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ROBUST ESTIMATION OF AVERAGE TWITCH CONTRACTION FORCES OF
POPULATIONS OF MOTOR UNITS IN HUMANS

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1 ABSTRACT

2 The characteristics of motor unit force twitch profiles provide important information for
3 the understanding of the muscle force generation. The twitch force is commonly estimated
4 with the spike-triggered averaging technique, which, despite the many limitations, has been
5 important for clarifying central issues in force generation. In this study, we propose a new
6 technique for the estimation of the average twitch profile of populations of motor units
7 with uniform contractile properties. The method encompasses a model-based
8 deconvolution of the force signal using the identified discharge times of a population of
9 motor units. The proposed technique was validated using simulations and tested on signals
10 recorded during voluntary activation. The results of the simulations showed that the
11 proposed method provides accurate estimates (relative error $<25\%$) of the main parameters
12 of the average twitch force when the number of identified motor units is between 5% and
13 15% of the total number of active motor units. It is discussed that current detection and
14 decomposition methods of multi-channel surface EMG signals allow decoding this relative
15 sample of the active motor unit pool. However, even when this condition is not met, our
16 results show that the estimates provided by the new method are anyway always superior to
17 those obtained by the spike triggered average approach, especially for high motor unit
18 synchronization levels and when a relatively small number of triggers is available. In
19 conclusion, we present a new method that overcome the main limitations of the spike-
20 triggered average for the study of contractile properties of individual motor units. The
21 method provides a new reliable tool for the investigation of the determinants of muscle
22 force.

23

1 INTRODUCTION

2 The contraction of a muscle is the result of the summation of sequences of twitch
3 forces of the muscle units. The superimposition of all motor unit (MU) twitches is the total
4 force generated by the muscle (Heckman and Enoka, 2004). In general, the identification
5 of the twitch temporal profiles at the motor unit level provides important information on
6 the characteristics of the investigated motor units (Bagust et al., 1973; Burke et al., 1974;
7 Heckman and Enoka, 2004; Macefield et al., 1996; McNulty and Macefield, 2005; Thomas,
8 1995). Unfortunately, due to the fusion and summation of hundreds of motor unit forces,
9 the extraction of the motor unit twitch characteristics is a complex problem. Currently,
10 there are no robust methods for estimating the contractile properties of individual muscle
11 units, therefore our understanding of acute and chronic adaptations of muscular and neural
12 mechanisms of movement is limited (Enoka and Fuglevand, 2001). For example, during
13 sustained contractions, muscle fatigue determines changes in both the discharge rate of the
14 motor neurons (Carpentier et al., 2001; Gandevia, 2001) and the contractile properties of
15 the muscle fibers (Nordstrom and Miles, 1990), although the links between these two
16 effects are debated (Fuglevand, 1996).

17 The only available method for the estimation of the motor unit temporal twitch
18 profiles *in vivo* during voluntary contractions is the spike-triggered averaging (STA)
19 (Gossen, 2003; Milner-Brown et al., 1973; Thomas, 1995) technique. Under the
20 assumptions of uncorrelated activity of the motor units, the STA performs an average of
21 the force signal triggered by the discharge times of one motor unit, usually identified by
22 decomposition of intramuscular EMG (Farina et al., 2008, 2005; Milner-Brown et al.,
23 1973; Roatta et al., 2008). The averaging process increases the signal-to-noise ratio (SNR)

1 for the force generated by the triggered unit with respect to the summation of the forces
2 produced by the other active motor units. One of the main problems in this approach is that
3 the average inter-spike interval of MU during voluntary contraction is smaller than their
4 twitch duration. On this basis, the STA estimation is always biased (Gossen et al., 2003;
5 Negro et al., 2014). This effect alters the STA estimates substantially, especially during the
6 relaxation time (Kossev et al., 1994). Another limitation of the STA for twitch estimation
7 is the need for relatively long signal recordings (usually >2 min) to obtain stable estimates
8 (Nordstrom and Miles, 1990). This impedes the use of STA for assessing changes of twitch
9 force over short time intervals, for example during fast fatigue development. Finally, the
10 synchronization between motor unit spike trains also negatively influences the STA
11 estimates of motor unit twitch forces (Keen, 2004; Kutch et al., 2007). Because of these
12 important limitations, a few alternative methods and models have been proposed to extract
13 the contributions of individual MU to the force profile in human (Andreassen and Bar-On,
14 1983; Kutch et al., 2010; Lim et al., 1995) and animal recordings (Drzymala-Celichowska
15 et al., 2016), but without an extensive validation and with very limited use.

16 In this study, we approach the problem of estimating twitch forces in motor units
17 by deconvolution. To overcome the problem of low SNR for individual motor unit
18 twitches, our approach will aim at the estimation of the average twitch in populations of
19 motor units having similar recruitment thresholds and hence presumably similar force
20 twitches. We extensively validate the method on simulated and experimental signals and
21 prove that its application is feasible in a variety of conditions. The method provides for the
22 first time the possibility of accurately estimate the twitch contractile properties of motor
23 units in voluntary contractions.

