HIGHLIGHTED TOPIC | Neural Control of Movement

Low-threshold motor unit membrane properties vary with contraction intensity during sustained activation with surface EMG visual feedback

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Farina, Dario, Marco Gazzoni, and Federico Camelia. Low-threshold motor unit membrane properties vary with contraction intensity during sustained activation with surface EMG visual feedback. J Appl Physiol 96: 1505–1515, 2004. First published December 12, 2003; 10.1152/japplphysiol.01047.2003.—Single-motor unit (MU) activities were detected from the abductor pollicis and abductor digiti minimi muscles providing the subjects with visual feedback of multichannel surface electromyogram (EMG) signals. The subjects could modulate the force to observe on the surface recordings a single dominant MU and modulate its firing rate for contractions of 300 s with a noninvasive EMG feedback. The firing rate was maintained at ~8 pulses per second [low-frequency (LF) contraction] and at ~12 pulses per second [high-frequency (HF) contraction]. Single-MU conduction velocity (CV) decreased slightly but significantly over time, and it was possible to identify a significantly larger rate of decrease of CV during the HF with respect to the LF contractions. CV initial value significantly increased with the average firing rate, and CV values were significantly correlated to the instantaneous firing rate (R ranging from 0.21 to 0.39). Both additional MU recruitment and substitution were observed during the contractions. The study provides evidence that 1) it is possible to follow the same MU in a hand muscle at two different intensities (HF and LF) for 300-s durations by using visual feedback of surface EMG, 2) low-threshold single-MU CV changes over time since the beginning of the contraction, and 3) it is possible to distinguish between CV changes of the same MU at slightly different firing rates. The technique provides a practical method for the noninvasive assessment of both control and membrane properties of single MUs.

MOTOR UNITS (MUs) are the basic functional blocks of the neuromuscular system. The analysis of their properties leads to the understanding of motor control strategies (6, 9, 17, 32) and their adaptations to specific conditions, such as pathology, fatigue, pain, or exercise (5, 11, 16, 30, 31, 41). Historically, single-MU analysis was performed by invasive recordings, which have the selectivity needed for the decomposition of the interference electromyogram (EMG) signal (23). Decomposition of intramuscular signals provides the times of occurrence of the detected MU action potentials (MUAPs) and thus allows the investigation of MU firing rates and recruitment strategies. On the other hand, invasive recordings do not directly provide information on the muscle fiber membrane properties. Thus, with these techniques, it is not possible to directly investigate the peripheral properties of the neuromuscular system. MU control properties may change as a consequence of changes of the contractile and membrane muscle fiber properties. Thus the analysis of the peripheral mechanisms may be important for a better understanding of the relations between control strategies and muscle properties (10, 11). The analysis of muscle fiber contractile properties by the averaging of the force signal (42) is very critical (43). On the contrary, conduction velocity (CV) of single MUs can be estimated at any physiological firing rate, without the limitations of the twitch-averaging technique. Muscle fiber CV is correlated to the peak twitch force exerted by the MU in the fresh muscle (1).

The indirect analysis of MU control and membrane properties by global surface EMG variables, that is without decomposing the surface EMG into the constituent MUAP trains, is limited in many respects (13). Techniques for the analysis of the central control on the basis of surface EMG are being currently used, but many of them should be carefully evaluated and discussed because of limitations (13).

Multichannel surface EMG allows the analysis of anatomic and membrane MU properties (27, 45, 46). The joint intramuscular and multichannel surface EMG recording may be used to assess central and peripheral neuromuscular system properties with the possibility of analyzing the adaptation of MU control strategies to the peripheral modifications of the system (10). A complete noninvasive approach to MU characterization has been the aim of a part of surface EMG research in the last 15 yr (7, 26, 28, 37, 38, 45, 46). The main issue when surface EMG is used for the identification of single MUs is the low spatial selectivity (36). Spatial selectivity depends on the subcutaneous layer thickness, depth of the sources, and detection system adopted, which are factors of variability among different subjects and experimental conditions. Although isolated occurrences of MUAPs can be detected in specific conditions and by means of selective detection systems (8), the detection of almost all the occurrences of action potentials of specific MUs, with the possibility of estimating the average and instantaneous MU firing rate, is in general not feasible with surface techniques.

