Summation of Forces From Multiple Motor Units in the Cat Soleus Muscle

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[1]*Department of Physiology, Northwestern University Medical School, Chicago, Illinois 60611; and* [2]*Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta T2N 4N1, Canada*

Perreault, Eric J., Scott J. Day, Manuel Hulliger, Charles J. Heckman, and Thomas G. Sandercock. Summation of forces from multiple motor units in the cat soleus muscle. *J Neurophysiol* 89: 738–744, 2003; 10.1152/jn.00168.2002. Nearly all muscle models and most motor control concepts assume that forces from individual muscle fibers and motor units sum in an additive manner once effects of in-series tendon compliance are taken into account. Due to the numerous mechanical linkages between individual fibers, though, it is unclear whether this assumption is warranted. This work examined motor unit force summation over a wide range of muscle forces in the cat soleus. Nonadditive summation implies a nonlinear summation of motor unit forces. Summation nonlinearities were quantified during interactions of 10 individual motor units and 4 motor unit bundles containing approximately 10 units each. These protocols allowed motor unit force summation to be examined from approximately 0 to 25% of tetanic muscle force. Nonlinear summation was assessed by comparing the actual forces to the algebraic sum of individual units and bundles stimulated in isolation. Superadditive summation meant that the actual force exceeded the algebraic sum, whereas subadditive summation meant that the actual force was smaller than the algebraic sum. Experiments tested the hypothesis that superadditive summation occurs at low force levels when few motor units are recruited, whereas subadditive summation prevails above 10% of tetanic force. Results were consistent with this hypothesis. As in previous studies, nonlinear summation in the soleus was modest, but a clear transition from predominately superadditive to predominately subadditive summation occurred in the range of 6–8% of tetanic force. The largest nonlinearities were transient and appeared at the onset of recruitment and derecruitment of groups of motor units. The results are discussed in terms of the mechanical properties of the connective tissue forming the tendon and linking muscle fibers.

INTRODUCTION

During normal activity, increasing neural activation of a muscle results in a complex pattern of recruitment and rate modulation of many motor units. To understand the role of muscle properties in the neural control of movement, it is necessary to first understand how the actions of individual motor units sum to generate whole muscle forces under natural conditions. The standard assumption of nearly all muscle models and most motor control concepts is that individual muscle fibers and individual motor units interact only via the in-series

compliance of the tendon, but there are also numerous mechanical linkages between individual muscle fibers. Fibers of different motor units are interspersed within a given muscle (Burke 1981) and are mechanically linked via the extracellular matrix (Thornell and Price 1991; Trotter et al. 1995). Similar links may exist between tendon fibrils (Cribb and Scott 1995). As a result, there is a substantial compliance held in common by muscle fibers (Sandercock 2000).

Recent studies demonstrate significant nonlinear interactions between small numbers of motor units. For the most part, motor unit force summation is superadditive with the actual force sum during simultaneous stimulation of the units being greater than the algebraic sum of each unit's force measured in isolation (Clamann and Schelhorn 1988; Emonet-Dénand et al. 1990; Powers and Binder 1991; Troiani et al. 1999). This superadditive summation may be due to the thixotropic nature of the passive fibers mechanically linked to the fibers of the active motor unit under study. In contrast, if the mechanical links between muscle fibers occur predominantly through an elastic tendon and the muscle is on the ascending limb of its length-tension relation, force summation should be subadditive with the actual sum being less than the algebraic sum. Subadditive summation is indeed observed when a muscle's innervation is divided into two portions with each producing approximately half of the total force (Sandercock 2000) or when multi-unit bundles of motor units are combined (Brown and Mathews 1960; Hunt and Kuffler 1954). These results suggest that force summation undergoes a transition from superadditive to subadditive summation as force levels rise due to progressive recruitment and rate modulation of the motor unit population. We hypothesized that superadditive summation would only occur when a few motor units were active, so that subadditive summation would prevail above about 10% of tetanic force.

To test this hypothesis, we examined force summation over a wide range of forces in the cat soleus muscle. Motor unit summation nonlinearities were quantified during interactions of 10 individual motor units and 4 bundles of motor units each containing approximately 10 individual units, allowing motor unit force summation to be examined from approximately 0 to 25% of tetanic muscle force. Normal recruitment and rate

modulation were simulated by ordering the unit activation sequence according to unit force and by linearly increasing the stimulus rate of each unit. As in previous work, nonlinear summation in the soleus muscle was modest, but a clear transition from predominately superadditive to predominately subadditive summation occurred in the range of 6–8% of tetanic force. The largest observed summation nonlinearities were transient and appeared at the onset of recruitment and derecruitment of groups of motor units. Regardless of force level, these transient nonlinearities always corresponded to subadditive summation. The results are discussed in terms of the mechanical properties of the connective tissue forming both tendon and the links between muscle fibers.

