Submitted to: *JEK*

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

Francesco Negro* & Claudio Orizio

Department of Clinical and Experimental Sciences, University of Brescia, 25123 Brescia, Italy

Running title: Robust estimation of motor unit twitch

Keywords: motor unit, twitch, iEMG, deconvolution, force
Total number of words:

Corresponding author:

* Francesco Negro, PhD
Department of Clinical and Experimental Sciences
Università degli Studi di Brescia
Viale Europa 11
25123 Brescia
Italy
Tel: +39 0303717452
Fax: +39 0303717443
Email: francesco.negro@unibs.it

ABSTRACT

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

INTRODUCTION

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

The only available method for the estimation of the motor unit temporal twitch profiles *in vivo* during voluntary contractions is the spike-triggered averaging (STA) (Gossen, 2003; Milner-Brown et al., 1973; Thomas, 1995) technique. Under the assumptions of uncorrelated activity of the motor units, the STA performs an average of the force signal triggered by the discharge times of one motor unit, usually identified by decomposition of intramuscular EMG (Farina et al., 2008, 2005; Milner-Brown et al., 1973; Roatta et al., 2008). The averaging process increases the signal-to-noise ratio (SNR) for the force generated by the triggered unit with respect to the summation of the forces produced by the other active motor units. One of the main problems in this approach is that the average inter-spike interval of MU during voluntary contraction is smaller than their twitch duration. On this basis, the STA estimation is always biased (Gossen et al., 2003; Negro et al., 2014). This effect alters the STA estimates substantially, especially during the relaxation time (Kossev et al., 1994). Another limitation of the STA for twitch estimation is the need for relatively long signal recordings (usually >2 min) to obtain stable estimates (Nordstrom and Miles, 1990). This impedes the use of STA for assessing changes of twitch force over short time intervals, for example during fast fatigue development. Finally, the synchronization between motor unit spike trains also negatively influences the STA estimates of motor unit twitch forces (Keen, 2004; Kutch et al., 2007). Because of these important limitations, a few alternative methods and models have been proposed to extract the contributions of individual MU to the force profile in human (Andreassen and Bar-On, 1983; Kutch et al., 2010; Lim et al., 1995) and animal recordings (Drzymała-Celichowska et al., 2016), but without an extensive validation and with very limited use.

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

MATERIALS & METHODS

Theory

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

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

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

We can rewrite Eq. (1) as

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

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

$$SNR = 10log_{10}\left(\frac{P_M}{P_W}\right) \qquad (3)$$

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

Description of the method

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

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

where M is the number of known motor unit spike trains. In general, the problem of estimation the twitch parameters has an unbiased solution only if the activation of all sources is known and a correct parameterization of the sources is available. However, only in the case of electrically stimulated twitches, the full activation pattern is known. Additionally, the frequency and variability in the discharge timing of individual motor units in physiological conditions does not allow the correct estimation of the twitch waveform, as previously demonstrated (Negro et al., 2014). Finally, the estimation of each individual motor unit twitch waveform is not feasible due to the low SNR (see Results section). For this reason, we focused this study on the estimation of the properties of the *average* twitch of the motor units identified. Therefore the estimation provided is related to an averaged twitch extracted using the composite spike train (CST) (Castronovo et al., 2015; Farina et al., 2014; Farina and Negro, 2015; Negro et al., 2016b; Francesco Negro and Farina, 2011; Negro and Farina, 2012) of the M identified motor units. This is not a limiting factor since, for low contraction forces, the motor units have similar recruitment threshold and therefore similar parameters. The set of parameters θ in Eq. (4) includes three variables to characterize the average twitch profile and one to provide a better estimation in case of underdetermined systems. The twitch response was modeled using a modified version of the impulse response described by Fuglevand et al. (Fuglevand et al., 1993) that has been proposed by Roatta and Farina (Roatta and Farina, 2011) in a previous study:

$$g_i(t) = \begin{cases} P_i \dfrac{t}{T_i^1} e^{1-\frac{t}{T_i^1}} & t \le T_i^1 \\ P_i \dfrac{t - T_i^1 + T_i^2}{T_i^2} e^{\left(1-\frac{t-T_i^1+T_i^2}{T_i^2}\right)} & t > T_i^1 \end{cases} \tag{5}$$

