects at Northwestern University.

Experimental Setup

Participants were seated upright in a Biodex chair with their upper arm comfortably resting on a plastic support. To standardize hand position and to minimize contributions of unrecorded muscles, the forearm was cast and placed in a ring-mount interface attached to an elbow rest. This rest was securely mounted with magnetic stands to a heavy steel table. The forearm was placed in full pronation, and the wrist was held neutral with respect to flexion/extension. The little, ring, and middle fingers were extended away from the index finger and strapped to the support surface. The thumb was secured at an ~60° angle to the index finger. The index finger was placed in line with the second metacarpal and the long axis of the forearm, creating a 0° or neutral (abduction/adduction) MCP joint angle (Fig. 1A). The proximal phalanx of the
Data processing.

The index finger was cast and fixed to a ring-mount interface attached to a 6 degrees-of-freedom load cell (ATI; no. 3226). The recorded forces from the $x$ (abduction/adduction) direction were low-pass filtered (cutoff = 200 Hz) and digitized at a sampling frequency of 1 kHz. The subjects were instructed to produce required abduction forces while minimizing the off-axis forces. The force in the $y$ direction (flexion/extension), which was outside the desired force generation direction, was also monitored by the experimenter.

**EMG Recordings**

The subject's skin was prepared by cleaning the superficial layers with adhesive tape, and the skin was then cleaned with alcohol pads to ensure proper electric contact and low baseline noise. sEMG was recorded from the FDI (Fig. 1B) using a surface sensor array (Delsys) that consisted of five cylindrical probes (0.5-mm diameter) as shown in Fig. 1C. The probes are located at the corners and at the center of a $5 \times 5$-mm square. Pairwise differentiation of the five electrodes yields four channels of sEMG signals (Fig. 1D). The sEMG sensor and a reference electrode were connected to four channels of a Bagnoli sEMG system (Delsys). The signals were amplified and filtered with a bandwidth of 20 Hz to 2 kHz. The signals were sampled at 20 kHz and stored on a computer for decomposition processing.

**Procedures**

For the first trial of the experimental session, subjects were asked to perform maximal voluntary contractions (MVCs) for 3 s. This maximum contraction was repeated three times in total, with 1 min of rest between trials. The largest value of the three trials was designated as the MVC. The rest of the session consisted of a series of isometric voluntary contractions during which the subject was asked to follow trapezoid force trajectories displayed on a computer screen, each at a different varying percentage of the MVC. The force output in one exemplar trial is shown in Fig. 1E. The trapezoid trajectory contains five segments: a 5-s quiescent period for baseline noise calculation, an up-ramp increased at a rate of 10% MVC/s, a constant force of the prescribed %MVC for 12 s, a down-ramp decreased at 10% MVC/s, and a 3-s quiescent period. To ensure that the subjects could trace the trapezoid trajectory closely, subjects practiced five trials of a 30% MVC constant-force trapezoid before performing the main experiment. For the main part of the experiment, the subjects performed four blocks of trials with five repetitions of each block. Four constant force levels (20%, 30%, 40%, and 50% MVC) were tested, and each block contained one force level. The order of the force levels was randomized for each subject. A 30-s rest period between repetitions was provided.

**Data Analysis**

Data processing. The sEMG and force trials were selected for further analysis on the basis of the following criteria: 1) there was no sudden change (i.e., larger than 20% MVC/s) in the up-ramp force, 2) the force variability during the steady-state hold was low (within ±2SD of background force level), and 3) the sEMG signal had a peak-peak baseline noise $<$20 μV and signal-to-noise ratio (SNR) $>$5. The SNR was calculated on the basis of the peak-peak amplitude of the baseline noise and peak-peak amplitude of the EMG signal at steady state.

These criteria were based on the Delsys recommendations for robust MU discrimination using the sEMG decomposition system (version 1.0.0.28). For each subject, based on the preceding criteria, two trials were selected at each force level for further analysis.

For the EMG data recorded from these trials, the decomposition algorithm was used to extract discriminable MUs. The decomposition algorithm consists of two stages. The first stage involves MUAP template creation, matching, and updating. First, the algorithm identifies as many template shapes as possible from the peaks of the EMG data. When similar shapes are identified consistently for a large
number of times, a template is initiated by averaging the similar shapes. Second, the matching of MUAP templates goes through a maximum a posteriori probability classifier that uses the information of the correlation between the templates and the EMG signal, the amplitude of action potential, and the remaining energy of the EMG signal. Last, when a new instance of MUAP is identified through the matching procedure, the MUAP templates are updated through a weighting process, in which a larger weighting is assigned to the existing old template and a smaller weighting is assigned to the new action potential shape. The second stage identifies MUAPs within complex superposition in the EMG signal. Multiple MUAP templates are iterated through a discrimination analysis to determine the combination of MUAP templates that matches the signal shape, and, meanwhile, the coefficient of variation of the interspike interval (ISI) of these MUs reaches a minimum. It is worth noting that not all the MUAPs embedded in the EMG signal are identifiable, especially for those small MUAPs under the influence of background noise and extensive superposition. Detailed information for the decomposition algorithms is described by Nawab et al. (2010).

For each identified MU, the output from this algorithm consisted of the firing times for the rising, falling, and steady phases of the isometric force tasks. In addition, for each identified MU, four MUAP templates (from each of the 4 recorded sEMG channels) were also available. The decomposed MU templates, utilized for MU discrimination in the algorithm, were used in this study to provide information about the MU shapes but are not necessarily representative of the actual MUAP signal amplitude (Fig. 2D). Therefore, we utilized the MU events output by the algorithm to derive an STA MU template, a means by which we could also confirm the accuracy of the decomposition output addressing concerns raised in earlier publications (Farina and Enoka 2011).