METHODS

Surgical procedures

An acute soleus nerve-muscle preparation, as fully described elsewhere (Day and Hulliger 2001; Hulliger et al. 2001), was used in 12 cats deeply anesthetized with pentobarbitone (initial dose: 40 mg/kg). The hind limb was completely denervated except for the soleus muscle. The distal 2 cm of the soleus nerve was dissected and freed from connective tissue to permit neurogram recordings. The soleus muscle was exposed by removing both heads of the gastrocnemius and the plantaris muscle. The fine fascia surrounding the soleus was resected to fully expose the posterior surface of the muscle belly and its tendon. A surface electrode was sutured to the soleus epimysium, and a pair of fine wire electrodes was inserted into the muscle belly for recording electromyograms (EMGs). The distal muscle tendon was attached to the force transducer of a linear actuator using a bone chip from the calcaneus. After this macroscopic dissection was completed, the hip, knee, and ankle were rigidly fixed to a stereotactic frame using steel pins.

After a lumbo-sacral laminectomy, the ventral roots (L_5–S_3) were exposed and cut. The roots containing motor axons to the soleus muscle (L_7, S_1) were divided into smaller filaments with the goal of isolating either single motor units (SMUs) or multi-motor unit (MMU) bundles containing approximately 10 SMUs (6–8% of whole muscle tetanic force). Four criteria were used to determine whether a filament contained a SMU: all-or-none force steps, all-or-none surface EMG potentials, all-or-none indwelling EMG potentials, and all-or-none neurogram potentials as recorded from the distal muscle nerve (see Day and Hulliger 2001). Each test was performed when the filament was stimulated at about five times activation threshold using a stimulation rate of 30 pulses/s.

Apparatus

The ventral root filaments selected for each experimental trial were ordered according to increasing tetanic force and mounted on separate stimulating electrodes that were housed in one of two multi-channel electrode arrays (Hulliger et al. 2001). Isolated voltage-controlled stimulators were used to stimulate each filament. Each stimulator was triggered independently using TTL pulses generated by a custom-designed 16 channel digital I/O board. Pulse outputs were synchronized to the master data acquisition clock to eliminate the jitter associated with summing data from successive trials.

The soleus forces resulting from the ventral root stimulation were measured using a load cell (Kistler) with a resolution of 1 mN. Force signals were low-pass filtered at 100 Hz and EMG signals at 1,000 Hz. The force data were sampled at 250 Hz and EMG data at 2 kHz using a custom A/D system with gains optimized to permit distortion-free recording of the largest signals expected within each trial.

Protocols

Two separate protocols were used to assess motor unit force summation. Both were performed under isometric conditions, with a muscle length corresponding to 90° ankle dorsiflexion. Between trials the muscle was shortened to 5 mm below acquisition length to minimize tendon creep and maximize circulation. The first protocol evaluated the summation properties of 10 SMUs, whereas the second evaluated the summation properties of 4 MMU bundles, each containing approximately 10 SMUs. In all trials, the smallest ventral root filament (SMU or MMU) was recruited first and the largest filament last, broadly imitating physiological recruitment order (Henneman et al. 1965a,b). SMU and MMU force summation were evaluated by stimulating each ventral root filament in isolation and comparing the algebraic sum of these individually measured forces to that measured from a single trial in which all filaments were stimulated. For each filament, the stimulation patterns used for the individual stimulation trials and the combined stimulation trials were identical.

Figure 1 illustrates the two stimulation patterns used in these experiments. The first mimicked the normal patterns of recruitment *and* rate modulation seen during slow increases in muscle force (Fig. 1, *A* and *B*); the second pattern incorporated only changes in recruitment, maintaining a constant average activation rate for all active fibers (Fig. 1, *C* and *D*). All SMU protocols used the recruitment and rate modulation patterns, whereas the MMU protocols also used the recruitment only protocols. Each pattern was characterized by a background component (Fig. 1, *A* and *C*), representing the mean stimulation rate and by an added component of Gaussian noise (coefficient of variation, CV, 12.5%), which varied the timing of successive stimulus events. The combination of these components is shown in Fig. 1, *B* and *D*. The addition of Gaussian noise altered the duration of inter-stimulus intervals up to approximately 40% of the background interval. This variability is consistent with that observed in human MU firing patterns during voluntary contractions (CV 12.5% or larger) (Dorfman et al. 1988, 1989; Person and Kudina 1972). More recent data suggest average inter-stimulus interval variability near 20% (Erim et al. 1999; Laidlaw et al. 2000); nevertheless, the chosen variability is within the physiological range for humans.

Several measures were used to ensure that the state of the muscle remained constant throughout the course of each experiment. First, isometric tetanic force measurements for each ventral root filament under study were made at a stimulation rate of 30 pulses/s prior to the beginning of each stimulation protocol and immediately after each protocol was completed. Trials were kept for further processing only if the initial and final tetanic measurements for each filament differed by less than 5%. Second, two trials were collected during which all
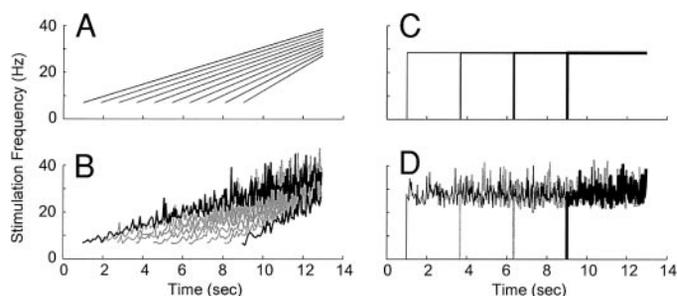

