

Force-Frequency and Fatigue Properties of Motor Units in Muscles That Control Digits of the Human Hand

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Fuglevand, Andrew J., Vaughan G. Macefield, and Brenda Bigland-Ritchie. Force-frequency and fatigue properties of motor units in muscles that control digits of the human hand. *J. Neurophysiol.* 81: 1718–1729, 1999. Modulation of motor unit activation rate is a fundamental process by which the mammalian nervous system encodes muscle force. To identify how rate coding of force may change as a consequence of fatigue, intraneural microstimulation of motor axons was used to elicit twitch and force-frequency responses before and after 2 min of intermittent stimulation (40-Hz train for 330 ms, 1 train/s) in single motor units of human long finger flexor muscles and intrinsic hand muscles. Before fatigue, two groups of units could be distinguished based on the stimulus frequency needed to elicit half-maximal force; group 1 ($n = 8$) required 9.1 ± 0.5 Hz (means \pm SD), and group 2 ($n = 5$) required 15.5 ± 1.1 Hz. Twitch contraction times were significantly different between these two groups (group 1 = 66.5 ms; group 2 = 45.9 ms). Overall 18% of the units were fatigue resistant [fatigue index (FI) > 0.75], 64% had intermediate fatigue sensitivity ($0.25 \leq \text{FI} \leq 0.75$), and 18% were fatigable (FI < 0.25). However, fatigability and tetanic force were not significantly different among groups. Therefore unlike findings in some other mammals, fast-contracting motor units were neither stronger nor more susceptible to fatigue than slowly contracting units. Fatigue, however, was found to be greatest in those units that initially exerted the largest forces. Despite significant slowing of contractile responses, fatigue caused the force-frequency relation to become displaced toward higher frequencies ($44 \pm 41\%$ increase in frequency for half-maximal force). Moreover, the greatest shift in the force-frequency relation occurred among those units exhibiting the largest force loss. A selective deficit in force at low frequencies of stimulation persisted for several minutes after the fatigue task. Overall, these findings suggest that with fatigue higher activation rates must be delivered to motor units to maintain the same relative level of force. Questions regarding classification of motor units and possible mechanisms by which fatigue-related slowing might coexist with a shift in the force-frequency curve toward higher frequencies are discussed.

INTRODUCTION

Motor commands generated in the CNS of mammals ultimately are translated into skeletal muscle force through two interrelated processes, 1) by varying the number of motor units that participate in a contraction (recruitment) and 2) by modulating the rate at which action potentials drive active motor units (rate coding). Of the two processes, rate coding appears to play a more significant role in terms of the overall capacity to grade muscle force (Botterman et al. 1986; Kernell 1992). The transformation of discharge rate into force by motor units therefore represents a fundamental feature by which the ner-

vous system controls skeletal muscle (Heckman and Binder 1991).

Whereas the relationship between activation rate and isometric force is known to have a sigmoid form (Bigland and Lippold 1954; Cooper and Eccles 1930), the specific shape depends in a complex way on the contractile speed of a motor unit (Botterman et al. 1986; Kernell et al. 1983b). Because of their protracted time course, slow twitch units summate individual force impulses more readily than do fast twitch units. Consequently, the activation rate needed for half-maximal or maximal force is usually lower for slow than for fast twitch units (Botterman et al. 1986; Kernell et al. 1983b). With fatigue, not only does force magnitude decline but also contractile speed may decrease (e.g., Sahlin et al. 1981). Such a fatigue-related change in contractile speed should, in theory, reduce the motoneuron discharge rates required to maintain maximal force (Bigland-Ritchie et al. 1983). Indeed, motor unit discharge rate decreases during sustained maximum voluntary contractions (Bigland-Ritchie et al. 1983; Grimby et al. 1981). Accordingly, it was hypothesized that the reduction in motor unit discharge rates seen during prolonged activity may help to optimize force output as the contractile properties of the motor units change rather than directly contribute to force decline, a phenomenon termed muscular wisdom (Bigland-Ritchie et al. 1983; Marsden et al. 1983).

Few studies, however, actually examined how the force-frequency property changes with fatigue in mammalian motor units. Initial studies on motor units in the cat hindlimb (Powers and Binder 1991) and in thenar muscles of the human hand (Thomas et al. 1991a) suggest that fatigue-related adaptation in the force-frequency relation may diverge for different classes of motor units. Fatigable units tended to require higher rates, whereas fatigue-resistant units either showed little change or required lower rates to attain half-maximal force after fatigue from intermittent stimulation.

To understand better how rate coding of force is modified with fatigue, intraneural microstimulation was used to elicit force-frequency responses in single motor units of extrinsic and intrinsic muscles of the human hand before and after a standard fatigue protocol (Burke et al. 1973). For the majority of units tested, higher stimulus frequencies were needed to maintain the same relative level of force after the fatigue protocol. Furthermore, the degree of shift in the force-frequency relation toward higher frequencies was directly related to the fatigability of the motor unit. Preliminary account of this work was presented previously as an abstract (Fuglevand et al. 1995).

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METHODS

Nineteen experiments were performed on 11 healthy human volunteers (4 female, 7 male, ages 23–55 yr). The Institutional Human Investigation Committee approved the procedures, and all subjects gave their informed consent to participate in the study. The method of intraneural stimulation was described in detail elsewhere (Macefield et al. 1996; Westling et al. 1990). Subjects reclined in a dental chair with the upper arm abducted to $\sim 70^\circ$, the elbow was extended to $\sim 150^\circ$, and the forearm and hand were supinated and stabilized in a vacuum pillow. A rubber sphere, mounted on an adjustable metal frame, was pressed down in the center of the palmar surface to secure the hand in place. Force and electromyographic (EMG) responses to intraneural stimulation of single axons in the median and ulnar nerves were recorded from long flexor muscles of the digits [flexor digitorum superficialis (FDS), flexor digitorum profundus (FDP), and flexor pollicis longus (FPL)] and from intrinsic hand muscles controlling the thumb [adductor pollicis (AdP) and abductor pollicis brevis (AbPB)].

Force and EMG recording

The distal interphalangeal joint of the test digit was enclosed in a cylindrical fitting 2 cm in length. Fittings of different internal diameters were used to accommodate fingers of various sizes. A Velcro strap spanned a small gap in the cylinder to secure the digit in the fitting. An orthogonal, biaxial force transducer was then attached to the fitting. One axis of the isometric transducer was aligned to detect flexion forces, and the other axis was aligned to detect abduction and adduction forces. Digits other than the test digit were immobilized with straps. Force was reset 10 ms before the delivery of stimuli to the nerve by means of an analogue circuit to minimize the effect of baseline fluctuations caused by respiration and arterial pulse pressure waves. Stimuli were triggered and delayed from the R-wave of the electrocardiogram to evoke twitches during the plateau phase of the pulse pressure cycle (Westling et al. 1990). In some cases subjects were also encouraged to breath-hold during brief stimulation sequences to lessen baseline oscillations caused by respiration.

EMG signals were detected by five sets of surface bipolar electrodes (Ag-AgCl, 4-mm diam, ~ 2 -cm interelectrode spacing). These were located 1) on the proximal forearm ~ 3 cm medial to the midline to record FDS, 2) on the distal forearm ~ 2 cm medial to midline to record FDP, 3) on the distal forearm ~ 2 cm lateral to midline to record FPL, 4) over the thenar eminence to record AbPB, and 5) on the palmar surface of the hand just distal to the thenar eminence, to record AdP.

Stimulation procedures

A tungsten microelectrode (1- to 5- μ m tip diam, 5- to 10- μ m uninsulated length, 250- μ m shaft diam, 100–300 k Ω impedance after insertion) was inserted through the skin on the medial surface of the upper arm to penetrate either the median or ulnar nerve. Low-intensity negative pulses (1–5 V, 0.2 ms, ~ 1 Hz) were delivered through the electrode via an isolated constant voltage stimulator. An adjacent uninsulated electrode inserted subdermally served as the anode. The intraneural electrode position was adjusted manually until a site was found that elicited motor responses in one of the target muscles. Activation of flexor digitorum profundus was distinguished from that of flexor digitorum superficialis by the presence of evoked movements in the distal phalanx. The force transducer was then fixed to the digit exhibiting the largest responses. The microelectrode site was then tested to determine whether a single motor axon could be activated in isolation. This procedure involved a gradual increase in stimulus pulse intensity until all-or-nothing responses were seen simultaneously in EMG and force. If these responses were stable over a range of intensities above threshold (termed the safety margin), it was assumed that a single motor axon was stimulated (cf. Macefield et al. 1996). If

a clear safety margin could not be demonstrated, the electrode position was readjusted, and the process was repeated.