One of the strengths of the decomposition-derived MU template is that it is not as susceptible to the waveform distortions as the STA of the recorded EMG signal would be, such as the influence of signal correlated “noise.” In contrast, the STA MUAP representation is derived directly from the recorded raw EMG signal, thereby providing us with MUAP amplitude information. The raw EMG is a stochastic signal, the amplitude of which can be represented by a Gaussian distribution. Thus, on average, the EMG signal portion that is not time locked to MU events will contribute minimally to the MUAP estimate.

To combine the advantages of MUAP estimates derived from both methods, as detailed in STA template estimation, we derive a group of MUAPs for each trial that could be used to assess the relationship between MUAP amplitude, recruitment threshold, and MU firing rate.

**STA template estimation.** To characterize the MU waveform (amplitude and shape) recorded from the electrodes, a STA was performed on each of the four channels of the raw sEMG signals, resulting in four representative waveform estimates for each MU identified by the decomposition procedure. The identified firing times for each MU were used as triggers for the STA calculation. The time interval used to derive the template estimate was set at 10 ms before and after the firing time; the firing time was at the (algebraic) center of the time window. The firing events of the entire trial were used for the STA template estimation.

To ensure reliable estimate of MUAP P-P amplitude, we then performed two separate tests to determine which MUs would be retained for further analysis. These tests were designed to assess the stability of the waveform over the trial duration.

First, the STA template variation over time was calculated based on a window length of 8 s. This window yielded ~100–200 firing events in each window, and the window was shifted over the sEMG signal, using a step size of 1 s. The coefficient of variation (CV) of the P-P amplitude of the STA MUAP templates, for each step change, was calculated as a measure of the stability of the waveform average and the firing time estimation. The templates estimated at different windows are shown in Fig. 2C (average CV of P-P across the 4 channels is 0.04 for the exemplar MU that was selected).

Second, the MUAP estimates derived using STA were compared with the decomposition-estimated
templates. The maximum linear correlation coefficient between the STA estimate (calculated over the entire trial duration) and the decomposition-estimated templates was computed as a second measure of the reliability of the STA estimates of the MUAP.

A high correlation between these two estimates signifies the reliability of the STA waveform average. The STA template was shifted 10 ms backward and forward relative to the decomposition template to find the maximum correlation coefficient. The four channels of templates of three exemplar MUs are shown in Fig. 2A (average correlation coefficient across 4 channels is 0.96), 2B (average correlation coefficient is 0.79), and 2D (average correlation coefficient is 0.89).

A low correlation between the STA and decomposition estimates can arise from multiple factors. The discrepancy could reflect errors in the STA template, which could be due in turn to a large number of superpositions that contaminate the STA derived estimate, usually affecting the smallest MUAPs. Spurious timing errors or inaccurate positioning of the MU timings from the decomposition system could also distort our STA estimates, in which case we would not be able to recreate the template using the STA method. Finally, the weighting of templates by the sEMG decomposition algorithm takes into consideration the varying nature of templates, weighted according to the most likely template shape for a given MUAP. Thus our nonweighting procedure in STA could contribute to the discrepancies between STA and decomposition templates for those MUAPs with some amount of variability.

A large number of identified MUs exhibited a strong correlation between the STA-derived MU waveform average and the MU template given by the decomposition algorithm (see RESULTS) for every subject at each of the specified force tasks.

**MU selection criteria.** To avoid bias of the signal amplitude distribution, the average correlation coefficient between the STA MUAP and MU template, as well as the average CV of P-P amplitude derived from each of the four channels, was used to qualify each MU for the process of selection of the set of MUs that were used for further analysis. For each identified MU, the combined results from all four channels was used only for the MU selection process; note that subsequent analysis of the selected MUs was performed on a per channel basis.

The MUs with a correlation coefficient (between the STA MUAP estimate and the decomposition MU template) >0.7 and CV of P-P <0.3 across all four channels were selected for later analysis (see Sensitivity analysis of the selection criteria and DISCUSSION for the criteria selection justification). The correlation coefficient as a function of CV of P-P is shown in Fig. 3. For each channel, one open symbol represents one MU and different types of open symbols represent different force-level conditions. The black dots represent the excluded MUs, and the solid lines represent the inclusion criteria. The four channels represent the four different templates of the same MUs. Note that the selected MUs from a particular channel may have a correlation coefficient ≤0.7 or CV of P-P ≥0.3, because the selection criteria were based on the average of four channels. A sensitivity analysis was performed to examine the selection criteria in relation to the dependent variables.

**Recruitment threshold and mean firing rate.** To estimate the recruitment threshold, the threshold force of the selected MU was calculated from the averaged isometric force data (as a percentage of MVC) over an interval (~50 to 150 ms relative to the instance of the first firing event). An averaged force was calculated to reduce the influence of force fluctuations registered at the load cell. The window was asymmetric relative to the firing time because of the delay between the occurrence of an action potential and a registered force increment. The mean firing rate (MFR) was calculated from a 4-s averaging window in which the firing rate was stable and the force was relatively constant at the steady stage. The P-P amplitude of the STA template was computed as a measure of the estimated size of the MU.

The relation between the size (P-P amplitude of the MUAP) of the MU and the recruitment threshold of the
Statistical analysis. Given that there were four different P-P amplitude estimates (one from each channel), and that the four P-P amplitudes may differ depending on the relative position of the electrodes over the FDI, a least-squares linear regression of the P-P MUAP amplitude as a function of threshold force was performed for each channel. To avoid potential bias (e.g., the recording fidelity of each electrode for a given MU may differ) of an individual channel, the average of the fit parameters (e.g., slope or r²) from the four channels was reported as the representative parameter at a particular force level.