FIG. 1. Motor unit (MU) stimulation patterns. *A*: variation of mean stimulation rates with time for the stimulation protocol designed to mimic changes in recruitment and firing rate of multiple MUs. Each line represents the average stimulation rate of single or multi-MU (SMU or MMU) bundle. *B*: example of the actual stimulation pattern for simulated recruitment rate modulation. Same as *A* with Gaussian noise added to the mean stimulation rates. The firing rates for the 1st and 10th recruited MUs are shown in black for clarity. *C*: mean stimulation rates for the recruitment only stimulation protocol employed for MMU bundles. *D*: actual stimulation pattern for recruitment only protocol. Same as *C* with added Gaussian noise.
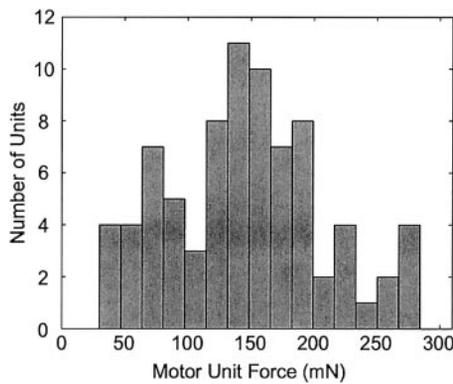
FIG. 2.   MU force distribution. Distribution of tetanic MU forces used for analysis. Represents a total of 80 SMUs collected from 8 cats.

filaments were stimulated. These measurements were made immediately before and after the force measurements for the individual filaments. Again, trials were analyzed further only if the two combined stimulation trials differed by less than 5% at the end of the stimulation protocol; the terminal forces of the trials meeting this criterion differed by only 0.6 ± 1.8% (mean ± SD), indicating that fatigue was not a significant factor. All subsequent analyses used the second of the two combined stimulation trials. From the 12 cats used in this study, 12 data sets for the SMU summation protocol and 6 data sets for the MMU summation protocols were collected. Of these, eight SMU data sets and five MMU data sets met the preceding selection criteria.

Analysis

The goal of these experiments was to determine whether the force generated by individual MUs and groups of MUs summed in an additive manner over a broad range of muscle forces. The degree of nonlinear summation (F_{NL}) at a given force level was quantified as the difference between the force generated by the combined stimulation (F_{CMB}) of several ventral root filaments and the algebraic sum of the forces generated when the filaments were stimulated in isolation (F_i), as summarized by *Eq. 1*. These values were also normalized by the instantaneous muscle force during combined stimulation to provide a relative measure of nonlinear summation as different numbers of MUs were activated, as shown in *Eq. 2*

$$F_{NL}(t) \equiv F_{CMB}(t) - \sum_{i=1}^{N} F_i(t) \qquad (1)$$

$$\tilde{F}_{NL}(t) \equiv \frac{F_{NL}(t)}{F_{CMB}(t)} \qquad (2)$$

In *Eq. 2*, $\tilde{F}_{NL}$ is the normalized nonlinearity. We define superadditive summation as occurring when the actual sum was greater than the algebraic sum [$F_{NL}(t) > 0$]. This corresponds to a positive summation nonlinearity. Subadditive summation was defined as $F_{NL}(t) < 0$, corresponding to a negative summation nonlinearity.

RESULTS

Stimulation of SMUs

A total of 80 individual MUs were characterized in this study, divided into eight groups of 10. Tetanic MU forces ranged from approximately 30 to 300 mN with a mean value of 146 ± 62 mN, as shown in the histogram of tetanic forces of Fig. 2. This range is nearly identical to that reported by

McPhedran et al., who used similar experimental techniques (McPhedran et al. 1965).

Motor-unit force summation was remarkably additive in response to the recruitment and rate modulation stimulation pattern (see Fig. 1, *A* and *B*). In the example illustrated in Fig. 3, the total muscle force resulting from the combined stimulation of the unit forces was very close to the algebraic sum (*B*). Summation nonlinearities for this set of units remained less than 50 mN during active contractions (*C*). However, in all trials, a transient increase in summation nonlinearity magnitude was observed during force relaxation after the end of stimulation (*C*). This relaxation nonlinearity was always negative, indicating that the sum of the forces from the individually measured units was greater than that from the units stimulated together. These results suggest that the relaxation time of individually activated MUs is longer than that of groups of MUs activated at the same time. This was quantified by examining the half-relaxation time of all SMUs and groups of MUs. Half-relaxation time is defined as the time required for force to decay to half of its maximum value. Although there was considerable spread in the relaxation times of the SMUs within each group (average SD = 14.7 ms), the average results for all groups were very consistent. Across all groups of SMUs, the average half relaxation time of the 10 individual MUs was 15.3
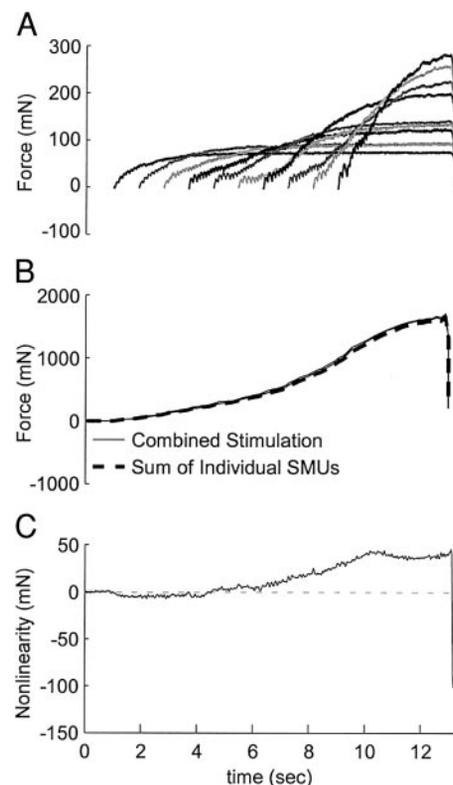