1

2 *MATERIALS & METHODS*

3 *Theory*

4 The generation of the muscle force signal can be modelled as a spatial and temporal
5 summation of motor unit twitch forces, as:

$$6 \quad Y(t) = \sum_{i=1}^N X_i(t) * g_i(t) \quad (1)$$

7 where $X_i(t)$ and $g_i(t)$ are respectively the spike train and the twitch force of the i -th motor
8 unit and N is the total number of active motor units (Negro et al., 2014). In general, it is
9 not possible to identify all motor units that are active during a voluntary contraction, even
10 at very low contraction levels (Farina et al., 2010). However, recent advances in the
11 detection and decomposition of intramuscular (iEMG) and surface EMG (sEMG) allow to
12 the reliable identification of several (>10) concurrently active motor units (Farina et al.,
13 2016; Holobar et al., 2010; Nawab et al., 2008; Negro et al., 2016a, 2009; Francesco Negro
14 and Farina, 2011).

15 We can rewrite Eq. (1) as

$$16 \quad Y(t) = \sum_{i=1}^M X_i(t) * g_i(t) + W(t) \quad (2)$$

17 where $M \ll N$ is the number of spike trains identified reliably from EMG decomposition
18 and $W(t)$ is the activity of the remaining motor units that can be seen as additive noise. In
19 general, we are interested in the estimation of the twitch force waveforms $g_i(t)$. From this
20 equation, we can define a signal-to-noise (SNR) measure as

$$21 \quad SNR = 10 \log_{10} \left(\frac{P_M}{P_w} \right) \quad (3)$$

22 where P_M is the power of the force signal generated by the M detected units and P_w the
23 power of the additive noise introduced by the force of the remaining motor units.

1

2 *Description of the method*

3 In order to solve this underestimated problem, we implemented a time-domain
 4 deconvolution technique. The optimization technique estimates a model with a set of
 5 parameters θ (the parameters describe the average twitch of the M motor units, see below)
 6 in order to solve the least-mean-square (LMS) problem

$$7 \quad \min_{\theta} \left\| \left[\sum_{i=0}^{M-1} X_i(t) * g_{\theta}(t) \right] - \hat{Y}(t) \right\|_2^2 \quad (4)$$

8 where M is the number of known motor unit spike trains. In general, the problem
 9 of estimation the twitch parameters has an unbiased solution only if the activation of all
 10 sources is known and a correct parameterization of the sources is available. However, only
 11 in the case of electrically stimulated twitches, the full activation pattern is known.
 12 Additionally, the frequency and variability in the discharge timing of individual motor units
 13 in physiological conditions does not allow the correct estimation of the twitch waveform,
 14 as previously demonstrated (Negro et al., 2014). Finally, the estimation of each individual
 15 motor unit twitch waveform is not feasible due to the low SNR (see Results section). For
 16 this reason, we focused this study on the estimation of the properties of the *average* twitch
 17 of the motor units identified. Therefore the estimation provided is related to an averaged
 18 twitch extracted using the composite spike train (CST) (Castronovo et al., 2015; Farina et
 19 al., 2014; Farina and Negro, 2015; Negro et al., 2016b; Francesco Negro and Farina, 2011;
 20 Negro and Farina, 2012) of the M identified motor units. This is not a limiting factor since,
 21 for low contraction forces, the motor units have similar recruitment threshold and therefore
 22 similar parameters. The set of parameters θ in Eq. (4) includes three variables to
 23 characterize the average twitch profile and one to provide a better estimation in case of

1 underdetermined systems. The twitch response was modeled using a modified version of
 2 the impulse response described by Fuglevand et al. (Fuglevand et al., 1993) that has been
 3 proposed by Roatta and Farina (Roatta and Farina, 2011) in a previous study:

$$g_i(t) = \begin{cases} P_i \frac{t}{T_i^1} e^{1-\frac{t}{T_i^1}} & t \leq T_i^1 \\ P_i \frac{t - T_i^1 + T_i^2}{T_i^2} e^{\left(1-\frac{t-T_i^1+T_i^2}{T_i^2}\right)} & t > T_i^1 \end{cases}$$

(5)

6 where P_i , T_i^1 and $\frac{5}{3}T_i^2$ are the peak amplitude, the rising time and the half
 7 relaxation time (HR) for the motor unit i . Figure 1 shows two examples of twitch
 8 waveforms described by this model.

FIGURE 1 HERE

10 An additional parameter was added for the estimation of the bias of the unidentified
 11 motor units. This model was used since it allowed having a direct measure of the three
 12 parameters independently. The optimization problem of Eq. (4) was performed using an
 13 iterative algorithm for time-domain deconvolution (trust-region-reflective, (Absil et al.,
 14 2007)). The Levenberg–Marquardt algorithm (Levenberg, 1944) provided similar results.
 15 In order to limit the number of iterations, upper and lower ranges for each parameter should
 16 be set. In the simulations, the upper and lower limits for P , T_1 , T_2 and the Bias were
 17 respectively: 0.1-100 AU, 30-120 ms, 30-120 ms, 0-Max_Force. For the experimental

signals, they were: 0 Nm – 0.03 Nm, 30-120 ms, 30-120 ms and 0-Max_Force. The starting point was chosen as a random variable uniformly distributed between the range values.