One possible approach to identify single-MU activities during voluntary muscle contractions is to provide the subject with a feedback of the EMG signal and ask him or her to train until a few MU activities are visible on the recording. This approach was applied in previous studies that used intramuscular record-
ings as feedback (3). In this case, it is relatively easy to discriminate single MUAPs because of the small detection volume of the recording system. It is also possible to real-time decompose the intramuscular signals and online estimate the average firing rate of a few selected MUs (34, 35).

In the present work, we demonstrate that it is possible to follow single MUs for long periods via visual feedback of the surface EMG signal and, thus, to noninvasively analyze changes in motor control and muscle fiber membrane properties during sustained activation of low-threshold MUs. A selective multichannel surface EMG detection system will be used for the purpose.

MATERIALS AND METHODS

Subjects. Eleven male subjects [age 27.2 ± 2.0 (SD) yr, height 1.76 ± 0.07 m, body mass 72.1 ± 8.7 kg] took part in this study. None of the subjects reported symptoms of neuromuscular disorders. The study was approved by the Local Ethics Committee, and written informed consent was obtained from all participants before inclusion.

Surface EMG recording. Surface EMG signals were detected using a linear array of 16 silver electrodes (point electrodes, 1-mm diameter, 2.5-mm interelectrode distance, Fig. 1A) (27) from the abductor pollicis and abductor digitii minimi muscles of the dominant hand. The small interelectrode distance ensured high spatial selectivity of the recordings (24). The EMG signals were amplified (gain 2,000–5,000) by a multichannel surface EMG amplifier (EMG 16. LISiN-Prima Biomedical & Sport, Treviso, Italy), band-pass filtered (−3-dB bandwidth, 10–500 Hz), sampled at 2,048 samples/s, and converted to digital data by a 12-bit analog-to-digital converter board. The acquisition software allowed the real-time display of the 15 single differential surface EMG signals during the recording.

The two muscles were assessed in a few test contractions to identify the locations of the innervation zone(s) and tendon regions (25). The array was then located to cover the distal semifiber. Because the semifiber length was always shorter than the length of the array, some of the electrodes covered the innervation zone(s) and the proximal semifiber (Fig. 1, B and C). The orientation of the array was selected on the basis of visual signal analysis, choosing the angle of inclination that led to most similar potentials traveling along the array from the innervation zone to the distal tendon. The parts of the skin where the array was located were slightly abraded with abrasive paste (Meditec-Every, Parma, Italy). The array was fixed at the skin surface by adhesive tape. A reference electrode was placed at the right wrist.

General procedures. Five of the 11 subjects participated in both sessions. Three subjects participated in the test for the abductor digitii minimi, and three subjects participated in the test for the abductor pollicis. Thus eight subjects were investigated for each muscle. After electrode placement, the hand of the subject was fixed in specifically designed ergometers for measuring the force of the two muscles (Fig. 2). The first phalanx of the thumb or of the fifth finger touched a force sensor, in case of recordings from the abductor pollicis and digitii minimi, respectively (Fig. 2). The force signal was amplified (Force Amplifier, MISO-II, LISiN, Torino, Italy) and stored for later analysis. Before each signal recording, the force sensor output was set to zero in resting conditions.

The subject then performed three maximal voluntary contractions (MVCs), separated by a 2-min rest in between. During each MVC contraction, verbal encouragement was provided to the subject. After the MVCs, a 5-min rest was allowed. The subject was then given ~20 min to train with the visual feedback. The surface EMG signals detected in single differential configuration by the array were real-time displayed on the screen of a personal computer put in front of the subject, 1 m away. The display presented 500-ms-long portions of signals, as representatively shown in Fig. 1, B and C. The amplification factor was adjusted to optimize the quality of the feedback. The instructions given to the subject were to modulate the force to see on the screen of the personal computer clear MUAPs repeating over time.
and belonging to the same MU, with the smallest number of contributions from MUs other than the selected one. In most tests, a single MU was detected in this phase (Fig. 1, B and C). In case the identification of a single MU was not feasible with the selected array location, the array was slightly moved and the training repeated with the new array position. Once a MU was identified, the subject trained in modulating its firing rate at two values, 8 pulses per second (pps) and 12 pps. To maintain an approximately fixed firing rate, the subject was asked to modulate the force to observe four or six action potentials of the selected MU in the window of 500 ms (Fig. 1, B was asked to modulate the force to observe four or six action potentials that at the high rate as HF (high frequency). In the following, the contraction at the low firing rate will be indicated as LF (low frequency) and that at the high rate as HF (high frequency).

