Inference of motor unit recruitment order in voluntary and electrically elicited contractions

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KNAFLITZ, MARCO, ROBERTO MERLETTI, AND CARLO J. DE LUCA. *Inference of motor unit recruitment order in voluntary and electrically elicited contractions.* J. Appl. Physiol. 68(4): 1657–1667, 1990.—The relationship between surface myoelectric signal parameters and the level of voluntary or electrically elicited contractions was studied in 32 experiments on the tibialis anterior muscle of 22 healthy human subjects. Contractions were performed at 20 and 80% of the maximum voluntary contraction torque. Two levels of stimulation current were used, yielding, respectively, a maximum M wave and an M wave ~30% of the maximum. A four-bar electrode probe was used to detect single- and double-differential signals from which mean and median frequency of the power spectrum and average muscle fiber conduction velocity were estimated. Measurements obtained from voluntary contractions showed a positive correlation between contraction levels and both conduction velocity and spectral parameters. Conduction velocity increased by 21.2 ± 10.9% when voluntary contraction level increased from 20 to 80% of the maximal value. Spectral parameters increased by similar amounts. Tetanic electrical stimulation was applied to a muscle motor point for 20 s via surface electrodes. Rectangular current pulses with 0.1-ms width and frequencies of 20, 25, 30, 35, and 40 Hz were used. Four types of behavior were observed with increasing stimulation level: *1*) the two spectral parameters and conduction velocity both increased with stimulation in 15 experiments, *2*) the two spectral parameters decreased and conduction velocity increased in 8 experiments, *3*) the two spectral parameters and conduction velocity both decreased in 6 experiments, and *4*) the two spectral parameters increased and conduction velocity decreased in 3 experiments. Conduction velocity increased with increasing stimulation current in 72% of the experiments, indicating a recruitment order similar to that of voluntary contractions, although it decreased in the other 28% of the cases, indicating a reverse order of recruitment. Contrary to what is observed in direct stimulation of nerves, motor units are not in general recruited in reverse order of size during electrical stimulation of a muscle motor point. This discrepancy may be the result of geometric factors or a lack of correlation between axonal branch diameter and the diameter of the parent motoneuron axon. Changes of conduction velocity and spectral parameters in opposite directions may be the result of the combined effect of the motor unit recruitment order and of the different tissue filtering function associated with the geometric location of the recruited motor units within the muscle.

human muscle; tibialis anterior; myoelectric signal; electrical stimulation; electromyography; spectral parameters; conduction velocity

THE ADVENT AND INCREASING popularity of functional electrical stimulation for augmenting the force generation capacity of fully or partially paralized muscles after stroke or spinal cord lesions has reawakened interest in electrical stimulation of muscles. Some of the functional electrical stimulation approaches use surface electrodes because of the convenience and simplicity they present over invasive indwelling electrodes. When surface electrodes are used there is a concern about the characteristics of the muscle fibers that are excited and the order of recruitment of the motor units within the muscle. It is well known that during voluntary contractions the motor unit recruitment order progresses as a function of increasing size, both in number of muscle fibers in the motor unit and diameter of the muscle fibers.

It is also well known that motor units with larger fibers are innervated by larger axons, which have a lower threshold of electrical excitability (10, 13, 33, 35). Therefore, it seems reasonable to expect that during electrically elicited contractions larger motor units might be recruited at lower stimulation levels. This has been shown to be the case if direct nerve stimulation is applied by means of implanted electrodes (10, 13, 33, 35), but no direct evidence exists indicating if such is the case when surface stimulation is used.

This study was undertaken to investigate the recruitment order of motor units in contractions electrically elicited by means of surface electrodes. The motor unit recruitment order was monitored by measuring the conduction velocity (CV) and two spectral parameters of the myoelectric signals, the mean (MNF) and the median frequency (MDF). These parameters were obtained for both electrically stimulated and voluntary contractions for comparison. The relationship between the two spectral parameters and CV has been reviewed by De Luca (12).

Several noninvasive methods have recently been developed to estimate average CV by detecting the myoelectric (ME) signal with multiple surface electrodes and employing zero-crossing delay or cross-correlation techniques (5, 6, 21, 30, 32). Correlation between CV and spectral parameters of the ME signal during voluntary contractions has been shown in both theoretical (20) and experimental (1, 4, 12) work and is particularly evident when the surface electrodes used to detect the signal can be located away from the innervation zone (28, 32). Thus the ME signal spectral parameters provide indirect information about the average CV value and may be used to monitor its changes when direct measurements are not possible.

A number of articles have provided evidence that CV, MNF, and MDF increase with increasing levels of voluntary contraction and decrease during constant-force sustained isometric contractions (1, 2, 4, 6, 12, 23). According to Hakansson (14), who worked with frog muscles, and other researchers who worked with the human biceps or tibialis anterior muscles (6, 17, 23), the increase of the initial value of spectral parameters with increasing levels of voluntary contractions may be attributed to the recruitment of motor units consisting of progressively larger fibers with progressively higher CV. Evidence for this interpretation has also been provided by animal models. Solomonow et al. (33) were able to reproduce the physiological order of recruitment by modulating a sciatic nerve conduction block in cats. These authors observed an increase of ME signal MDF of the gastrocnemius muscle as recruitment was increased. Further support for this interpretation comes from the work of Westbury and Shaughnessy (34), who reported a MNF decrease with an increase of voluntary contraction of the human masseter and associated such behavior to the biopsy finding that the studied muscle has type II fibers with smaller diameter than type I fibers.

METHODS

The tibialis anterior muscle was selected for this study because of the relatively extensive body of data available on its structure and behavior (6, 16, 23, 28). This muscle is also particularly suitable for CV measurements because of a relatively long region between the motor point(s) and the lower tendon that is sufficient to accommodate the detection electrode (28, 32). Thirty-two experiments were performed on 22 volunteer subjects (17 males and 5 females) with no history of orthopedic or neurological disorders. Ages ranged from 18 to 41 yr, with a mean of 29 ± 6.9 (SD) yr. The leg of the dominant side was always used.

Stimulation Technique

Attempts to stimulate the tibialis anterior muscle via the peroneal nerve, at the fibular head, led to considerably unstable M waves at low stimulation levels. Such instability was probably the result both of the difficulty in generating a sufficiently uniform current field near the nerve and to the movement of the muscle during contraction. Furthermore, we were concerned about the possible interference as the result of cross-talk from the peroneii muscles, which would be recruited together with the tibialis anterior. Therefore, this technique was discarded.