FIG. 3.   Summation of SMUs. Typical data from a SMU experiment. *A*: the force measured from 10 SMUs activated with mean stimulation rates that increased with time (see Fig. 1*B*). *B*: the algebraic sum of these individually measured forces (thick dotted line) and the force measured from the combined stimulation of these units (thin solid line). Note that the timing of the stimulation applied to each unit was identical whether the unit was stimulated in isolation or in combination with other units. Two combined stimulation trials are shown, these correspond to measurements made before and after the individual measurements for all MUs. *C*: summation nonlinearities, defined as the difference between the force measured during the combined stimulation and the algebraic sum of the individually measured SMU forces.
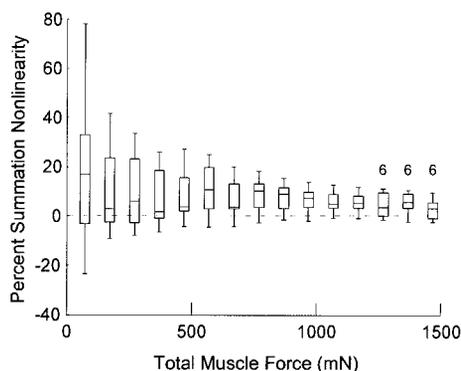
FIG. 4. Summation nonlinearities for all SMU trials. Box and whisker plots show the variation in normalized summation nonlinearities (*Eq. 2*) as a function of total muscle force. Pooled results for all 8 sets of 10 units are plotted for force levels ranging from 20 to 1,520 mN in increments of 100 mN. The box and central line represent the upper and lower quartiles as well as the data median within each range; the whiskers show the extent of the remaining data. Not all data sets had forces in each of the ranges shown in Fig. 4. For ranges that did not include all 8 data sets, the number of sets included is shown above the box and whisker plot (see right-hand boxes).

ms greater than that of the same MUs when stimulated together ($P < 0.001$; $n = 8$).

To summarize the data for all eight sets of SMU bundles, the instantaneous normalized summation nonlinearities (*Eq. 2*) for all sets were pooled. Instantaneous summation nonlinearities were only computed when more than one SMU or MMU bundle was stimulated, thereby specifically excluding the transient nonlinearities observed during relaxation. The pooled data were divided into 15 equal force ranges, extending from 20 to 1,520 mN; each range had a width of 100 mN. The box and whisker plots shown in Fig. 4 characterized the data within each range. The line in each box gives the median, the box borders shows the quartiles, and the vertical whiskers indicate the full range of the data. The average median nonlinearity across all trials was 5%, and this value remained relatively constant at each force level except for 20- to 120-mN range, which had a median nonlinearity of 16.7%. For all force ranges, the median nonlinearity was positive, indicating that the force generated by stimulating all recruited MUs together was greater than that obtained by summing their individually measured tensions. In contrast to the median, the range of observed summation nonlinearities had a stronger dependence on total muscle force. At low force levels (20–120 mN), there was a high degree of inter-animal variability, with the range of observed nonlinearities extending from –23 to 78%. This variability decreased as the total muscle force increased. At the highest force levels (1,420–1,520 mN) the range extended only from –3 to 9%. Much of the variability observed at lower force levels was due to the normalization process. The *unnormalized* summation nonlinearities had inter-quartile ranges that increased only modestly with total muscle force, extending from 24 mN at the lowest force range to 93 mN at the highest force range shown in Fig. 4. This resulted in a *normalized* variability that decreased with increases in total muscle force.

Stimulation of MMU bundles

Summation of MMU bundles (each producing between 6 and 8% of tetanic muscle force and thus corresponding to approximately 10 SMUs) allowed investigation of summation

over a wider range of forces than the SMU summations illustrated in Figs. 3 and 4. Figure 5 illustrates the application of the simulated recruitment and rate modulation scheme (Figs. 1, *A* and *B*) to a set of four MMU bundles. This stimulation generated a force profile that exceeded 5,000 mN (compare with 1,500 mN in Fig. 3*B*). Note that, as for the SMUs, nonlinear summation was small, with the traces for the actual and algebraic sums in Fig. 5*B* being nearly indistinguishable; summation nonlinearity magnitudes were less than 100 mN throughout the stimulation period (Fig. 5*C*). Note that there was a small, transient increase in the magnitude of the summation nonlinearity in the subadditive direction as each bundle of MUs was initially recruited. Strong subadditive summation was also present in this data at the termination of stimulation, similar to that shown in Fig. 3, but the data following the termination of stimulation are not shown in Fig. 5 to emphasize the force transients as each bundle was initially recruited. As for the SMU bundles, this relaxation nonlinearity was due to a decreased relaxation time as more MUs were recruited. The average half relaxation time of the four MMU bundles was 14.4 ms greater than that of the same MMU bundles when stimulated together ($P < 0.001$; $n = 5$).

