AMPLITUDE CANCELLATION OF MOTOR UNIT ACTION POTENTIALS
IN THE SURFACE ELECTROMYOGRAM CAN BE ESTIMATED WITH
SPIKE-TRIGGERED AVERAGING

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ABSTRACT

The study presents an analytical, simulation, and experimental analysis of amplitude cancellation of motor unit action potentials in the interference EMG and its relation to the size of the spike-triggered average (STA) EMG. The amount of cancellation of motor unit action potentials decreases monotonically as a function of the ratio between the root mean square (RMS) of the motor unit action potential and the RMS of the interference EMG signal. The theoretical derivation of this association indicates a method to measure cancellation in individual motor units by STA of the interference and squared EMGs. The theoretical relation was examined in both simulated EMG signals generated by populations of 200 motor units and experimental recordings of 492 and 184 motor unit action potentials in the vastus medialis and abductor digiti minimi muscles, respectively. Although the theoretical relation predicted \( R^2 = 0.95; \ P < 0.001 \) the amount of cancellation in the simulated EMGs, the presence of motor unit synchronization decreased the strength of the association for small action potentials. The decrease in size of the STA obtained from the squared EMG relative to that extracted from the interference EMG was predicted by the experimental measure of cancellation \( R^2 = 0.65; \ P < 0.001 \), for vastus medialis; \( R^2 = 0.26; \ P < 0.05 \), for abductor digiti minimi. The results indicate that cancellation of action potentials in the interference EMG can be analytically predicted and experimentally measured with STA from the discharge times of the motor units into the surface EMG.
INTRODUCTION

Spike-triggered averages (STA) can be derived from the surface electromyogram (EMG) by triggering from the discharge times of a single motor neuron and averaging into the concurrently recorded surface EMG signals (Kakuda et al. 1991). STA into the interference and rectified EMGs has been used to extract single motor unit potentials from the surface EMG to measure motor unit synchronization (Milner-Brown et al. 1973; 1975), to estimate the number of motor units in a muscle (McComas et al. 1971; Stein & Yang 1990; Slawnych et al. 1990), to assess cross-talk in the surface EMG (Farina et al. 2003), and to quantify motor unit conduction velocity (Farina et al. 2002). It has often been assumed that STA into the rectified or squared EMG is not influenced by amplitude cancellation (Milner-Brown et al. 1975). However, cancellation of EMG amplitude, which is the result of the algebraic summation of positive and negative phases of motor unit action potentials (Day & Hulliger 2001; Keenan et al. 2005), occurs before the interference signal is rectified (Keenan et al. 2006).

Simulation analysis has shown that there is a link between the area of the STA obtained from the rectified (or squared) EMG and amplitude cancellation (Keenan et al. 2006). This observation suggested that estimating the strength of motor unit synchronization from the size of the STA derived from the rectified EMG (Milner-Brown et al. 1975) is limited by the unpredictable influence of amplitude cancellation. However, the association between amplitude cancellation and STA has not been demonstrated theoretically or confirmed experimentally. The purpose of this study was to derive an analytical expression for the amount of amplitude cancellation experienced by motor unit potentials in the surface EMG and to examine its association with the size of the STA.
extracted from the surface EMG. The analytical derivations were evaluated with a computer model in which the amount of amplitude cancellation could be quantified and experimental recordings of motor unit potentials from the vastus medialis and abductor digiti minimi muscles.

MATERIALS & METHODS

The approach begins with the derivation of an analytical expression for the degree of cancellation of motor unit action potentials in the surface EMG and an explanation of how the amount of cancellation can be measured experimentally with STA. The analytical results are validated with a computational model and experimental recordings.

Cancellation of motor unit action potentials

The RMS of the summation of two signals $x_1[n]$ and $x_2[n]$ is obtained as:

$$RMS = \sqrt{\frac{1}{M} \sum_{n=1}^{M} (x_1[n] + x_2[n])^2} = \sqrt{\frac{1}{M} \sum_{n=1}^{M} x_1^2[n] + \frac{1}{M} \sum_{n=1}^{M} x_2^2[n] + \frac{2}{M} \sum_{n=1}^{M} x_1[n]x_2[n]}$$

where $M$ is the number of samples in the signals. The term $\frac{2}{M} \sum_{n=1}^{M} x_1[n]x_2[n]$ tends to zero for large $M$ under the assumptions that $x_1[n]$ and $x_2[n]$ are uncorrelated and jointly ergodic for the cross-correlation. With these assumptions:

$$RMS = \sqrt{RMS_1^2 + RMS_2^2}$$

where $RMS_1$ and $RMS_2$ are the RMS values of the two signals. The property in Eq. 1 is used in all of the following derivations, which limits them to low levels of motor unit synchronization. The effect of synchronization on these derivations is addressed by the modeling approach and by experimental results on two muscles with different amounts of synchronization.
When the action potential of the generic motor unit $m$ is summed with an interference EMG signal, the RMS of the combined signal is:

$$RMS_{S+m} = \sqrt{RMS_{S}^2 + RMS_{R+1}^2}$$

(2)

where $RMS_{S+m}$ is the RMS of the EMG signal after the summation of the action potential, $RMS_{S}$ is the RMS of the interference signal before the addition of the action potential and $RMS_{R+1}$ is the RMS of the added action potential.