In the clinical environment, muscle contractions are usually elicited with a pair of electrodes placed on the muscle motor points. With this technique the electric field within the muscle is highly nonuniform and mostly superficial, making the M wave amplitude unstable. To improve field uniformity, especially in the deeper part of the muscle, a monopolar technique was used with a negative rectangular sponge-electrode (2 × 3 cm) placed on the most proximal motor point of the muscle and a large (8 × 12 cm) positive electrode placed on the gastrocnemius muscle. Therefore, the current lines would traverse a roughly conical space across the leg along the tibia. Although some pulse-to-pulse variability could not be eliminated, the submaximal M wave amplitude was found to be much more stable than that obtained with bipolar techniques or with nerve stimulation.

Avoiding or removing the large stimulation artifact present between the ME signal detection electrodes is a common problem in these types of experiments. Estimates of CV are detrimentally affected by stimulation artifacts simultaneously present on both double-differential channels. To avoid this problem an original artifact suppression technique was implemented. The details of such technique have been described elsewhere (18).

Pulse duration has been shown to affect both the selectivity of stimulation as well as the relationship between stimulus current and muscle force. It is known that narrow pulses allow more gradual recruitment of nerve fibers (10, 13). A rectangular current pulse with a time width of 0.1 ms was selected as a compromise between such requirements and the rise time and slew rate of the stimulator's output stage. Stimulation frequencies of 20, 25, 30, 35, and 40 Hz were selected to study the effect of electrical stimulation frequency on spectral parameters and CV.

Myoelectric Signal Detection Technique

The ME signal was detected with the four-bar electrode technique described by Broman et al. (5, 6). The single differential output was obtained from the two central bars and was used to compute the ME signal spectral parameters. The detection technique provided two double-differential outputs from which the average muscle fiber CV was estimated (see *Signal Processing*). Common mode signals (such as power line interferences) and differential signals (such as volume-conducted signals from neighboring muscles) simultaneously present on the three electrode pairs were rejected electronically. A "hybrid" output stage, slew rate limiting, and time windowing (signal blanking) were used to eliminate the stimulation artifact (18). The stimulation and ME signal detection circuits were fully isolated. The three ME signals were low-pass filtered with a cut-off frequency of 480 Hz (120 dB/decade rolloff) and recorded on analog magnetic tape together with the force signal and a timing signal from the stimulator and were processed off-line.

Experimental Protocol

The experimental protocol consisted of a preparatory phase and an experimental phase. Each subject was seated comfortably in a dental chair with the ankle joint at ~90°. The foot was bound in an isometric brace equipped with a torque transducer. The motor points of the muscle were identified as those with the lowest stimulation threshold. The number of motor points ranged from one to three. The stimulation electrode was moved over the motor point area until a location was found that provided the greatest muscle contraction force and the highest M wave within pain tolerance.

The detection electrode was applied on previously shaved skin cleaned with alcohol. No conductive paste was necessary. The electrode was moved over the muscle in the area between the most distal motor point and the tendon and was positioned, with an elastic strap, with the four bars perpendicular to the muscle fibers. Correct alignment was indicated by maximally delayed and highly correlated double-differential signals during voluntary and stimulated test contractions. The test contractions were intense but lasted only a few seconds. About 4 min were allowed before beginning the experimental phase to avoid any effect of the test stimulation on the voluntary order of recruitment. A skin temperature sensor, with a resolution of 0.1°C, was fixed on the skin near the detection electrode.

The experimental phase consisted of three maximal voluntary contractions (100% MVC) lasting 5–10 s and spaced 3 min apart. The highest of the three maximal values was taken as the 100% MVC value. A 20% MVC contraction followed by an 80% MVC contraction were then performed. These voluntary contractions lasted 20 s, were spaced 3 min apart, and were performed with visual feedback that was used by the subject to match and maintain a force target level shown on an oscilloscope screen. Stimulated contractions were then performed with the subject relaxed and physically passive. Such condition was indicated by the absence of voluntary ME signals. Two stimulation levels were used. A supramaximal stimulation (~10% above the level generating the maximal M wave) was defined as the high-level stimulation (HLS). A stimulation level eliciting an M wave with an average peak-to-peak amplitude of 25–30% of the maximal M wave was defined as the low-level stimulation (LLS). At LLS, the M wave amplitude showed some pulse-to-pulse instability; attempts to stabilize it were unsuccessful. Twenty experiments were performed on 10 subjects. Experiments were repeated in two different days. In each experiment two 20-s contractions were performed at LLS at 3-min intervals at each stimulation frequency. They were followed by two HLS contractions at the same stimulation frequencies and time intervals. In an additional group of 12 experiments on 12 subjects, stimulated contractions were performed at 20 Hz only.

Signal Processing

ME and force signals played back from analog tape were sampled at 1,024 Hz for each ME channel and at 85 Hz for the force channel. The samples were digitized by a 12-bit analog-to-digital converter and stored on the disk of a Micro PDP 11/23 computer. ME spectral parameters and CV were then computed with numerical algorithms. Power spectra of voluntary contractions were calculated from the single differential ME signal over two 0.5-s subepochs and averaged providing a frequency resolution of ±2 Hz. Power spectra of stimulated contractions were calculated from the electrically elicited responses averaged over a 1-s signal epoch. Zero padding in the time domain was used to obtain a frequency resolution of ±1 Hz. MNF and MDF of the power spectrum were then computed. The time epoch was then shifted by 1 s, and the process repeated so that 20 values for each parameter were obtained over the 20-s contraction time.

CV was computed as d/τ, where d = 10 mm was the interelectrode distance and τ was the time delay between the two double-differential signals. This delay was obtained by identifying the time shift required to minimize the mean square error between the two double-differential signal Fourier transforms using the method outlined by McGill and Dorfman (22). This estimation technique appeared to be more robust than the cross-correlation method and led to stable CV estimates even when the correlation coefficient between the two double-differential signals was as small as 0.6.

MNF, MDF, average rectified value and root mean square value, CV, correlation coefficient, and force were then tabulated and plotted vs. time for each contraction. The time course of these parameters was fitted with a least-square regression line or a least-square exponential curve. The curve that showed the lowest residual standard deviation was selected and its intercept with the y-axis was taken as the initial value. Initial values of spectral parameters and CV were corrected for skin temperature variations >0.5°C taking place during the experiment. The correction factor was 3.5%/°C obtained from our previous work (24).