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

FIGURE 1 HERE

An additional parameter was added for the estimation of the bias of the unidentified motor units. This model was used since it allowed having a direct measure of the three parameters independently. The optimization problem of Eq. (4) was performed using an iterative algorithm for time-domain deconvolution (trust-region-reflective, (Absil et al., 2007)). The Levenberg–Marquardt algorithm (Levenberg, 1944) provided similar results. In order to limit the number of iterations, upper and lower ranges for each parameter should be set. In the simulations, the upper and lower limits for P, T1, T2 and the Bias were 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 typical experimentally recorded level of force used for the estimation of the twitch properties with the STA technique (Roatta et al., 2008).

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

Simulation analysis

For assessing the performance of the proposed technique, the composite spike train of 5, 15, 25, 50, 75 and 100 % of the active motor units was estimated by the proposed algorithm. The duration of the analyzed segment was set to 5, 10 and 30 s. For each case, 100 simulations were performed with a random selection (normal distribution) of the motor units in each simulation. The results were compared with the parameters extracted from the average twitch force of all motor units selected for the calculation. For each simulation, the STA twitch force was extracted for the motor unit with the lowest discharge rate (~ 8 pps) and compared with the estimated parameters calculated by the algorithm. The duration of the segment used for the calculation of the twitch parameters using the STA technique was 120 s, much longer than the interval used for the deconvolution methods. The STA 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 ± SD, age: 26.1 ± 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 ± 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 motor units were used for the estimate (18 motor units in these simulations). Conversely, the estimates obtained with the STA were biased in all cases.

FIGURE 4 HERE

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

FIGURE 5 HERE

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

FIGURE 6 HERE

Experimental results

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.

FIGURE 7 HERE

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.

FIGURE 8 HERE

DISCUSSION

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

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

During voluntary contractions, the minimal discharge rate of the motor units ranges from ~7 to ~20 pps (first dorsal interosseous) (Moritz, 2005) with a CoV of 15-20 %. In general, 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 rate, required by the STA, this implies hundreds of seconds of recording with no changes in the contractile properties of the motor unit, a condition presumably not met in practice. Again, the high demands of the STA technique of estimating individual motor unit twitch properties implies strong limitations in applicability, e.g. too long estimation intervals. The proposed method, on the other hand, has good convergence properties as long as a relatively small population of motor units is used for the calculation, even for short segments of signals (Figure 3). In the simulations, the proposed algorithm demonstrated robustness in the estimation of the commonly used parameters P, T, and HR, even for intervals of 5-s duration (Figure 4).

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

The experimental results calculated during voluntary contractions provided 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 unit twitch parameters reliably, even in relatively short segments. Therefore, the proposed method opens a new opportunity for neuromuscular assessments in clinical applications.

ACKNOWLEDGEMENTS

Francesco Negro has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 702491 (NeuralCon).

REFERENCES

Absil, P.A., Baker, C.G., Gallivan, K.A., 2007. Trust-region methods on Riemannian manifolds, Foundations of Computational Mathematics. doi:10.1007/s10208-005-0179-9

Andreassen, S., Bar-On, E., 1983. Estimation of Motor Unit Twitches. IEEE Trans. Biomed. Eng. BME-30. doi:10.1109/TBME.1983.325189

Bagust, J., Knott, S., Lewis, D.M., Luck, J.C., Westerman, R.A., 1973. Isometric contractions of motor units in a fast twitch muscle of the cat. J. Physiol. 231, 87–104. doi:10.1113/jphysiol.1973.sp010221

Burke, D., Skuse, N.F., Lethlean, A.K., 1974. Isometric contraction of the abductor digiti minimi muscle in man. J. Neurol. Neurosurg. Psychiatry 37, 825–34. doi:10.1136/JNNP.37.7.825

Carpentier, A., Duchateau, J., Hainaut, K., 2001. Motor unit behaviour and contractile changes during fatigue in the human first dorsal interosseus. J. Physiol. 534, 903–912. doi:10.1111/j.1469-7793.2001.00903.x