The contribution of the action potential to the RMS of the combined signal is smaller than the RMS of the action potential due to cancellation (Eq. 2). The increase in RMS of the combined signal by the addition of the action potential of motor unit $m$ is the difference between $RMS_{S+m}$ and $RMS_{S}$:

$$\Delta RMS_{C} = RMS_{S+m} - RMS_{S} = \sqrt{RMS_{S}^2 + RMS_{R+1}^2} - RMS_{S}$$

(3)

The increase in RMS of the combined signal in the absence of cancellation ($\Delta RMS_{NC}$) is equal to $RMS_{R+1}$. The contribution of the action potential $m$ to the RMS of the combined signal, as a percentage of its contribution without cancellation, is:

$$\Delta RMS_{\%} = \frac{\Delta RMS_{C}}{\Delta RMS_{NC}} \cdot 100 = \frac{\sqrt{RMS_{S}^2 + RMS_{R+1}^2} - RMS_{S}}{RMS_{R+1}} \cdot 100$$

(4)

Thus, the amount of cancellation of the motor unit action potential is:

$$C_{MU} = 100 - \Delta RMS_{\%} = 1 - \frac{\sqrt{RMS_{S}^2 + RMS_{R+1}^2} - RMS_{S}}{RMS_{R+1}} \cdot 100 = \left[1 - \sqrt{1 + \frac{1}{\alpha^2}} + \frac{1}{\alpha}\right] \cdot 100$$

(5)
where $\alpha = \frac{RMS_{R+1}}{RMS_S}$ is the ratio between the RMS of the action potential of motor unit $m$ and the RMS of the initial interference signal (Fig. 1).

**Experimental measurement of cancellation with STA**

The ratio $\alpha = \frac{RMS_{R+1}}{RMS_S}$ in Eq. 5 can be computed experimentally by STA into the interference EMG ($STA_{int}$) to provide the action potential $s[n]$ of the triggering motor unit (Keenan et al. 2006), and from which $RMS_{R+1}$ can be computed ($RMS_{R+1} = RMS_{STA_{int}}$).

The number of triggers used experimentally should be sufficient to reduce the contribution of the other motor units to a negligible value with respect to $s[n]$, and thus it depends on the amplitude of the interference EMG with respect to the amplitude of the motor unit action potential. The number of triggers for small motor unit action potentials should be greater than for bigger motor unit action potentials for a given amplitude of the interference EMG. The denominator of the ratio can be determined by taking the square root of the STA obtained from the squared EMG as follows:

$$
STA_{sa}[n] = \sqrt{\frac{1}{M} \sum_{i=1}^{M} [s[n] + v_i[n]]^2} = \sqrt{s^2[n] + \frac{1}{M} \sum_{i=1}^{M} v_i^2[n] + 2s[n] \frac{1}{M} \sum_{i=1}^{M} v_i[n]} \tag{6}
$$

where $v_i[n]$ is the interference signal without the motor unit action potential $s[n]$. The term $\frac{1}{M} \sum_{i=1}^{M} v_i[n]$ in Eq. 6 tends to zero when $M$ is large because it is the mean value of the EMG signal, whereas the term $\frac{1}{M} \sum_{i=1}^{M} v_i^2[n]$ is the RMS of the interference signal without the action potential of the motor unit $m$ ($RMS_S$). With a sufficient number of triggers (Keenan et al. 2006), Eq. 6 reduces to:
\[
ST_{\text{sq}}[n] = \sqrt{s^2[n] + \text{RMS}_S^2} \quad \text{for} \quad M \to \infty
\]  

(7)

where the baseline of \( ST_{\text{sq}}[n] \) represents the RMS of the interference signal without the action potential of motor unit \( m \) (Fig. 1). Thus, STA into the interference EMG and into the squared EMG provide experimental estimates of \( \text{RMS}_{R+1} \) and \( \text{RMS}_S \), that can be used in Eq. 5 to determine the amount of amplitude cancellation of the motor unit action potential.

The size of the STA obtained from the squared EMG (RMS of \( ST_{\text{sq}}[n] \)) can be related to amplitude cancellation as follows:

\[
\text{RMS}_{ST_{\text{sq}}} = \sqrt{\frac{1}{N} \sum_{n=1}^{N} ST_{\text{sq}}^2[n]} = \sqrt{\frac{1}{N} \sum_{n=1}^{N} s^2[n] + \text{RMS}_S^2} = \sqrt{\text{RMS}_{R+1}^2 + \text{RMS}_S^2}
\]

(8)

Combining Eqs. 4 and 8:

\[
\Delta \text{RMS}_S = \frac{\text{RMS}_{ST_{\text{sq}}} - \text{RMS}_S}{\text{RMS}_{ST_{\text{sq}}}} \cdot 100
\]

(9)

and combining Eqs. (5) and (9):

\[
C_{MU} = \left[ 1 - \frac{\text{RMS}_{ST_{\text{sq}}} - \text{RMS}_S}{\text{RMS}_{ST_{\text{sq}}}} \right] \cdot 100
\]

(10)

The term \( \frac{\text{RMS}_{ST_{\text{sq}}} - \text{RMS}_S}{\text{RMS}_{ST_{\text{sq}}}} \), which is equivalent to the ratio investigated by Keenan et al. (2006) in simulation and used by Milner-Brown et al. (1975) to estimate motor unit synchronization, is linearly related to cancellation (the complement to 1 indicates the amount of cancellation; Eq. 10). Because cancellation can be directly computed from only the RMS of the action potential and of the interference signal (Eq. 5), the RMS of the STA into the squared EMG is a redundant measure: the size of the STA into the
squared EMG can be obtained from other measures (in the absence of synchronization) and is the result of cancellation. This association was tested in experimental recordings. Eqs. 5 and 10 indicate that the size of the STA for the squared EMG can be predicted from the size of the action potential and the amplitude of the interference EMG (Fig. 1):

\[ RMS_{STA_{sq}} - RMS_{S} = \sqrt{RMS_{S}^2 - RMS_{R+1}^2} - RMS_{S} \]  

(11)

Simonulation procedure and analysis

The simulations were based on an anatomical model that comprised a cylindrical volume conductor (Farina et al. 2004) with an anisotropic muscle layer, and isotropic bone, subcutaneous, and skin layers. The model is similar to one used in a previous study (Keenan et al. 2005), and the parameters are reported in Table 1.