Figure 6 summarizes the summation nonlinearities for all five MMU data sets with the graded recruitment and rate modulation stimulation pattern of Fig. 1, *A* and *B*. Boxes and whiskers are plotted for force levels ranging from 1,000 to 8,000 mN in increments of 1,000 mN; below this range, there was typically only a single bundle active. In all cases, median summation nonlinearities were less than 5%. At the lowest force levels (1,000–2,000 mN), summation was superadditive
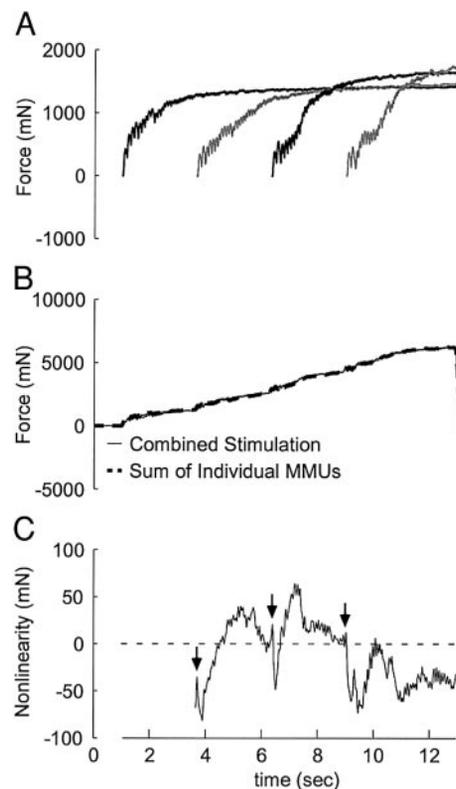

FIG. 5. Summation of MMU bundles during simulated recruitment and rate modulation. Typical data from a MMU experiment using the recruitment and rate modulation stimulation protocol (Fig. 1*B*). Format is identical to that of Fig. 3.
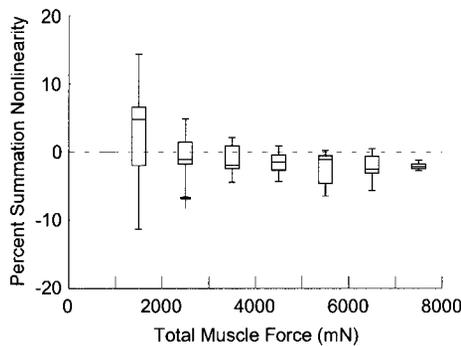
FIG. 6. Summation nonlinearities for MMU trials. Box and whisker plots show variation in summation nonlinearity as a function of total muscle force in five animals. Results are plotted for force levels ranging from 1,000 to 8,000 mN in increments of 1,000 mN. Format is identical to Fig. 4. Crosses indicate outliers, defined as points more than 1.5 times the inter-quartile range beyond the box, which is the maximum whisker length. When no outliers are present, the whiskers indicate the extent of the data. All data sets are represented in each force range.

and the nonlinearities were similar to those at the largest forces recorded during the stimulation of individual MUs (see Fig. 4). However, force summation became subadditive above approximately 2,000 mN (approximately 10% of tetanic force), indicating that the force obtained when stimulating all bundles together was lower than that obtained by summing the individually measured contributions of each bundle. This result supports our hypothesis. The transient nonlinearities at recruitment of each bundle illustrated in Fig. 5C are included in the preceding overall assessments. A separate analysis of these transients was performed by comparing the nonlinearity in the 400-ms period after recruitment of a new bundle to that 400 ms before recruitment of the next bundle, when force was nearly constant. The transient nonlinearity was significantly different from the steady-state nonlinearity at all force levels ($P <$ 0.001), although these values differed by less than 0.6% when more than two MU bundles were active. In contrast, on recruitment of the second MMU bundle, the average transient summation nonlinearity was −1.15% (subadditive summation) and that prior to recruitment of the third MMU bundle was +2.75% (superadditive summation).

To further analyze the transient summation nonlinearities observed at the onset of stimulation of MMU bundles, data were also recorded during stepwise recruitment of the MU bundles (this pattern is shown in Fig. 1, C and D). Figure 7 shows the results of a typical experiment. Steady levels of summation nonlinearity were similar to those for the graded recruitment and rate modulation of Figs. 5 and 6. However, summation nonlinearities increased dramatically as each bundle was recruited. The transient nonlinearities were significantly larger than the steady nonlinearities across all force levels ($P <$ 0.001). The average transient summation nonlinearity was −3.13% and the average steady state nonlinearity was −0.78%. There was little variation in the magnitude of these nonlinearities with increased force level, and both remained small, being less than 10% of the total muscle force. In all cases, these transient nonlinearities were negative (subadditive), as was the case for the transient nonlinearities shown after cessation of stimulation to the individual MUs and on the initial recruitment of MU bundles during graded stimulation.