Similarly, an inverse power function was fit to the MFR as a function of the MU size:

\[
MFR = b \cdot (P-P \text{ amplitude})^a
\]

where \( b \) is the scale factor and \( a \) is the power or rate of decay (reduction). Similarly, the averaged fit parameters over the four channels were reported at the different force levels.

RESULTS

Our key findings are that for our pool of simultaneously recorded MUs in the FDI muscle, the P-P amplitude of MUAPs recorded from the skin surface increased in an orderly manner with increasing recruitment force, affirming a key attribute of the size principle. Furthermore, the MFR at a specified force level decreased as a function of P-P MUAP amplitude (i.e., MU size). Consistent with these results, we also found that the firing rate of the smaller (earlier recruited) units was systematically higher than that of the larger (later recruited) units for individual force tasks at virtually all forces examined, affirming the onion-skin property.
The total numbers of MUs that were decomposed by the algorithm are shown in parentheses. On average, 90.28%, 85.94%, 87.26%, and 82.98% of the decomposed MUs were analyzed at 20%, 30%, 40%, and 50% MVC, respectively.

Single Trial vs. Single Force Level Analysis

During initial analysis, regression of relations between the dependent variable pairs (P-P amplitude vs. MU recruitment, P-P amplitude vs. MU firing rate, and MU recruitment vs. firing rate) was performed for individual trials that consisted of concurrently active MUs as well as at a single force level that pooled active MUs from two trials at the same contraction level. Single-trial analysis leads to two sets of regression parameters at each force level for each examined functional relationship. Averaging of the two sets of parameters (i.e., slope and \( r^2 \)) was then performed at each force level. In contrast, the single-force level analysis only leads to half the set of regression parameters, simplifying the data reporting for four channels and eight subjects at four contraction levels. The fit parameters in single-trial and single-force level regression between the P-P amplitude and threshold force are shown in Table 2.

The regression parameters were similar between these two analyses, and, in some cases, the single-trial regression even exhibited a stronger fit than the single-force level regression. To simplify the presentation of the results, only statistical reports using the single-force level regression are presented; however, in describing some of our results, we will refer to single-trial outcomes.

Threshold Force vs. P-P Amplitude

Figure 4 shows the threshold force as a function of the P-P amplitude at the four force levels for two individual subjects. The fit lines at each force level of each channel are also plotted. There was a strong linear relation between the threshold force and P-P amplitude across different force-level conditions and in each of the four channels. MUs with larger P-P amplitudes and higher threshold forces were recorded regularly at higher levels of muscle contraction. At large force levels, the smallest MUAPs as observed in low-force tasks were not found, possibly because of the influence of background noise and extensive superposition. Nonetheless, the linear relation still held as shown by the \( r^2 \) values at the four force levels.

The average slopes and \( r^2 \) value over the four channels and eight subjects are shown in Fig. 5, A and B. The error bars represent the SE across subjects. All the fit slopes were significantly different from zero (\( P < 0.05 \)). The slopes were also significantly different between force levels [\( F(3,21) = 4.26; P = 0.017 \)]. A post hoc analysis revealed that the slope at 20% MVC was significantly shallower than that at 40% and 50% MVC conditions (\( P < 0.05 \)). The fit \( r^2 \) values at the four force levels were 0.46 ± 0.03, 0.53 ± 0.02, 0.52 ± 0.03, and 0.55 ± 0.05, respectively.

MFR vs. P-P Amplitude

Figure 6 depicts the firing behavior in relation to the size of the MUAP at the four force levels for two exemplar subjects. The fit inverse power functions determined using Eq. 1 at each force level of each channel are also plotted. The results show that the MFR across the detected MUs decreased at a decelerating rate with increasing P-P amplitude of the MUAP. The rate of decay of the MFR as a function of the P-P amplitude also tended to differ between the different force levels. For instance, in subject 2 (Fig. 6B), the MFR at 40% and 50% MVC conditions decreased more slowly than the 20% MVC condition, which was consistent across the four different channels. The slower rate of decay of the MFR as a function of the P-P amplitude at higher levels of muscle contraction resulted in an overall higher firing rate for the same sizes of MUs.

The average fit parameters for the MFR vs. P-P amplitude relation, including the rate of MFR reduction, the scale factor (coefficient \( b \) as in Eq. 1), and \( r^2 \) over the four channels and eight subjects are shown in
The rate at which the MFR decreased as a function of P-P amplitude (Fig. 7A) differed significantly between contraction levels \( F(3,21) = 5.21; P = 0.008 \). Specifically, the reduction of the MFR vs. P-P relation in the 20% and 30% MVC conditions was significantly larger than for the 40% and 50% MVC conditions \( (P < 0.05) \).

Similarly, the scale factors (Fig. 7B) at the 20% and 30% MVC contractions were also significantly smaller than those derived for the 40% and 50% MVC contractions \( (P < 0.05) \). The larger scales at higher force-level conditions indicated that the firing rates of the MN pool covered a larger range, which could potentially result from a larger range of recruited MUs. The fit \( r^2 \) values (Fig. 7C) at the four force levels were 0.41 ± 0.03, 0.52 ± 0.04, 0.45 ± 0.05, and 0.49 ± 0.07, respectively.

**MFR vs. Threshold Force**

**Figure 8.** A and B, shows the firing rate as a function of the threshold force at the four force levels for two exemplar subjects. The MFR decreased with higher threshold forces across the different contraction levels. Combining these findings with the size principle results (Fig. 4) shows that the earlier-recruited smaller MUs tended to fire at higher rates than the later-recruited larger MUs. Additionally, a slower (i.e., shallower slope) rate of reduction of MFR as a function of the threshold force was evident at higher force levels.