The muscle had an elliptical shape and was 30 mm in the transverse direction and 25.4 mm thick. The thickness of the subcutaneous layer was 1 mm. The intracellular action potentials of the muscle fibers were derived from the analytical description developed by Rosenfalk (1969). The innervation zones of the motor units \((n = 200)\) were located at the center of the muscle. Average fiber length was 120 mm for all motor units and the locations of the end-plates and tendon endings varied randomly (uniform distribution) over a range of 5 mm. The motor unit territories were circular and distributed randomly throughout the muscle. The fibers of each motor unit were
distributed with a density of 20 fibers/mm² (Armstrong et al. 1988) and interdigitated with the fibers of many other units to yield a fiber density in the muscle of 200 fibers/mm². When a portion of the motor unit territory was constrained by the muscle boundary, the territory of the unit was modified to fit the muscle cross-section (Keenan et al. 2005). Innervation numbers ranged from 15 to 1500, based on a ~100-fold range of twitch forces (Elek et al. 1992), with an exponential distribution across the population (Fuglevand et al. 1993a).

The motor units had a mean muscle fiber conduction velocity of 4.0 ± 0.35 m/s (Farina et al. 2000; Troni et al. 1983), with the slowest conduction velocity assigned to the smallest motor unit (Andreassen and Arendt-Nielsen 1987). The surface-recorded, motor unit potential comprised the sum of the action potentials of the muscle fibers belonging to the motor unit. The simulated EMG signals were detected with bar electrodes (5×1 mm), arranged in bipolar configurations with 5-mm distance between electrodes. The center of the detection system was between the innervation zone and tendon. EMG signals were computed at 4096 samples/s.

Activation of the motor unit pool was modeled as a constant force contraction for 20 s. The distribution of recruitment thresholds for the motor neurons was determined from an exponential function with many low-threshold neurons and progressively fewer high-threshold neurons (De Luca et al. 1982; Moritz et al. 2005). Each motor unit began discharging at 8 pulses per second (pps) once excitation exceeded the assigned recruitment threshold of the unit, and discharge rates increased linearly with increased excitation; the rate was 3 pps per 10% increase in excitation. Excitation corresponded to the simulated net synaptic input onto the motor neuron pool (Heckman and Binder 1991),
and it was assumed that all neurons received the same level of excitation. As a consequence, the simulated input-output functions of the neurons matched the observed relations between discharge rate and injected current (Kernell 1965).

Maximal excitation was denoted as the level of input necessary to bring the last recruited motor neuron to its assigned peak discharge rate, and values of excitation were expressed as a percent of this maximum. As described by Fuglevand et al. (1993a), the first recruited unit had a maximal discharge rate of 35 pps, whereas peak discharge rate decreased linearly with increasing recruitment threshold so that the last recruited unit had a peak discharge rate of 25 pps. The last unit was recruited at 50% of maximal excitation. The discharge rate was modeled as a random process with a Gaussian distribution, with the coefficient of variation for discharge rate set at 20%. Activation levels from 2.5% to 25% (2.5% increments) of the maximum excitation were simulated. Ten motor unit populations were simulated at each excitation level with a random location of the motor units within the muscle tissue.

Motor unit synchronization was examined by adjusting the timing of selected action potentials to impose a temporal association with action potentials discharged by different motor neurons, as described by Yao et al. (2000). Briefly, motor unit synchronization was imposed by applying a function that selected a percentage of the action potentials discharged by each motor unit to be synchronized with the discharges of 6 other motor units. Discharges of the reference motor unit were randomly selected, and when a discharge of another randomly selected motor unit occurred within ±30 ms of the discharge by the reference motor unit, this discharge time was aligned with the reference unit with a Gaussian distribution that had an SD of 1.67 ms centered on the reference
unit. This process was repeated until the discharges of 6 other motor units were aligned with the discharge of the reference motor unit for each discharge of the reference motor unit selected for this adjustment, and *ad seriatim* for all motor units in the pool. The percentage of discharges of the reference motor unit to be synchronized with other units was determined in order to obtain specified levels of common input strength (*CIS*), which is the frequency of extra synchronous discharges (Nordstrom et al. 1992). To determine the level of motor unit synchronization across the motor unit pool, *CIS* values were calculated for every motor unit with the 15 preceding and the 15 succeeding motor units (Keenan et al. 2006). In this way, *CIS* values were fixed in a range 0-1, regardless of excitation level.