Simulations

The simulations performed on this study were based on a model of integrate-and-fire motor neurons with discharge characteristics similar to a previous model by Fuglevand et al. (Fuglevand et al., 1993). Each motor neuron received a synaptic input current that was a linear combination of shared and independent Gaussian stochastic processes (0-50 Hz). The shared synaptic input simulates the summation of all inputs that project commonly to the motor neuron pool (Farina and Negro, 2015). On the other hand, the independent noise resembles the fluctuation of the membrane potential generated by the summation of synaptic inputs that are individual for each motor neuron. The motor neuron pool include a number of motor neurons equal to 300, similar to histological findings in the abductor digiti minimi (ADM) muscle (Santo Neto et al., 1985), which was the muscle used for the experimental analyses (see “experimental recordings” section below). The model described the motor units with an exponential variation of the recruitment threshold, peak-to-peak and twitch forces. The range of discharge rate was set between 8 and 35 pps. The level of force at which all the motor units were recruited corresponded to 50 % (Burke et al., 1974; Milner-Brown et al., 1973) of maximal synaptic input. The rising time of the smallest motor unit was set to 90 ms. The range of twitch tensions was set to 100 and the range of contraction times was set to 3 (Fuglevand et al., 1993).

The proposed technique was applied on simulated forces corresponding to 5% of synaptic input (equivalent to approximately 3% MVC). This level was chosen to be similar to the

1 typical experimentally recorded level of force used for the estimation of the twitch
2 properties with the STA technique (Roatta et al., 2008).

3 To study the influence of motor unit synchronization on the twitch estimation, we
4 performed simulations with a moderate level of synchronization among the spike trains.
5 The synchronization was imposed injecting correlated synaptic noise into the motor
6 neurons (Negro et al., 2016b; F. Negro and Farina, 2011; Negro and Farina, 2012). Motor
7 unit synchronization was quantified with the common input strength (CIS) index as the
8 frequency of extra synchronous discharges (Nordstrom et al., 1992) between all pairs of
9 active motor units. The proportion of shared synaptic noise was selected in order to obtain
10 physiological levels of synchronization (Keen et al., 2012).

11 *Simulation analysis*

12 For assessing the performance of the proposed technique, the composite spike train of 5,
13 15, 25, 50, 75 and 100 % of the active motor units was estimated by the proposed algorithm.
14 The duration of the analyzed segment was set to 5, 10 and 30 s. For each case, 100
15 simulations were performed with a random selection (normal distribution) of the motor
16 units in each simulation. The results were compared with the parameters extracted from
17 the average twitch force of all motor units selected for the calculation. For each simulation,
18 the STA twitch force was extracted for the motor unit with the lowest discharge rate (~ 8
19 pps) and compared with the estimated parameters calculated by the algorithm. The duration
20 of the segment used for the calculation of the twitch parameters using the STA technique
21 was 120 s, much longer than the interval used for the deconvolution methods. The STA
22 twitch was measured 50 ms prior to and 150 ms after the reference action potential. The

intervals preceding and succeeding the reference action potential were required to have durations of ≥ 110 ms (Nordstrom et al., 1989; Taylor et al., 2002).

Experiments

Subjects. 5 healthy men participated in the main experiment (mean \pm SD, age: 26.1 \pm 2.5 yrs; range, 25-31 yrs).

The experiments were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee (approval number N-20090019 and 01/10/12). All participants self reported to be right handed and signed a written informed consent form before inclusion.

Recordings. Individual motor unit action potentials were recorded from the abductor digiti minimi muscle with Teflon-coated stainless steel wires (diameter 0.1 mm; A-M Systems, Carlsborg, WA) inserted into the muscle with 25-gauge hypodermic needles. Each wire was cut to expose the cross section of the tip without insulation. In order to increase the number of identified motor units, two pairs of wires were placed approximately 1 cm apart in the transverse direction in the proximal portion of the muscle. The needles were inserted and removed after the insertion, leaving the wires inside the muscle. The two bipolar intramuscular EMG signals were amplified (Counterpoint EMG, Dantec Medical, Skovlunde, Denmark), band-pass filtered (1000 Hz to 5 kHz), and sampled at 10 kHz.

Procedures. The subject was seated on an adjustable chair with the right arm extended in a force brace (Aalborg University). The fifth finger was fixed in the isometric device for the measurement of finger-abduction forces. The forearm and the four digits were secured with Velcro straps. The force produced by the fifth finger was measured using

two force transducers (Interface, Arizona USA), one in the transverse plane (abduction force) and the other in the sagittal plane (flexion force). The force signal was sampled at 10 kHz. Visual feedback on the finger abduction force was provided on an oscilloscope.

The subjects performed three maximal voluntary contractions (MVCs) of finger abduction with a rest of 3 min in between. The force generated during the maximal contractions was considered as the reference MVC. Afterward, the subject performed one contraction of 60-s duration at 5% MVC with visual feedback. During each contraction, the flexion force was also monitored and contractions with not significant flexion force were repeated.

Experimental signal analysis.