Nonparametric tests were used to estimate the statistical significance of differences (31). The one-sample or two-sample (paired when appropriate) Wilcoxon tests were used because of their independence from the prob-

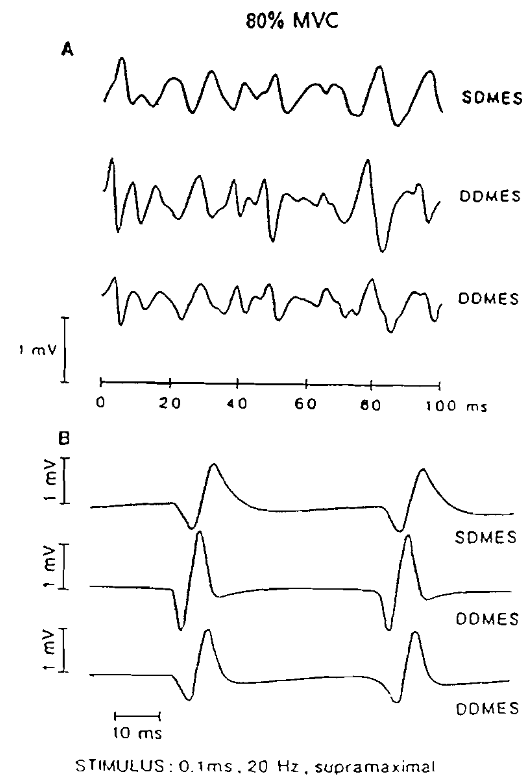

FIG. 1. Voluntary and electrically elicited single- (SDMES) and double-differential myoelectric signals (DDMES) provided by detection system. *A*: voluntary signals at 80% MVC; *B*: electrically elicited maximal M waves.

ability distribution function of the sample.

Figure 1*A* shows the single- and double-differential ME signals obtained during a voluntary contraction at 80% MVC. Figure 1*B* shows the single- and double-differential M wave responses at 20-Hz HLS. Figure 2 shows the time course and the interpolation curves of MDF and CV during voluntary and stimulated contractions in a typical set of measurements. Similar data were obtained for MNF. The intercepts of the interpolation curves with the vertical axis were taken as the initial values of the variables.

RESULTS

Figure 3 shows the initial values of MDF, MNF, and CV corresponding to voluntary contractions and to contractions electrically elicited at different stimulation frequencies in two subjects. The behavior of MDF and MNF was always similar, with MNF always slightly higher than MDF because of the skewed shape of the ME signal power spectrum. A relationship between the initial values of MNF, MDF, CV, and the stimulation frequency was not evident in most contractions. Only few contractions showed a statistically significant positive or negative correlation as indicated by the coefficient of linear regression (see DISCUSSION and Fig. 7). Therefore, the initial values at LLS and HLS were defined as the average of the values at all stimulation frequencies.

Random fluctuations of the parameters were always much smaller during stimulated contractions, as evident from Fig. 2. Intraexperiment repeatability was usually within a few percent, as evident from Fig. 3.

The force levels attained with our stimulation technique are a small fraction of the maximal voluntary force.

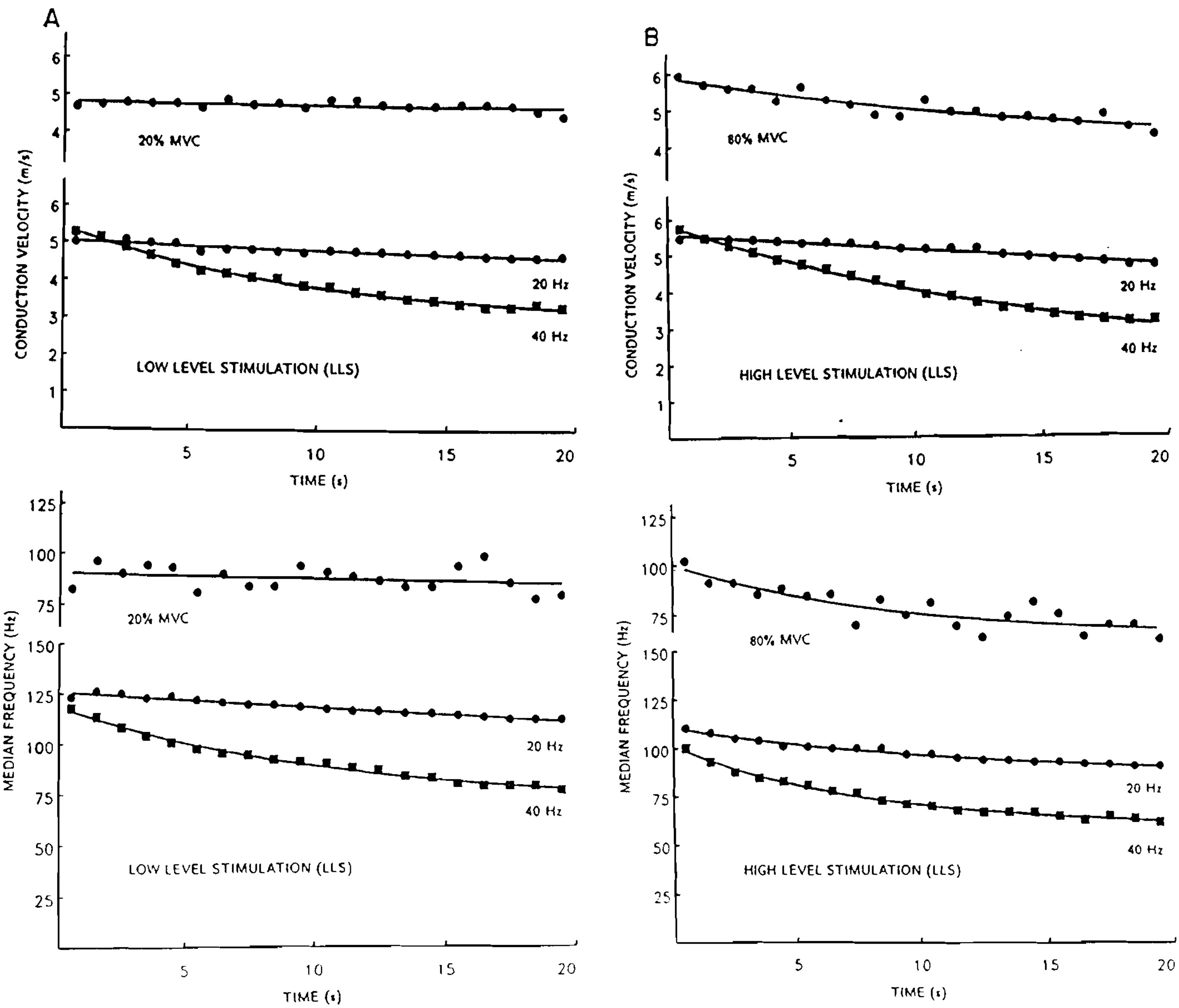

FIG. 2. Examples of plots of MDF and CV vs. time during voluntary (20 and 80% MVC) and electrically elicited contractions at LLS and HLS. See text for details. Regression lines and regression exponential curves are shown. Intercepts of such lines or curves with vertical axis are taken as initial values of MDF. Similar plots are obtained for mean frequency.