The group-average fit parameters are shown in Fig. 8, C (slopes), D (y-intercept), and E \( (r^2) \). All the fit slopes were significantly different from zero \( (P < 0.05) \). ANOVA results revealed that the slopes were significantly shallower at higher force levels \( F(3,21) = 15.99; P = 0.001 \). In particular, the slope at 20% MVC was significantly steeper than at other force levels \( (P < 0.05) \), and the slope at 50% MVC was significantly shallower than at other force levels \( (P < 0.05) \). The y-intercept at 50% MVC was also significantly higher than 20% MVC \( (P < 0.05) \), which means that the fit line was shifted upward and the MFR was higher at higher force levels. The fit \( r^2 \) value was consistently high across force levels and was 0.74 ± 0.05, 0.70 ± 0.03, 0.73 ± 0.02, and 0.72 ± 0.05 at the four force-level conditions, respectively.

**MUAP Duration vs. Threshold Force**

To examine whether the estimated MU size and recruitment threshold were associated with the depth of the MU within the muscle, as estimated from action potential duration, the P-P duration of the MUAP was estimated and compared with the recruitment threshold. The P-P durations as a function of the threshold force at the four force levels for two exemplar subjects are shown in Fig. 9, A and B. There was no visible correlation between the MUAP duration and threshold force, suggesting that depth of MU location within the muscle, which is linked to the P-P duration, did not bias our assessment of MU size. The average correlation across all the subjects was −0.058 ± 0.035. The two-tailed \( t \)-test showed that the correlation was not significantly different from zero \( (P > 0.05) \).

**DISCUSSION**

The objective of this study was to examine the relation between MU size and the recruitment and firing properties of a substantial population of MUs recorded simultaneously from the FDI muscle at different isometric force contraction levels. Our results show that the MU size, estimated from the P-P amplitude of the MUAP, increased linearly with MN recruitment threshold (estimated from the recorded muscle force at MU recruitment). Conversely, the MFR at a specified force level decreased as a function of P-P MUAP amplitude (i.e., MU size).

**Orderly Recruitment Based on MU Size**

After the landmark study articulating the size principle (Henneman 1957), a series of investigations have
been conducted examining MU recruitment order during force generation in human muscles. In one such study, Milner-Brown et al. (1973) estimated the twitch forces generated by individual MUs in the human FDI muscle during steady-state isometric force tasks, using very low MU firing rates to minimize the degree of fusion of the tetanic unit contraction. They reported that the amplitude of the MU twitch force scaled linearly with the total muscle force at MU recruitment. Utilizing the STA method, triggered by MU event times recorded (simultaneously) from intramuscular electrodes, the same group observed that the P-P amplitude of the MUAP varied as the square root of the MU threshold force (Milner-Brown and Stein 1975). (Note that the linear relation in Fig. 2 of their report was in double logarithmic scale.)

Consistent with these earlier reports, our current study confirms the orderly recruitment of MUs according to their size, based on MUAP size. However, unlike the square root scaling shown by Milner-Brown and Stein (1975), our results show a linear scaling relation between P-P amplitude and threshold force. This apparent steeper scaling relation (linear vs. square root) found in our current study could have arisen from several factors.

First, in our current study, the maximum threshold forces (which are smaller than the task force) were between 12 and 16 N. In contrast, in the Milner-Brown study, the threshold force reached up to 20 N. It is possible that the linear scaling we report holds at a limited range (low to moderate) of force levels, and with further increases in force, the estimated size of the MU may increase more slowly and eventually plateau, leading to an exponent lower than 1.

Second, in our current study, each linear function (P-P amplitude vs. threshold force) was fit for a single force task performed by an individual subject, allowing us to derive an amplitude-threshold relation for a substantial group of units recorded at the same time. In contrast, the log-linear function (Milner-Brown and Stein 1975) was fit for MUs that were recorded during various tasks at different force levels and thus used MUs recorded across different trials. In short, the different ways of grouping the data may have contributed to the apparent discrepancies observed between these two studies.

**Inverse Relation Between MFR and MU Size**

Although MU size increases with recruitment threshold, our data also shows an inverse relation between MFR and MU size as well as between MFR and MU recruitment threshold, affirming the presence of “onion skinning,” as described in earlier studies (De Luca and Hostage 2010; De Luca et al. 1982b; Tanji and Kato 1973). This inverse relation between MFR and MU recruitment threshold holds across a large force range and across different muscles.

It is important to also note that the decomposition algorithm itself does not impart the inverse MFR vs. recruitment threshold structure (De Luca and Contessa 2012). Specifically, when simulated MU firing times were shuffled to remove the onion-skin property, the decomposition algorithm did not produce an onion-skin output (De Luca and Hostage 2010).

The onion-skin property, which shows that for a given force during an isometric ramp task, mean firing rates are higher in lower-threshold MUs than in higher-threshold MUs, has not been universally supported in earlier studies of human muscles. Several studies have shown that MU firing rates correlate positively with the recruitment threshold in that later-recruited larger MUs tend to fire at a higher rate (Grimby et al. 1979; Moritz et al. 2005; Oya et al. 2009). Essentially, this latter organization would maximize total force generation by driving putative fast-twitch units at higher rates. Thus firing rate modulation would be well-matched to the contractile properties of the MU muscle fibers.

On the other hand, the fast-twitch units are more fatigable than the slow ones, and high firing rates may not be sustained. De Luca and Hostage (2010) hypothesized that the onion-skin organization leads potentially to the existence of force reserves and to reduced fatigue, since the larger (potentially fast-fatigable fibers)
MUs are driven at a submaximal rate.