Individual motor units were identified from the intramuscular EMG signals recorded from the two locations in the muscle by the use of a decomposition algorithm (McGill et al., 2005). Each motor unit spike train was manually edited by an experienced operator and any unusually long (>250 ms) or short (<20 ms) inter-spike intervals (ISIs) were manually inspected for checking potential discrimination errors. From the results of the decomposition, spike trains of individual motor units were calculated with a sampling rate of 1000 Hz. The CST (composite spike train) was defined as the sum of the individual spike trains, as for the simulated signals. The calculation of the STA twitch was performed, for each contraction, on the motor units with the lowest discharge rate using a duration of 60s.

Statistical analysis. All variables were tested for normality prior statistical analysis. The experimental results are reported as mean \pm SD. Comparison of the estimates performed with the proposed algorithm, the simulated values, and the STA technique were

analyzed using ANOVA. Statistical analyses were performed using Matlab[®] (Mathworks, Natick, Massachusetts, United States) and statistical significance was set at $P < 0.05$.

RESULTS

Simulations

In general, the greater the number of motor units, the higher was the probability of convergence of the algorithm. This was due to the increase in the SNR defined in Eq. 3, as shown in Figure 2.

FIGURE 2 HERE

The proposed method was tested on simulated segments of 5, 10 and 30 s duration. At the end of the iterations, if anyone of the parameters had the value set for upper or lower bound, it was assumed that the algorithm did not converge. Figure 3 shows the percent of times in which the algorithm converged as a function of the number M of motor units used in the estimation.

FIGURE 3 HERE

The deconvolution method outperformed standard STA estimation in simulated contractions. Figure 4 shows the comparison between the simulated (correct) values for P, T and HR, the estimated ones using the proposed method and those obtained using the traditional STA technique. The values for all variables estimated with the proposed method were not statistically different ($P > 0.05$) from the simulated ones when at least 15 % of the

1 motor units were used for the estimate (18 motor units in these simulations). Conversely,
2 the estimates obtained with the STA were biased in all cases.

3
4 **FIGURE 4 HERE**

5
6 The error in the estimation was always lower with the proposed algorithm compared
7 with standard STA estimation. Figure 5 shows the relative error in the estimation for the
8 proposed algorithm and the STA technique compared with the simulated one. In this case,
9 all three variables were statistically different ($P < 0.05$) from the ones estimated with the
10 STA technique when more than 15 % of available motor units were included in the analysis.
11 In this case, the STA technique had an error approximately double than the one provided
12 by the proposed method.

13
14 **FIGURE 5 HERE**

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16 If the assumption of uncorrelated discharge timings is violated, the estimation of
17 STA technique is always biased. Figure 6 shows the influence of the different levels of
18 synchronization on the estimation performed with the proposed algorithm and the STA
19 technique. The total amount of motor units used was fixed to 15 % and the duration of the
20 segment was set to 30 s. The relative error of all parameters was found to be considerably
21 lower than the estimation performed with the STA technique. The parameter that was most
22 affected by the synchronization level was the peak amplitude.

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FIGURE 6 HERE

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4 *Experimental results*

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The experimental results confirm the conclusions derived from the simulated signals. Figure 7 shows the estimated twitch using the STA and the proposed technique in three representative subjects. Ten segments of 5 s and 10, 12, 19 motor unit spike trains were used respectively. The STA estimations were calculated using motor units with an averaged inter-spike interval higher than 90 ms.

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FIGURE 7 HERE

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The direct comparison of the twitch estimated with the proposed and STA techniques is shown in Figure 7A in one representative subject. The results for all subjects are reported individually in Table 1 and averaged in Figure 7B.

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FIGURE 8 HERE

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18 **DISCUSSION**

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In this study, we proposed a new method for the estimation of the twitch forces of groups of motor units *in vivo* during voluntary isometric contractions. The STA technique has been proposed several decades ago for estimating the twitch force of individual motor units (Goldberg and Derfler, 1977; Stein et al., 1972), and it is still the only available

1 method to estimate the contractile properties of the muscle units (Enoka and Duchateau,
2 2016) from voluntary contractions. Several studies have underlined the limitations of this
3 technique that are mostly related with the fusion of the twitch forces (Negro et al., 2014; C
4 K Thomas et al., 1990) and motor unit synchronization (Taylor et al., 2002). Both effects
5 produce a bias in the estimation of the peak force, time to peak and, especially, half
6 relaxation time of the motor units (Gossen et al., 2003; Nordstrom et al., 1989; Thomas,
7 1995). These limitations are so severe that the results of the STA approach are often
8 considered qualitative rather than quantitative.

9 The convolution between the neural drive to the muscle and an average force twitch profile
10 can be seen, in the frequency domain, as a sampling process. For example, a periodic spike
11 train generates, in the Fourier domain, a line spectrum with equally spaced lines. Therefore,
12 when the discharge rate is very high, the only frequency line with non-negligible amplitude
13 may be the DC. In this case, the trains of twitches do only contain the information on the
14 average force level. Practically, this means that the fusion of twitches is complete to
15 generate a constant force value, from which there is not sufficient information to extract
16 the individual components. However, if some ISI variability is introduced, an additional
17 component (continuous spectrum) will be present and this component has values for any
18 frequency since it is not a line spectrum (Dideriksen et al., 2012). Therefore, even in the
19 case of a spike train with a relatively high discharge rates and a slow twitches, the
20 reconstruction is still possible with a proper amount of variability (Negro et al., 2014).

