Nonuniform Fatigue Characteristics of Slow-Twitch Motor Units Activated at a Fixed Percentage of Their Maximum Tetanic Tension

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SUMMARY AND CONCLUSIONS

1. The endurance of slow-twitch motor units from the soleus (SOL) and medial gastrocnemius (MG) muscles of the cat were tested by determining the length of time (endurance time, \(E_t\)) that a unit could maintain its tension output at 85% of maximum. Motor-unit tension was clamped at the target level by altering the stimulation rate of a unit’s motor axon through computer feedback control. Tested in this way, units of both muscles displayed a wide range of \(E_t\)s, ~40- to 50-fold.

2. Electromyographic (EMG) waveforms of motor units subjected to force-clamp contractions were analyzed to assess whether any activity-dependent changes in their waveform shape might predict \(E_t\). Three measurements of waveform shape were determined: baseline-to-baseline duration, peak-to-peak amplitude, and area. Typically, amplitude decreased and duration increased as a contraction proceeded, whereas area remained fairly constant. Because changes in each measure were very similar for units of widely different \(E_t\)s, it was concluded that neuromuscular junction failure and changes in the excitability of the sarcolemma (excluding the t-tubule system) play a minor role in determining \(E_t\).

3. \(E_t\) was highly correlated with the mean stimulation rate (\(E_t/\text{number of stimuli}\)) used during the force-clamp contractions. Mean rate was seen to progressively decrease with increasing \(E_t\). This correlation could not be explained by measures of isometric contractile speed or relaxation (e.g., twitch contraction time or half-relaxation time) measured before the force-clamp contractions. Both contraction time and half-relaxation time were found to be unrelated to both \(E_t\) and the rate used to stimulate the unit during the force-clamp contraction.

4. Among type S units of SOL and MG, maximum tetanic tension and \(E_t\) were not related. A significant relation \((r = -0.49)\) was found between axonal conduction velocity and \(E_t\) for SOL units \((n = 38)\). In addition, a significant correlation \((r = 0.47)\) was found between conduction velocity and tetanic tension for SOL units. Perhaps because of the small sample of type S units from MG \((n = 10)\), conduction velocity was found not to be related to either \(E_t\) or tetanic tension.

5. Others have shown that a motor unit’s maximum tetanic tension and axonal conduction velocity are correlated with its order of recruitment among motoneurons innervating a muscle. Recent work has further shown that among type F units the order in which a motoneuron is recruited is highly correlated with the fatigue resistance of its muscle unit. In contrast, the present study suggests that among type S units a much weaker relationship exists between a unit’s recruitment order and its fatigue resistance, if the interrelationships among conduction velocity, tetanic tension, and \(E_t\) can be used as a guide. The functional significance of \(E_t\) in relation to the recruitment order of type S units is discussed.

INTRODUCTION

The fatigue resistance of single motor units has been studied primarily as it pertains to the classification of motor units into groups. Typically a unit’s fatigability is expressed as a ratio of its tension output before and after 2 min of intermittent stimulation delivered in short trains at 40 imp/s. The traditional view is that there are generally two groups of units, those that are fatigue resistant (type S and FR) and those that are highly fatigable (type FF). In many muscles another group of fast-twitch units (type FI) is well represented and is described as having intermediate fatigability. Although various classification schemes are beneficial for identifying organization among motor-unit properties, not only within a muscle but also across muscles and animal species, the classification process has the disadvantage of minimizing differences within groups of units. For example, within the FR group, units that have equivalent fatigue indexes can have highly variable resistances to fatigue when compared under different test conditions (Botterman and Cope 1988a; Burke and Tsairis 1974). For purposes of classification, type S units also have fatigue indexes similar to those of type FR units, but are known to be much more fatigue resistant than type FR units (Botterman and Cope 1988a; Burke et al. 1974; Burke and Tsairis 1974). Type S units generally have been treated as a homogenous population of units with respect to fatigue. Very little, however, is known of their relative fatigability.