**Task-Related Onion Skinning**

The contrasting results between studies that show onion skinning, including the present study, and those that show the reverse could be due to differences in data collection and analysis methods. In particular, in our study, the relations between MFR and recruitment threshold, as well as MFR and estimated MUAP amplitude, are derived from a single (linearly increasing and hold) force task performed by an individual subject. We assess the relative activation of simultaneously driven MUs, using a subset of the MU pool that is activated for a given task. The firing rate patterns recorded between lower and higher threshold units may therefore reflect a neural control strategy for an individual ramp tracking task and/or the synaptic drive required to generate an isometric force trapezoid.

In contrast, when multiple MUs are grouped together across multiple force tasks, the onion-skin property is not as clear. Analysis of our present data suggests that this very property holds in our case. For example, as shown in Fig. 10, the high-threshold MUs in box B at a high force level discharged at the same rate as the low-threshold MUs in box C at a low force level. Similarly, MUs with high recruitment threshold in box B discharged at even higher rates than lower-threshold MUs in box A. Indeed, when the plot of MFR vs. recruitment threshold is generated by utilizing grouped MUs from multiple force levels, MUs of different recruitment thresholds discharge at similar rates, and in certain cases, MUs recruited at higher %MVCs in one task can discharge at higher rates than MUs recruited at lower %MVCs in another task. In short, alterations in the normal onion-skin order occur when MUs are compared across force tasks, rather than within a task (De Luca and Hostage 2010).

**Validity of P-P MUAP as an Estimate of MU Size**

In human muscles, MU size is typically estimated using indirect measures, such as the estimated MU force-twitch amplitude (Burke 1967; McPhedran et al. 1965; Milner-Brown et al. 1973), the amplitude of the MUAP (Olson et al. 1968), or the conduction velocity of motor axons (Kadrie and Brown 1978). In the current study, we used the P-P amplitude of the MUAP derived from the sEMG as an estimate of the MU size. One potential confound is the depth of the MU within the muscle, in that deeper MUs may show smaller action potential sizes recorded at the skin surface. To counter this concern, using intramuscular recordings in human FDI muscle, Milner-Brown and Stein (1975) have shown that MUs of different sizes are fairly uniformly distributed throughout the muscle and that the duration of the MUAP, a measure of depth, is independent of the recruitment threshold. Similar results from a variety of animal studies have also shown that muscle fibers belonging to a single MU are distributed in a relatively uniform manner throughout a broad region and are interspersed with fibers from other MUs (Armstrong et al. 1988; Bodine et al. 1987; Buchthal et al. 1957).

One relevant MUAP attribute to help assess the role of MU depth is the reported increase in MUAP duration recorded on the surface as a function of recording depth (Fuglevand et al. 1992). Our results show that there was no correlation between MUAP duration and threshold for all of our identified MUs across all force levels, which would suggest that for this study, the recorded MUAP amplitude is legitimately a representative of MU size.

Our STA-derived MUAP estimates could be subject to additional error due to superimposed MUAPs attributable to synchronized firing across MUs (i.e., overestimating the MUAP amplitude). To combat this possibility, our MU selection criteria were designed to reduce the potential distortions due to the influence of superposition. For example, we performed waveform averages using a moving window that covered a segment of the EMG signal. We would expect to see an increase in the MUAP amplitude in a particular window if superposition of multiple MUs occurred in that window, and subsequently, an increase in the CV
of the P-P amplitude across windows (segments of EMG) would also be expected. Those MUs with unstable MUAPs due to superposition would be removed from further analysis due to a CV of P-P greater than the acceptance criterion detected for the MU.

In addition, the emergence of a distorted MUAP shape due to superposition would also reduce the correlation between the STA-derived and decomposition algorithm-derived MUAP templates, since the MUAP templates have a much reduced chance of the same distortion arising due to superposition.

**Decomposition Accuracy and STA Estimates**

The main advantage of the current sEMG decomposition method is that it yields a large number of MUs simultaneously and thus allows for a systematic investigation of the organizational properties of a given MN pool. To guarantee accurate results of the study, it is important, therefore, that the decomposition method be seen as trustworthy.

The accuracy of the decomposition method used in the current study has been validated previously using a two-source procedure ([De Luca et al. 2006](#)) and also by a reconstruct-and-test procedure ([De Luca and Hostage 2010](#); [Nawab et al. 2010](#)) in which the event times were shuffled ([De Luca and Contessa 2012](#)). Specifically, in the two-source procedure, surface and intramuscular EMG signals were recorded simultaneously, and the degree of match of common MU firings between the two sources of EMG was calculated as an accuracy index. In the reconstruct-and-test procedure ([Nawab et al. 2010](#)), the EMG signal was first synthesized from the firing times and MUAP shapes of the decomposed MUs with Gaussian noise added, the reconstructed signal was decomposed again, and the firing times were compared with the original ones. In the shuffled reconstruct-and-test method ([De Luca and Contessa 2012](#)), the onion-skin property was demonstrated to be robust, and not an artifact of the decomposition algorithm. Namely, the MU event times recovered from the decomposition algorithm were in the same “shuffled” order as they were in the reconstructed signal.

Our preliminary two-source testing has also shown that the decomposition algorithm was able to identify the spike trains with high accuracy (see APPENDIX). As shown in the exemplar trials, the algorithm was able to identify the spike timing reliably despite superpositions that would likely have increased the difficulty of identifying the individual action potentials in the raw EMG signal.

Both validation methods provide confidence in the decomposition methods, although each has limitations. For example, the two-source procedure only validates the common MUs, which represent only a small portion of the detected MUs in the sEMG recording, and the degree of accuracy of the identification of the noncommon MUs is unknown. Nonetheless, it is still an unbiased sample. Alternatively, the reconstruct-and-test validation depends on the amplitude of the Gaussian noise added ([Farina and Enoka 2011](#)); namely, larger noise amplitude leads to poorer validation results.