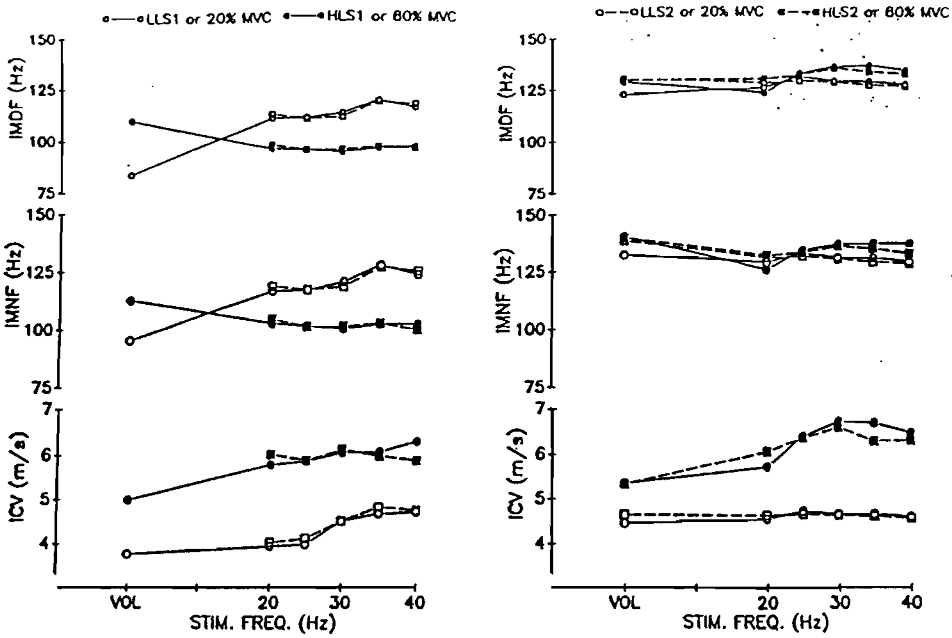

FIG. 3. Examples of initial values of median frequency (IMDF), mean frequency (IMNF), and conduction velocity (ICV) obtained during voluntary and electrically elicited contractions at different frequencies. LLS1, LLS2, HLS1, and HLS2 are contractions from the same experiment. Good repeatability between 2 contractions within the same experiment is evident. Similar behavior was seen in all experiments. Correlation between initial values and stimulation frequency is evident in some plots. The first subject shows a *type 2* behavior, whereas the second shows *type 1* behavior. See text for details.

At HLS force ranged between 7.5 and 38% with an average of 26.5 ± 4.6% of the MVC. This is the result of the selective stimulation of a fraction of the tibialis anterior muscle, although the entire anterior compartment of the leg is activated during MVCs. Therefore, it may be reasonable to expect different results in experiments in which different fiber sets of the muscle may be activated by stimulation.

As observed in previous studies, both spectral parameters and CV increased with increasing level of voluntary contraction (6, 23). This behavior was seen in each of the 32 experiments. Increasing intensity of stimulation current did not lead to a consistent behavior. As stimulation was increased from LLS to HLS, CV increased by an average of 16% in 23 cases and decreased by an average of 7.6% in 9 cases, whereas spectral parameters increased by an average of 10.4% in 18 cases and decreased by an average of 9.9% in 14 cases. Four types of behavior were identified during electrically elicited contractions with regard to spectral parameters and CV: *type 1*, both spectral parameters and CV increase with increasing level of stimulation; *type 2*, spectral parameters decrease and CV increases with increasing level of stimulation; *type 3*, both spectral parameters and CV decrease with increasing level of stimulation; and *type 4*, spectral parameters increase and CV decreases with increasing level of stimulation.

Five of the 10 subjects on whom the experiment was

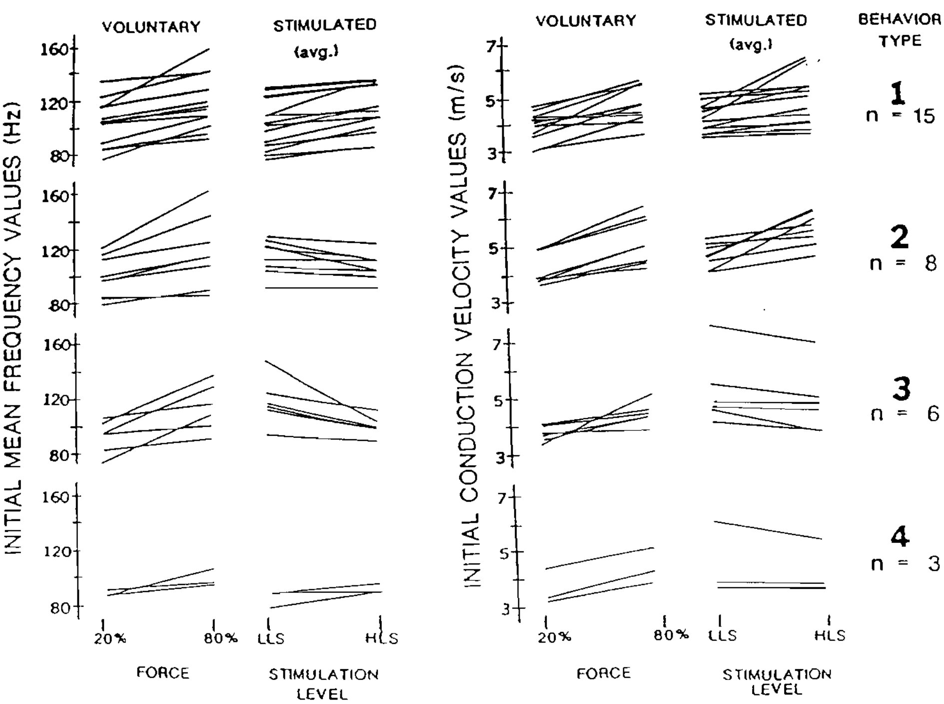

FIG. 4. Change in initial value of mean frequency and CV between 20 and 80% MVC and between LLS and stimulation HLS. The 32 experiments are divided into 4 types of behavior according to direction of change of initial mean frequency and conduction velocity when stimulation is increased from LLS to HLS. Although not presented here, median frequency behaved similarly to mean frequency.

repeated were classified differently in the two experiments, indicating a high interexperiment and intrasubject variability probably as the result of the recruitment of different muscle fiber sets in different experiments.