Once a site was found that yielded unitary responses, the axon was stimulated in the following order: 1) individual pulses triggered and delayed from the R-wave of the electrocardiogram at ~ 1 Hz to obtain twitch responses, 2) tetanic stimulation involving a 3.1-s train of stimuli to potentiate the force responses in which stimulus frequency increased smoothly from 5 to 80 Hz and then returned to 5 Hz, 3) pulses triggered on the R-wave to obtain posttetanic twitch responses, 4) a series of constant-frequency trains, 5 s at 2 Hz, 2 s at 5 Hz, and then 1 s at 8, 10, 15, 20, 30, 50, 80, and 100 Hz to obtain force-frequency responses, 5) a standard fatigue protocol involving 330-ms trains at 40 Hz, 1 train/s for 2 min (Burke et al. 1973), 6) immediately after the fatigue protocol, the force-frequency sequence was reapplied, 7) the stimulus safety margin was reevaluated with ~ 1 -Hz stimuli, and 8) after 10 min of rest, stimulus sequences 1–4 were repeated. Individual trains in the force-frequency sequence were triggered and delayed from the R-wave such that there was a 1- to 3-s delay between trains.

Data acquisition and analysis

Force, EMG, and stimulus pulse signals were digitally sampled at 0.8, 3.2, and 12.8 kHz, respectively, by a computerized data acquisition and analysis system (SC-Zoom, University of Umeå, Sweden). Resultant forces were computed from the vector addition of the two orthogonal force signals. For FDS and FDP muscles the resultant force was practically equivalent to the flexion force vector. Twitch parameters were measured from the ensemble average of five twitch responses obtained before and after tetanic stimulation. These parameters included peak force, contraction time, half-relaxation time, time constant of relaxation after half-relaxation time, total duration, area, and the maximum rates of rise and relaxation of force (normalized to peak twitch force). For the force-frequency sequence, mean force was computed over the final 250 ms for each train for stimulus frequencies ≥ 10 Hz. For trains of lower-stimulus frequency, mean force was computed over an epoch equivalent to twice the interstimulus interval. From the fatigue protocol, peak force, half-relaxation time, time constant of force decay after half-relaxation time, and the maximum rates of rise and relaxation of force (normalized to peak force) were measured from the first five and last five trains. Changes in tetanic response during the fatigue protocol were then computed as the ratio of the average values obtained from the first five trains to that of the last five trains. Paired *t*-tests and linear regression analysis were used to evaluate whether the fatigue protocol caused significant changes in the mechanical properties of motor units. Values are expressed as means \pm SD, and differences are considered significant at $P < 0.05$.

RESULTS

Experiments typically lasted 2.5–4.0 h. Most subjects reported experiencing light tingling sensations in small regions of skin in one or two fingers or in the palm when the electrode was moved or during electrical stimulation. On occasion, while manipulating the electrode, some subjects reported a local dull ache in the vicinity of the electrode. In those cases, the electrode was withdrawn slightly, and the angle of penetration was adjusted to alleviate the discomfort.

Reliable unitary responses to intraneural stimulation were obtained for 13 motor units. Eleven of these were supplied by axons in the median nerve, and two were supplied by axons in the ulnar nerve. The larger proportion of median nerve-supplied units reflects the preponderance of experiments involving the median nerve (15/19). Ten units belonged to extrinsic, long-flexor muscles (7 FDS, 2 FPL, and 1 FDP), whereas three

units were from intrinsic muscles controlling the thumb (2 AbPB and 1 AdP). Because of the relatively small sample of units from individual muscle or muscle groups, intermuscle comparisons were not performed.

The mean (\pm SD) stimulus intensity for eliciting a threshold response in the 13 motor units was 701 ± 558 mV. The increase in stimulus intensity above threshold within which unitary responses were obtained was 225 ± 200 mV. F waves (sporadic delayed responses to stimulation caused by antidromic activation of the motoneuron) were seen in two units (1 FDS and 1 AbPB). The EMG waveforms for these F-wave responses were identical to those of the direct responses, which provided additional supportive evidence for unitary stimulation (Westling et al. 1990).

Twitch properties

Twitch responses potentiated on average by $29.5 \pm 30.7\%$ after 3.1 s of tetanic stimulation. Posttetanic twitch amplitude varied over a wide range of values, from 2.6 to 135.3 mN with a mean value of 70.7 ± 41.3 mN. Twitch contraction times were unimodally distributed and ranged from 43 to 91 ms (mean 57 ± 14 ms). Twitch half-relaxation times ranged from 50 to 92 ms (mean 71 ± 12 ms), the time constant of force decay varied from 21 to 56 ms (mean 40 ± 10 ms), and total twitch duration ranged from 156 to 301 ms (mean 224 ± 43 ms). The maximum rates of rise and relaxation of force during the twitch, normalized to twitch amplitude, varied from 19 to 53 s^{-1} (mean $28 \pm 9 \text{ s}^{-1}$) and from -7 to -33 s^{-1} (mean $-12 \pm 7 \text{ s}^{-1}$), respectively. Twitch area values extended from 0.3 to 14.3 mN/s (mean 7.7 ± 4.6 mN/s).

Contraction time correlated significantly with half-relaxation time ($r = 0.64$), twitch duration ($r = 0.80$), and normalized maximum contraction rate ($r = -0.60$) but not with normalized relaxation rate or the time constant of force decay. As in previous reports on human motor units (Macefield et al. 1996; Sica and McComas 1971; Thomas et al. 1990), no significant associations were found between twitch amplitude and temporal features of the twitch nor was twitch amplitude correlated with force-rate parameters. As would be expected, however, twitch amplitude was positively correlated with twitch area ($r = 0.98$).

Prefatigue force-frequency relation

Force and EMG responses to different stimulus frequencies from 2 to 100 Hz are shown for an FPL unit in Fig. 1. Figure 1, *inset*, depicts five superimposed twitch responses. Maximum force was attained in this unit at a stimulus rate of 80 Hz, whereas the greatest increases in force occurred for stimulus frequencies between 10 and 30 Hz. The ratio of twitch to maximum tetanic force for this unit was 0.17. Fusion (absence of stimulus-related force fluctuations) was nearly complete in this unit at a stimulus frequency of 15 Hz, well before the stimulus frequency required for maximum force. This was typical of other units tested in this study and is similar to that reported previously for human toe extensor motor units (Macefield et al. 1996). Occasionally, a stimulus pulse failed to evoke a response. This can be seen in the 20-Hz train of Fig. 1 (sixth train from the left) as a small gap in EMG record and a transient fall in force. At high stimulus frequencies (80 and 100

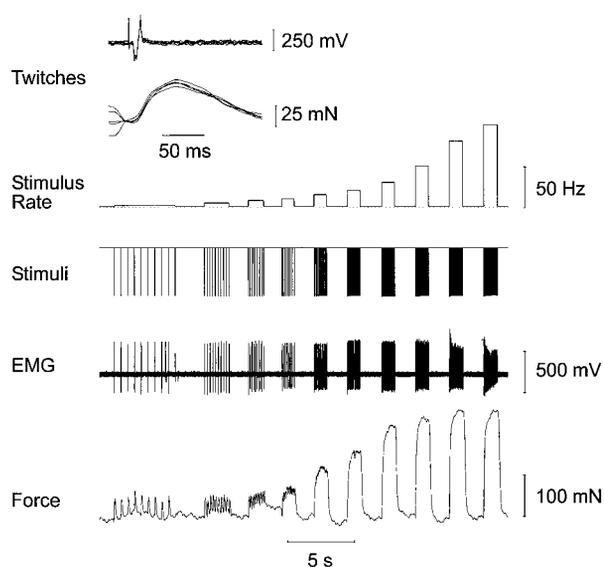


FIG. 1. Example force and electromyographic (EMG) responses in a flexor pollicis longus (FPL) motor unit to trains of stimuli delivered at different frequencies (2–100 Hz) before fatigue. *Top inset*: 5 superimposed twitch (twitch amplitude 44 mN, contraction time 54 ms, half-relaxation time 58 ms) and EMG responses to 1-Hz stimuli. Maximal force in this unit was attained with a stimulus rate of 80 Hz, and half-maximal force was attained with a stimulus rate of ~ 15 Hz.