DISCUSSION

The goal of this study was to examine MU force summation in the cat soleus over a wide range of muscle forces using physiologically relevant patterns of MU recruitment and firing. MU summation nonlinearities remained small over the tested force range. At low forces, summation was predominantly superadditive, indicating that simultaneously active units generated more force than the sum of the individually stimulated units. In contrast, summation became subadditive as the total muscle force exceeded approximately 6–8% of tetanic force. This transition in summation nonlinearity indicates a shift in the dominant mechanism influencing the summation of MU forces. We propose that when more than a few MUs are active, stretch of the common tendon shared by all MUs and the resulting changes in fiber length and velocity dominate the summation properties of soleus MUs.

Summation of SMU forces

The summation nonlinearities between individual MU forces remained small across a wide range of muscle forces. The median nonlinearity across all trials was 5%, similar to that reported by Clamann and Schelhorn (1988) and Emonet-Dénand et al. (1990) for the soleus muscle. While the nonlinear summation was predominantly superadditive, instances of subadditive summation also were observed. Subadditive summation has been reported previously, most notably in cat ocular muscles (Goldberg et al. 1997). The variability across force levels and different animals was appreciable. Similar variability has been noted (Emonet-Dénand et al. 1990; Powers and
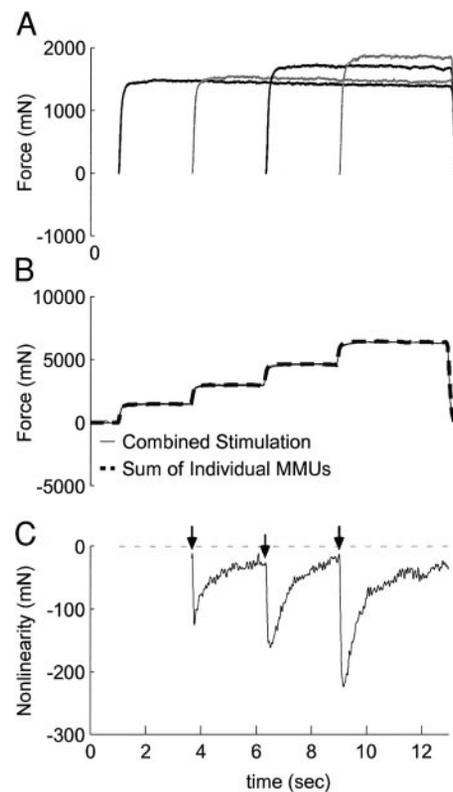

FIG. 7. Summation of MMU bundles during simulated recruitment. Typical data from a MMU experiment using the recruitment only stimulation protocol (Fig. 1D). Format is identical to that of Fig. 3.

Binder 1991; Troiani et al. 1999) and may be due to differences in the proximity of active MUs during different trials.

Many factors could contribute to the nonlinear summation of SMU unit forces. The elegant studies by Street (1983) demonstrated that force could be transmitted between adjacent muscle fibers. Costameres, which are regularly spaced subsarcolemmal rings of proteins, appear to play a role in this transmission by connecting the filamentous actin to the collagenous extracellular matrix (Patel and Lieber 1997; Trotter et al. 1995). These connections between fibers could complicate individual MU force measurements when the fibers of adjacent MUs are passive. Passive muscle exhibits thixotropic behavior, resisting small movements while yielding and providing less resistance to larger movements, a phenomenon that may result from the stable, slowly cycling cross bridges of passive muscle (Hill 1968; Proske and Morgan 1999). Because active muscle fibers must shorten slightly before force is measured, the links to neighboring passive fibers could result in passive fiber compression. Such compression, acting in parallel with the force generating mechanisms of active MUs, would decrease the amount of force transmitted to the muscle tendon. This would result in an underestimation of MU force capabilities for units activated in isolation. Our results suggest that in the soleus muscle, these effects are small and decrease with increasing unit size, most likely because larger units would provide additional force to compress the passive fibers. Further decreases in nonlinear force summation are likely when the fibers adjacent to the MU under study are active, because the rapidly cycling cross-bridges in the active fiber would prevent the generation of significant compression forces. Hence, although the cross-links between fibers can cause an underestimation of the forces generated by individual MUs, they are not likely to have a large impact on muscle force production when more than a few MUs are active.

Strong summation nonlinearities were also noted on derecruitment of the MUs, with the algebraic sum of the individual MU forces always greater than the force measured from the simultaneously active units. This was shown to result from a decreased force relaxation time with increasing numbers of active MUs. This phenomenon may arise from the increased tendon stretch encountered with simultaneously active units (Sandercock 2000). Once force begins to decline, previously stretched tendon will begin to shorten, thereby increasing muscle fiber length. Fiber length changes during force relaxation increase the rate of calcium release from bound cross-bridges, thereby providing a positive feedback situation that accelerates force decay (Caputo et al. 1994).