Figure 4 shows the initial MNF and CV values corresponding to 20% MVC, 80% MVC, LLS, and HLS. The experiments are grouped according to the four types of behavior shown by spectral parameters and CV when stimulation level was increased from LLS to HLS. Figure 5*A* shows the initial MNF values vs. the initial CV values detected at 20 and 80% MVC. Figure 5*B* shows the initial MNF values vs. the initial CV values detected at LLS and at HLS.

The different behavior observed in voluntary and stimulated contractions is even more clearly evident in Fig. 6, *A* and *B*, which shows the percent changes of initial MNF and CV when contraction level is increased from 20 to 80% MVC (Fig. 6*A*) and from LLS to HLS (Fig. 6*B*) in each experiment. The four behavior types are identified with different symbols and belong to different quadrants in Fig. 6*B* (stimulated contractions) but only to the first quadrant in Fig. 6*A* (voluntary contractions).

Types 1 and *2* are the most frequent outcomes, both implying CV increase with increasing stimulation intensity and indicating a recruitment in order of increasing CV.

Figure 7 shows data from the 10 subjects on whom

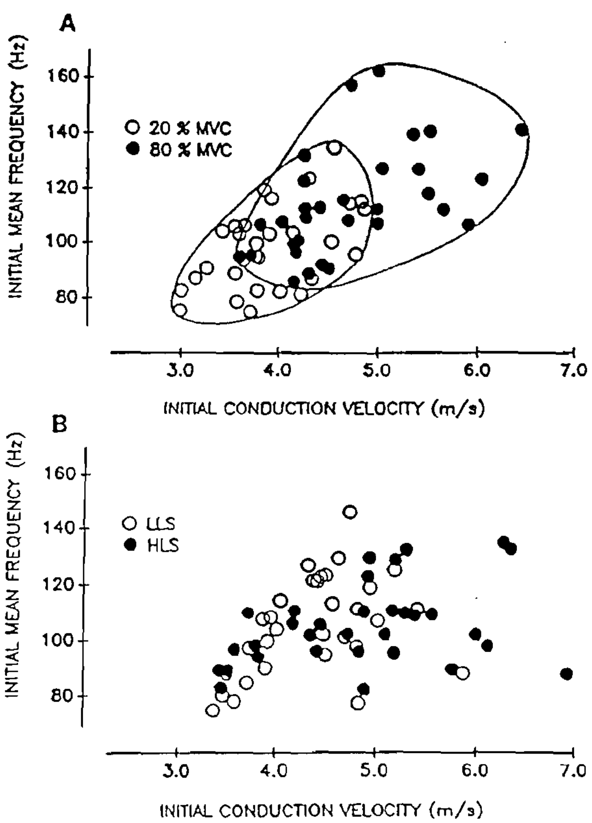

FIG. 5. Initial MNF vs. initial CV values. *A*: 20% MVC (o) and 80% MVC (•). Regression line of initial MNF vs. initial CV has a slope of 16.33 $Hz \cdot (m/s)^{-1}$ (significantly >0 with $P \leq 0.01$) and a correlation coefficient of 0.63. *B*: LLS and HLS. Regression line of initial MNF vs. initial CV has a slope of 4.9 $Hz \cdot (m/s)^{-1}$ (significantly >0 with $P \leq 0.05$) and a correlation coefficient of only 0.27. Note set separation in voluntary contractions (*A*) and not in stimulated contractions (*B*).

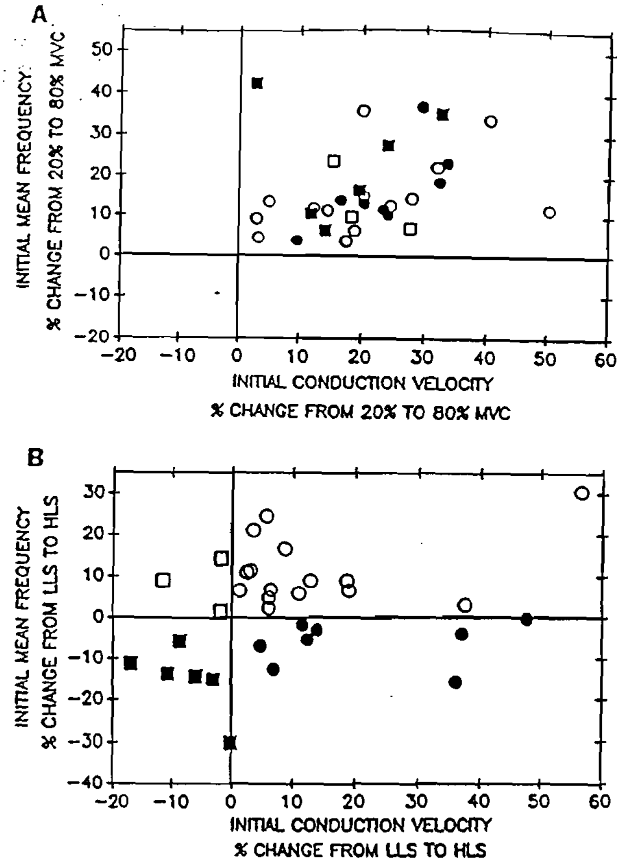

FIG. 6. *A*: percent variation of initial mean frequency vs. percent variation of initial conduction velocity for voluntary contraction level increasing from 20 to 80% MVC. *B*: percent variation of initial mean frequency vs. percent variation of initial CV for stimulation level increasing from LLS to HLS. Points belonging to different quadrants define different behavior types. See text for details. o, *Type 1*; •, *type 2*; ■, *type 3*; □, *type 4*.

stimulation was applied at different frequencies. Linear regression of the initial values of MNF, MDF, CV vs. stimulation frequency was performed. Sufficient evidence was not available, in most cases, to indicate a regression coefficient different from zero at the 0.05 significance level. Therefore, our results are not in complete agreement with previous papers (26, 29) that report an increase of CV with increasing firing rate of a motor unit. However, our data might have been contaminated by cumulative fatigue effects (see the DISCUSSION for details).