A motor unit’s relative fatigability in relation to its recruitment order is of obvious importance to muscle function. Motor units are generally recruited in order of increasing fatigability (Botterman and Tansey 1989; Stephens and Usherwood 1977; Tansey and Botterman 1989; Zajac and Faden 1985; cf. Nordstrom and Miles 1990), with the highly fatigue-resistant type S units recruited before type FR units, followed by type FF units (Botterman and Tansey 1989; Tansey and Botterman 1989; Zajac and Faden 1985; see also Bawa et al. 1984). Therefore within the S group it might be expected that motor units recruited early in a contraction would also have the greatest resistance to fatigue, as is the case for type F units when traditional measures of fatigue resistance have been correlated with recruitment order (Botterman and Tansey 1989; Zajac and Faden 1985). Among type F motor units recent experiments have shown that there is a highly significant relationship between maximum tetanic tension (\(P_{\text{max}}\)) and the length of time (endurance time, \(E_t\)) that a unit can maintain tension at 25%
of maximum (Botterman and Cope 1988b). To a lesser degree, a significant correlation was also found between axonal conduction velocity and \( F_t \). These observations take on additional significance because it has been shown that the recruitment order of type S units is highly correlated with both their axonal conduction velocity and \( P_c \) (Botterman and Tansey 1989; Clark and Cope 1990; Tansey and Botterman 1989; Zajac and Faden 1985; see also Bawa et al. 1984).

The present experiments were undertaken to address two questions. First, it was of interest to see whether type S units display a variable resistance to fatiguing contractions under conditions in which their force output was "clamped" at 85% of maximum. This output level was selected because previous experiments had shown that type S units could maintain 25% of their maximum output well in excess of 3,000 s. This made study of their relative fatigability impractical at this lower output level. A second question of interest was to see whether there was a significant correlation between \( F_t \) and \( P_c \) or axonal conduction velocity. If such correlations were found, it would lend indirect support to the notion that type S units are recruited in order of increasing fatigability. An account of this work has been presented in abstract form (Webb et al. 1987).

**METHODS**

Experiments were performed on six cats (2.0–3.6 kg) anesthetized with halothane in a 2:1 mixture of \( \text{N}_2\text{O} \) and \( \text{O}_2 \) during surgical procedures and replaced with pentobarbital sodium (35 mg/kg) during the recording session. A deep level of anesthe sia was maintained by supplemental doses of pentobarbital, as judged by a stable mean blood pressure (carotid) between 80 and 140 mmHg. End-tidal \( \text{CO}_2 \) was monitored and kept in the range of 3.5–4.5% by adjusting ventilation rate when necessary. Core temperature and that of the muscle and spinal oil-pools were maintained at 36–37°C.

The methods for isolating and identifying motor axons have been described in detail, as well as the procedures used to characterize the physiological properties of motor units (Botterman et al. 1985; Botterman and Cope 1988a). Slow-twitch motor units from either soleus (SOL) or medial gastrocnemius (MG) were functionally isolated by dissection of ventral root filaments. Motor units in MG were identified by type on the basis of criteria established by Burke et al. (1973). Units isolated from MG were also subjected to a conventional fatigue test (Burke et al. 1973) after contractions in which their tension output was clamped at 85% of maximum (see below). All units tested in this way were fatigue resistant (i.e., fatigue indexes >0.75).

For each unit studied, an active length-tension curve was generated and used to find a unit's optimal muscle length for tension development. All reported tension measurements were made at this optimal length. Muscle unit tension was clamped at 85% of maximum by altering the stimulation rate of its axon through computer feedback control. Effectiveness of the feedback algorithm in regulating tension output is shown for a soleus (SOL) motor unit. Dashed lines indicate a window of ±1% of maximum tension around the target tension. Top curve represents tension averaged during each interstimulus interval. Unit was stimulated 3,378 times during the contraction and had an endurance time \( (E_t) \) of 99.5 s. Bottom curve illustrates the instantaneous stimulation rate. Mean instantaneous rate for the contraction was 34 imp/s.

This output level was derived by activating the muscle unit with stimulus trains of various frequencies, beginning with 100 imp/s for slow-twitch units and 200 imp/s for fast-twitch units. \( P_c \) was defined as the peak force obtained during the initial, high-frequency train. The duration of the trains was 1,500 ms for slow-twitch units and 600 ms for fast-twitch units.

In experiments involving MG small fluctuations in tension (10–20 mN) were frequently observed at optimal length (passive tension, ~500 mN). These fluctuations were often time-locked to heart rate or respiration. In other instances, passive tension would slowly change over a period of several hundred seconds. Because a relatively small change in passive tension during the test contraction could have a noticeable effect on stimulation rate, passive tension was recorded at optimal length before and after each force-clamp contraction. If passive tension deviated by >2% from its initial value 5 min after the end of the contraction, data derived from that contraction were discarded. Perhaps because of the lower passive tensions corresponding to unit optimal length (~250 mN), as well as differences in muscle architecture (Spector et al. 1980), such fluctuations in passive tension were rarely seen in SOL muscles.