Given that our STA estimate uses the firing times of the decomposition algorithm as the triggering signal, the accuracy of the decomposition can potentially influence the STA estimate of the MUAP. First, a spike omission will not influence the STA estimate, because that action potential is missed and will not be taken into account during the averaging calculation. In contrast, a false-positive error (i.e., a spurious spike) will influence the reliability of the STA estimate. The potential influences are twofold: 1) spurious spikes may increase the variability of the MUAP amplitude estimate. To minimize this possibility, during the calculation, MUs with high CV of P-P were excluded. Our sensitivity analysis also shows that using a tighter criterion (i.e., smaller CV) does not alter the main findings of this study. Nevertheless, further investigation is necessary to examine the impact of accuracy of firing spike detection in relation to the variability and reliability of the STA estimates. 2) The spurious spikes may also bias the estimate of the P-P amplitude of the MUAP. Namely, smaller MUs have a higher chance of including a larger action potential during the average calculation because a small MU sits at the lower end of the rank-ordered MU sizes, and
larger MUs have a higher chance of including a smaller action potential. As a result, smaller MUAPs may be larger than their actual sizes, and larger MUAPs may be smaller than their actual sizes. However, such biased estimates will make our basic results even stronger, because one would expect a steeper slope capturing the size principle in an ideally unbiased estimate.

Conversely, consistent MUAP shape series derived from the STA method can provide validation about the firing times of the identified MUs, because one would expect inconsistent MUAP shapes if the firing times have been incorrectly identified (i.e., false-positive errors). As stated in METHODS, only MUs that have low variability in P-P amplitudes were included. Thus only reliably discriminated MUs whose firing times are largely correctly identified were used.

In addition to the CV criterion, the degree of match between the STA-estimated MUAP shape and the decomposed MUAP shape was used as an MU selection criterion. The correlation measure between the STA and decomposed MUAPs takes into account the MUAP information that is largely ignored in the previous validation techniques described by Nawab et al. (2010). A high correlation is expected when the firing times are largely identified correctly and when the decomposed MUAP templates represent the actual MUAP shape. The numbers of MUs selected and decomposed as shown in Table 1 reveal that most of the MUs passed the selection criteria and that STA estimates of MUAPs were reliable for most of the MUs.

The analytical method (i.e., STA combined with selection criteria) used in the current study also provides general information about the reliability of the decomposition method used. The MUs used for our analysis were derived from the raw EMG waveform based on the decomposed firing events, and for a large percentage of decomposed events, the STA-based MUAP waveform was highly correlated with the MUAP provided by the decomposition template. Although this does not constitute validation, it is difficult to obtain such results without a robust and accurate output from the decomposition algorithm (Hu et al. 2013).

There also exists the possibility that the decomposition algorithm can confuse two different MUs that exhibit similar shapes on the recorded signal due to the low-pass filtering effect of passive tissues and that this error will remain undetected by the STA assessment. However, this error is unlikely due to the following reasons. First, the algorithm uses multiple features of a MUAP template vector of an MU across four channels to distinguish one MU from another. The distinguishability of different MUAPs increases when more features are used to capture specific signatures of the MUAP (see APPENDIX). Additionally, even if all derived MUAP features are similar from one recorded channel, the similarity would have to exist between various features of the MUAP shapes across all four channels. To date, we have not observed such an event and thus consider it to be unlikely. Second, mixing one spike train with another will likely produce very short ISIs. Thus an ISI distribution skewed to the short intervals is to be expected. However, such frequent short ISIs in the identified MUs with highest firing rates were not observed in our data (see APPENDIX for the exemplar ISI distributions).

**Conclusion**

Our main finding is that the MU size, as estimated using the STA, increased in an orderly manner with increasing recruitment force during individual isometric force production tasks. We also found that the steady-state firing rate of the smaller and earlier-recruited MUs was systematically higher than that of larger and later-recruited MUs. The physiological properties that have been reported using intramuscular recording also hold here in the sEMG recording and decomposition techniques. Most importantly, the size principle is clearly evident, and the rate of decay of the MFR as a function of P-P amplitude is slower at higher force levels. Finally, these techniques appear to be sufficiently robust to allow analyses of motor unit firing patterns in other muscles, and potentially even in disease states.

**GRANTS**
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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

X.H., W.Z.R., and N.L.S. conception and design of research; X.H. and N.L.S. performed experiments; X.H. and N.L.S. analyzed data; X.H., W.Z.R., and N.L.S. interpreted results of experiments; X.H. prepared figures; X.H. drafted manuscript; X.H., W.Z.R., and N.L.S. edited and revised manuscript; X.H., W.Z.R., and N.L.S. approved final version of manuscript.

**APPENDIX**

We performed three sets of preliminary tests to examine the reliability of the identified spike trains.

**Two-Source Testing Comparing Intramuscular MU Recordings With sEMG Recordings** We performed two-source testing in two subjects using paired intramuscular fine-wire electrodes recorded simultaneously with the sensor array electrode placed over the first dorsal interosseous muscle. Decomposition of the intramuscular recording was accomplished using EMGLab (version 1.03) with rigorous manual corrections, and decomposition of the sensor array data was accomplished using the sEMG decomposition algorithm ([De Luca et al. 2006](#); [Nawab et al. 2010](#)).

The discrimination was treated as accurate if the difference in spike timing between the unit discriminated in the sEMG and in the intramuscular EMG (iEMG) record was less than 5 ms. The percentage of agreement was above 97% in the first exemplar trial at ~5% of maximum contraction ([Fig. A1](#)) and was above 90% in the second exemplar trial at ~20% of maximum contraction ([Fig. A2](#)).
Intramuscular and surface EMG signals and identified spike train segment at a 5% of maximum contraction of the FDI.  