DISCUSSION

Voluntary Contractions

Motor unit recruitment order. It is generally accepted that during a voluntary muscle contraction, recruitment of motor units progresses from small (lesser number of fibers, smaller fiber diameter, more fatigue resistant) units to large (greater number of fibers, larger fiber diameter, more fatigable) units (35). It has been shown that muscle fibers of larger motor units have higher CV than those of smaller units (see Ref. 19 among others). It has also been shown that the spectral parameters of the surface ME signal are positively correlated with the

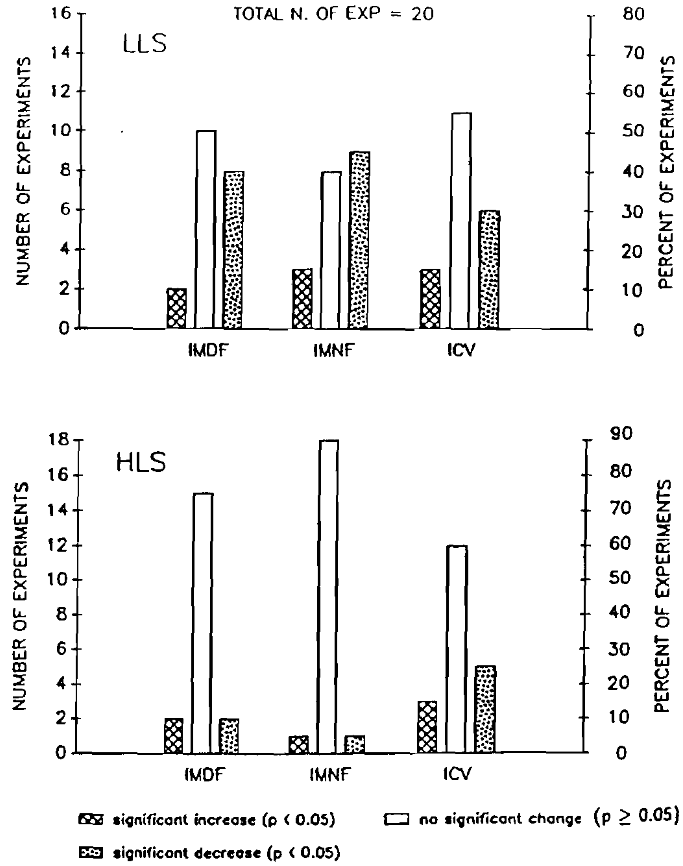

FIG. 7. Distribution of experiments showing a regression coefficient of initial mean frequency (IMNF), initial median frequency (IMDF), and initial conduction velocity (ICV) vs. stimulation frequency different from zero at LLS and HLS. Evidence sufficient to indicate correlation is not available in most cases.

average muscle fiber CV (1, 4, 28). Specifically, in the tibialis anterior muscle the first motor units recruited are smaller, fire at higher rates, and contract more slowly than those recruited at higher contraction levels (9). Direct correlation of these properties with histological types I and II is still controversial (9) but not relevant for the points addressed in this paper.

The morphological and histochemical properties of human tibialis anterior have been studied in detail on six cadavers by Henriksson-Larsen et al. (16). They found that in the superficial part of the muscle, the fiber size distribution ranged from 1,000 to 7,000 μm^2 for type I fibers and from 2,000 to 9,000 μm^2 for type II fibers, indicating an overall diameter range of 3:1. Helliwell et al. (15) also found type II fibers larger than type I fibers in the human tibialis anterior and an overall area range of 3,520–9,614 μm^2 in normal males (15). These data suggest that with proper detection techniques (28) both spectral parameters and CV of the surface ME signal should increase with increasing level of voluntary contraction. This association is valid because, during voluntary contraction, MDF has been shown to be positively correlated with CV, which in turn is positively correlated with the size of the active fibers, whose average value increases with increasing level of contraction.

In our work the initial values of MDF, MNF, CV at 80% MVC were found to be higher than those at 20% MVC in all cases ($P \le 0.01$ for each of the three parameters). Increase of voluntary contraction level from 20 to 80% MVC caused an increase of initial CV in the range of 2.7–50.5% with an average and standard deviation of 21.2 ± 10.8%, although the increase of initial MDF was 18.4 ± 13.6% and that of initial MNF was 16.2 ± 10.8%. All were significantly greater than zero ($P \le 0.01$), and the increase of initial CV was significantly larger than that of either initial MDF or initial MNF ($P \le 0.01$ and $P \le 0.05$, respectively, paired test). Broman et al. (6) found the same results in all eight cases they studied. Merletti et al. (23) showed the same behavior for MDF and MNF values in all seven studied cases. Similar results were reported by Arendt-Nielsen and Mills (1).

It can be concluded that our results, as well as those reported in literature, are consistent with the hypothesis that, in voluntary contractions, motor units of the human tibialis anterior muscle are recruited in ascending order of muscle fiber CV values, indirectly reflecting an ascending order of fiber size.

Conduction velocity and spectral parameters. Roy et al. (28) reported a high correlation ($r = 0.98$) between tibialis anterior initial MDF and CV values in contractions showing highly correlated double-differential signals. Our results, as well as those from Broman et al. (6), show a lower correlation between either initial values of MNF or MDF and CV ($r = 0.67$). It may be concluded that, although a positive correlation between initial values of MNF or MDF and CV is evident, the correlation between these variables may be lowered by random factors such as the individual differences between the tissue layers interposed among the signal sources and the detection electrodes (11) or differences in spatial pattern of motor unit recruitment.

The relationships between the initial values of spectral parameters and CV may be interpreted in terms of anatomic properties of the muscle. Consider the following. *1*) In all cases the initial values of CV, MNF, and MDF increased with increasing level of voluntary contraction, as represented in Fig. 4. This observation may be explained by assuming that the distribution of the recruited muscle fibers across the muscle cross section does not change substantially when the contraction level is increased. In fact, the initial CV estimate is not substantially affected by the position of the recruited muscle fibers in the muscle section, although the spectral parameters are greatly affected by the tissue filtering function (11, 20). *2*) The average percent changes of the initial spectral parameters are significantly smaller than those of the initial CV ($P \le 0.05$). This finding may be explained by assuming that in the deeper portions of the muscle the percent of high CV motor units is slightly higher than in the more superficial ones. The greater low-pass filtering effect of the tissue on the motor unit action potentials generated in the deeper portions of the muscle induces the smaller percent changes of the initial spectral parameters. Such an explanation is supported by the findings of Henriksson-Larsen et al. (16), who reported an average ratio of deep to superficial diameters of 1.13 in the human tibialis anterior.

Stimulated Contractions

Effect of the stimulation frequency. A theoretical study of the dependence of impulse propagation velocity on firing rate has been proposed by Miller and Rinzel (25). Starting from the Hodgkin-Huxley equations they demonstrated that the CV of unmyelinated fibers depends on the firing rate, and that particular combinations of firing rates and membrane parameters may be associated with supernormal CV.