Hz), the decay in EMG amplitude was likely caused by signal cancellation associated with temporal overlap of long-duration, biphasic action potentials (~ 16 ms) detected with surface electrodes (Fuglevand 1995).

Maximum force within the range of stimulus frequencies tested was attained for all units between 50 and 100 Hz (mean 85 ± 21 ; median 100 Hz). It is possible that even greater forces might have been exerted had we stimulated at frequencies greater than 100 Hz; however, above 30 Hz force increased very gradually with increased frequency. Force was normalized to the maximum force generated by each unit. Stimulation at 50 Hz elicited on average 91% of maximum force. The relation between force (relative to maximum force) and stimulus frequency is shown in Fig. 2 over the frequency range from 2 to 50 Hz to highlight the range of frequencies within which most force was developed. Two groups of units could be clearly distinguished based on the stimulus frequency needed for half-maximum force (estimated by linear interpolation; Fig. 2, dashed arrows); group 1 ($n = 8$) required 9.1 ± 0.5 Hz, and group 2 ($n = 5$) required 15.5 ± 1.1 Hz. Twitch contraction times were significantly different between these two groups as were the normalized maximum contraction rates (Table 1). Indeed, there was no overlap in the range of values between the two groups for contraction time and minimal overlap for normalized contraction rate.

Parameters related to the relaxation phase of the twitch, although tending to be slower in group 1 units (those units requiring lower stimulus rates to attain half-maximum force), were not significantly different between the two groups. For both groups of units combined, the stimulus frequency required to generate half-maximal force was significantly correlated to twitch contraction time ($r = -0.69$) but not to other twitch properties. This finding is similar to that shown previously for human toe extensor motor units (Macefield et al. 1996) and suggests that the time course of the rising phase of the twitch

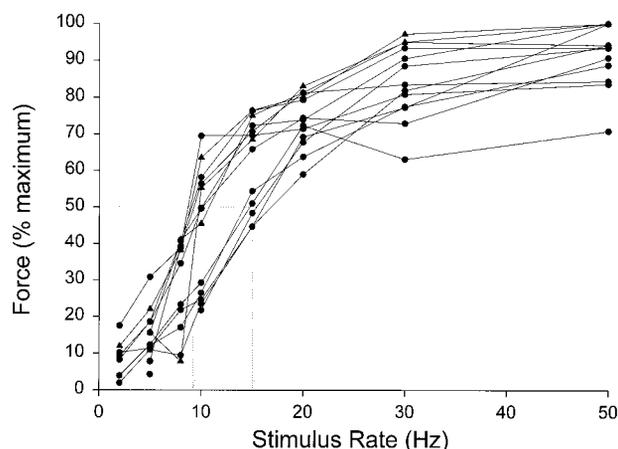


FIG. 2. Force-frequency curves obtained for 13 motor units before fatigue test. Two groups of units (group 1, $n = 8$; group 2, $n = 5$) were identified on the basis of the stimulus frequency needed for half-maximal force (indicated by arrows). Group 1 required 9.1 ± 0.5 Hz, and group 2 required 15.5 ± 1.1 Hz. Solid triangles: motor units from intrinsic muscles of the hand; solid circles: motor units from extrinsic muscles.

appears to be the most important determinant of the position (along the frequency axis) of the force-frequency relation (cf. Kernell et al. 1983b).

The peak slope (% maximum force/Hz) of the force-frequency curve was twice as steep on average for group 1 units compared with group 2 units and occurred at significantly lower stimulus frequencies (Table 1). There was no overlap in the range of values between group 1 and group 2 units for the stimulus frequency of half-maximum force, peak slope, or for the stimulus frequency of peak slope. Maximal tetanic force and the frequency of maximal force, however, were not different between the two groups. The ratio of twitch amplitude to maximal tetanic force was significantly larger for group 1 (0.36 ± 0.08) units compared with group 2 units (0.25 ± 0.06). These values for twitch-tetanus ratio are similar to that previously reported for human toe extensor motor units (0.28) (Macefield et al. 1996).

TABLE 1. Twitch and force-frequency parameters of two groups of motor units distinguished on the basis of stimulus frequency for half-maximum force

Twitch	Group 1 ($n = 7$)		Group 2 ($n = 5$)
Contraction time, ms	65.6 ± 12.4 (55.9–90.7)	*	45.9 ± 4.5 (43.1–53.7)
Half-relaxation time, ms	75.8 ± 9.0 (61.6–91.6)		64.1 ± 14.1 (50.5–84.5)
Time constant of terminal relaxation, ms	42.5 ± 6.6 (34.5–51.7)		37.3 ± 13.6 (20.7–56.3)
Total duration, ms	242.5 ± 40.1 (177.4–301.0)		198.1 ± 34.2 (155.8–235.3)
Amplitude, mN	82.9 ± 30.4 (36.7–121.6)		53.6 ± 51.7 (2.6–135.3)
Area, mN · s	9.5 ± 3.1 (5.5–13.5)		5.2 ± 5.5 (0.3–14.3)
Normalized maximum contraction rate, s^{-1}	23.3 ± 2.1 (18.7–24.8)	*	34.5 ± 11.4 (24.8–53.3)
Normalized maximum relaxation rate, s^{-1}	-9.0 ± 1.4 (-7.3–10.9)		-16.0 ± 9.9 (-8.5–33.3)
Force frequency	Group 1 ($n = 8$)		Group 2 ($n = 5$)
Frequency for half-maximum force, Hz	9.1 ± 0.5 (8.3–10.1)	*	15.5 ± 1.1 (14.2–16.9)
Peak slope, % maximum force/Hz	9.9 ± 2.8 (7.4–15.1)	*	4.9 ± 0.6 (4.2–5.6)
Frequency of peak slope, Hz	8.2 ± 1.2 (6.5–9.0)	*	13.5 ± 2.2 (12.5–17.5)
Maximum force, mN	200.2 ± 58.7 (110.4–293.0)		222.6 ± 220.1 (8.4–570.5)
Frequency of maximum force, Hz	82.5 ± 21.9 (50–100)		90.0 ± 22.4 (50–100)
Twitch-tetanus ratio	0.36 ± 0.08 (0.20–0.43)	*	0.25 ± 0.06 (0.17–0.31)
Force at 8 Hz, % maximum force	39.3 ± 2.6 (34.6–42.6)	*	17.2 ± 5.6 (9.4–23.3)

Values are means \pm SD. *Significant difference between groups ($P < 0.05$).

The larger value of the twitch-tetanus ratio for group 1 units suggests that these units may have less capacity to vary force by rate modulation. The difference between the two groups of units in this regard is even greater when the rate of discharge at recruitment threshold is considered (Fuglevand et al. 1993). The minimal rate of discharge during human voluntary contraction appears to be similar for most motor units in a muscle and is normally ~ 8 Hz (De Luca 1982; Milner-Brown et al. 1973b; Monster and Chan 1977). The average force produced with 8-Hz stimulation was $\sim 40\%$ of maximal force for group 1 units and 17% of maximal force for group 2 units (Table 1). Therefore the capacity to grade force by rate coding is substantially narrower for group 1 than for group 2 motor units.

Fatigue

Eleven units were followed for the duration of the fatigue protocol. Figure 3A shows force and EMG responses of one of those units (for comparison, the same unit as depicted in Fig. 1) during 2 min of intermittent stimulation at 40 Hz. Force declined in this unit by $\sim 50\%$ during this period [fatigue index (FI) = 0.52] with little change in the magnitude of the EMG responses.

In this study, the distribution of motor units with respect to fatigability was continuous and without distinct groupings of units. Nonetheless, when conventional criteria were applied (Burke et al. 1973), 2 of 11 (18%) were categorized as fatigue resistant ($FI > 0.75$), 7 of 11 (64%) units were of intermediate fatigability ($0.25 \leq FI \leq 0.75$), and 2 of 11 (18%) were considered fatigable ($FI < 0.25$). The two units identified as fatigable were from extrinsic flexors muscles (FPL and FDS).