21 During voluntary contractions, the minimal discharge rate of the motor units ranges from
22 ~7 to ~20 pps (first dorsal interosseous) (Moritz, 2005) with a CoV of 15-20 %. In general,
23 such ISI variability is not enough for a complete sampling of the twitch transfer function

(Negro et al., 2014). However, the concurrent activation of many motor units would improve the estimation since the continuous spectra will linearly sum. In this case, the result will be the average twitch of the decoded motor units. For these reasons, the method that we propose focuses on the estimation of the average twitch of a population of motor units, instead of the single motor unit twitch estimation. The underlying assumption is that the population of motor units selected is sufficiently homogeneous so that the differences between the individual twitches and the average twitch over this sub-population are negligible (at least with respect to the estimation errors). This practically implies to consider an average twitch of at least 10-15 motor units (see simulation results). Although the estimation of the average twitch in small populations of motor units may seem a very important drawback with respect to the scope of the STA approach to estimate the twitch properties of individual motor units, the estimated twitch with the current approach is much more representative of the individual twitches than any estimates provided by the STA method. Indeed, the estimation errors in the STA approach are so large (as also evidenced in previous literature, e.g. (C. K. Thomas et al., 1990; Thomas, 1995) that the result is only a crude approximation of the twitch properties of the active units (Figure 7 and 8).

An additional reason for the population estimation has to be found in the low signal-to-noise ratio of the force produced by the single motor unit when other motor units are active. Even at relatively low force levels, tens of motor units are concurrently active, resulting in a SNR for the single motor unit force of ~ -25 dB (Figure 2). There is no denoising technique that can work in these conditions unless the observation window is extremely long. For example, the STA technique needs at least a thousand triggers to have a correct estimation of the single motor unit twitch (Roatta et al., 2008). Assuming a low discharge

1 rate, required by the STA, this implies hundreds of seconds of recording with no changes
2 in the contractile properties of the motor unit, a condition presumably not met in practice.
3 Again, the high demands of the STA technique of estimating individual motor unit twitch
4 properties implies strong limitations in applicability, e.g. too long estimation intervals. The
5 proposed method, on the other hand, has good convergence properties as long as a
6 relatively small population of motor units is used for the calculation, even for short
7 segments of signals (Figure 3). In the simulations, the proposed algorithm demonstrated
8 robustness in the estimation of the commonly used parameters P, T, and HR, even for
9 intervals of 5-s duration (Figure 4).

10 In comparison with the STA technique, the average error of the estimations provided by
11 the proposed method was lower in most conditions (Figure 5). For example, in the case
12 where the 15 % of the active motor units was used, the average error was approximately
13 half of the one given by the STA technique for the P and T parameters. For this simulated
14 contraction level, the average firing rate of the motor unit was 9.1 pps. However, if the
15 discharge rates are forced to higher values (~ 30 pps) the estimation dramatically improves
16 (0.5 % of error for 100 % MUs). The reason is that the continuous spectrum of the motor
17 unit spike train is weighted by the average discharge rate frequency, therefore higher values
18 implies better estimation of the twitch waveforms in the case of the same ISI variability.
19 The presence of synchronization introduces a bias in the estimation of the different
20 parameters for both techniques. However, the proposed method maintained a lower error
21 for HR compared with the classic method (Figure 6).

22 The experimental results calculated during voluntary contractions provided
23 estimations that differ substantially from the ones derived with the classical approach

(Figure 7). In these conditions, it is not possible to know the real values, but the comparison with the simulation results gives us confidence that the estimations with the proposed method are reasonable (Figure 8).

The main requirement of the method described in this study is the need of decoding the activity of a relatively large number of motor units (10-15). However, recently, many techniques have been proposed to increase the number of motor unit spike trains that is possible to extract from iEMG (Farina et al., 2016; Florestal et al., 2009; Ge et al., 2011, 2008; Marateb et al., 2011; McGill et al., 2005; Muceli et al., 2015; Negro et al., 2016a, 2009) and sEMG (Castronovo et al., 2015; Holobar et al., 2009; Negro et al., 2016a, 2009; Yavuz et al., 2015) during voluntary contractions. Moreover, the combination of the proposed technique with high-density sEMG recordings can provide the possibility to track the twitch properties of the same motor units across several recording sessions (E. Martinez-Valdes et al., 2017; Eduardo Martinez-Valdes et al., 2017) and/or compare them with other peripheral characteristics (Del Vecchio et al., 2017a, 2017b). Finally, the proposed methodology can be usefully applied together with the maximal twitch scan suggested by Orizio et al (Orizio et al., 2016) to evaluate the prevalence of fast or slow contribution to the mechanical output of a specific muscle during stimulated contraction and the interpolated twitch technique (Maffiuletti et al., 2016; Zarkou et al., 2017).

In conclusion, we showed that the proposed method could provide reliable average motor unit twitch estimations of the muscle *in vivo* during voluntary isometric contractions at moderate force levels. The combination of recent multi-unit EMG recordings and the optimization technique described in the present study offer the possibility to quantify motor

1 unit twitch parameters reliably, even in relatively short segments. Therefore, the proposed
2 method opens a new opportunity for neuromuscular assessments in clinical applications.