Data were collected on magnetic tape (bandwidth DC–5 kHz) and in computer memory (LSI 11/23) after digitization by a 12-bit analog-to-digital converter at 100 or 200 μs/pt. In addition to tension, recordings were made of unit electromyographic (EMG) activity, the muscle-nerve neurogram, and the computer-generated stimulus train. Unit EMG activity was recorded from bipolar silver-ball electrodes positioned on the surface of the muscle to maximize the amplitude of the EMG waveform.

Individual EMG waveforms were collected off-line from magnetic tape and stored in computer disk memory after digitization at 40 μs/pt. The collected signals included the first EMG waveform of a force-clamp contraction and other waveforms that occurred at selected percentages of \( E_t \), or at a fixed interval of activations during the contraction (e.g., 500). Three characteristics of the EMG waveform were determined: baseline-to-baseline duration, peak-to-peak amplitude, and area. The duration of the waveform was determined by selecting the beginning and ending points from which the waveform departed and then returned to baseline. Because most waveforms were triphasic (e.g., Fig. 6), an end point was selected when the waveform entered and remained within an appropriate voltage time window around baseline. Window val-
UCS for voltage were typically <1% of peak-to-peak amplitude and between 0.1 and 1.0 ms in duration for time. Peak-to-peak amplitude was the difference between the absolute maximum and minimum points of the waveform. After waveform rectification from baseline, area (nV·ms) of the waveform was determined by digital integration between the beginning and ending points found for waveform duration. All waveform measurements for a given contraction were expressed as percentage of the first (control) waveform in a contraction.

RESULTS

The results are drawn from four experiments in which 38 SOL motor units were isolated (8–10 units/animal) and from two experiments in which 10 slow-twitch units from MG were studied (5 units/animal). All slow-twitch units were tested by clamping their force output at 85% of Po. To further illustrate differences between the slow-twitch populations of MG and SOL and the fast-twitch population of MG, data derived from the experiments of Botterman and Cope (1988a,b) are presented to compare the performance of slow-twitch units clamped at 85% of Po with that of fast-twitch units clamped at 25% of Po.

Stimulation patterns during force-clamp contractions

The overall stimulation pattern for SOL type S units was similar among the four tested muscles. As illustrated in Fig. 2, SOL units responded with a brief decrease in firing rate, followed by a sharp increase in rate, before they reached a phase during which rate increased much more gradually. When units could no longer maintain their tension within the target window, they all displayed rapid increases in discharge rate as they approached the 100-imp/s upper limit set for stimulation rate. An exception to this trend was seen for a few units in which stimulation rate continually increased during the test contraction. An example of such a unit is shown in Fig. 2. This unit, as well as other units like it, had a relatively brief contraction time (58 ms) and Et (14 s).

Compared with their counterparts in SOL, type S units in MG required a similarly shaped activation curve to main-

FIG. 2. Stimulation patterns and endurance of SOL motor units, the force output of which was clamped at 85% of maximum. Units were isolated from 1 muscle. Note the stimulation pattern close to the left axis ( ). This unit had a contraction time of 58 ms and a maximum tetanic tension (Po) value of 93 mN. The other 9 units had contraction times ranging between 73 and 90 ms. Po ranged between 59 and 155 mN for the 10 units.

tain tension but differed in the amount of fluctuation around this curve (Fig. 3). The presence of these rate fluctuations was associated with specific muscles and generally was more prevalent in the least forceful units. Because of the difficulties encountered with passive tension (see METHODS), type S units were reliably studied in only two MG muscles.

As a population, MG type S units did differ from SOL units in that they required a significantly (P < 0.05) higher stimulation rate to maintain 85% of Po (Table 1). This was true not only at the beginning of a contraction, when target tension was initially reached, but throughout the contraction. The higher stimulation rates partially reflect the shorter contraction times and half-relaxation times of MG type S units (Table 1).

Three fast-twitch units from MG, including one type FR unit, were also tested at 85% of Po. As expected, these MG units fatigued rapidly, requiring a sharp increase in stimulation rate to maintain tension at the selected target level (Fig. 3).

Endurance of slow-twitch units

Slow-twitch units from both muscles displayed a broad range of Es, ~40- to 50 fold, when their outputs were...
TABLE 1. Properties of slow-twitch motor units clamped at 85% of their maximum tension