An amplitude cancellation index was calculated for all simulated motor units at each excitation level (Keenan et al. 2006): 1) the RMS was calculated from the motor unit action potential; 2) the difference between the RMS of the interference EMG before and after removing all the action potentials of the reference motor unit divided by the number of action potentials of the reference unit was subtracted from the value derived from *step 1*; and 3) the value from *step 2* was divided by the value from *step 1* and multiplied by 100. These steps correspond to a direct calculation of cancellation, which is not possible in experimental conditions. Amplitude cancellation was also computed from the simulated signals using Eq. 5, which corresponds to a measure that can be made in experimental conditions, and compared with the direct measure of cancellation from steps 1-3. The direct measure of cancellation and that derived from Eq. 5 were compared by linear regression analysis. The simulations served to prove the validity of Eq. 5 in realistic synthetic signals. This relation cannot be examined in experimental recordings.
because the direct measure of cancellation is not available. However, the association between Eq. 5 and the reduction in size of the STA into the squared EMG was experimentally tested in two muscles. The association between Eq. 10 (reduction in size of the STA into the rectified EMG) and amplitude cancellation has been established with simulations (Keenan et al. 2006; their Fig. 3); the results from that study were confirmed with the present simulation analysis and are not reported here.

**Experimental procedures and signal analysis**

Two groups of 10 (5 men; age, mean ± SD, 29.1 ± 5.8 yr) and 8 (6 men; age, mean ± SD, 30.3 ± 4.2 yr) healthy subjects participated to the study. One muscle (vastus lateralis or abductor digiti minimi) was investigated in each group. The study was conducted in accordance with the Declaration of Helsinki, approved by the local Ethics Committee, and written informed consent was obtained from all subjects prior to participating in the study.

Because the theoretical derivations are limited to low levels of motor unit synchronization, two muscles with different amounts of synchronization were selected: the vastus medialis obliquus muscle \((n = 10 \text{ subjects})\) and the abductor digiti minimi muscle \((n = 8 \text{ subjects})\). Motor unit synchronization is generally greater in hand muscles compared with large limb muscles (Datta et al. 1991; De Luca et al. 1993; Kim et al. 2001).

The measurements on the vastus medialis were obtained with the subject seated comfortably on a chair with a belt fixing the position of the hip and the right thigh. The knee joint was flexed to 90°. A strap connected by a chain to a load cell was attached to the ankle to measure the extension force exerted by quadriceps femoris. The
measurements on the abductor digiti minimi were obtained by fixing the fifth finger in a
custom-made brace to record the force exerted during an isometric contraction of the
muscle (Politecnico di Torino, Torino, Italy).

The subjects performed three maximal voluntary contractions (MVC) with the
knee extensors or abductor digiti minimi, with each trial separated by 2 min of rest. The
greatest force was used as the reference MVC for the submaximal contractions. Surface
and intramuscular EMG electrodes were then mounted.

A linear array of equally spaced surface electrodes (vastus medialis: 8 bar
electrodes 5-mm long, 1-mm diameter, 5-mm inter-electrode distance; abductor digiti
minimi: 7 circular electrodes, 1-mm diameter, 2.5-mm inter-electrode distance) was
located between the most distal innervation zone and the distal tendon region of each
muscle (Masuda et al. 1985). The array electrodes were used so that the maximal bipolar
EMG amplitude could be detected (Merletti et al. 2003). The orientation of the array was
based on visual inspection of the surface EMG signals detected in test contractions with
the criterion of minimal change in the shape of the action potentials across channels. The
skin was lightly abraded at the location selected for array placement. Surface EMG
signals were amplified and a bipolar signal was derived from the center of the array
(EMG16, LISiN – Ottino Bioelettronica, Torino, Italy, bandwidth 10-500 Hz), sampled at
2048 Hz, and stored after 12 bit A/D conversion.

Two pairs of wire electrodes made of Teflon coated stainless steel (A-M Systems,
Carlsborg, WA, USA; diameter 50 µm) were inserted into the muscle with 25 G needles
at locations 10-20 mm proximal to the surface array for both muscles. One needle was
inserted in line with the direction of the array, the other at ~10 mm in a transverse
distance. The insulated wires were cut to expose only the cross section at the tip. The needles were inserted to a depth of a few millimeters below the muscle fascia and removed to leave the wire electrodes inside the muscle. The intramuscular EMG signals were amplified and provided a bipolar recording (Counterpoint EMG, DANTEC Medical, Skovlunde, Denmark) that was band-pass filtered (500 Hz–5 kHz). A reference electrode for both the surface and intramuscular recordings was placed around the ankle (for vastus medialis) or wrist (for abductor digiti minimi).

The subject performed a series of 30-s contractions at forces that ranged from 2.5%-25% MVC (2.5% increments) for the vastus medialis and from 2.5%-12.5% MVC (2.5% increments) for the abductor digiti minimi, in a randomized order. The rest periods varied with the target force and were the same for both muscles: 3 min for forces of 2.5 – 10 %MVC, 15 min for 12.5 – 20 % MVC, and 20 min for 22.5 – 25 % MVC. The different ranges of force analyzed for the two muscles were due to a greater complexity of the signals from the abductor digiti minimi muscle that impeded accurate decomposition of the intramuscular signals at higher force levels.

In a separate experimental session, the level of motor unit synchronization in the two muscles was measured from the two groups of subjects. The subjects were provided with feedback of the intramuscular signal recorded from one of the two locations and were asked to activate a motor unit at ~8 pps for 2 min (Semmler et al. 1999). The task was repeated for both wire locations and for as many motor units as could be controlled with the feedback.