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1 FIGURE CAPTIONS

2 Figure 1. Twitch model used for validating the proposed technique.

3

4 Figure 2. Signal-to-noise ratio for different percentages of motor units (the 0 % value shows
5 the SNR calculated for one motor unit only).

6

7 Figure 3. Convergence rate for the algorithm applied to simulated contractions of 5 (dark
8 blue), 10 (blue) and 30 s (light blue) duration.

9

10 Figure 4. P, T and HR for the simulated motor units when 5, 15 etc. % of the active motor
11 units was used in the calculation. The values are reported for the average of the simulated
12 twitch (red), estimated twitch with 5 (dark blue), 10 (blue), 30 s (light blue) segments and
13 STA technique (black).

14

15 Figure 5. Percent of error estimated for the variables P, T and HR in simulated conditions.
16 Colors represent the variables extracted as described before.

17

18 Figure 6. Influence of synchronization on the estimation of the twitch force provided by
19 the proposed algorithm. The estimated parameters using a segment of 30 s and 15 % of the
20 active motor units (dark blue) and the estimated values using STA technique (light blue).

21

22 Figure 7. Estimation of twitch parameters using the STA and the proposed technique during
23 sustained contractions at 5 % MVC in three representative subjects. Upper panels show the
24 recorded (black) and estimated force (blue) signals. Middle panels show the twitches
25 estimated with the proposed technique in several segments of 5 s duration and the averaged
26 twitch (blue). Bottom panels show the STA estimations of the motor units with averaged
27 inter-spike interval higher than 90 ms.

28

29 Figure 8. Comparison of the STA and the proposed technique during sustained contractions
30 at 5 % MVC. A. Twitch estimations calculated using the proposed method (blue), average

1 value (red) and the STA twitch estimation (black) for one representative subject. B.
2 Averaged values of P, T, and HR for all subjects.

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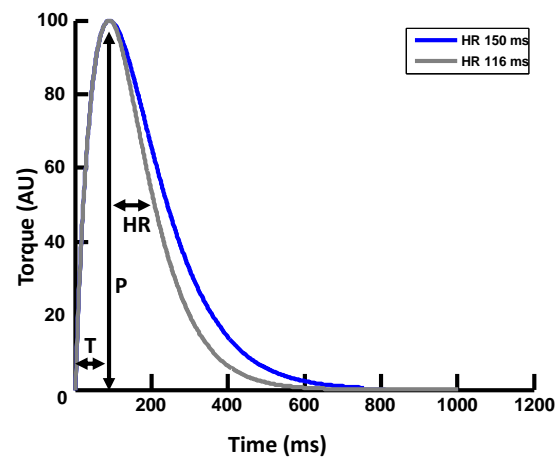
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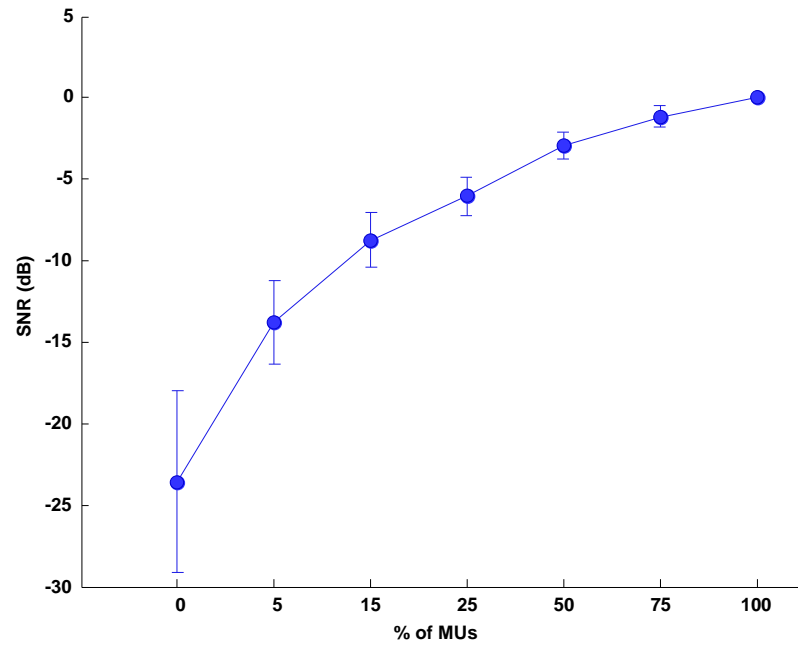
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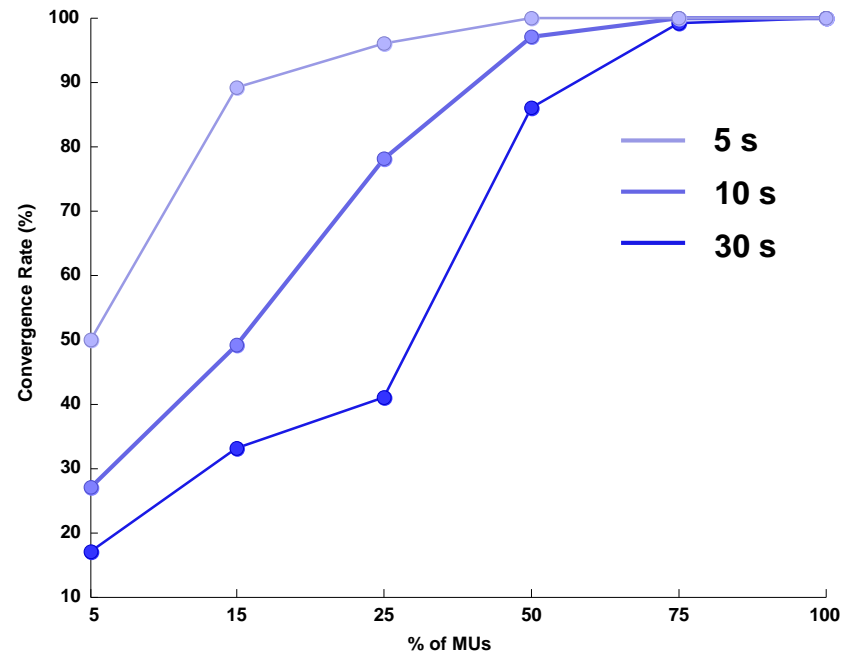
TABLE 1. Parameters estimated for the five subjects during voluntary contractions.