**A:** 4 channels of sEMG and 1 channel of intramuscular (iEMG).  

**B:** from top to bottom are a zoomed segment of the 4 channels of sEMG signal, the discriminated spike timing from the common MU in sEMG (vertical bars) and iEMG (circles), and a zoomed segment of iEMG signal. Exemplar superimposed APs of this common MU in the sEMG are signified in the dashed boxes.
Intramuscular and surface EMG signals and identified spike train segment at a 20% of maximum contraction of the FDI.

Fig. A2. A: 4 channels of sEMG and 1 channel of iEMG. B: from top to bottom are a zoomed segment of the 4 channels of sEMG signal, the discriminated spike timing from the common MU, and a zoomed segment of iEMG signal. Exemplar superimposed action potentials of this common MU in the sEMG are signified in the dashed boxes.

Most importantly, our analysis shows that there was no inappropriate selection of MUAP templates in any of the data records collected at different force levels. As indicated in the dashed box on the sEMG signal (Figs. A1B and A2B), despite the superimposed action potentials from other MUs, the sEMG decomposition algorithm can still identify these action potentials reliably, as indicated in the spike timing results.

**Distinguishability of Concurrent Active MU** Four features of an MU pair were extracted.

*Feature 1* is the maximum correlation between action potentials: \( \text{max}[\text{corr}(\text{MU}_i, \text{MU}_j)] \), where \( i \) and \( j \) denote the motor unit index; “corr( )” denote the lagged correlation between the action potential templates.

*Feature 2* is the normalized difference between the P-P amplitude of the action potential: \( 1 - \frac{|P_i - P_j|}{P_i + P_j} \), where \( P_i \) and \( P_j \) denote the P-P amplitude of MUs \( i \) and \( j \). A value of 1 represents the same P-P amplitude, and a value close to 0 represents very different amplitudes between MUs.

*Feature 3* is the normalized difference between the maximum peak amplitude of the action potential pair: \( 1 - \frac{|M_{i} - M_{j}|}{M_i + M_j} \), where \( M_i \) and \( M_j \) denote the maximum peak amplitude of MUs \( i \) and \( j \). A value of 1 represents the same maximum peak amplitude, and a value close to 0 represents very different peaks.

*Feature 4* is the normalized difference between the absolute value of the maximum trough amplitude of the action potential pair: \( 1 - \frac{|M_{i} - M_{j}|}{|M_i| + |M_j|} \), where \( |M_i| \) and \( |M_j| \) denote the absolute maximum trough amplitude of MUs \( i \) and \( j \). A value of 1 represents the same trough amplitude, and a value close to 0 represents very different troughs.
An MU similarity matrix was constructed, based on the different combinations of the four features. The different features were multiplied at individual channels when multiple features were used. The similarity index matrices of two sets of concurrent active MUs are illustrated in Figs. A3 (8 MUs during a 20% of maximum contraction) and A4 (17 MUs during a 40% of maximum contraction). As more features were used, the difference between MUs became stronger (i.e., the color gets darker). The four-channel information also improves the distinguishability between different MUs. For example, MU pair 4 and 8 in channel 1 (Fig. A3C) were similar even when four features were combined; however, the difference in the other three channels can distinguish the different MUs. Similar cases were also evident in MU pair 7 and 13 in channel 1 (Fig. A4C) and pair 7 and 14 in channel 4 (Fig. A4C).

Fig. A3.

![Action potential similarity matrix of 8 concurrent active MUs from a single contraction.](image)

- **A**: similarity matrix based on feature 1 (shape correlation). Only the upper section was calculated due to matrix symmetry. MU index represents the motor unit number.
- **B**: similarity matrix based on features 1 and 2 (shape correlation and difference in P-P amplitude).
- **C**: similarity matrix based on features 1–4 (shape correlation, difference in P-P amplitude, difference in maximum peak, and difference in absolute maximum trough).
Statistics of Single MU Discharge

The distribution of the ISIs of the discriminated MU discharge was examined. At steady muscle contractions, the peak discharge rates of MUs range from 20 to 35 Hz, which correspond to minimum spike intervals ranging from 28.6 to 50 ms. Therefore, if the sEMG decomposition algorithm confuses two MUs with similar action potential shapes, similar recruitment threshold, and similar firing rates, one would expect to see very short ISIs observed frequently.

At high contraction levels, the small MUs are more likely to be influenced by superpositions of other larger MUs; therefore, the identified spike trains of these MUs are more prone to error.

As shown in Figs. A5 and A6, the interval distribution largely followed a Gaussian distribution with longer tails at larger intervals. More importantly, there are virtually no short intervals at the 14.3- to 25-ms interval range, which is half of the minimum typical interval range. This would be expected if there were spurious identifications of two unit trains as one train.
**Fig. A5.**

Distribution of the interspike interval (ISI) of the first 4 MUs during a 40% of maximum contraction.

**Fig. A6.**

Distribution of the ISI of the first 4 MUs during a 50% of maximum contraction.

**REFERENCES**


De Luca CJ, Nawab SH. Reply to Farina and Enoka: The reconstruct-and-test approach is the most appropriate validation for surface EMG signal decomposition to date. J Neurophysiol 105: 983–984, 2011


Figures and Tables
Experimental setup, exemplar surface electromyogram (sEMG), and force display. 

A: sEMG and force data are collected while the subject performs isometric abduction of the index finger. 

B: placement of the sEMG electrode above the first dorsal interosseous (FDI) muscle. 

C: the 5-cylinder probe array. 

D: 4 channels of EMG recorded during an exemplar trial (top) and a zoomed display of individual action potentials (APs; bottom). 

E: force feedback during trapezoid force production.
Exemplar spike-triggered average (STA) and decomposed motor unit action potential (MUAP) template comparisons. 

A: 4 channels (Ch 1–4) of APs of a single MU with high correlation between template pairs. The STA and decomposed AP templates (Delsys) are shown in blue and red, respectively. 