Castronovo, A.M., Negro, F., Conforto, S., Farina, D., 2015. the Proportion of Common Synaptic Input To Motor Neurons Increases With an Increase in Net Excitatory Input. J. Appl. Physiol. 119, jap.00255.2015. doi:10.1152/japplphysiol.00255.2015

Del Vecchio, A., Negro, F., Felici, F., Farina, D., 2017a. Associations between Motor Unit Action Potential Parameters and Surface EMG Features. J. Appl. Physiol. jap.00482.2017. doi:10.1152/japplphysiol.00482.2017

Del Vecchio, A., Negro, F., Felici, F., Farina, D., 2017b. Distribution of muscle fiber conduction velocity for representative samples of motor units in the full recruitment range of the tibialis anterior muscle. Acta Physiol. doi:10.1111/apha.12930

Dideriksen, J.L., Negro, F., Enoka, R.M., Farina, D., 2012. Motor unit recruitment strategies and muscle properties determine the influence of synaptic noise on force steadiness. J. Neurophysiol. 107, 3357–3369. doi:10.1152/jn.00938.2011

Drzymała-Celichowska, H., Kaczmarek, P., Krutki, P., Celichowski, J., 2016. Summation of slow motor unit forces at constant and variable interpulse intervals in rat soleus muscle. J. Electromyogr. Kinesiol. 30, 1–8. doi:10.1016/j.jelekin.2016.05.001

Enoka, R.M., Duchateau, J., 2016. Chapter 1 Physiology of Muscle Activation and Force Generation. Surf. Electromyogr. Physiol.

Enoka, R.M., Fuglevand, A.J., 2001. Motor unit physiology: Some unresolved issues. Muscle and Nerve 24, 4–17. doi:10.1002/1097-4598(200101)24:1<4::AID-MUS13>3.0.CO;2-F

Farina, D., Arendt-Nielsen, L., Graven-Nielsen, T., 2005. Spike-triggered average torque and muscle fiber conduction velocity of low-threshold motor units following submaximal endurance contractions. J. Appl. Physiol. 98, 1495–1502. doi:10.1152/japplphysiol.01127.2004

Farina, D., Arendt-Nielsen, L., Roatta, S., Graven-Nielsen, T., 2008. The pain-induced decrease in low-threshold motor unit discharge rate is not associated with the amount of increase in spike-triggered average torque. Clin. Neurophysiol. 119, 43–51. doi:10.1016/j.clinph.2007.10.003

Farina, D., Holobar, A., Merletti, R., Enoka, R.M., 2010. Decoding the neural drive to muscles from the surface electromyogram. Clin. Neurophysiol. 121, 1616–1623. doi:10.1016/j.clinph.2009.10.040

Farina, D., Negro, F., 2015. Common synaptic input to motor neurons, motor unit synchronization, and force control. Exerc. Sport Sci. Rev. 43, 23–33. doi:10.1249/JES.0000000000000032

Farina, D., Negro, F., Dideriksen, J.L., 2014. The effective neural drive to muscles is the common synaptic input to motor neurons. J. Physiol. 592, 3427–3441. doi:10.1113/jphysiol.2014.273581

Farina, D., Negro, F., Muceli, S., Enoka, R.M., 2016. Principles of Motor Unit Physiology Evolve With Advances in Technology. Physiology 31, 83–94. doi:10.1152/physiol.00040.2015

Florestal, J.R., Mathieu, P.A., McGill, K.C., 2009. Automatic decomposition of multichannel intramuscular EMG signals. J. Electromyogr. Kinesiol. 19, 1–9. doi:10.1016/j.jelekin.2007.04.001

Fuglevand, A.J., 1996. Neural Aspects of Fatigue. Neurosci. 2, 203–206. doi:10.1177/107385849600200407

Fuglevand, a J., Winter, D. a, Patla, a E., 1993. Models of recruitment and rate coding organization in motor-unit pools. J. Neurophysiol. 70, 2470–2488.