More advanced feedback systems, based on the automatic decomposition of the signal and on the online computation of the instantaneous firing rate, were not implemented in the present study. These systems may be applied to intramuscular recordings for which optimized, fast-decomposition algorithms are available. However, this is not yet possible with surface EMG.

A thermometer was attached at the skin surface next to the array, and the skin temperature was maintained constant during the experiment with the resolution of ±0.5°C by means of a fan blowing air at adjustable temperature.

After the training, the subject was asked to maintain the selected MU active for 300 s at the first randomly selected average firing rate (LF or HF contraction). A 5-min resting period was given to the subject after the first contraction. A second contraction lasting 300 s was performed at the other average firing rate. The subject was asked to maintain the same MU active during the two contractions. Before the beginning of the second contraction, the signals acquired in the first contraction were shown to the subject, when necessary, to facilitate the recruitment of the same MU on the basis of the shape of its MUAPs.

Signal analysis. The recorded signals were offline decomposed into the constituent single MUAPs with a segmentation-classification approach (17a). The segmentation phase, which extracts MUAPs from the multichannel signals, is performed by the continuous wavelet transform while the extracted MUAPs are classified by means of a multichannel neural network. The neural network adapts to slow changes in MUAP shape over time to account for modifications in the muscle fiber membrane properties during sustained contractions. Extensive validation of the method has been provided (17a). The algorithm does not resolve superimposed MUAPs. However, with the experimental procedure designed for the present study, the number of superpositions was negligible (Fig. 1, B and C), and almost complete firing patterns could be reliably recovered (Fig. 3). The few missed firings that may have occurred in some conditions were in a negligible number (see also RESULTS). The signals from the two contractions (LF and HF) were decomposed independently. The presence of the same MU was checked by comparison of the shape of the average templates (Fig. 4). The classification of MUAPs was on the basis of the spatiotemporal information of the multichannel surface EMG recordings. The detection of many signals over the skin surface allowed to reliably discriminate among potentials whose shape was similar in some of the channels (17a).

The number of missed firings was roughly estimated from the number of potentials whose distance from the preceding one was larger than twice the average inter-pulse interval. Because the firing rate could oscillate during the contraction, this number is larger than the actual number of missed firings.

Single-MU CV was computed over time by applying a multichannel estimator (14) to double-differential derivations of each detected firing. The double-differential signals used for the CV estimates were manually selected as those distally detected from the innervation zone with clear propagation and small shape changes. Instantaneous firing rate was computed as the inverse of the time interval between subsequently detected firings.

The CV values of the MU used as feedback were fitted by a regression line. The intercept of the regression line was assumed as the initial value of CV. The slope of the regression line indicated the rate of change of CV over time. The percent decrease of CV was defined as the difference between initial and final (at the end of the contraction) CV value, divided by the initial value, and expressed as a percentage. The normalized rate of change was defined as the slope of the regression line divided by the initial value expressed in percentage. The average CV values from firings detected within intervals of 2 s at the beginning, middle, and end of the contraction were also computed.

In addition to single-MU CV, the global CV was computed from signal epochs of 1 s (300 epochs for each contraction) from the same double-differential signals used for the estimate of CV of the MU used as feedback. The algorithm described in Farina et al. (14) was applied
for the global CV estimates. Global CV is the weighted average of the CVs of the active MUs.

**Statistical analysis.** The hypothesis that single-MU CV rate of change was different from zero was tested by linear regression analysis for each MU. Two-way repeated-measures ANOVA was applied to analyze the influence of the average firing rate and of the interval of observation (beginning, middle, and end of the contraction) on CV values. The post hoc Student-Newman-Keuls (SNK) test for pairwise comparisons followed the ANOVA, when required. The comparison between CV rates of change and normalized CV decrease at LF and HF was performed by paired Student’s t-tests. To assess correlation between instantaneous firing rate and CV, Pearson’s correlation coefficient was calculated. Statistical significance was set to \( P = 0.05 \). Data are presented as means ± SE.