<table>
<thead>
<tr>
<th></th>
<th>Endurance Time, s</th>
<th>Number of Stimulations</th>
<th>Initial Stimulation Rate, imp/s</th>
<th>Mean Stimulation Rate, imp/s</th>
<th>Contraction Time, ms</th>
<th>1/2RT, ms</th>
<th>Maximum Tetanic Tension, mN</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOL</td>
<td>38</td>
<td>272 ± 210</td>
<td>6467 ± 3580</td>
<td>16.5 ± 6.5*</td>
<td>28.2 ± 7.3*</td>
<td>77.3 ± 11.3*</td>
<td>94.7 ± 17.9</td>
</tr>
<tr>
<td>(14–807)</td>
<td>(757–14839)</td>
<td>(10–41)</td>
<td>(16.7–54.2)</td>
<td>(43.3–101.5)</td>
<td>(39.2–117.6)</td>
<td>(19.6–212.7)</td>
<td></td>
</tr>
<tr>
<td>MG</td>
<td>10</td>
<td>1/1 ± 19.3</td>
<td>4666 ± 3687</td>
<td>25.1 ± 7.7*</td>
<td>40.0 ± 14.6</td>
<td>62.3 ± 14.7</td>
<td>84.9 ± 22.7</td>
</tr>
<tr>
<td>(13–526)</td>
<td>(575–11896)</td>
<td>(14–34)</td>
<td>(17.0–61.1)</td>
<td>(46.2–83.2)</td>
<td>(62.4–132.8)</td>
<td>(43.1–174.5)</td>
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Values are means ± SD with ranges in parentheses. Stimulation rates were derived from constant-tension contractions. Initial stimulation rate is the rate at which tension first reached and remained within the target window (e.g., <1% of maximum tension). Mean stimulation rate is the number of stimuli that a unit sustained during a run divided by its endurance time. 1/2RT = twitch half-relaxation time, measured as the time for tension to drop to one-half of peak tension. *Indicates significant difference between soleus (SOL) and medial gastrocnemius (MG) groups at \( P < 0.05 \) using t test.

Changes in motor-unit EMG waveforms during force-clamp contractions

One possible source of the wide variation of \( E_t \) is transmission failure at the various sites leading up to calcium release from the sarcoplasmic reticulum. More fatigable motor units require higher stimulation rates to maintain

![Graph A](http://www.jn.physiology.org/lookup/doi/10.1152/jn.00230.2017)

![Graph B](http://www.jn.physiology.org/lookup/doi/10.1152/jn.00230.2017)
their tension output at 85% of \( P_0 \) and, therefore, may be more susceptible to transmission failure. As recorded in the present experiments, EMG waveforms reflect the overall electrical excitation of muscle fibers within the muscle unit. Alterations in the EMG signal may reflect failure of electrical transmission presynaptically, diminished release of acetylcholine at the junction, and altered excitability of the sarcolemma.

Analysis of SOL EMG waveforms revealed that motor units with widely different fatigabilities underwent similar changes in shape as units approached their respective \( E_s \) (Fig. 6). This point was tested by placing motor units in either a "low-" or "high-" endurance group based on whether their \( E_s \) were greater than or less than 200 s. This arbitrary grouping divides the SOL population into approximately equal numbers (low group = 18; high group = 20). Only the instantaneous stimulation rate was found to differ between the two groups as \( E_s \) was approached (Fig. 7). On average, the peak-to-peak amplitude of the EMG waveform declined while its duration increased during the course of a contraction for units of both groups. As a consequence of these changes, area remained fairly constant during most test contractions. Only after units reached >95% of their \( E_s \) did area decline. With the exception of the last 5% of these contractions, rate did not seem to be a major factor in bringing about changes in the EMG waveform. Only when stimulation rate was sharply increased to maintain tension did EMG waveforms show abrupt changes in amplitude and area (cf. Clamann and Robinson 1985; Sandercock et al. 1985).

Additional evidence that low and high endurance units responded in a comparable fashion is indicated by the parallel changes in their EMG waveforms over the same number of stimuli. Over the first 4,500 stimuli, waveform area remained fairly constant for both groups. Expressed as a percentage of the first EMG waveform in a contraction, area ranged between 102 and 107% for high-endurance units and 92 and 113% for low-endurance units. For example, after 3,000 stimuli the low-endurance group (14 of 18 units) had a mean area of 103% compared with 107% for

![Comparison of electromyographic (EMG) waveforms from 2 motor units isolated from the same SOL muscle.](http://jn.physiology.org/)

**FIG. 6.** Comparison of electromyographic (EMG) waveforms from 2 motor units isolated from the same SOL muscle. Top and bottom panels: profiles of a low- and a high-endurance motor unit, respectively. Left: 3 superimposed waveforms representing the 1st waveform in each contraction (control), 1 at 50% of \( E_s \), and 1 at 95% of \( E_s \). Right: changes in waveform area (■), peak-to-peak amplitude (▲), and duration (▲) as the contraction progressed for each unit. Instantaneous stimulation rate is indicated by squares. See text for explanation of low- and high-endurance units.
Relationships among conduction velocity, $P_o$, and $E_t$

Because $E_t$ was highly correlated with $P_o$ among fast-twitch units when $E_t$ was determined at 25% of $P_o$ (Fig. 8B; Botterman and Cope 1988,a,b), it was of interest to see whether these properties were related among slow-twitch units when tension was clamped at 85% of $P_o$. Figure 8 compares the responses of fast- and slow-twitch units at the two output levels. Among slow-twitch units of MG and SOL, no relationship was found between $P_o$ and $E_t$. This was so despite the fact that $E_t$ essentially spans the same range for the two types of units at the two output levels.