The motor unit action potentials were identified in the intramuscular recordings with a decomposition algorithm (McGill et al. 2005). The discharge times of the
identified motor units from the wire in line with the array were used as triggers to average into the interference surface EMGs obtained with the array electrode during the constant force contractions. The RMS of the STA into the interference and squared (with subsequent square root) EMG signals was computed for each motor unit. The degree of cancellation was measured for each detected motor unit with Eq. 5. The relation between amplitude cancellation and STA size was investigated by comparing the degree of cancellation estimated with Eq. 5 and the STA ratio defined in Eq. 10.

Peak-to-peak amplitude of the action potential and muscle fiber conduction velocity were estimated from the STA into the interference EMG for each motor unit and compared across contraction levels with a one-way ANOVA. The amount of cancellation observed in the experimental measurements with Eq. 5 was compared to the Eq. 10 ratio by linear regression analysis.

The amount of motor unit synchronization was quantified from cross-correlation histograms (±100 ms relative to the reference motor unit discharge, with bin width of 1 ms) (Nordstrom et al. 1992) of all pairs of motor units identified from separate insertion sites during the 2-min feedback contractions. Histograms with a mean bin count <4 were excluded from further analysis (Semmler and Nordstrom 1997). The cumulative sum function was used to identify the location of the peak in the histogram (Ellaway 1978). A significant peak in the cumulative sum was defined as an increase >3 times the standard deviation with respect to the mean over the first 50 bins (Davey et al. 1986). Synchronization was quantified with the CIS index (Nordstrom et al. 1992), as for the simulated signals.

RESULTS
Eq. 5 indicates that amplitude cancellation decreases monotonically with an increase in $\alpha$. Motor unit action potentials that are large relative to the amplitude of the interference signal experience less amplitude cancellation, whereas small motor units are almost completely cancelled in the interference signal. Motor unit action potentials with an RMS value less than that for the interference EMG ($\alpha < 1$) are cancelled by more than 60% (Fig. 2).

Measurement of cancellation in the simulated signals

Fig. 3A shows the comparison between the direct computation of cancellation, which is not feasible with experimental recordings, and the estimated cancellation using Eq. 5 for all motor units in the simulated conditions in the absence of synchronization ($CIS = 0$). There was a strong association ($R^2 = 0.95; P < 0.001$) between the theoretically derived relation and the simulation results, with some variability around the predicted values due to the standard deviation in the estimation of RMS values from 20-s simulated signals. The results indicate that Eq. 5 provides a measure of amplitude cancellation in the absence of motor unit synchronization, as predicted theoretically.

The presence of synchronized discharges decreased the strength of the association between the theoretically derived and the computed cancellation because synchronization was not included in the analytical analysis. Eq. 5 overestimated cancellation for moderate levels of synchronization ($CIS = 0.2$), especially for small motor unit action potentials (Fig. 3B). The same trend was observed when the degree of synchronization was further increased to a $CIS$ value of 1 (Fig. 3C). Accordingly, there was a decrease in
the strength of the association between Eq. 5 and the computed cancellation ($R^2 = 0.42$; $P < 0.001$ for $CIS = 0.2$; $R^2 = 0.22$; $P < 0.01$ for $CIS = 1$). However, the theoretical analysis was inaccurate only for small motor unit action potentials (Fig. 3B,C). For values of $\alpha$ greater than 0.2, the estimate of cancellation from Eq. 5 was reasonable even for large levels of synchronization (Fig. 3). There was a strong association between Eq. 5 and amplitude cancellation for both synchronization levels for $\alpha$ values >0.2 ($R^2 = 0.92$; $P < 0.001$ for $CIS = 0.2$; $R^2 = 0.79$; $P < 0.001$ for $CIS = 1$), which indicates that the degree of cancellation can be estimated from Eq. 5 even for high levels of motor unit synchronization when the motor unit action potentials are large enough ($\alpha > 0.2$).

Insert Figure 3 and Table 2

Experimental Recordings

The simulation results confirmed the analytical prediction that cancellation can be estimated using Eq. 5. Thus, this relation was used in the experimental recordings to analyze the association between amplitude cancellation and the size of the STA from the squared EMG. According to the theoretical derivation and the simulation results reported by Keenan et al. (2006), the size of the STA from the squared EMG should be predicted by the measure of amplitude cancellation provided by Eq. 5.

The intramuscular recordings yielded 492 motor units identified from the vastus medialis muscle of the 10 subjects at 10 target forces and 184 motor units from the abductor digiti minimi at 5 target forces. Out of the total number of identified motor units, many units were the same motor unit identified at more than one contraction force; this did not affect the analysis of results. The number of identified motor units tended to
increase with contraction force, but there was no difference in the number of detected discharges during the 30-s contractions (Table 2). As expected (Hedayatpour et al. 2007), the peak-to-peak amplitude of the surface potentials, muscle fiber conduction velocity, and discharge rate of the identified motor units increased from the low to the high target force (P < 0.05; Table 2).

The average force exerted during the 2-min contractions for the measurement of synchronization was 5.4 ± 2.4 % MVC and 4.1 ± 2.6 % MVC for the vastus medialis and abductor digiti minimi, respectively. These contractions yielded 82 and 94 motor unit pairs from the vastus medialis and abductor digiti minimi, respectively. Only 35 of the 82 pairs of motor units from the vastus medialis showed significant peaks in the cross-histogram, whereas 90 of the 94 pairs of units had significant cross-histogram peaks in the abductor digiti minimi. The CIS values from the pairs that showed significant peaks were 0.10 ± 0.06 for the vastus medialis and 0.92 ± 0.31 for the abductor digiti minimi. Thus, the two muscles presented two substantially different levels of motor unit synchronization.