In addition to changes in force magnitude, the time courses of the tetanic responses were slower at the end of the fatigue protocol compared with the beginning (Fig. 3B). For the 11 motor units, the mean value of the first five tetani differed significantly from that of the last five for normalized maximum contraction rate (11.1 ± 2.2 vs. $8.6 \pm 2.2 s^{-1}$), normalized

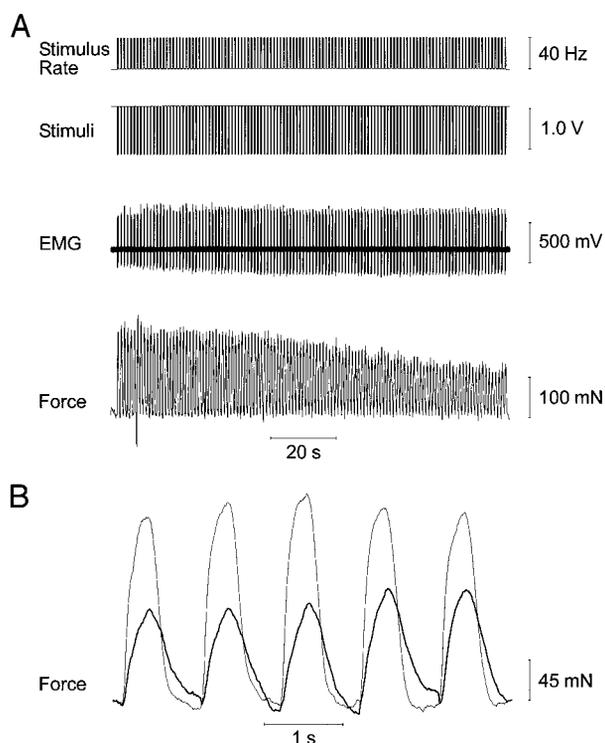


FIG. 3. *A*: example force and EMG responses in a FPL motor unit (same unit as in Fig. 1) to 2 min of intermittent stimulation (40-Hz trains for 330 ms, 1.0 train/s). *B*: initial (light line) and final (dark line) force responses from fatigue test shown in *A*. Fatigue index (FI, average peak force for final 5 responses/average peak force for initial 5 responses) for this unit was 0.52. The rate of rise and rate of relaxation of force were reduced in the final 5 tetani compared with the initial 5.

maximum relaxation rate (-6.7 ± 3.0 vs. -4.9 ± 1.9 s⁻¹), half-relaxation time (142.2 ± 62.2 vs. 208.6 ± 62.1 ms), and time constant of force decay after half-relaxation (72.4 ± 36.5 vs. 117.9 ± 44.2 ms). There was no significant correlation, however, between any of these parameters or change in these parameters and FI. For the current population of units, therefore, the degree of slowing in the tetanic response was unrelated to change in force magnitude (cf. Thomas et al. 1991b).

Relationship between fatigability and pre-fatigue contractile properties

FI was significantly correlated with both twitch amplitude ($r = -0.80$) and maximal tetanic force ($r = -0.71$) (Fig. 4A) but not with contraction time (Fig. 4B) or any other contractile speed property measured before fatigue. This finding is consistent with that previously reported by Thomas et al. (1991b) for human thenar motor units, namely, fatigue was greater in those units that initially generated the largest forces but was unrelated to contractile-rate parameters. In other mammals, fatigability also is consistently correlated with motor unit force (Kernell et al. 1975; Lev-Tov et al. 1988; Olson and Swett 1966; Reinking et al. 1975; Tötösy de Zepetnek et al. 1992) and in some studies correlated with contractile speed (Bakels and Kernell 1993; Gardiner 1993; Lev-Tov et al. 1988; Reinking et al. 1975), although this is not always the case (Bigland-Ritchie et al. 1998). There was no difference between group 1 and group 2 units with regard to fatigability (mean FI: group 1 = 0.51 ± 0.30 ; group 2 = 0.46 ± 0.22).

Fatigue-related change in the force-frequency relation

Immediately after the fatigue protocol, motor units were activated with the same sequence as before fatigue with brief trains from 2 to 100 Hz (Fig. 5). The most striking feature of the postfatigue responses depicted in Fig. 5 (same unit as in Figs. 1 and 3) was the absence of force at low frequencies of stimulation (i.e., low-frequency fatigue) (Edwards et al. 1977; Jami et al. 1983). This deficit occurred despite the presence of fully developed motor unit action potentials. Only for stimulus frequencies greater than or equal to 15 Hz were detectable force responses elicited. Maximal tetanic force after fatigue, however, was only moderately diminished from that obtained in this unit before fatigue (215 vs. 252 mN, or 85% of pre-fatigue level). For the 11 motor units, the average maximal tetanic force immediately after fatigue was $81 \pm 31\%$ of that before fatigue. This change with fatigue, however, did not attain statistical significance ($P = 0.06$).

The relationship between stimulus frequency and normalized force, derived from the recordings of the unit depicted in Figs. 1 (before fatigue) and 5 (immediately after fatigue), are shown in Fig. 6A. The force-frequency curve shifted to the right (i.e., toward higher frequencies) with fatigue in this unit. The frequency needed for half-maximal force was 16.9 Hz before fatigue and increased to 24.5 Hz after fatigue (Fig. 6A, arrows), a 45% increase in this parameter. The fatigue-related

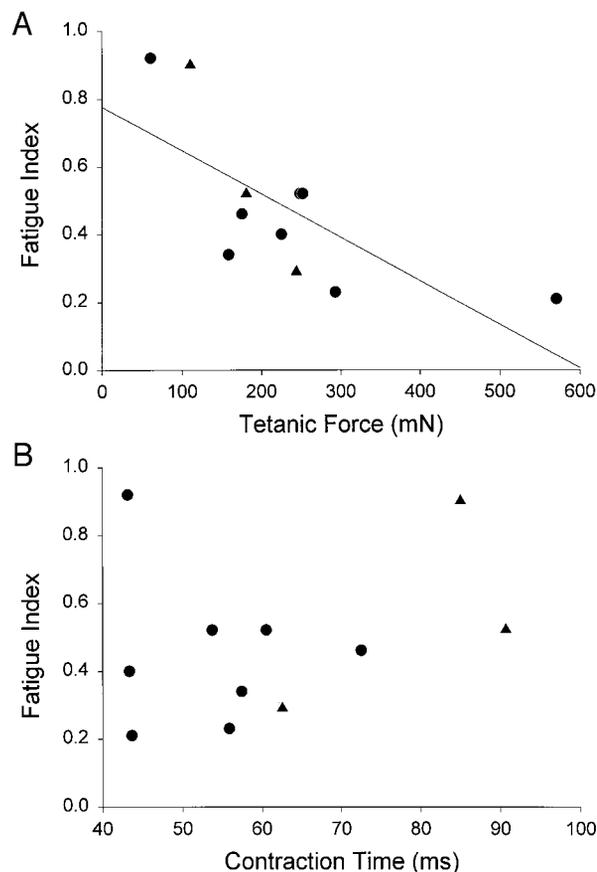


FIG. 4. Plot of FI as a function of pre-fatigue maximum tetanic force (*A*) and as a function of pre-fatigue twitch contraction time (*B*) for 11 motor units. Regression line is shown for significant correlation between FI and tetanic force ($r = -0.71$, *A*). Fatigue was greater in those units that initially generated the largest forces but was unrelated to contractile speed. \blacktriangle : motor units from intrinsic muscles of the hand. \bullet : motor units from extrinsic muscles.