In contrast to the findings with $P_o$, $E_t$ was found to be correlated with conduction velocity (Fig. 9B). This was true only for the SOL population, possibly because of the small MG sample size ($n = 10; r = 0.276; P > 0.44$). As others have found (Emonet-Denand et al. 1988; Jami and Petit 1975), axonal conduction velocity is correlated with $P_o$ among SOL units (Fig. 9A). No relationship was found for MG units ($r = 0.323; P > 0.36$). Emonet-Denand and co-workers (1988) have shown that the relationship between conduction velocity and $P_o$ among type S units is not as strong as found for other hindlimb muscles. Al-

![Diagram of EMG waveform characteristics](image)

**FIG. 7.** Changes in soleus EMG waveform characteristics as a function of $E_t$. Waveform area (●), peak-to-peak amplitude (△), and duration (●) are shown as percentages of values found for the 1st action potential of the force-clamp contraction. Because there was no difference between waveform means for low- ($n = 18$) and high-endurance units ($n = 20$), the 2 groups were combined and plotted as 1 group. Instantaneous stimulation rate is indicated by squares (●, low-endurance group; △, high-endurance group). With the exception of rates at 5% of $E_t$, there were significant differences between the 2 groups at the remaining points ($t$ test, $P < 0.05$). Dividing line between low- and high-endurance groups was an $E_t$ of 200 s for the high-endurance group (20 units). This difference was not significant ($P > 0.45$). As stimulation number approaches 4,500, progressively fewer low-endurance units contribute to mean area, because many of them had reached their $E_t$s. Only four low-endurance units were activated >4,500 times. These four units had a mean area of 92%, compared with 106% for the high-endurance group. Again, the mean areas of the two groups were not significantly different ($P > 0.22$).

Recovery of tension after force-clamp contractions

The maximum tension of which a muscle unit was capable was determined 2 min after the completion of its force-clamp contraction (100 imp/s, 1,500-ms-duration train). Units of both muscles were able to produce ~95% of $P_o$ (range 82–100%). Moreover, no difference was seen between the low- and high-endurance groups ($<200$ s: $\bar{x} = 94.7 \pm 5.3$, mean ± SD, $n = 25$ vs. >200 s: $\bar{x} = 95.3 \pm 4.7$, mean ± SD, $n = 23$, $P > 0.65$). As might be expected, EMG waveform characteristics had returned to control values. This generally occurred within the first 30 s.

Because peak tension almost fully recovered during the 2-min rest period, a second force-clamp contraction was made in five units to assess whether these units could approach their previous $E_t$s. These units were selected at random and allowed to rest for an additional 30 min before the second contraction. Two SOL units with $E_t$s <100 s were able to sustain contractions lasting ~70% of their initial $E_t$s at 85% of $P_o$. In contrast, a MG unit that had an $E_t$ of 220 s was able to maintain a second contraction for only 25 s. In two other soleus units, target tension was reduced to 65% of $P_o$. Both units were able to increase their $E_t$s at the lower output level (from 141 to 998 s and 115 to 276 s).

![Graphs of $P_o$ and $E_t$](image)

**FIG. 8.** Relationships between $P_o$ and $E_t$ for type S units of SOL and MG when output of units was clamped at 85% of $P_o$ (A) and for type F units of MG when output was clamped at 25% of $P_o$ (B). A: correlation analysis indicated that no relationship existed between $P_o$ and $E_t$ at 85% of $P_o$ for either the 38 SOL units or the 10 MG type S units. B: there was a significant correlation ($r = 0.82; n = 53; P < 0.001$) between $P_o$ and log $E_t$ at the lower output level. For clarity 1 point, which had an $E_t$ of 2,063 s and a $P_o$ value of 228 mN, was excluded from the plot. Data in B are taken from the results of Botterman and Cope (1988b).
within known physiological ranges (10–40 imp/s; Hennig 1981; P < 0.01) and log $J$ ($r = -0.49; n = 38; P < 0.01$) were significantly lower in the study of fatigue resistant. To our knowledge, however, no formal attempt has been made to fully characterize this population with regard to their fatigue properties. Most attention has been paid to differences between SOL and MG type S units with regard to such properties as specific tension, twitch speed, and posttetanic effects on twitch amplitude (Burke 1981; Burke et al. 1974).