Gandevia, S.C., 2001. Spinal and supraspinal factors in human muscle fatigue. Physiol. Rev. 81, 1725–89. doi:citeulike-article-id:1572911

Ge, D., Le Carpentier, E., Farina, D., Idier, J., 2008. Unsupervised Bayesian EMG decomposition algorithm using tabu search. IEEE - Biomed. Commun. Technol. 57, 1–5. doi:10.1109/ISABEL.2008.4712579

Ge, D., Le Carpentier, E., Idier, J., Farina, D., 2011. Spike sorting by stochastic simulation. IEEE Trans. Neural Syst. Rehabil. Eng. 19, 249–259. doi:10.1109/TNSRE.2011.2112780

Goldberg, L.J., Derfler, B., 1977. Relationship among recruitment order, spike amplitude, and twitch tension of single motor units in human masseter muscle. J. Neurophysiol. 40, 879–890.

Gossen, E.R., 2003. The time course of the motoneurone afterhyperpolarization is related to motor unit twitch speed in human skeletal muscle. J. Physiol. 552, 657–664. doi:10.1113/jphysiol.2003.048132

Gossen, E.R., Ivanova, T.D., Garland, S.J., 2003. Factors affecting the stability of the spike-triggered averaged force in the human first dorsal interosseus muscle. J. Neurosci. Methods 126, 155–164. doi:10.1016/S0165-0270(03)00077-3

Heckman, C.J., Enoka, R.M., 2004. Physiology of the motor neuron and the motor unit, Handbook of Clinical Neurophysiology. Elsevier B.V. doi:10.1016/S1567-4231(04)04006-7

Holobar, A., Farina, D., Gazzoni, M., Merletti, R., Zazula, D., 2009. Estimating motor unit discharge patterns from high-density surface electromyogram. Clin. Neurophysiol. 120, 551–562. doi:10.1016/j.clinph.2008.10.160

Holobar, A., Minetto, M.A., Botter, A., Negro, F., Farina, D., 2010. Experimental analysis of accuracy in the identification of motor unit spike trains from high-density surface EMG. IEEE Trans. Neural Syst. Rehabil. Eng. 18, 221–229.

doi:10.1109/TNSRE.2010.2041593

Keen, D.A., 2004. Distribution of Motor Unit Force in Human Extensor Digitorum Assessed By Spike-Triggered Averaging and Intraneural Microstimulation. J. Neurophysiol. 91, 2515–2523. doi:10.1152/jn.01178.2003

Keen, D.A., Chou, L.-W., Nordstrom, M.A., Fuglevand, A.J., 2012. Short-term synchrony in diverse motor nuclei presumed to receive different extents of direct cortical input. J. Neurophysiol. 108, 3264–3275. doi:10.1152/jn.01154.2011

Kossev, A., Elek, J.M., Wohlfarth, K., Schubert, M., Dengler, R., Wolf, W., 1994. Assessment of human motor unit twitches--a comparison of spike- triggered averaging and intramuscular microstimulation. Electroencephalogr. Clin. Neurophysiol. 93, 100–105.

Kutch, J.J., Kuo, A.D., Rymer, W.Z., 2010. Extraction of individual muscle mechanical action from endpoint force. J. Neurophysiol. 103, 3535–46. doi:10.1152/jn.00956.2009

Kutch, J.J., Suresh, N.L., Bloch, A.M., Rymer, W.Z., 2007. Analysis of the effects of firing rate and synchronization on spike-triggered averaging of multidirectional motor unit torque. J. Comput. Neurosci. 22, 347–361. doi:10.1007/s10827-007-0023-0

Levenberg, K., 1944. A method for the solution of certain non-linear problems in least squares. Q. Appl. Math.

Lim, K.Y., Thomas, C.K., Rymer, W.Z., 1995. Computational methods for improving estimates of motor unit twitch contractions properties. Muscle Nerve 18, 165–174.

Macefield, V.G., Fuglevand, a J., Bigland-Ritchie, B., 1996. Contractile properties of single motor units in human toe extensors assessed by intraneural motor axon stimulation. J Neurophysiol 75, 2509–2519.