The effect of motor unit firing rate on CV has also been reported in other studies. Recently Morimoto and Masuda (26) studied the correlation between individual motor unit interspike intervals and CV during voluntary ramp force contractions in the human vastus medialis. They observed a CV increase of 15% when firing rate increased from 6.6 to 20 pulses/s. Also Sadoyama and Masuda (29), by using a surface ME signal decomposition technique, observed a positive correlation between CV and firing rate in individual motor units during voluntary ramp force contractions of the biceps brachii. CV of individual motor units increased by an average of 19% (range of 9–29%) when the firing rate increased from ~12 to ~25 pulses/s.

As already pointed out in RESULTS (see Fig. 7), our data do not completely agree with those reported by previous investigators (26, 31), mainly because in several of our cases a negative correlation was found between the initial values of MNF, MDF, CV, and the stimulation frequency. A possible explanation of this apparent disagreement is that in our experimental protocol electrically elicited contractions might have induced a cumulative fatigue effect. As a consequence, the initial values might have been affected by at least two factors: the stimulation frequency and the cumulative effect of fatigue. The decrease of the initial values as the result of cumulative fatigue might have partially masked (or reversed) their increase as the result of the increased stimulation frequency. The fact that similar initial values were obtained from the voluntary contractions performed at the beginning and at the end of each experiment does not rule out the above explanation because our data show that different muscle portions may be involved in voluntary and stimulated contractions.

Motor unit recruitment order. Data describing the behavior of CV and spectral parameters of the ME signal during electrical stimulation (8, 27) are not as abundant as those relating to voluntary contractions. It is generally accepted that, with direct nerve stimulation by means of implanted electrodes, the order of recruitment of motor units with increasing stimulation level is the opposite of the one occurring during voluntary recruitment (10, 13, 33). However, it has not yet been established whether motor units are recruited in a given order during surface electrical stimulation of a motor nerve or of a muscle motor point. Brown et al. (7) and separately Bergmans (3) found that when graded electrical stimulation was applied to the median or ulnar nerve via surface electrodes, the first units recruited had the smallest M waves and the longest latencies, suggesting recruitment in order of size. The data shown in Fig. 4 suggest that the geometric location and the orientation of the nerve fibers or of the terminal axonal branches in relation to the current field may be more important than their electrical excitability threshold in determining the recruitment order. If this were the case, the order of motor unit recruitment during surface stimulation would not necessarily be related to muscle fiber size or CV.

According to known physiology and field theory, three factors may have a role in determining the order of recruitment (as indicated by muscle fiber CV changes) during surface electrical stimulation of muscle motor points: *1*) the size of the motoneuron (known to be related to excitability threshold, to the size of the motor unit, and to the average size of its muscle fibers), *2*) the size of the motoneuron branches (the diameter of which is related to excitability threshold), and *3*) the location and the orientation of the motoneuron branches in the current field.

Our data indicate that *factors 2* and *3* play an important, if not dominant, role. This finding may be explained as follows. *1*) If the current field were nonhomogeneous (as in our experimental setup), the superficial motor units (axon branches size notwithstanding) would be recruited first as the result of higher local current density. Henriksson-Larsen et al. (16) showed that motor units in the deep region of the tibialis anterior have an average diameter 13% larger than that of the superficial motor units. Increasing pulse amplitude might induce recruitment of progressively deeper axonal branches innervating larger and faster conducting muscle fibers. Such an occurrence would explain the 13.4% and 21.4% average increase in CV from LLS to HLS observed respectively in *type 1* and *type 2* behavior. Contributing factors include the spatial distribution, orientation, and size of the axons and of their terminal branches in the muscle. *2*) If larger axons had a significant number of branches smaller than those of smaller axons, then motor units with small fibers (lower CV) could be recruited first in a homogeneous current field. This occurrence would explain the recruitment in increasing order of muscle fiber CV.

The fact that 2 of 10 subjects showed CV increasing with increasing stimulation level in one experiment and decreasing with increasing stimulation level in the second experiment implies that the exact position of the stimulation electrode, and therefore geometric factors, are indeed critical in determining recruitment order.

In experiments classified as *type 1* and *2*, CV increased 20.7% and 23.6%, respectively, when the voluntary level of contraction increased from 20% to 80% MVC, but it increased only 13.4% and 21.4% when stimulation level increased from LLS to HLS. In these experiments the size principle is more strictly respected during voluntary contractions (see Figs. 8 and 9). The role played by axon diameter (excitability threshold) during electrical stimulation is relatively minor and only mitigates the size principle without reversing it in 72% of the experiments. Statistical significance of these increases was high for both types of behavior ($P \leq 0.01$).

The behavior of the CV during voluntary and electrically elicited contractions can be studied by plotting the initial CV values with respect to each other, as shown in

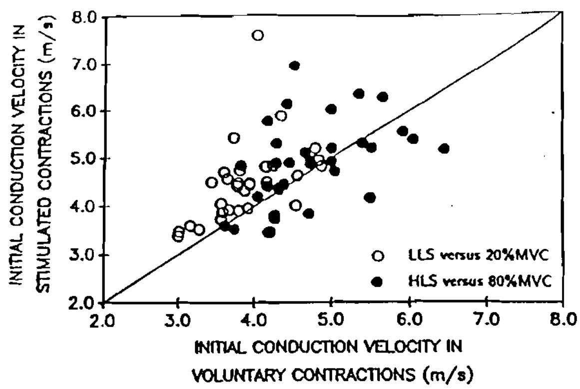

FIG. 8. Plot of initial conduction velocity at LLS vs. initial CV at 20% MVC and plot of initial CV at HLS vs. initial CV at 80% MVC. Diagonal line, locus of equal values in the 2 conditions. Data show that initial CV at LLS is higher than initial CV at 20% MVC ($P \leq 0.01$), whereas it is not significantly different at HLS and at 80% MVC. See text for further explanation.

Fig. 8. (Each point corresponds to one experiment.) LLS values are plotted against 20% MVC values and HLS values are plotted vs. 80% MVC values. It is evident that initial CV values at LLS are higher than those at 20% MVC ($P \leq 0.01$), whereas HLS values are not significantly different from those at 80% MVC. This finding strongly suggests that electrically induced recruitment at LLS begins at some intermediate value of fiber size, whereas at HLS it involves a motor unit population similar to the one recruited at 80% MVC. Such initial intermediate value of fiber size is determined by the geometric and threshold factors described above and most of the times is close to the low end of the size range. This concept is illustrated in Fig. 9.