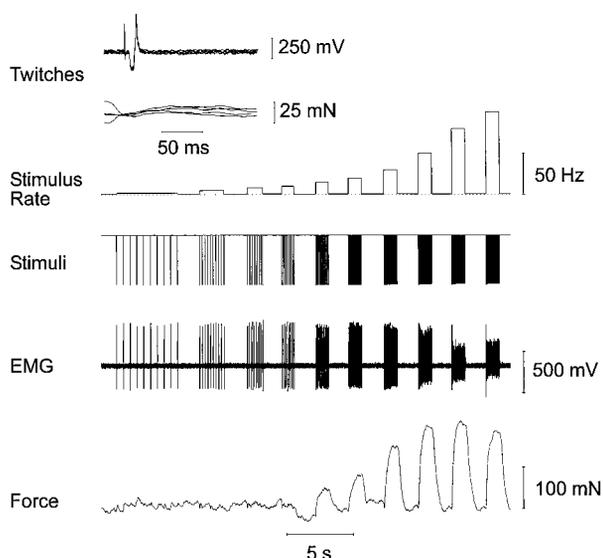


FIG. 5. Example force and EMG responses in FPL motor unit (same unit as in Figs. 1 and 3) obtained immediately after the 2-min fatigue protocol. Frequency of stimulus trains were from 2 to 100 Hz. *Inset*: 5 superimposed twitch and EMG responses that were obtained during \sim 1-Hz stimulation directly after the force-frequency sequence. Note the absence of force at low-stimulus rates and that EMG responses were fully developed.

change in the frequency for half-maximal force, expressed as a percentage of the pre-fatigue value, is plotted for the 11 units as a function of FI in Fig. 6B. For all units but one, the frequency for half-maximal force increased (positive change), indicating a rightward shift in the force-frequency relation with fatigue. The average percentage change in the frequency for half-maximal force was 44% (\pm 41%). In addition, there was a marked correlation ($r = -0.80$) between the FI of the unit and the degree of shift in the force-frequency curve. Those units that were most fatigable also exhibited the greatest rightward shift in the force-frequency relation.

One parameter that has been used extensively to characterize low-frequency fatigue is the ratio of force elicited at a low-stimulus frequency to that elicited by a high frequency (e.g., Edwards et al. 1977). The 15-Hz-maximal tetanic force ratio was significantly smaller after the fatigue task (0.44 ± 0.22) compared with before (0.62 ± 0.12). This change was consistent with the observed shift in the force-frequency curves toward higher frequencies. Furthermore, the 15-Hz-maximal tetanic force ratio was strongly correlated with the frequency for half-maximal force both before ($r = -0.98$) and after the fatigue protocol ($r = -0.87$). In addition, the regression equations fitting these data were practically identical in fresh ($Y = -0.036X + 1.04$) and fatigued motor units ($Y = -0.031X + 0.96$). These findings, therefore, indicate that the 15-Hz-maximal tetanic force ratio, which can be determined with relatively few measurements, could be used as a robust predictor of the position of the force-frequency curve along the frequency axis.

Interestingly, despite the shift in the force-frequency curve to higher frequencies with fatigue (as indicated by the frequency for half-maximal force and the 15-Hz-maximal tetanic force ratio), the frequency that elicited maximal force decreased with fatigue (from 85.4 ± 21.5 Hz, median 100 Hz, before fatigue to 64.5 ± 23.4 Hz, median 80 Hz, with fatigue; $P < 0.05$). This change was mainly due to a slight decline in

force at the highest stimulus frequencies for 9 of the 11 units (e.g., Fig. 5). Nevertheless, the combined effects of a decrease in frequency for maximal force and an increase in the frequency for half-maximal force meant that the slope of the force-frequency curve was steeper in the upper-frequency range with fatigue. Indeed, the *average* slope of the curve between 15 Hz and the frequency for maximal force was significantly steeper with fatigue ($1.3 \pm 0.7\%$ maximum force/Hz) compared with before fatigue ($0.6 \pm 0.3\%$ maximum force/Hz).

Persistence of low-frequency fatigue

The contractile responses of four motor units were reexamined 10 min after the fatigue protocol. All four units exhibited low-frequency fatigue (i.e., marked depression in force responses to low-stimulus frequencies) at this time. For two of these units, however, the specific deficit in force

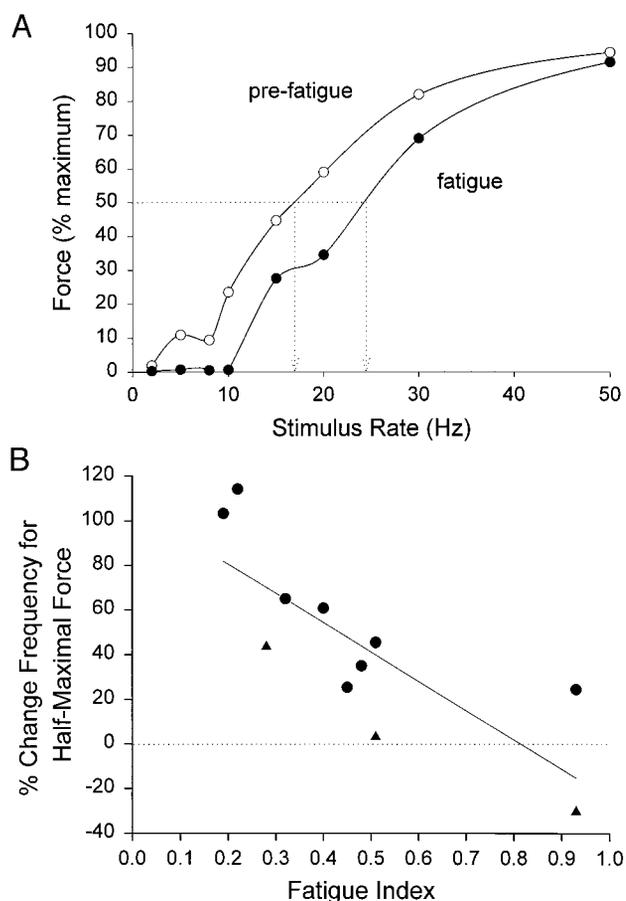


FIG. 6. A: relation between relative force and stimulus frequency for FPL motor unit (same unit shown in Figs. 1 and 5) before and immediately after the 2-min fatigue test. The force-frequency curve shifted toward higher frequencies with fatigue. The stimulus frequency needed to attain half-maximal force (dashed arrow) increased from 16.9 Hz before fatigue to 24.5 Hz after fatigue, a 45% increase in this parameter. B: relation between FI and fatigue-related change in the frequency required for half-maximal force (percentage of the pre-fatigue value) for 11 motor units. Positive values of percentage change indicate a rightward shift of the force-frequency relation, whereas negative values indicate a leftward shift with fatigue. A significant negative correlation ($r = -0.80$) was found between FI and the degree of shift of force-frequency relation. Motor units that were most fatigable had the greatest rightward shift in the force-frequency relation with fatigue. Solid triangles: motor units from intrinsic muscles of the hand. Circles: motor units from extrinsic muscles.