### Fatigue properties of type S units in SOL and MG muscles

How one views the fatigue properties of a population of motor units depends in large measure on the stimulation protocol employed to bring about the fatigue. Fast-twitch units have traditionally been divided into two groups on the basis of the bimodal distribution of fatigue indexes. This physiological result is satisfying because, from qualitative histochemical analyses, fast-twitch muscle fibers can be separated into two groups from differences on their reliance of glycolytic and oxidative processes. However, analytical measures of oxidative capacity have shown fast-twitch fibers to have primarily a unimodal distribution (Blanco et al. 1982; Hintz et al. 1980; Sieck et al. 1986; Spamer and Pette 1977). By evaluating the fatigue properties of fast-twitch units at a constant output level (25% of $P_o$) and allowing stimulation rate to vary as required, we have shown previously that $E_t$ has a skewed, continuous distribution with no obvious bimodality (Botterman and Cope 1988a). Like fast-twitch units, the distribution of $E_t$ values for type S units was found to be a continuum. There was no evidence for unit subgroups.

The marked resistance of slow-twitch muscle fibers to fatigue appears to be related to their high oxidative capacity and the efficiency with which they utilize ATP (Goldspink et al. 1970; Kugelberg and Lindegren 1979; Martin et al. 1988; Nemeth et al. 1987). Biochemical analysis of single fibers from rat SOL has shown that there is a continuous distribution of activities for several oxidative enzymes and that the range of activities is about fourfold (Hintz et al. 1980, 1982). A similar range and distribution was found for slow-twitch fibers in cat (Martin et al. 1988; Sieck et al. 1986). This compares with the 40- to 50-fold range in $E_t$. It is clear that other factors must be involved in determining the range of values found for $E_t$.

There are a number of possible explanations for the broad range of $E_t$ seen in these experiments. Aside from the energy supply for contraction and the efficiency with which energy stores are used, type S motor units may show differences in fatigue related to neuromuscular junction transmission, propagation of action potentials, and excitation-contraction coupling. Each of these factors could interact to produce the broad range seen for $E_t$. It was not possible in the present experiments to distinguish which of these factors or combination of factors is most important in determining $E_t$. However, on the basis of our analysis of EMG waveforms, it would seem that events leading up to and including propagation of action potentials along the sarclemma are not important factors. It should be noted that changes in regenerative activity in the tubule system would go undetected with the present recording arrangement.

The progressive changes in EMG waveform shape have been reported by many investigators (for reviews, see Hannaut and Duchateau 1989; MacLaren et al. 1989). The increase in duration and decrease in amplitude of the EMG

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**DISCUSSION**

The main finding of this study is that type S motor units from both SOL and MG displayed a broad range of endurance when their tension output was clamped at 85% of maximum. The stimulation rates necessary to maintain this relatively high output level were for the most part within known physiological ranges (10–40 imp/s; Hennig and Iømo 1985; Hoffer et al. 1987; Zajac and Young 1980). Most previous studies that have examined the fatigue properties of single motor units have done so in the context of separating units into different types (i.e., S, FR, and FF) by use of the intermittent stimulus protocol of Burk and colleagues (1973). Principally through the use of this protocol (Burke et al. 1973, 1974; Burke and Tsairis 1974; Cope et al. 1986; cf. Botterman and Cope 1988a), it has long been appreciated that type S motor units are highly fatigue resistant. To our knowledge, however, no formal attempt has been made to fully characterize this population with regard to their fatigue properties. Most attention has been paid to differences between SOL and MG type S units with regard to such properties as specific tension, twitch speed, and posttetanic effects on twitch amplitude (Burke 1981; Burke et al. 1974).