The CV was estimated by performing the best alignment (in the mean square error sense) of the two double-differential signals (see METHODS). Any misalignment between the probe and the muscle fibers leads to an overestimation of the CV. During preliminary intense short test contractions the probe was oriented for maximal delay between the double-differential signals (see METHODS). During LLS contractions the recruited fiber population might be more superficial than that recruited during HLS or voluntary contractions. Consequently, as fiber orientation might have been somewhat different, CV might have been overestimated during LLS contractions. If so, the recruitment scheme seen with electrical stimulation could be even closer to that seen during voluntary contractions.

Our findings indicate that, in general, the axonal diameter (that is, motoneuron electrical excitability threshold) is not a major factor in determining motor unit recruitment order during surface stimulation of a muscle motor point. It is suggested that terminal branches of large motoneurons could be either smaller or more deeply located than those of smaller motoneurons and, therefore, may be excited at higher current levels. Such hypothesis would explain the finding of similar recruitment order during voluntary and electrically elicited contractions observed in 23 of 32 experiments.

Conduction velocity and spectral parameters. The consistent relation between CV and spectral parameters found for voluntary contractions by other authors as well as in our experiments does not seem to hold during stimulated contractions.

For high signal-to-noise ratios the estimate of CV is not greatly affected by the depth of the muscle fibers, whereas the estimate of spectral parameters is highly affected by the low-pass transfer function of the tissue interposed between the signal sources and the detection electrodes. According to the model developed by Lindstrom and Magnusson (20) a ME signal component at 100 Hz would be attenuated 12 dB by a 2-mm-thick tissue layer and 29 dB by a 10-mm-thick layer. Deeper muscle fibers, even if they had higher CV, would contribute predominantly to the low part of the ME signal spectrum therefore lowering the value of spectral parameters.

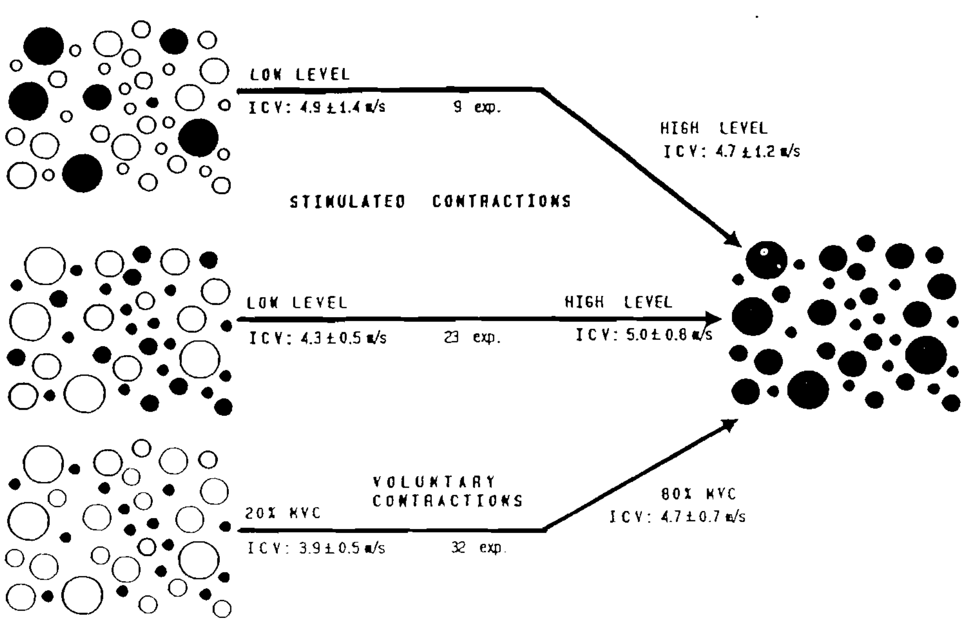

FIG. 9. Schematic representation of recruitment order during voluntary and electrically elicited contractions. For increasing level of voluntary contraction, recruitment proceeds in ascending order of CV values, reflecting ascending order of fiber size. At LLS, initial CV is higher than at 20% MVC, indicating that recruitment begins from larger fibers. Value of initial CV (and therefore of fiber size) at LLS is near lower end of range in 23 cases (*types 1* and *2*) and near higher end of range in 9 cases (*types 3* and *4*). See also Fig. 4 and text for details.

In the light of these considerations, the four types of behavior observed in these experiments may be explained as follows.

Type 1 behavior. Consider an innervation zone that has small axonal branches located more superficially than large axonal branches so that the geometric factor dominates the stimulation threshold factor. Furthermore, consider that the region of muscle activated by electrical stimulation has small and large muscle fibers uniformly distributed across its cross section. Motor units would then be recruited in a similar order during either voluntary or electrically elicited contractions and, being uniformly distributed, would lead to a similar behavior of spectral parameters in the two cases.

Type 2 behavior. Consider an innervation zone that has small axonal branches located more superficially than large axonal branches so that the geometric factor dominates the stimulation threshold factor. Furthermore consider that the region of muscle activated by electrical stimulation has a higher percentage of large fibers in the deep regions of the muscle. The order of recruitment during voluntary or stimulated contractions would be similar in terms of CV, but progressively deeper motor units would be recruited with increasing stimulation level. Such units would contribute energy to the low end of the spectrum, thus reducing the values of spectral parameters.

Type 3 behavior. This is the behavior that would be expected if motor units were recruited according to the excitability threshold of their axons and if slow- and fast-conducting units were uniformly distributed within the cross section of the activated muscle region. The fact that the average decrease of initial CV from LLS to HLS was only 7.7% indicates that electrical excitability was the dominant but not the only factor determining recruitment. Uniform distribution of motor units with different CV would make spectral parameters dependent on CV only. The fact that in four of these six experiments, CV at LLS was higher than at 20% MVC and 80% MVC indicates that the motor units recruited at LLS were indeed those with the highest CV.

Type 4 behavior. Consider an innervation zone that has superficial axonal branches mostly innervating relatively fast conducting muscle fibers. Further consider that in the muscle cross section under the detection probe, fast muscle fibers are deeper than the slower ones. At LLS mainly fast muscle fibers will be recruited, although at HLS the fiber population will be mixed. Because of the hypothesized spatial fiber distribution, the initial CV-decreasing behavior when increasing the stimulation level would be associated with an increasing behavior of the spectral parameters. As indicated in Fig. 4, this behavior was observed in only 3 of 32 experiments.

From these considerations it may be concluded that, during surface electrical stimulation, the location of the recruited motor units with respect to the stimulating and detection electrodes, and the location, orientation, and diameter of the motoneuron branches, have an important role in determining the relationship between CV and spectral parameters.

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