From the 30-s contractions, the times of occurrence of motor unit action potentials were used to trigger the averaging of the surface EMG without pre-processing (interference EMG) and after squaring the signal (Fig. 4). The amount of amplitude cancellation for each motor unit was quantified with Eq. 5. As theoretically predicted (Fig. 2), the amount of cancellation for a single motor unit (Fig. 5) and for all motor units in each subject (Fig. 6) increased with target force. For example, the average amount of cancellation for the individual shown in Fig. 6 (vastus medialis muscle) increased from ~20% at 2.5% MVC force to ~80% at 25% MVC force. The average (across all
contraction levels and motor units) amount of cancellation varied substantially across subjects: the range across subjects was 49.7 - 77.8 % for the vastus medialis and 45.6 – 80.1 % for the abductor digiti minimi.

Insert Figures 4, 5 and 6

The amount of amplitude cancellation obtained from Eq. 5 was compared with the ratio in Eq. 10, which involves the size of the STA relative to the squared EMG. Within the limits of experimental accuracy in estimating the STA from a limited number of triggers, the ratio in Eq. 10 approximated the expected amount of amplitude cancellation in both muscles (Fig. 7). For small values of $\alpha$, however, the estimated size of the STA obtained from the squared EMG signal was rather variable because the action potential was almost completely cancelled in the summed signal. Moreover, according to the simulation analysis, synchronization of motor units may increase the variability for small values of $\alpha$, as observed experimentally especially for the abductor digiti minimi (Fig. 7). The experimental measurements of cancellation with Eq. 5 and the STA ratio in Eq. 10 were linearly correlated, although the strength of the correlation was greater for the vastus medialis muscle ($R^2 = 0.65; P<0.001$) compared with abductor digiti minimi ($R^2 = 0.26; P<0.05$; Fig. 7). When considering only $\alpha$ values larger than 0.5 ($n = 129$), however, the strength of the correlation increased substantially in the abductor digiti minimi ($R^2 = 0.63; P<0.001$). The correlation between Eq. 5 and Eq. 10 experimentally demonstrated that the size of the STA from the squared EMG is associated to the amount of cancellation.
DISCUSSION

The amount of amplitude cancellation experienced by the action potentials of individual motor units has been theoretically proven to depend only on the ratio between the RMS of the motor unit action potential and the RMS of the interference signal. According to this theoretical derivation, small surface action potentials are cancelled more than large action potentials in the interference signal. The derivation indicates that the amplitude cancellation of individual motor units can be experimentally estimated by the STA of the interference and squared EMGs and that the relative reduction in the size of the STA extracted from the squared EMG is attributable to amplitude cancellation.

The analytical derivation of cancellation provides the means for predicting the amount of amplitude cancellation in a population of motor units. The amount of cancellation at each amplitude of the interference EMG depends on the size of the motor unit action potential. Because low-threshold motor units tend to produce smaller surface action potentials than high-threshold units (Keenan et al. 2006b), it is the surface action potentials of the low-threshold motor units that are reduced more by amplitude cancellation (Fig. 2). As a consequence of this effect, the surface EMG amplitude can be relatively insensitive to changes in the activity of low-threshold motor units. For example, Mottram et al. (2005) found that the discharge rate of low-threshold motor units decreased more rapidly during one type of fatiguing contraction compared with another type, whereas the change in the amplitude of the surface EMG was similar during the two tasks. Similarly, indexes of neural drive to the muscle based on EMG amplitude, such as
neuromuscular efficiency (Deschenes et al. 2002), are largely biased toward changes in
the activity of high-threshold motor units.

The sensitivity of EMG amplitude to the recruitment of motor units depends on
the ratio ($\alpha$) of the RMS values for the action potential and the interference EMG. Due
to this relation, a doubling of the amplitude of the interference EMG will reduce the
index $\alpha$ for a motor unit action potential by one half. For a motor unit with $\alpha = 1$, a
doubling of the interference EMG amplitude will increase the amplitude cancellation of
its surface action potential from ~60% to ~75% (Fig. 2). If $\alpha$ is reduced to one third of
the initial value, amplitude cancellation will increase further to ~85%. The relation
between the ratio $\alpha$ and amplitude cancellation explains why simulation results (Keenan
et al. 2005) indicated that a doubling in the number of motor units in a muscle produced
only a limited increase in surface EMG amplitude. Furthermore, the theoretical relation
serves that surface EMG amplitude is relatively insensitive to the changes in motor unit
activity at high levels of excitation: when $\alpha$ is much smaller than 1, cancellation is close
to 100% (Eq. 5; Fig. 2).

When an individual sustains a submaximal contraction, the amplitude of the
surface EMG increases due to the recruitment of additional motor units (Garland et al.
1994; Person & Kudina 1972; Riley et al. in press), the decrease in muscle fiber
conduction velocity (Bigland-Ritchie et al. 1981; Merletti et al. 1990), and the change in
the shape of the intracellular action potential (Dimitrova & Dimitrov 2002; Hanson &
Persson 1971). According to the relation in Eq. 5, the amount of amplitude cancellation
for a motor unit action potential will increase with the progressive increase in the
amplitude of the surface EMG. Because the relation represented in Eq. 5 is non linear
(Fig. 2), however, an increase in the amplitude of the interference signal has a different influence on the amount of amplitude cancellation experienced by each motor unit. The relative contribution of different motor units to the signal amplitude changes with increases in EMG amplitude. Similarly, the amount of cancellation in motor unit action potentials may differ in fatigued and unfatigued states due to differences in the RMS of the action potentials (Dimitrova & Dimitrov 2002; Hanson & Persson 1971), which is one of the factors that may contribute to the surface EMG at the end of a sustained submaximal contraction not reaching the amplitude achieved during a control MVC (Fuglevand et al. 1993b).