Summation of MMUs

MU summation properties at higher force levels were investigated using MMU bundles containing approximately 10 SMUs. At the lowest force levels, summation was superadditive with nonlinearities around 5%, as observed in the SMU study. As total muscle force exceeded about 2,000 mN, the average summation became subadditive, indicating that the combined stimulation of several MMU bundles produced less force than the sum of the forces measured when stimulating each bundle in isolation. This switch from superadditive to subadditive responses indicates a change in the dominant mechanism influencing MU summation. Similar subadditive

summation has been reported previously (Brown and Mathews 1960; Hunt and Kuffler 1954), although no study has examined the factors influencing MU summation over the range of forces studied in this work, which spanned about 0–25% tetanic muscle force. Sandercock (2000) recently demonstrated that subadditive summation between two halves of the cat soleus could be accounted for by the series elasticity of the muscle-tendon complex. We propose that a similar mechanism dominates soleus MU force summation above approximately 6–8% of total muscle force. Subadditive summation can occur when the force produced by any given MU stretches the series elasticity, thereby shortening the length of all MUs. For MUs operating on the ascending limb of the length-tension curve, this shortening decreases their force generating capacity. If the soleus was studied at a longer length than used in these experiments, so as to operate on the descending limb of the length tension curve, superadditive summation would be expected. Such effects could be described by Hill-type muscle models incorporating muscle length-tension properties and a series-elastic element (Zajac 1989), although these models cannot account for the observed transition from superadditive to subadditive summation. This length-tension effect above 6–8% of total muscle force is likely to be especially important in naturally activated muscle, where the physiological rates of MU activation result in steeper length-tension curves than typically measured using tetanic stimulation (Rack and Westbury 1969; Sandercock and Heckman 2001).

Large transient summation nonlinearities were observed on rapid recruitment of MMU bundles (Fig. 7) and also would likely be observed for rapidly activated SMUs, although this was not explored. Similar recruitment nonlinearities have been reported for force summation between two halves of the cat soleus (Sandercock 2000), and these were shown to be related directly to the common tendon shared by both muscle halves. A similar mechanism likely contributed to the transient recruitment nonlinearities observed in this study. Rapid recruitment of a MMU bundle causes rapid stretch of the common tendon linking all MUs and a corresponding shortening of all muscle fibers. The force generated by active fibers decreases rapidly with shortening (Hill 1938), which would account for the observations in this and previous studies. Hill-type models incorporating muscle force-velocity properties and a series-elastic element (Zajac 1989) can account for much of the observed recruitment nonlinearities. However, it is interesting to note that the recruitment nonlinearities observed at the low force levels of this study were slower than those reported when two halves of the soleus were summated. This suggests that additional mechanisms may influence recruitment nonlinearities when small numbers of MUs are recruited. Nevertheless, the nonlinearities during rapid recruitment coupled with those seen on rapid derecruitment emphasize that the dynamics of muscle force generation need to be considered when examining MU force summation.

Dynamic movement effects

This study examined MU summation properties during dynamic changes in MU firing rate, but was restricted to isometric conditions. Most functionally relevant behaviors, however, involve dynamic changes in muscle length and activation. Powers and Binder (1991) demonstrated that summation non-

linearities between pairs of MUs decreased significantly during movement of the cat tibialis posterior. Similarly, Heckman et al. (1992) showed that slow shortening movement could actually increase the force of small single MUs in the cat medial gastrocnemius. These effects may be due to movement-related decreases in the viscoelastic connections between fibers or changes in the properties of passive fibers. If this finding holds true for other muscles, the isometrically measured summation nonlinearities observed at low forces in this study as well as other studies would represent an upper limit on the degree of nonlinearity that could be expected during normal movement conditions. Decreased mechanical coupling between fibers would also imply that the common tendon shared by all MUs is the dominant source of coupling during movement. To further explore these possibilities, it is necessary to extend the results of the present study to nonisometric conditions.

Summary

The nonlinear summation of MU forces in cat soleus was minimal over the range of studied forces, spanning approximately 0–25% of tetanic muscle force. For small numbers of MUs, the summation of MU forces was on average superadditive, indicating that the simultaneously active MUs generated more force than the sum of the individual MUs. This appears to result from errors in the measurement of single MU forces due to connective tissue links to passive muscle fibers. As larger portions of the muscle were stimulated, nonlinear summation became subadditive, apparently due to stretch of the common tendon and a shift in fiber length to a shorter and less optimal place on the length-tension curve. The common tendon linking all fibers is also thought to be primarily responsible for the large transient summation nonlinearities observed on rapid recruitment and derecruitment of MUs. These results suggest that the tendon linking all muscle fibers and dynamics of muscle force generation needs to be considered when assessing the summation of MU forces and when modeling the effects of this summation on whole muscle force production.

REFERENCES

Brown MC and Mathews PBC. An investigation into the possible existence of polyneuronal innervation of individual skeletal muscle fibers in certain hind-limb muscles of the cat. *J Physiol (Lond)* 151: 436–457, 1960.

Burke RE. Motor units: anatomy, physiology, and functional organization. In: *Handbook of Physiology. The Nervous System. Motor Control.* Bethesda, MD: Am Physiol Soc, 1981, sect. 1, vol. II, p. 345–422.

Caputo C, Edman KA, Lou F, and Sun YB. Variation in myoplasmic Ca^{2+} concentration during contraction and relaxation studied by the indicator fluo-3 in frog muscle fibers. *J Physiol (Lond)* 478: 137–148, 1994.