**RESULTS**

The MVC force was (means ± SE; \( n = 8 \)) 39.3 ± 2.9 N for the abductor pollicis and 16.2 ± 1.1 N for the abductor digiti minimi. All subjects were able to identify a single MU and activate it at LF and HF, in the conditions described above. The force level (means ± SE; \( n = 8 \)) at which the MUs were recruited for the LF contractions (recruitment threshold) was 3.2 ± 0.7% MVC for the abductor pollicis and 5.1 ± 0.7% MVC for the abductor digiti minimi. At the beginning of the HF contractions, the exerted force was 6.3 ± 1.0% MVC for the abductor pollicis and 7.2 ± 0.8% MVC for the abductor digiti minimi.

The estimated percentage number of missed firings for the feedback MUs (means ± SE, over the 2 muscles, \( n = 16 \)) was 5.2 ± 0.8% for the LF contractions and 6.0 ± 1.1% for the HF contractions. As indicated above, this is an overestimation of the actual number of missed firings. The average (over the 300 s) firing rate of the MUs used as feedback at LF (target firing rate 8 pps) was (means ± SE, over the 2 muscles, \( n = 16 \)) 7.5 ± 0.2 pps, whereas at HF (target firing rate 12 pps) it was 10.5 ± 0.2 pps. The firing rates in the two conditions were significantly different (Student’s t-test for dependent samples).

The number of double-differential derivations selected for the multichannel CV estimation was (means ± SE, \( n = 8 \)) 5.4 ± 0.6 for the abductor pollicis and 5.1 ± 0.4 for the abductor digiti minimi.

**Changes of single-MU CV during sustained activation.** The number of feedback MUs for which CV decreased over time (slope of the CV regression line statistically different from zero and negative, linear regression analysis) was seven of eight and six of eight for the abductor pollicis and abductor digiti minimi, respectively.
A two-way ANOVA (factors: contraction level, LF or HF, and time during the contraction, initial, middle, end) of CV of the abductor pollicis was significant for both factors \( (F = 5.10, \ p < 0.05; \ F = 11.57, \ p < 0.01, \) respectively). The LF contractions resulted in significantly lower CV than the HF ones. Moreover, the three instants of time during the contraction (initial, middle, and end) resulted in significantly different CV values among each other (SNK post hoc test, \( P < 0.05), \) with CV decreasing over time.

A two-way ANOVA (factors: contraction level, LF or HF, and time during the contraction, initial, middle, end) of CV of the abductor digiti minimi was significant for both factors \( (F = 6.64, \ p < 0.05; \ F = 7.59, \ p < 0.01, \) respectively). The LF contractions showed lower CV than the HF ones and the three instants of time during the contraction (initial, middle, and end) resulted in significantly different CV values among each other (SNK post hoc test, \( P < 0.05), \) with CV decreasing over time.

Table 1 reports the initial CV values, slopes, and percent decrease of CV for the two muscles, at the two contraction intensities. For both muscles, the slope and normalized CV decrease at HF were significantly larger (absolute values) than those at LF (Student’s \( t\) test for dependent samples, \( P < 0.05). \)

Figure 5 shows an example of single-MU CV decrease over time during the LF and HF contractions. Note the small standard deviation of estimation and the larger CV decrease at HF with respect to LF.

**Table 1.** CV initial values, slopes, and CV percent decrease for the two muscles and the two contraction levels analyzed

<table>
<thead>
<tr>
<th></th>
<th>Abductor Pollicis</th>
<th>Abductor Digitii Minimi</th>
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<tbody>
<tr>
<td></td>
<td>LF</td>
<td>HF</td>
</tr>
<tr>
<td>CV initial values, m/s</td>
<td>3.07 ± 0.19</td>
<td>3.18 ± 0.19</td>
</tr>
<tr>
<td>CV slopes, m/s²</td>
<td>−0.11 ± 0.03 × 10⁻³</td>
<td>−0.25 ± 0.09 × 10⁻³</td>
</tr>
<tr>
<td>CV decrease, from initial value, %</td>
<td>−1.09 ± 0.29</td>
<td>−2.15 ± 0.77</td>
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Values are means ± SE for 8 subjects. The conduction velocity (CV) initial values reported here are the intercept of the regression line at time \( t = 0). \)

Relationship between firing rate and CV. Figure 6 shows a scatterplot between CV and instantaneous firing rate for the two muscles of one subject. The relation between CV and firing rate was significant and positive (linear regression analysis, \( P < 0.05) \) in six of eight and in five of eight MUs for the abductor pollicis and abductor digitii minimi, respectively. Cross-correlation coefficient between firing rate and CV was \( (means \pm SE, n = 8) 0.21 \pm 0.09 \) at LF and \( 0.39 \pm 0.10 \) at HF for the abductor pollicis, whereas it was \( 0.21 \pm 0.12 \) and \( 0.20 \pm 0.11 \) at LF and HF, respectively, for the abductor digitii minimi.