The simulation results demonstrated the validity of the theoretical derivations in the absence of motor unit synchronization. The simulations also allowed the inclusion of synchronization, which was not investigated theoretically. The results indicated that the presence of synchronization has the greatest influence on the cancellation experienced by small motor units (Fig. 3B,C), consistent with the observation that the summing of motor unit action potentials in the presence of synchronization depends on the duration of the action potentials (Zhou & Rymer 2004). The amount of cancellation is, in general, smaller than predicted by Eq. 5 when motor units are synchronized (Fig. 3B,C); however the difference is relatively small for $\alpha$ values larger than 0.2. The presence of synchronization reduces, on average, amplitude cancellation for small motor units, although it increases the total cancellation in the signal by decreasing the $\alpha$ value (compare the $\alpha$ axis in Fig. 3A with Fig. 3B,C), as observed in a previous simulation study (Keenan et al. 2006). The two muscles investigated experimentally exhibited different degrees of synchronization: it was low in vastus medialis, as previously reported.
(Kim et al. 2001), and high in abductor digiti minimi as reported for other hand muscles (Semmler & Nordstrom 1999). The simulation analysis indicated a weaker correlation between the STA-derived and the analytical estimates of synchronization for abductor digiti minimi (Fig. 7).

Extraction of the STA from the squared EMG followed by taking the square root of the signal is a similar operation to obtaining a STA from the rectified EMG; the latter has been used to assess the amount of motor unit synchronization (Milner-Brown et al. 1973; 1975). However, the theoretical analysis proved that the size of the STA from the squared EMG is fully explained by the amount of amplitude cancellation even in the absence of motor unit synchronization: the larger the cancellation, the smaller the size of the STA. This association was experimentally proved by predicting the reduction in size of the STA from the squared EMG with Eq. 5 (Fig. 7). Because cancellation depends on the size of the motor unit action potential, the size of the STA from the squared EMG relative to that from the interference EMG differs among motor units in a predictable way. Moreover, the STA from the squared EMG will change with the amplitude of the surface EMG because this influences cancellation, which explains previous results (Yue et al. 1995).

In summary, amplitude cancellation of motor unit action potentials in the surface EMG can be analytically predicted and experimentally measured, within the limitations of the STA approach (Keenan et al. 2006) and for low levels of motor unit synchronization. The size of the STA extracted from the squared EMG can be largely predicted from the size of the surface action potential of the motor unit and the amplitude of the interference EMG. The amount of amplitude cancellation is greatest for low-
threshold motor units that produce small surface action potentials, although the presence of synchronization may decrease the amount of cancellation experienced by these motor units. These effects confound the interpretation of changes in the amplitude of the surface EMG as an index of the activation of a motor unit population.

GRANTS

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REFERENCES


Table 1. Parameters of the cylindrical volume conductor model.

<table>
<thead>
<tr>
<th>Model Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue Conductivities (S/m)</strong></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>0.02</td>
</tr>
<tr>
<td>Muscle (radial and transverse)</td>
<td>0.1</td>
</tr>
<tr>
<td>Muscle (longitudinal)</td>
<td>0.5</td>
</tr>
<tr>
<td>Subcutaneous tissue</td>
<td>0.05</td>
</tr>
<tr>
<td>Skin</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Muscle Properties</strong></td>
<td></td>
</tr>
<tr>
<td>Number of motor units</td>
<td>200</td>
</tr>
<tr>
<td>Number of muscle fibers</td>
<td>119,634</td>
</tr>
<tr>
<td>Number of fibers in a motor unit (range)</td>
<td>15 - 1500</td>
</tr>
<tr>
<td>Muscle cross-sectional area</td>
<td>598 mm²</td>
</tr>
<tr>
<td>Average fiber length</td>
<td>120 mm</td>
</tr>
<tr>
<td>Skin thickness</td>
<td>1 mm</td>
</tr>
<tr>
<td>Subcutaneous tissue thickness</td>
<td>1 mm</td>
</tr>
<tr>
<td>Bone (radius)</td>
<td>20 mm</td>
</tr>
<tr>
<td>Tendon ending spread</td>
<td>5 mm</td>
</tr>
<tr>
<td>Innervation zone spread</td>
<td>5 mm</td>
</tr>
<tr>
<td>CIS</td>
<td>0-1</td>
</tr>
<tr>
<td><strong>Electrodes</strong></td>
<td></td>
</tr>
<tr>
<td>Rectangular, size</td>
<td>5×1 mm</td>
</tr>
<tr>
<td>Interelectrode distance</td>
<td>5 mm</td>
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</tbody>
</table>
Table 2. Motor unit characteristics at the low and high target forces for each muscle.