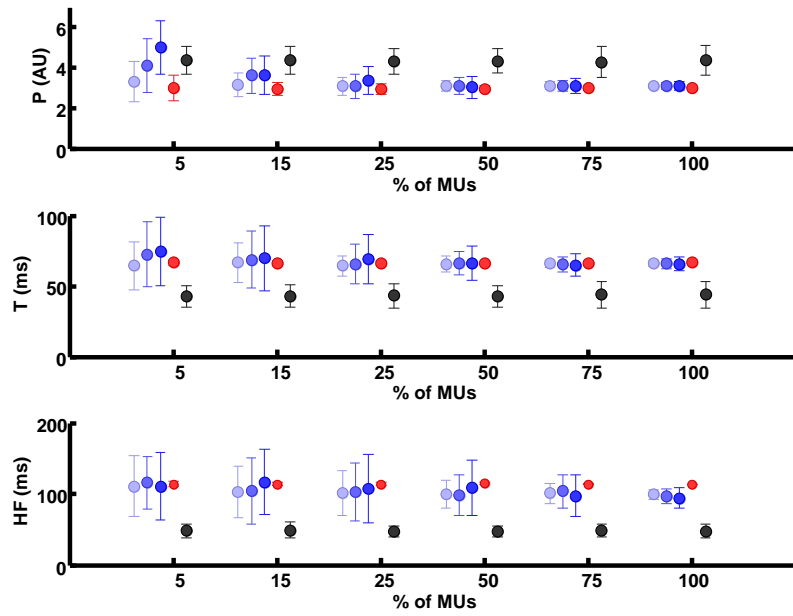
| | P (1e-4) | T | HR |
|--------|-----------------------|------------------------|------------------------|
| Sub. 1 | 1.31 (PM), 1.25 (STA) | 112.6 (PM), 58.0 (STA) | 142.1 (PM), 47.0 (STA) |
| Sub. 2 | 1.31 (PM), 1.40 (STA) | 93.1 (PM), 61.0 (STA) | 146.3 (PM), 45.0 (STA) |
| Sub. 3 | 1.00 (PM), 2.10 (STA) | 102.2 (PM), 52.0 (STA) | 120.6 (PM), 33.0 (STA) |
| Sub. 4 | 1.20 (PM), 1.98 (STA) | 99.6 (PM), 55.0 (STA) | 115.3 (PM), 46.0 (STA) |
| Sub. 5 | 1.21 (PM), 1.95 (STA) | 99.3 (PM), 56.0 (STA) | 113.2 (PM), 43.0 (STA) |

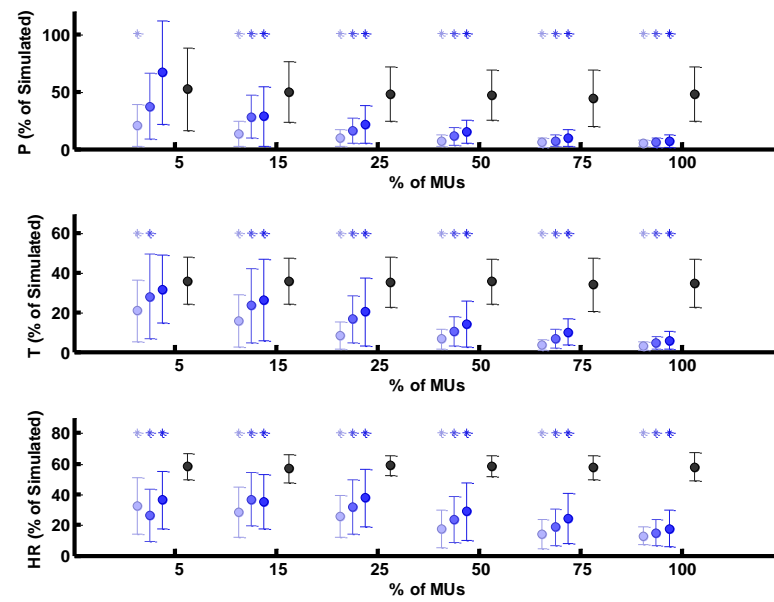
P: Peak amplitude (Nm). T: Time peak (ms); HR: Half relaxation time (ms); PM: Proposed method; STA: Spike triggered averaging technique.