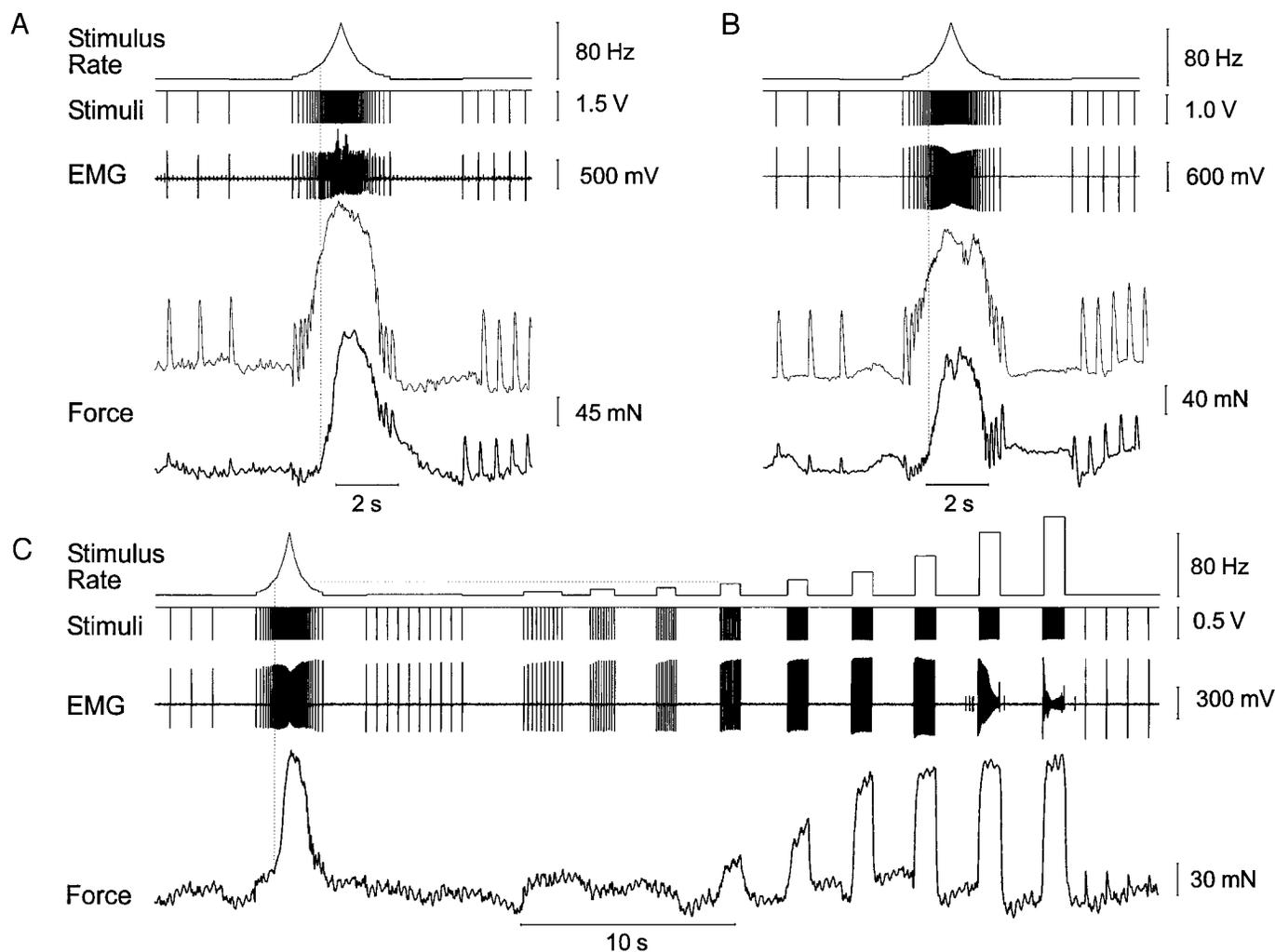


FIG. 7. Force and EMG responses to stimulus trains in which frequency continuously increased from 5 to 80 Hz and then decreased back to 5 Hz after 10 min of recovery from the fatigue task. *A–C*: responses for 3 motor units. The force response to a similar stimulation sequence delivered before fatigue (light line) is shown for the motor units in *A* and *B* (the timing of the posttetanus stimuli used to elicit twitches was slightly different for the 2 time periods). Twitches elicited in the motor units shown in *A* and *B* were markedly depressed before the triangular train of stimuli but recovered to approximately one-half the prefatigue level after the tetanus. In these 2 units force was abruptly restored during the triangular stimulus train when stimulus frequency exceeded ~ 15 Hz (dashed vertical line). Before fatigue, force at this same frequency was $\sim 75\%$ of the peak force (light traces, *A* and *B*). Similarly, force was promptly reestablished in the unit shown in *C* when stimulus frequency increased to > 15 Hz. However, force responses at low frequencies were practically absent both before and after the tetanus. In subsequent constant frequency trains, noticeable force was developed only for stimulus frequencies that were ≥ 15 Hz. After the last train in the sequence (100 Hz), twitches were momentarily visible but decayed rapidly and were difficult to resolve from baseline fluctuations within a few seconds.

at low-stimulus frequencies was largely eliminated by a brief period of tetanic stimulation. Force and EMG responses to low-frequency stimulation before and after a 3.1-s train of stimuli in which stimulus frequency increased from 5 to 80 Hz and then returned to 5 Hz are shown for these two units in Fig. 7, *A* and *B*. The upper force trace (light line) in each record depicts the response of the unit obtained before fatigue. For both of these units, at 10 min after the fatigue protocol (dark lines), the twitch responses associated with 1-Hz stimulation were markedly diminished before the tetanus but were restored to approximately one-half the prefatigue level after the tetanus. The transition from depressed to restored force was abrupt and occurred as stimulus frequency increased above ~ 15 Hz (Fig. 7, *A* and *B*, dashed vertical lines). Before fatigue (light tracing), the

force developed at this same frequency was $\sim 75\%$ of the peak force attained during the tetanus.

In a third unit, force was also abruptly restored as stimulus frequency increased above 15 Hz (Fig. 7*C*, dashed vertical line). However, force responses at low-stimulus frequencies were practically absent both before and after the tetanic stimulation in this unit. Noticeable force was developed during the subsequent constant-frequency trains only for stimulus frequencies greater than or equal to 15 Hz. Immediately after the last train in the series (100 Hz, Fig. 7*C*), twitches were momentarily visible, but these responses decayed rapidly and were difficult to resolve from baseline fluctuations within a few seconds. In a fourth unit (not shown), the constant-frequency trains were not preceded by tetanic stimulation. The responses in that unit to constant-frequency trains were similar to those

shown in Fig. 7C, namely, detectable force was developed for stimulus frequencies greater than or equal to 15 Hz, and twitch responses were restored for only a few seconds after the 100 Hz train.

DISCUSSION

Intraneural microstimulation of motor axons, mainly supplying long flexor muscles of the fingers, was used to examine changes in contractile properties of human motor units after a fatigue protocol that has been used extensively to study motor units in other mammals (Burke et al. 1973). The only comparable previous investigation in humans was carried out for motor units of the thenar muscles (Thomas et al. 1991a). Two groups of motor units could be distinguished in the present study based on the stimulus frequency required to attain half-maximal force. Those units with brief contraction times needed higher-stimulus frequencies to achieve the same relative force compared with units with longer-duration contraction times. Unlike findings in other mammalian muscles, fast-contracting motor units were neither stronger nor more susceptible to fatigue than slowly contracting units. Fatigue, however, was found to be greatest in those units that initially exerted the largest forces. After the fatigue protocol, the force-frequency relation was displaced toward higher frequencies with the greatest shift occurring among those units exhibiting the largest force loss. The significance of these observations is considered after a brief discussion of some technical limitations of the current investigation.

Limitations

The most significant limitation of the current study was the small sample of motor units from which a complete set of contractile properties could be measured. This problem should not be undervalued given the diversity of properties of motor units that normally exists within a single muscle, across different muscles, and across individuals. In this investigation, data from 13 motor units in 5 muscles from 11 subjects were pooled together for analysis. Clearly, this limits the generality of the conclusions that can be drawn about the organization of motor unit properties in these muscles.

The main factor that prevented successful isolation of more units was the difficulty in establishing and maintaining unitary stimulation of single motor axons. Obviously, it is not possible in human subjects to physically isolate motor axons by successive division of the motor nerve as is done in acute animal experiments. Consequently, successful stimulation with microelectrodes depends on the fortuitous placement of the electrode within a nerve fascicle such that a weak stimulus activates only one motor axon supplying a target muscle. Because of the relatively large size of the microelectrode in relation to axon diameter (Wall and McMahon 1985), such a site likely requires that all axons in the immediate vicinity of the target axon be either sensory fibers or motor axons supplying muscles whose mechanical action have minimal effect on the detected force. The probability of finding such a configuration improves with distance between the target muscle and electrode site because of the greater intermingling of sensory and motor axons supplying diverse targets within more proximal fascicles (Sunderland 1978). Also, with greater distance between electrode and

muscle, it is less likely that contraction within the target muscle will displace the electrode.

In a previous study, we examined twitch and force-frequency properties of motor units in human toe extensor muscles in response to intraneural stimulation in the common peroneal nerve (Macefield et al. 1996). We were unable, however, to measure fatigue properties in those units because safety margins invariably deteriorated during the fatigue protocol. This was likely due to the short distance between the electrode site and the muscles innervated by the peroneal nerve and associated greater density of motor axons within more distal fascicles. In the current study, the microelectrode was inserted into the median or ulnar nerve many centimeters proximal to the muscles under investigation. Both the mean threshold (701 mV) and mean safety margin (225 mV) for motor axons in the medial-ulnar nerves were nearly double those found in the peroneal nerve (411 and 116 mV, respectively) (Macefield et al. 1996). These differences probably reflect a lower density of motor axons in fascicles of the median-ulnar nerves. Consequently, minute alterations in electrode position or axon excitability in these nerves were less likely to activate neighboring motor axons or to interrupt activation of the target axon. Hence it was possible to maintain unitary stimulation of some motor axons for several minutes, which allowed us to record the contractile properties of their muscle units before and after fatigue. Therefore despite the limited sample size, these records represent some of the only data of this kind obtained in human subjects. Furthermore, the similarity of features of this small population of motor units with that of larger samples of human units obtained with intramuscular stimulation, e.g., absence of relation between twitch force and contraction time (Elek et al. 1992; Garnett et al. 1978; Mateika et al. 1998), and with that of nonhuman motor units, e.g., significant correlation between fatigability and tetanic force (Botterman et al. 1985; Fritz and Schmidt 1992; Goslow et al. 1977; Reinking et al. 1975; Tötösy de Zepetnek et al. 1992; Zajac and Faden 1985), suggests that the current collection of motor units did provide a coarse-grain view of motor unit organization in muscles that control the digits of the human hand.