<table>
<thead>
<tr>
<th></th>
<th>Vastus Medialis</th>
<th></th>
<th>Abductor Digiti Minimi</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5%</td>
<td>25%</td>
<td>2.5%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Motor units/Contraction</td>
<td>2.1 ± 0.3</td>
<td>6.5 ± 2.3*</td>
<td>2.5 ± 0.4</td>
<td>5.1 ± 1.7*</td>
</tr>
<tr>
<td>Action potentials/Contraction</td>
<td>217 ± 47.4</td>
<td>250 ± 92.5</td>
<td>245 ± 38.2</td>
<td>278 ± 45.1</td>
</tr>
<tr>
<td>Surface peak-to-peak amplitude (µV)</td>
<td>14.1 ± 5.3</td>
<td>38.3 ± 17.9*</td>
<td>22.2 ± 7.9</td>
<td>55.7 ± 21.6*</td>
</tr>
<tr>
<td>Muscle fiber CV (m/s)</td>
<td>3.78 ± 0.29</td>
<td>4.34 ± 0.76*</td>
<td>3.45 ± 0.22</td>
<td>3.79 ± 0.89*</td>
</tr>
<tr>
<td>Discharge rate (pps)</td>
<td>7.9 ± 1.3</td>
<td>10.3 ± 2.1*</td>
<td>8.1 ± 2.0</td>
<td>9.8 ± 2.4*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with 2.5%. CV = conduction velocity.
FIGURE CAPTIONS

FIG. 1. Estimation of amplitude cancellation for a motor unit action potential by using spike-triggered averaging. A motor unit was identified from the intramuscular EMG recording in the vastus medialis muscle during a contraction at 7.5% MVC. The action potentials of the motor unit were used to trigger averages from the interference (black area) and the squared EMGs. The average of the squared EMG was followed by computation of the square root (dashed area). With these measurements, the amount of amplitude cancellation can be estimated from Eq. 5.

FIG. 2. Theoretical analysis of amplitude cancellation for the action potentials of single motor units (Eq. 5). The percentage of cancellation decreases monotonically as a function of the ratio $\alpha$. $C_{MU}$: amplitude cancellation in individual motor unit action potentials; $\alpha$: ratio between the root mean square values of the motor unit action potential and of the interference EMG signal.

FIG. 3. Measurement of amplitude cancellation ($C_{MU}$) in the simulated EMG signals. Interference EMG signals were simulated at excitation levels that ranged from 2.5%–25%. A $C_{MU}$ value of 100% denotes complete cancellation of the motor unit potential in the interference signal. A: The motor unit discharge patterns were uncorrelated ($CIS = 0$). B: A moderate level of synchronization was included ($CIS = 0.2$). C: A high level of synchronization was simulated ($CIS = 1$). Cancellation of the motor unit action potential (circles) at each level of excitation was computed directly by comparing the increase in RMS in the interference signal when the motor unit was added to the sum of the RMS values for the interference signal and the motor unit action potential. This comparison
cannot be performed in experimental conditions. Cancellation was also computed from Eq. 5 (dashed line). The direct measurement of cancellation (circles) corresponded to the analytical prediction (dashed line) with some variability for high levels of cancellation due to the variance in the estimation of the signal RMS from 20-s simulated contractions. Note that this variability may cause the estimated cancellation to be higher than 100% in panel A, due to estimation variance.

FIG. 4. Spike-triggered averaging from the discharge of a single motor unit into the surface EMG. A: The discharge times of a single motor unit have been aligned (left-most tic mark), and subsequent discharges of the unit are shown in the same trace. B: Superimposed intramuscular EMG recordings aligned with respect to the identified action potentials. C: The shape of the intramuscular action potential. D: Spike-triggered averages from the interference (solid line) and the squared (dotted line) EMG signals.

FIG. 5. Estimation of amplitude cancellation for a motor unit as a function of contraction force with the vastus medialis muscle. A: The motor unit was identified from the intramuscular recordings obtained during 6 contractions (2.5-15 % MVC force). The similarity in shape of the intramuscular action potentials was used to ensure that the same motor unit was identified at all target forces. B: Corresponding surface EMG signals with the discharge times of the identified motor unit indicated with circles. The surface action potentials can be distinguished in the surface EMG for the contraction at 2.5% MVC force, but this was more difficult with an increase in the amplitude of the interference EMG. C: The spike-triggered averages from the interference and the squared EMGs were computed for the identified motor unit and the amount of cancellation was estimated with Eq. 5. At the target force of 15% MVC, the action
potentials of the identified motor unit are almost completely cancelled (~90% cancellation). The numerator in Eq. 10 is obtained by subtraction of the baseline value from the root mean square of the spike-triggered average into the squared EMG (with subsequent square root). The ratio between the result of this subtraction and the root mean square of the spike-triggered average into the interference EMG is proportional to cancellation (Eq. 10).

FIG. 6. Amount of amplitude cancellation for all motor units identified in the vastus medialis muscle during the 10 contractions of one subject. The dashed lines connect values for the same motor unit.

FIG. 7. Comparison of the ratio in Eq. 10 derived from the STAs into the interference and the squared EMGs (circles) with the amount of cancellation measured experimentally by Eq. 5 (solid line). A: Vastus medialis muscle. B: Abductor digiti minimi muscle. The motor unit data (n = 492 in A and n = 184 in B) are shown as a function of the ratio \( \alpha \) between the RMS of the motor unit action potential and the RMS of the interference signal.
\[
C_{MU} = \left[1 - \sqrt{1 + \frac{1}{\alpha^2} + \frac{1}{\alpha}}\right] \cdot 100 \\
\alpha = \frac{RMS_{R+1}}{RMS_S} = \frac{RMS_{STA_{int}}}{RMS_S}
\]
A scatter plot shows the relationship between Force (% MVC) and $C_{MU}$ (%). The data points are connected by dashed lines, indicating a positive correlation between the two variables.