**FIG. 9.** Relationship between axonal conduction velocity and $P_o$ (A) and between conduction velocity and $E_t$ (B). Both $P_o$ ($r = 0.47; n = 38; P < 0.01$) and log $E_t$ ($r = -0.49; n = 38; P < 0.01$) were significantly related to conduction velocity. If the 1 SOL unit with a conduction velocity of $\sim 50 \text{ m/s}$ was excluded from the analysis, $P_o$ ($r = 0.36; P < 0.05$) and log $E_t$ ($r = -0.44; P < 0.01$) were still significantly related to conduction velocity. Because of the limited number of type S units sampled in MG (68 units), the pooled data obscured the relationship.
signal seen in these experiments is a common feature of sustained activity (Bigland-Ritchie et al. 1982; Clamann and Robinson 1985; Cooper et al. 1988; Duchateau and Hainaut 1985; Enoka et al. 1989; Sandercock et al. 1985; Thomas et al. 1989). Waveform area was seen to increase initially, then remain fairly constant before declining. Most of these EMG changes are undoubtedly related to a decrease in conduction velocity of the sarcolemmal action potential (Lännenroen and Westerblad 1986, 1987; Radicheva et al. 1986; Ståhlberg 1966). This interpretation is consistent with the observation that waveform amplitude and area declined while tension and stimulation rate remained constant (see bottom panels of Fig. 6). In one dramatic example of such uncoupling between waveform area and tension, area decreased to 80% of control after 150 s of activity (≈50% of Et). Up to that point in the contraction, a stimulation rate between 22 and 24 imp/s was used to maintain tension within the target window. Over the next 150 s, tension was constant and rate increased to ≈42 imp/s, whereas area declined to ≈50% of control. That the decline in amplitude and area was not due to a conduction block involving a substantial number of fibers in the muscle unit is supported by the work of Sandercock and colleagues (1985), who showed that individual muscle fibers occasionally failed to propagate action potentials during low-frequency stimulation (=40 imp/s) only when tension fell below 20% of control values. Our results, largely confirmatory of others (Clamann and Robinson 1985; Sandercock et al. 1985; for review, see Clamann 1990), illustrate that marked changes in the EMG waveform can occur for some units during sustained activity without affecting tension production. Thus for type S units there appears to be a substantial safety factor for excitation-contraction coupling (Clamann and Robinson 1985).

The relatively quick recovery of tension after 2 min of rest suggests that excitation processes, such as impaired electrical transmission within the t-tubule system and failure of excitation-contraction coupling, are partially involved in establishing Et. A rapid recovery of surface EMG potentials and tension are hallmarks of excitation failure and have been used to differentiate the contribution of metabolic and excitationary factors to fatigue (MacLaren et al. 1989). Metabolic factors may also be involved, because units subjected to a second force-clamp contraction were unable to match their previous Et after 30 min of rest.

**Influence of stimulation rate on Et**

Another interesting result of the present experiments was the strong, negative relationship between Et and the mean stimulation rate applied during the force-clamp contraction. This is similar to the result seen for flexor carpi radialis fast-twitch units clamped at 25% of Pmax, but in contrast to MG fast-twitch units, for which no relationship was found (Botterman and Cope 1988a). At issue is whether some slow-twitch units are predisposed to lower Et because they have relatively brief contraction and half-relaxation times and must be activated at higher rates to achieve the same target tension as slower contracting units. Under this scheme the higher rates needed early in the contraction would hasten the fatigue process. Another possibility is that the higher stimulation rates associated with low-endurance units are a result of the fatigue process itself. Our results suggest that the latter case is likely, because no relationship was found between either twitch property and Et, or mean stimulation rate. Both contraction time and half-relaxation time were highly correlated with the stimulation rate needed to first reach target tension, but were not correlated with the rates observed after 5% of Et had elapsed. It is clear the frequency-tension relationship of a significant number of units was altered during the first 10 s of activity (e.g., Fig. 2). Such alterations were probably due to the competing processes of postactivation potentiation and fatigue, both of which are associated with changes in speed of contraction (Bigland-Ritchie et al. 1983; Binder-Macleod and Clamann 1989; Botterman and Cope 1988a; Dubose et al. 1987; Edwards et al. 1975; Gordon et al. 1990a).

Clamann and colleagues (Dubose et al. 1987) have shown that, after modest intermittent activity (Burke fatigue test for 30 s), type S units responded with increases in twitch speed while twitch amplitude remained unchanged (cf. Gordon et al. 1990b). These activity-dependent speed changes are probably responsible for the early increase in stimulation rate seen during the first 10–20 s of force-clamp contractions (Figs. 1 and 2). They occur during a period of time when the force-producing capability of the muscle unit is maximal. After this initial period, further increases in rate probably represent a diminished capability of the muscle unit to produce maximum force. Units that are more fatigable require stimulation rate to increase more quickly to maintain target tension; hence they have higher mean stimulation rates (number of stimuli/Et) than less fatigable units. An additional factor is that higher stimulation rates, even at the same output level, may require more energy expenditure (Loiselle and Walmsley 1982). Note that the abrupt increase in stimulation rate at the end of a force-clamp contraction makes a minor contribution to mean stimulation rate, even in highly fatigable units, because this period of stimulation generally lasts <2 s.