Clamann HP and Schelhorn TB. Nonlinear force addition of newly recruited motor units in the cat hindlimb. *Muscle Nerve* 11: 1079–1089, 1988.

Cribb AM and Scott JE. Tendon response to tensile stress: an ultrastructural investigation of collagen:proteoglycan interactions in stressed tendon. *J Anat* 187: 423–428, 1995.

Day SJ and Hulliger M. Experimental simulation of cat electromyogram: evidence for algebraic summation of motor-unit action-potential trains. *J Neurophysiol* 86: 2144–2158, 2001.

Dorfman LJ, Howard JE, and McGill KC. Influence of contractile force on properties of motor unit action potentials: ADEMG analysis. *J Neurol Sci* 86: 125–136, 1988.

Dorfman LJ, Howard JE, and McGill KC. Motor unit firing rates and firing rate variability in the detection of neuromuscular disorders. *Electroencephalogr Clin Neurophysiol* 73: 215–224, 1989.

Emonet-Dénand F, Laporte Y, and Proske U. Summation of tension in motor units of the soleus muscle of the cat. *Neurosci Lett* 116: 112–117, 1990.

Erim Z, Beg MF, Burke DT, and De Luca CJ. Effects of aging on motor unit control properties. *J Neurophysiol* 82: 2081–2091, 1999.

Goldberg SJ, Wilson KE, and Shall MS. Summation of extraocular motor unit tensions in the lateral rectus muscle of the cat. *Muscle Nerve* 20: 1229–1235, 1997.

Heckman CJ, Weytjens JL, and Loeb GE. Effect of velocity and mechanical history on the forces of motor units in the cat medial gastrocnemius muscle. *J Neurophysiol* 68: 1503–1515, 1992.

Henneman E, Somjen G, and Carpenter DO. Excitability and inhibitibility of motoneurons of different sizes. *J Neurophysiol* 28: 599–620, 1965a.

Henneman E, Somjen G, and Carpenter DO. Functional significance of cell size in spinal motoneurons. *J Neurophysiol* 28: 560–580, 1965b.

Hill AV. The heat of shortening and the dynamic constants of muscle. *Proc R Soc Lond B Biol Sci* 126: 136–195, 1938.

Hill DK. Tension due to interaction between the sliding filaments in resting striated muscle. The effect of stimulation. *J Physiol (Lond)* 199: 637–684, 1968.

Hulliger M, Day SJ, Guimares A, Herzog W, and Zhang YT. A new method for experimental simulation of EMG using multi-channel independent stimulation of small groups of motor units. *Mot Control* 5: 61–87, 2001.

Hunt CC and Kuffler SW. Motor innervaton of skeletal muscle: multiple innervation of individual muscle fibres and motor unit function. *J Physiol (Lond)* 126: 293–303, 1954.

Laidlaw DH, Bilodeau M, and Enoka RM. Steadiness is reduced and motor unit discharge is more variable in old adults. *Muscle Nerve* 23: 600–612, 2000.

McPhedran AM, Wuerker RB, and Henneman E. Poperties of motor units in a homogeneous red muscle (soleus) of the cat. *J Physiol (Lond)* 28: 71–84, 1965.

Patel TJ and Lieber RL. Force transmission in skeletal muscle: from actomyosin to external tendons. *Exerc Sport Sci Rev* 25: 321–363, 1997.

Person RS and Kudina LP. Discharge frequency and discharge pattern of human motor units during voluntary contraction of muscle. *Electroencephalogr Clin Neurophysiol* 32: 471–483, 1972.

Powers RK and Binder MD. Summation of motor unit tensions in the tibialis posterior muscle of the cat under isometric and nonisometric conditions. *J Neurophysiol* 66: 1838–1846, 1991.

Proske U and Morgan DL. Do cross-bridges contribute to the tension during stretch of passive muscle? *J Muscle Res Cell Motil* 20: 433–442, 1999.

Rack PMH and Westbury DR. The effects of length and stimulus rate on tension in the isometric cat soleus muscle. *J Physiol (Lond)* 204: 443–460, 1969.

Sandercock TG. Nonlinear summation of force in cat soleus muscle results primarily from stretch of the common-elastic elements. *J Appl Physiol* 89: 2206–2214, 2000.

Sandercock TG and Heckman CJ. Whole muscle length-tension properties vary with recruitment and rate modulation in areflexive cat soleus. *J Neurophysiol* 85: 1033–1038, 2001.

Street SF. Lateral transmission of tension on frog myofibers: a myofibrillar network and transverse cytoskeletal connections are possible transmitters. *J Cell Physiol* 114: 346–364, 1983.

Thornell LE and Price MG. The cytoskeleton in muscle cells in relation to function. *Biochem Soc Trans* 19: 1116–1120, 1991.

Troiani D, Filippi GM, and Bassi FA. Nonlinear tension summation of different combinations of motor units in the anesthetized cat peroneus longus muscle. *J Neurophysiol* 81: 771–780, 1999.

Trotter JA, Richmond FJ, and Purslow PP. Functional morphology and motor control of series-fibered muscles. *Exerc Sport Sci Rev* 23: 167–213, 1995.

Zajac FE. Muscle and tendon: properties, models, scaling, and application to biomechanics and motor control. *Crit Rev Biomed Eng* 17: 359–411, 1989.