Classification of motor units

This investigation was consistent with previous studies in both human and nonhuman muscle in that the distribution of motor unit contraction times was relatively continuous and unimodal (Elek et al. 1992; Goslow et al. 1977; Kernell et al. 1983a; Thomas et al. 1990). Consequently, motor unit populations typically cannot be separated readily into slow and fast twitch groups on the basis of twitch contraction speed (Reinking et al. 1975; Tötösy de Zepetnek et al. 1992). Indeed, one conventional criteria distinguishes fast from slow units not on the basis of twitch responses but on whether units exhibit a small loss in force during a short train of stimuli ("sag") (Burke et al. 1973). Although sag represents an important property for categorizing motor units in some muscles, e.g., cat gastrocnemius (Burke et al. 1973), cat tibialis posterior (McDonagh et al. 1980), cat tenuissimus (Lev-Tov et al. 1988), and rat gastrocnemius (Kanda and Hashizume 1992), it has also been shown to be poorly correlated with contractile speed in other muscles, e.g., rat tibialis anterior (Bakels and Kernell 1993; Tötösy de

Zepetnek et al. 1992) and cat extensor carpi ulnaris (Fritz and Schmidt 1992), and to be absent altogether in human motor units (Macefield et al. 1996; Thomas et al. 1990). When twitch contraction time itself was used to distinguish fast from slow units, the boundary delineating the two groups is often chosen arbitrarily (Olson and Swett 1966) or may be selected based on a property unrelated to contractile speed, such as fatigue resistance (Kernell et al. 1983a). In addition, the contraction time used to separate fast from slow units can vary over a wide range of values from as little as 14 ms in rat tibialis anterior (Bakels and Kernell 1993) to as much as 99 ms in human gastrocnemius (Garnett et al. 1978).

Therefore rather than impose an arbitrary criterion to segregate motor units into fast and slow categories based on twitch properties, we instead chose to classify motor units into two groups based on the configuration of the force-frequency relationship. Motor units recorded in this study clustered into two distinct groups based on the stimulus frequency required for half-maximal force (Fig. 2). Similar criteria were also used previously to classify motor units in nonhuman muscle (Botterman et al. 1985; Fritz and Schmidt 1992; Gardiner 1993). The force-frequency relation represents the fundamental input-output property of motor units; it indicates how the neural code, in terms of motor neuron discharge rate, is transformed into a mechanical response that can be applied to the external environment. The force-frequency property, therefore, would seem to possess greater functional significance than would twitch speed per se. Although features of the force-frequency relation can be predicted from the time course of the twitch (Botterman et al. 1986; Kernell et al. 1983b), the relationship between these two properties is not simple. For example, the stimulus frequency needed to attain half-maximal force can be markedly different for motor units with identical twitch contraction times but residing in different muscles (Kernell et al. 1983b). Consequently, we used a parameter that more directly denoted the configuration of the force-frequency relationship for different motor units, namely, the stimulus frequency for half-maximal force. On the basis of this parameter, we discerned two groups of units that differed in how they encode isometric force through rate modulation.

Distinct clustering of motor units based on fatigability was not observed. The majority of units had intermediate sensitivity to fatigue similar to what was described for some muscles in various species, e.g., rat tibialis anterior (Bakels and Kernell 1993; Tötösy de Zepetnek et al. 1992), cat tibialis anterior and extensor digitorum longus (Goslow et al. 1977), cat tenuissimus (Lev-Tov et al. 1988), and human first dorsal interosseous (Stephens and Usherwood 1977). This result, however, is the converse of that described for other muscles in which a clear bimodal distribution is found such that most units are either fatigue resistant or fatigable, and few units are categorized as having intermediate fatigue properties, e.g., cat medial gastrocnemius (Burke et al. 1973), cat peroneus longus (Kernell et al. 1983a), and cat tibialis posterior (McDonagh et al. 1980). Also, the current distribution of fatigue indices was different from that observed in human thenar muscle (Thomas et al. 1991b) and ankle extensors of the skunk (Van de Graaff et al. 1977) in which the largest proportion of motor units was fatigue resistant, a smaller proportion was classified as intermediate fatigable, and no units were categorized as fatigable. Therefore there seems to be several designs by which motor unit popu-

lations can be organized according to fatigability. These differences probably relate to variation in the repertoire of motor behaviors across muscles and species (Van de Graaff et al. 1977).

It is also possible that species differences in fatigability might arise because the same stimulation protocol has been applied to induce fatigue despite large differences in contractile speed (Bigland-Ritchie et al. 1998). For the relatively slow human motor units, 40-Hz stimulation produces near maximal force, whereas it elicits only partially fused, submaximal force in cat and rat motor units. Accordingly, it may be more appropriate to induce fatigue with a stimulus frequency selected for each motor unit that evokes a particular percentage of maximal force rather than use the same frequency for all units.

A consistent although unexpected feature of human motor units is the absence of a relationship between twitch speed and force magnitude. This was reported for a variety of human muscles, including those involved in posture and locomotion (Garnett et al. 1978; Macefield et al. 1996), intrinsic muscles of the hand (Elek et al. 1992; Thomas et al. 1990), intrinsic muscles of the foot (Sica and McComas 1971), respiratory muscles (Matioka et al. 1998), muscles involved in mastication (Nordstrom and Miles 1990), and extrinsic muscles that control the digits of the hand as found in the current study. In most nonhuman muscles, weak motor units tend to be slow (Bakels and Kernell 1993; Burke et al. 1973; Dum et al. 1982; Gordon et al. 1986, 1990; Kanda and Hashizume 1992; Kernell et al. 1983a). In contrast there is little evidence to suggest that weak motor units are inevitably slow or that strong units are necessarily fast in human muscle (Bigland-Ritchie et al. 1998).

Data from this study as well as from others (Nordstrom and Miles 1990; Thomas et al. 1991b) also indicate that contractile speed and fatigue sensitivity are poorly correlated among pools of human motor units. Interestingly, this is also the case for a wide array of nonhuman muscles, including rat tibialis anterior (Tötösy de Zepetnek et al. 1992), rabbit masseter (Kwa et al. 1995), cat flexor digitorum longus (Olson and Swett 1966), cat extensor digitorum (Fritz and Schmidt 1992), and cat tibialis anterior and extensor digitorum longus (Goslow et al. 1977). For some of these muscles, the absence of a correlation between contractile speed and fatigue was probably due in part to the preponderance of fast twitch units that are known to have a broad range of fatigabilities and a narrow range of contractile speeds (Fritz and Schmidt 1992; Goslow et al. 1977; Kwa et al. 1995). Along these lines, it is also conceivable that intraneural stimulation might have biased our sample toward those units with larger diameter (more excitable) axons that presumably supply fast, fatigable, and strong muscle units. However, the majority of units recorded in the present study could be considered slow based on the relatively long-duration twitches and the low-stimulus rates required to attain half-maximal force. Therefore it seems in some mammalian muscle and in particular human muscle that contractile speed is in no predictable way related to fatigability among a pool of motor units.