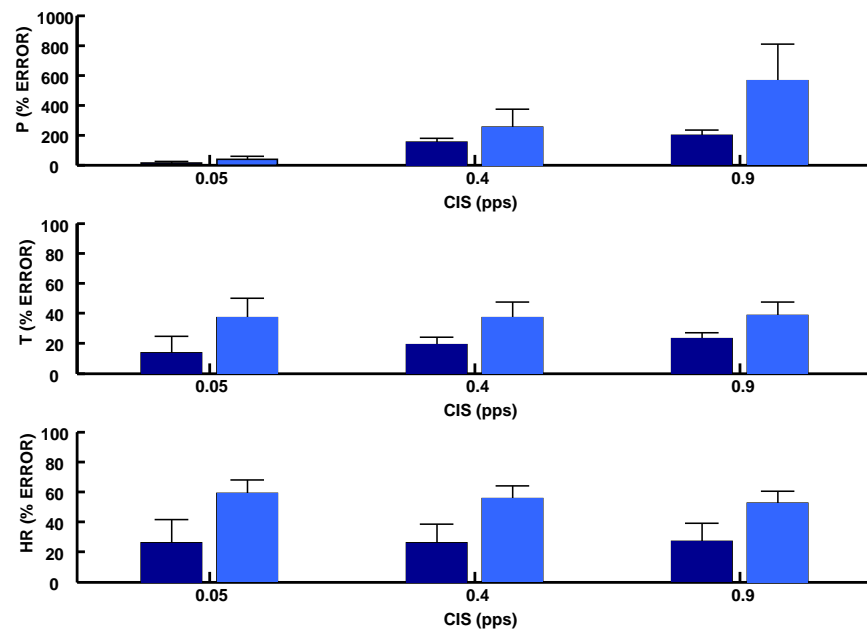


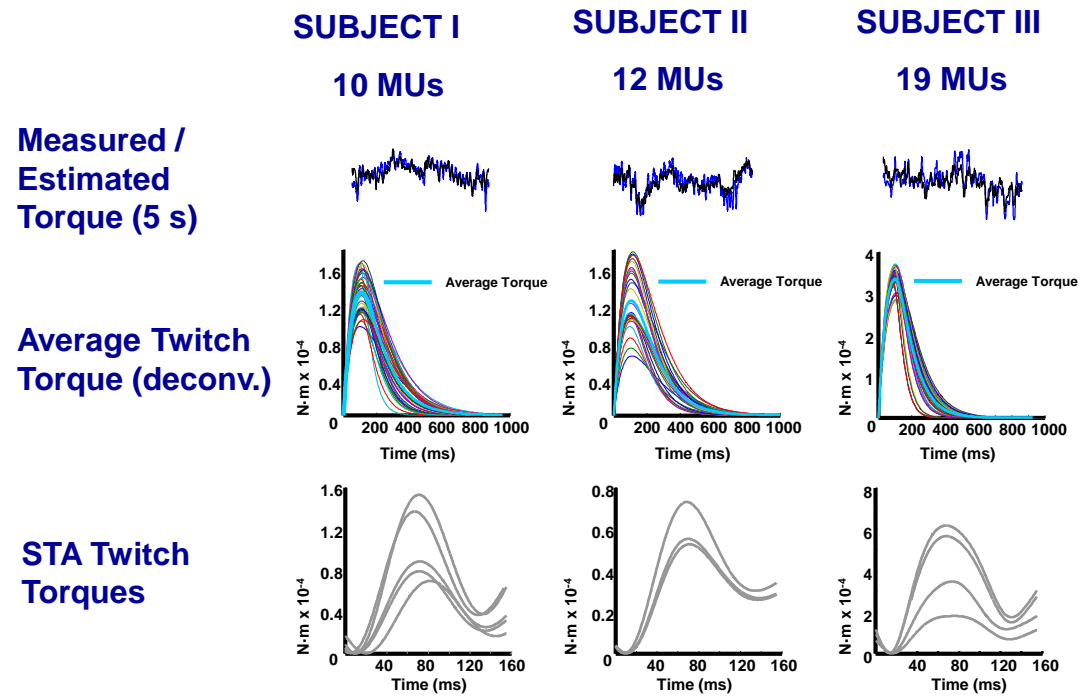


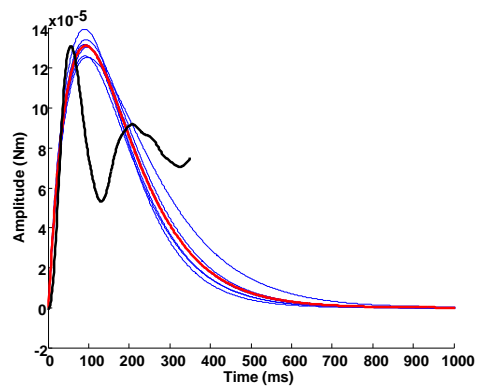










A**B**