**MU recruitment and substitution during sustained contractions.** Over all the subjects, the number of MUs not active at the beginning and recruited during the contraction was 3 and 11 at LF and HF, respectively, for the abductor pollicis. For the abductor digitii minimi, there were one and six MUs recruited at LF and HF, respectively. The CV and instantaneous firing rate of the MUs recruited after the beginning of the contraction was assessed only for HF, because only a few MUs were recruited during the LF contractions. At HF, the initial CV value of the additionally recruited MUs was \( (means \pm SE) 3.21 \pm 0.12 \) and \( 2.98 \pm 0.09 \) m/s for the abductor pollicis \( (n = 11) \) and abductor digitii minimi \( (n = 6), \) respectively. For both muscles, CV of the newly recruited MUs decreased, with a rate of change \( (means \pm SE) of −0.33 \pm 0.19 \times 10⁻³ \) m/s² for the abductor pollicis and \( −0.55 \pm 0.19 \times 10⁻³ \) m/s² for the other.
Fig. 5. Conduction velocity (CV) and instantaneous firing rate (IFR) of the same MU detected at LF (A) and HF (B) from the abductor pollicis of 1 subject. Note the decrease of CV over time, which can be clearly identified from the regression analysis. The initial CV value and the rate of change (absolute value) are larger at HF than at LF. Although the relative changes are rather small, it is thus possible to distinguish the fatigue development for the MU at the 2 contraction levels. The slow CV fluctuations resemble the slow instantaneous firing rate fluctuations (e.g., during the first half of the HF contraction), indicating correlation between IFR and CV (see also Fig. 6). The relation between fluctuations of the 2 variables can be detected because of the very small SD of CV estimation resulting from the multichannel estimation method. The force level at the beginning of the LF and HF contraction was 2.3 and 6.6% maximal voluntary contraction, respectively.

Fig. 6. Scatterplot of CV vs. IFR for the 2 contractions in 1 subject. Note the positive correlation between the 2 variables. A: results from the abductor pollicis. B: results from the abductor digiti minimi. The IFR values encircled by the dashed line are probably due to missed firings (indeed they approximately correspond to half of the target firing rate) and constitute a small percentage of the total detected firings. The force level at the beginning of the LF and HF contraction was 3.1 and 9.7% maximal voluntary contraction, respectively, for the abductor pollicis and 5.7 and 7.6% maximal voluntary contraction, respectively, for the abductor digiti minimi.
abductor digiti minimi. The average firing rate of the MUs recruited at HF was (means ± SE) 9.4 ± 0.2 pps for the abductor pollicis (n = 11) and 9.0 ± 0.3 pps (n = 6) for the abductor digiti minimi.

Both additional recruitment and substitution of MUs was observed. Figure 7 shows the instantaneous firing rate and CV estimated from two MUs (one is the feedback MU) during a contraction at HF. The MU recruited after ~75 s shows a clear decrease of CV and a larger CV initial value than the feedback MU. The average firing rates of the two MUs are similar. When the second MU was activated, it was not possible for the subject to turn it off and maintain only the first, despite the visual feedback. An example of MU substitution is shown in Fig. 8, where the MU on which the subject focused is derecruited and a new one takes its place for a limited interval of time. The newly recruited MU has CV lower than the MU recruited since the beginning of the contraction. Moreover, although the exerted forces are rather small, the CV values may largely differ between MUs. Figure 9 shows an example of recruitment with substitution. The CVs of the two MUs are markedly different, whereas the firing rates are similar.