One aspect of motor unit organization that seems consistent across muscles and species is that strong motor units invariably fatigue more rapidly than do weak motor units. A significant association between motor unit force (twitch or tetanic) and fatigability was found in practically every muscle in which the relation was examined in both nonhuman (e.g., Fritz and

Schmidt 1992; Gardiner 1993; Goslow et al. 1977; Kernell et al. 1975; Kugelberg and Lindgren 1979; Lev-Tov et al. 1988; McDonagh et al. 1980; Olson and Swett 1966; Reinking et al. 1975; Tötösy de Zepetnek et al. 1992) and human muscle (Garnett et al. 1978; Stephens and Usherwood 1977; Thomas et al. 1991b), including this study. This finding probably relates to systematic differences in the extent of daily activity across a pool of motor units. It is well established that motor units typically are recruited in an orderly sequence from those units that exert the smallest forces toward those that generate the largest forces (Henneman and Mendell 1981; Milner-Brown et al. 1973a; Zajac and Faden 1985). Most forms of muscular activity involve only a fraction of the motor unit population (Enoka 1995). Consequently, low-threshold, weak motor units will tend to be active for a much greater duration of a day compared with high-threshold, strong units. Because sensitivity to fatigue can be dramatically reduced by increasing the amount of chronic activity (Kernell et al. 1987), it follows that low-threshold motor units will tend to be more fatigue resistant than high-threshold motor units. In some respects then, categorization of motor units according to force capacity and fatigue sensitivity could be considered more physiologically relevant and more harmonious across species than prevailing classification schemes. In other words, it may be more meaningful to characterize motor units based on the conjunction of fatigability and contractile strength rather than that of fatigability and contractile speed.

These two schemes were compared for the current set of data in Fig. 4. When contraction time was plotted against FI, as is ordinarily done to classify motor units, no relation between the two variables was seen nor was there distinct clustering of data into subgroups (Fig. 4B). On the other hand, when tetanic force was plotted against FI (Fig. 4A), not only was the relationship between these two variables significant but three clusters of motor units also could be distinguished. One group was weak and fatigue resistant, a second (and most abundant) group was of intermediate strength and intermediate fatigue sensitivity, and a third group (one unit) was strong and fatigable. Because of the limited sample size, however, it is important that these findings not be overgeneralized until comparable criteria were applied to larger motor unit populations, including those from other muscles and species.

Fatigue-related change in the force-frequency relationship

Prolonged activation of muscle not only leads to loss in force but may also cause the contractile responses to slow. In the absence of other changes, slowing in contractile speed should cause the force-frequency curve to shift toward lower frequencies (Bigland-Ritchie et al. 1983). In this investigation, despite marked slowing in the contractile responses with fatigue (e.g., Fig. 3B), the force-frequency curve for most motor units was displaced toward higher, rather than lower frequencies. Similar findings were described previously for single muscle fibers of the mouse (Westerblad et al. 1993), motor units in the cat hindlimb (Powers and Binder 1991), motor units in human thenar muscles (Thomas et al. 1991a), and whole human muscle (Bergström and Hultman 1990; Binder-McLeod and McDermond 1992; Edwards et al. 1977).

How can fatigue-related slowing in contractile speed be reconciled with a shift in the force-frequency curve toward

higher frequencies? One possible explanation is that other modifications in neuromuscular function override the effect that contractile slowing alone would have on the force-frequency relation. One candidate for such a modification is a frequency-dependent impairment of excitation-contraction coupling. Virtually no force was developed in motor units at low-stimulus frequencies immediately after (Fig. 5) and for several minutes after the fatigue protocol (Fig. 7). This absence of force occurred, even though EMG responses were fully developed, suggesting that the site of impairment was distal to the sarcolemma. Because the deficit in force at high-stimulus frequencies was small, the intrinsic capacity of cross-bridges to generate force appears to have been minimally affected by the fatigue protocol. By exclusion then, it appears that impairment of intervening processes associated with excitation-contraction coupling were the principal contributors to fatigue. These arguments follow those previously articulated to explain similar findings, generally referred to as low-frequency fatigue, in a variety of preparations (Edwards et al. 1977; Grabowski et al. 1972; Jami et al. 1983; Powers and Binder 1991; Westerblad et al. 1993).

An intriguing aspect of low-frequency fatigue as revealed in this study was the abrupt transition from no force to near-normal force when stimulus frequency exceeded a particular value, usually ~ 15 Hz (Figs. 5 and 7). One interpretation of these findings is that excitation-contraction coupling was disrupted only at low-stimulus frequencies. Current theories, however, suggest that disturbances in the calcium-release channel of the sarcoplasmic reticulum lead to a decrease in the amount of calcium released per action potential, independent of frequency (Fitts 1996; Westerblad and Allen 1991; Westerblad et al. 1993). This implies that the concentration of myoplasmic calcium should be less for all stimulus frequencies up to that needed to just attain calcium saturation. As a consequence, the relation between relative force and frequency should have a lower slope over the entire rising phase of the curve, and the frequency needed for maximal force should be greater in low-frequency fatigue. However, our findings as well as those of others (Faulkner 1983; Powers and Binder 1991; Thomas et al. 1991a) indicated that the rate of rise of force with frequency once initiated was steeper and that there was a decrease in the frequency for maximal force with fatigue.

What then could account for the specific impairment of excitation-contraction coupling at low but not at high stimulus rates and the prolonged time course of this effect? Efficient communication between voltage-sensitive dihydropyridine receptors in the t-tubule and ryanodine-receptor calcium-release channels of the sarcoplasmic reticulum depends on the intimate juxtaposition of these molecules (Franzini-Armstrong and Protasi 1997). Recently, Gomez et al. (1997) demonstrated that reduced contractility in hypertrophied cardiac muscle cells was associated with an increased physical separation between adjacent dihydropyridine and ryanodine receptors. A similar alteration might occur in skeletal muscle with fatigue. One aftereffect of prolonged activity that could change the microgeometry between the t-tubule and sarcoplasmic reticulum and that follows a relatively long time course is postcontraction swelling of muscle fibers (Eisenberg and Gilai 1979; Lannergren et al. 1996). In swollen fibers, movement of the charged elements of the voltage-sensitive receptor in response to a single action potential, although normal (Gomez et al. 1997;

Györke 1993), might not be sufficient to provoke calcium release from the sarcoplasmic reticulum. However, if subsequent action potentials arrive before the charged elements fully relax to their initial state, summation of displacements could occur. With sufficiently brief time intervals between action potentials (i.e., higher stimulus rates), the overall displacement of the charged elements may be adequate to induce calcium release from the sarcoplasmic reticulum. Because the rate of calcium reuptake by the sarcoplasmic reticulum is slowed with fatigue (Westerblad and Allen 1993), myofibrillar calcium could accumulate to relatively high concentrations during trains of stimuli at activation rates in excess of that required to initiate calcium release. In this way fatigue-related slowing of the contractile response (caused by diminished rate of calcium uptake) feasibly could coexist with a steeper but rightward-shifted, force-frequency relation.

Functional consequences

Finally, it is of interest to consider the functional implications of fatigue-related alterations in the force-frequency relation. In this study, the stimulus frequency needed to attain half-maximal force in human motor units increased on average by >40% with fatigue. To varying degrees, similar findings were reported by others in a variety of preparations (Bergström and Hultman 1990; Binder-McLeod and McDermond 1992; Edwards et al. 1977; Powers and Binder 1991; Thomas et al. 1991a; Westerblad et al. 1993). In addition, there was a significant tendency for the most fatigable (and strongest) units to exhibit the greatest rightward shift in the force-frequency relation (Fig. 6B) (see also Thomas et al. 1991a). Therefore for the type of fatigue task used in these studies, greater activation rates would be required to maintain the same relative level of force with fatigue. Furthermore, the demand for higher activation rates would be greatest for those units least capable of maintaining force.

A crucial question then is whether the nervous system keeps pace with fatigue-related changes in contractile function of motor units during voluntary activity (Fuglevand 1996). The discharge rates of motor units decrease by ~50% from an initial level of ~30 Hz during sustained maximum voluntary contractions (Bigland-Ritchie et al. 1983). This finding together with the results of this study implies that fatigue-related adjustments in motor neuron discharge may fail to accommodate for changes in the mechanical properties of motor units and may directly contribute to force loss. However, two additional and interrelated factors require consideration. First, whereas intermittent activity was used to induce fatigue in the current investigation, little is known about how (if) motor neuron discharge adapts during intermittent voluntary contractions. Second, few studies have examined how the force-frequency relation changes as a consequence of sustained rather than intermittent contraction. Investigation into both these areas is needed to establish whether adaptation in motor neuron discharge during prolonged activity helps prevent or contribute to fatigue.

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