Muscular fatigue is characterized by loss of force and by a slowing of contractile speed (Edwards et al. 1975; Bigland-Ritchie et al. 1983). Bigland-Ritchie and co-workers (1983) have demonstrated in humans that, during a maximum voluntary contraction lasting 60 s, contractile speed progressively slowed and the stimulation rate needed to produce maximum activation actually decreased. During this period, force declined by 30–50%. This result is best explained by a shift in the frequency-tension relationship caused by slowing of contractile speed. In our experiments force remained constant; therefore a decrease in stimulation rate would indicate that potentiation occurred and/or that there was a slowing of contractile speed. It is well known that type S units in the cat show nominal amounts of potentiation after conditioning tetani or even depression in the case of SOL units (Burke et al. 1974). Any decrease in rate, then, would imply contractile slowing was of a magnitude to overcome the opposing trend to increase rate to compensate for force loss. We found no evidence for this. Stimulation rate progressively increased during force-clamp contractions (Figs. 1 and 2). Part of the reason for this result might lie in the relatively high output level selected for study. At lower output levels, where the tension...
profile demonstrates less fusion between stimuli, contractile speed may manifest itself as lower stimulation rates.

**Consequences of different recruitment-order schemes on muscle fatigue**

Henneman et al. (1965a,b) first clearly demonstrated that motor units have relatively fixed thresholds for activation and, as a consequence, motoneurons within a motor pool are generally recruited in the same order. With a fixed order of recruitment, the earliest recruited units in a muscle are invariably the most frequently used and should have the highest resistance to fatigue (Henneman and Olson 1965). This expectation has generally been confirmed by a number of laboratories using a variety of muscles, animal species, and stimulus protocols to induce fatigue (Botterman and Tanscy 1989; Stephens and Usherwood 1977; Tanscy and Botterman 1989; Zajac and Faden 1985; cf. Nordstrom and Miles 1990). What remains uncertain, however, is the degree to which recruitment order and fatigability are related. In animal studies, recruitment order has been determined by pair-wise recording of motor units, affording a high degree of precision, whereas the Burke fatigue test has been used to determine the units’ relative fatigability (Botterman and Tanscy 1989; Tanscy and Botterman 1989; Zajac and Faden 1985). Unfortunately, this test does not discriminate well among the most fatigue-resistant units (types S and FR; Botterman and Cope 1988a; Martin et al. 1988). In the two reports in which the relative recruitment thresholds and fatigabilities of human motor units were studied, fatigue was produced by continuous activity under voluntary control, lasting 5–15 min at a nominal rate of 10 imp/s (Nordstrom and Miles 1990; Stephens and Usherwood 1977). Threshold comparisons for pairs of simultaneously recorded units were not reported, and the recruited units were often restricted to the low-threshold range.

Although coupling between recruitment order and $E_r$ was not directly measured in this study, we can indirectly assess that relationship by looking at the correlation of $E_r$ with parameters that do vary with recruitment order. In the present study we have shown that $E_r$ is correlated with axonal conduction velocity, which is a good (see Henneman and Mendell 1981) if not a perfect (Bawa et al. 1984; Dick et al. 1987) predictor of recruitment order, particularly among low-threshold type S units. The association of $E_r$ with conduction velocity leads to the prediction that units recruited in order of increasing conduction velocity would also be progressively more fatigable. This organization of unit fatigability during recruitment is entirely consistent with that indicated in earlier studies mentioned above. A complication in these considerations arises, however, when unit tension is used as the index of recruitment. A number of studies have shown that $P_o$ is also a good (Botterman and Tanscy 1989; Clark and Cope 1990; Tanscy and Botterman 1989) if not a perfect (Zajac and Faden 1985) predictor of recruitment order among pairs of units in cat hindlimb muscles. But our data show that $E_r$ has no clear relationship with $P_o$. It seems, therefore, that determination of whether recruitment is ordered by $E_r$ will require direct examination. A less-than-precise coupling between recruitment order and fatigability among type S units may not be of critical importance for muscle performance, because as a group these units are highly fatigurable and show remarkably fast recovery after fatiguing contractions. Studies of muscle activity in bechaving cats, measured from EMG (Smith et al. 1977) and tension (Walmsley et al. 1978), have shown that the SOL is nearly as active during quiet standing as it is during jumping and running. Thus the order in which SOL motor units are recruited may be functionally irrelevant, because the entire SOL motor pool is often fully recruited regardless of the movement it supports.

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**REFERENCES**


ZAJAC, F. E. AND FADEN, J. S. Relationship among recruitment order, axonal conduction velocity, and muscle-unit properties of type-identi

ZAJAC, F. E. AND YOUNG, J. L. Discharge properties of hindlimb motorneu