**Global vs. single-MU CV.** The initial global CV was 3.38 ± 0.13 m/s (at LF) and 3.52 ± 0.15 m/s (at HF) for the abductor pollicis, whereas it was 3.02 ± 0.14 m/s (at LF) and 3.19 ± 0.17 m/s (at HF) for the abductor digiti minimi. In case of additional MU recruitment, the global CV was weighted by the contributions (amplitude and firing rates) of the different MUs. Because CV of the MUs recruited after the beginning of the contraction could be rather different from that of the feedback MU, the trends of global CV could be different from those of the single MUs. Figure 10 shows two examples of this issue. Note that the additional MU recruitment may mask the decreasing trend of CV when a global analysis is used instead of that at the single-MU level.

**DISCUSSION**

This study provides evidence that 1) it is possible to follow the same MU in a hand muscle at two different intensities (HF, LF) for 300-s durations by using visual feedback of surface EMG, 2) low-threshold-MU CV changes over time since the beginning of the contraction, and 3) it is possible to distinguish between CV changes of the same MU at slightly different firing rates. The technique proposed provides a practical method for the noninvasive assessment of both control and membrane properties of single MUs. However, the limitations of the method should be underlined for a proper interpretation of the results.

**Fig. 7.** CV and IFR of 2 MUs detected during a HF contraction of the abductor pollicis muscle. The MU recruited since the beginning of the contraction is that used as feedback. The second is additionally recruited, although the subject was trying to produce a selective activation of the first MU only. The average firing rate and CV over 20 firings are superimposed to instantaneous values. Note that there is a small percentage of missed firings (detected from an estimated instantaneous firing rate of ~6 pps), which do not affect significantly the average firing rate and are due to the inability of the algorithm to resolve superpositions. Some of the MUAPs of the 2 detected MUs are also shown (double-differential signals). The force level at the beginning of the contraction was 5.8% maximal voluntary contraction.
Fig. 8. Example of 3 MU activities (note the clear difference between their MUAP shapes; double-differential signals) detected during a 300-s-long HF contraction of the abductor pollicis. The feedback MU is active since the beginning of the contraction. A MU is additionally recruited with continuous activity after ~70 s of contraction. The first and second MUs are derecruited for ~20 s when a third MU is recruited, although the subject tried to maintain the feedback MU active. When the third MU stops firing, the feedback MU is recruited again. Some of the MUAPs of the 3 detected MUs are also shown (double-differential signals). The force level at the beginning of the contraction was 8.3% maximal voluntary contraction.

Fig. 9. Two MUs (with clearly different MUAP templates; double-differential signals) are detected during a LF contraction of 300 s of the abductor digiti minimi. The feedback MU is active since the beginning of the contraction. When the second MU is additionally recruited (after ~60 s), the feedback MU stops firing (at least in some of the intervals of activity of the second MU). The second MU fires for ~40 s, then stops firing, and is recruited again at the end of the contraction when the feedback MU is derecruited. Note the large difference between CV of the two MUs. Some of the MUAPs of the 2 detected MUs are also shown (double-differential signals). The force level at the beginning of the contraction was 3.9% maximal voluntary contraction.
The discriminative power of the recording was achieved partly because of the anatomy of the muscles selected. They present thin subcutaneous layers and fibers parallel to each other. In addition, the control of these muscles is probably more accurate than that of larger muscles. It is likely that the percentage of subjects being able to selectively activate single MUs, as requested in this study, would have been significantly smaller in case of other muscles. However, the application of a similar method to the tibialis anterior muscle showed promising results (D. Farina, unpublished observations). The non-invasive estimation of CV is possible only for a few muscles (26), which also limits the applicability of the method. Finally, the analysis is limited to the lowest-threshold, superficial MUs, and it is not possible to investigate high-threshold or deep units.

Besides the limitations, the technique proposed allowed the analysis of MU properties not directly accessible with other methods.

**Changes in single-MU CV during sustained activation.** Average muscle fiber CV decreases during sustained isometric contractions. Thus it has been used in past works to assess muscle fatigability under isometric, constant force contractions (2, 4, 29). CV of single MUs has been estimated in the past in the fresh (1, 14, 20, 40) and fatigued (12) muscle. Farina et al. (10) analyzed CV of low-threshold (25% MVC) MUs of the tibialis anterior muscle during 60-s-long contractions, tracking the MUs by intramuscular recordings and estimating their CV from spike-triggered averaged surface EMG signals. Changes of CV could be detected from the beginning of the muscle contraction. In the present study, the contraction levels were significantly lower than 25% MVC (3–7% MVC) because only the lowest-threshold units could be investigated. The analysis of membrane properties of these MUs is of importance for monitoring the development of neuromuscular disorders because of repetitive works at low-average force levels (19, 21).