B: 4 channels of APs of a single MU with low correlation between template pairs. 

C: STA template estimated using a window of 8 s. Window index represents the different windows used for the estimation. Consistent AP shapes of a single MU are shown. 

D: similar AP shape but different AP amplitudes between template pairs.
MU selection criteria. The correlation coefficient and the coefficient of variation (CV) of peak-peak (P-P) amplitude from 4 different force levels (% maximum voluntary contraction) of a particular subject are shown. Each symbol in each channel represents 1 MU, and the different open symbols represent the included MUs of different force levels. The black dots represent the excluded MUs. The solid lines above the data cloud represent the inclusion criteria.
Table 1.
Number of MUs analyzed and decomposed for each subject during individual force level

<table>
<thead>
<tr>
<th>Force Level</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
<th>Subject 6</th>
<th>Subject 7</th>
<th>Subject 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% MVC</td>
<td>40 (49)</td>
<td>44 (50)</td>
<td>33 (36)</td>
<td>46 (48)</td>
<td>37 (42)</td>
<td>35 (38)</td>
<td>41 (46)</td>
<td>45 (47)</td>
</tr>
<tr>
<td>30% MVC</td>
<td>39 (58)</td>
<td>47 (59)</td>
<td>38 (42)</td>
<td>42 (44)</td>
<td>38 (43)</td>
<td>42 (47)</td>
<td>37 (42)</td>
<td>56 (63)</td>
</tr>
<tr>
<td>40% MVC</td>
<td>39 (49)</td>
<td>43 (53)</td>
<td>30 (33)</td>
<td>48 (52)</td>
<td>47 (51)</td>
<td>35 (41)</td>
<td>43 (49)</td>
<td>48 (54)</td>
</tr>
<tr>
<td>50% MVC</td>
<td>38 (51)</td>
<td>53 (64)</td>
<td>30 (35)</td>
<td>48 (55)</td>
<td>48 (53)</td>
<td>36 (42)</td>
<td>30 (43)</td>
<td>49 (56)</td>
</tr>
</tbody>
</table>

Data are number of motor units (MUs; 2 trials combined) analyzed and number of MUs decomposed (in parenthesis) for each subject during individual force level (%MVC, percentage of maximum voluntary contraction).
Data grouping and regression analysis for single trials and single force levels (pooled MUs of 2 trials). Values are means ± SD of slope and fit $r^2$ of the regression analyses shown for the peak-peak amplitude vs. threshold force relation. SD was calculated across subjects.

<table>
<thead>
<tr>
<th>Regression Parameter</th>
<th>20% MVC</th>
<th>30% MVC</th>
<th>40% MVC</th>
<th>50% MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single trial</td>
<td>0.021 ± 0.009</td>
<td>0.029 ± 0.010</td>
<td>0.036 ± 0.013</td>
<td>0.033 ± 0.008</td>
</tr>
<tr>
<td>Single force level</td>
<td>0.019 ± 0.007</td>
<td>0.027 ± 0.011</td>
<td>0.032 ± 0.012</td>
<td>0.029 ± 0.008</td>
</tr>
<tr>
<td>$r^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single trial</td>
<td>0.51 ± 0.13</td>
<td>0.58 ± 0.10</td>
<td>0.62 ± 0.12</td>
<td>0.60 ± 0.15</td>
</tr>
<tr>
<td>Single force level</td>
<td>0.46 ± 0.09</td>
<td>0.54 ± 0.07</td>
<td>0.55 ± 0.08</td>
<td>0.56 ± 0.14</td>
</tr>
</tbody>
</table>
Fig. 4.

P-P amplitude vs. threshold force of individual subjects. Four channels of P-P amplitudes as a function of the threshold force of subject 1 (A) and subject 2 (B) are plotted. Individual linear regression was performed at each force level. Each symbol of each channel represents 1 MU. Note that the different channels represent the same set of MUs, because each channel captures separate AP shapes of the same MU.
Fit parameters of P-P amplitude vs. threshold force and recruitment threshold range of all the subjects. 

**A:** slope of the linear regression at individual task force levels. 

**B:** fit $r^2$ at each force level. Error bars represent standard error (SE) across subjects.
Fig. 6.

MFR vs. P-P amplitude of individual subjects. Four channels of mean firing rate (MFR) as a function of the P-P amplitude of subject 1 (A) and subject 2 (B) are plotted. An individual power function was fit to the data at each force level. Each symbol of each channel represents 1 MU. pps, Pulses per second.
Fig. 7.

Fit parameters of MFR vs. P-P amplitude of all the subjects. 

A: rate of decrease of MFR at individual task force levels. 
B: scale factor of the MFR at each force level. 
C: fit $r^2$ at each force level. 

Error bars represent SE across subjects.
Fig. 8.

MFR vs. threshold force of 2 individual subjects and fit parameters of all the subjects. MFR as a function of threshold force at individual task force levels is plotted with regression lines superimposed on the individual MUs of subject 1 (A) and subject 2 (B). C: slope of the regression of all the subjects. D: y-axis intercept of the regression of all the subjects. E: fit $r^2$ at each force level. Error bars represent SE across subjects.
Fig. 9.

P-P duration as a function of threshold force. A and B: P-P duration vs. threshold force of MUAPs at individual force levels of 2 exemplar subjects. The P-P duration did not show a correlation with the threshold force.
Task-modulated MFR: firing rate modulation of MU with different threshold force. MFR vs. threshold force of an individual subject is shown. Box A includes the MUs of low MFR and moderate recruitment threshold at low force levels. Box B includes the MUs of high MFR and relatively high recruitment threshold at high force levels. Box C includes the MUs of high MFR and relatively low recruitment threshold at low force levels.