The results shown prove that, even at low firing rates, the membrane fiber properties of low-threshold MUs change, starting from the beginning of the contraction. The percent changes of CV were rather small (on average, ~2–5% at HF), but they allowed us to distinguish two slightly different contraction levels (e.g., Fig. 5). Single-MU CV thus provides one of the earliest signs of modifications in the neuromuscular system induced by sustained contractions.

Global EMG analysis is critical in the conditions analyzed because it provides information on many active MUs that may be recruited at different instants of time (Fig. 10). Thus, in studies involving low contraction levels, single-MU analysis should be the method of choice rather than global EMG assessment. At low contraction levels, partial surface EMG decomposition is feasible even without a visual feedback, especially if high-density electrode grids are used for signal detection (17). A clear trend of CV decrease could be observed for the MUs recruited after the beginning of the contraction (e.g., Fig. 7). Even if the intervals of activity of the newly recruited MUs were shorter than the total contraction time, the rate of decrease of CV was significant.

**Relationship between firing rate and CV.** A small but significant correlation between instantaneous firing rate and CV was observed in many of the MUs analyzed (Fig. 6). Moreover, the initial MU CV was significantly higher for the HF than for the LF contractions in both muscles (Table 1). The percent increment of CV with firing rate, estimated from Table 1, is close to what was reported by Nishizono et al. (33) on the biceps brachii muscle, after microstimulation of single MUs. On the contrary, Kossev et al. (22) did not find any relation between firing rate and CV from spike-triggered averaged surface potentials. The differences between these studies may be related to the different signal-to-noise ratio when a single MU is stimulated and when its potentials are obtained by averaging. Moreover, different CV estimation methods, having different estimation variance, may account for the disagreement in the results. The present results show that CV is affected by firing rate, because it appeared even for small
fluctuations (Fig. 5). Given the small changes of CV, it is evident that an eventual relation with firing rate may be masked by methodological limitations.

Assuming a relation between firing rate and CV, the CV of a MU may significantly change with increasing muscle force. During increasing force contractions, the average muscle fiber CV increases because of the recruitment of additional MUs with larger CV (1) and the increase of the firing rate of the already active MUs. The relative weight of these two mechanisms on average muscle fiber CV during linearly increasing contractions is not fully clear. However, the use of average CV in voluntary contractions as an indicator of muscle fiber-type proportion should be discussed also on the basis of the relation between CV and firing rate (13). Indeed, at a given force level, the low-threshold MUs have higher firing rates than the high-threshold ones (15), which may affect the difference between CV values of MUs with different recruitment thresholds.

**MU recruitment and substitution during sustained contractions.** During constant-force, isometric contractions, MUs may be recruited in addition to the initially active ones (9, 17). The role of MU substitution or rotation is less clear. The Cinderella theory (19), for example, states that there are MUs always active and never substituted during a given task. In the present study, the force level exerted by the muscle was not constant. A single MU was activated by the subject who tracked it by a visual feedback. In these conditions, we observed both additional MU recruitment, and, in a few cases, MU substitution as also documented, in nonconstant force contractions, by Westgaard and De Luca (44). However, the present results relate to a limited number of MUs and to nonconstant force contractions. Moreover, the contraction modality with the feedback on one MU is very different from the normal operating conditions of the muscle. Finally, it cannot be excluded that the MU substitution reflected a small change in the activation strategy used by the subject, with, for example, a slight change in the direction of the exerted force.

The estimated CV of the MUs recruited after the beginning of the contraction was either larger or smaller than the CV of the previously active MUs. The difference could be rather large (Fig. 9). Although it is possible that the results are biased by different signal-to-noise ratios of the action potentials of the different MUs, this observation seems to indicate that the CVs of MUs with similar recruitment thresholds may be rather scattered, with implications that should be discussed when CV estimates or indirect CV assessment by spectral analysis are used to infer the MU type recruited (18, 39).

**Conclusion.** Properties of single MUs were analyzed by following their activity during time providing a feedback on the surface EMG signal to the subject and asking him to match the previously active MUs. It was shown that the method can be successfully applied to the two muscles investigated and allows the noninvasive analysis of both central and peripheral properties of the neuromuscular system at very low contraction levels.

**GRANTS**

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