Maffiuletti, N.A., Barbero, M., Cescon, C., Clijsen, R., Beretta-Piccoli, M., Schneebeli, A., Preiss, S., Togninalli, D., 2016. Validity of the twitch interpolation technique for the assessment of quadriceps neuromuscular asymmetries. J. Electromyogr. Kinesiol. 28, 31–36. doi:10.1016/j.jelekin.2016.03.002

Marateb, H.R., Muceli, S., McGill, K.C., Merletti, R., Farina, D., 2011. Robust decomposition of single-channel intramuscular EMG signals at low force levels. J. Neural Eng. 8, 66015. doi:10.1088/1741-2560/8/6/066015

Martinez-Valdes, E., Falla, D., Negro, F., Mayer, F., Farina, D., 2017. Differential Motor Unit Changes after Endurance or High-Intensity Interval Training. Med. Sci. Sports Exerc. 49, 1126–1136. doi:10.1249/MSS.0000000000001209

Martinez-Valdes, E., Negro, F., Laine, C.M., Falla, D., Mayer, F., Farina, D., 2017. Tracking motor units longitudinally across experimental sessions with high-density surface electromyography. J. Physiol. 595, 1479–1496. doi:10.1113/JP273662

McGill, K.C., Lateva, Z.C., Marateb, H.R., 2005. EMGLAB: An interactive EMG decomposition program. J. Neurosci. Methods 149, 121–133. doi:10.1016/j.jneumeth.2005.05.015

McNulty, P.A., Macefield, V.G., 2005. Intraneural microstimulation of motor axons in the study of human single motor units. Muscle and Nerve 32, 119–139. doi:10.1002/mus.20324

Milner-Brown, H.S., Stein, R.B., Yemm, R., 1973. The contractile properties of human motor units during voluntary isometric contractions. J. Physiol. 228, 285–306. doi:10.1113/jphysiol.1973.sp010087

Moritz, C.T., 2005. Discharge Rate Variability Influences the Variation in Force Fluctuations Across the Working Range of a Hand Muscle. J. Neurophysiol. 93, 2449–2459. doi:10.1152/jn.01122.2004

Muceli, S., Poppendieck, W., Negro, F., Yoshida, K., Hoffmann, K.P., Butler, J.E., Gandevia, S.C., Farina, D., 2015. Accurate and representative decoding of the neural drive to muscles in humans with multi-channel intramuscular thin-film electrodes. J. Physiol. 593, 3789–3804. doi:10.1113/JP270902

Nawab, S.H., Wotiz, R.P., De Luca, C.J., 2008. Decomposition of indwelling EMG signals. J. Appl. Physiol. 105, 700–10. doi:10.1152/japplphysiol.00170.2007

Negro, F., Farina, D., 2012. Factors Influencing the Estimates of Correlation between Motor Unit Activities in Humans. PLoS One 7, 44894. doi:10.1371/journal.pone.0044894

Negro, F., Farina, D., 2011. Linear transmission of cortical oscillations to the neural drive to muscles is mediated by common projections to populations of motoneurons in humans. J. Physiol. 589, 629–637. doi:10.1113/jphysiol.2010.202473

Negro, F., Farina, D., 2011. Decorrelation of cortical inputs and motoneuron output. J. Neurophysiol. 106, 2688–2697. doi:10.1152/jn.00336.2011

Negro, F., Holobar, A., Farina, D., 2009. Fluctuations in isometric muscle force can be described by one linear projection of low-frequency components of motor unit discharge rates. J. Physiol. 587, 5925–5938. doi:10.1113/jphysiol.2009.178509

Negro, F., Muceli, S., Castronovo, A.M., Holobar, A., Farina, D., 2016a. Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. J. Neural Eng. 13, 26027. doi:10.1088/1741-2560/13/2/026027

Negro, F., Yavuz, U.Ş., Farina, D., 2016b. The human motor neuron pools receive a dominant slow-varying common synaptic input. J. Physiol. 594, 5491–5505. doi:10.1113/JP271748

Negro, F., Yavuz, U.Ş., Farina, D., 2014. Limitations of the spike-triggered averaging for estimating motor unit twitch force: A theoretical analysis. PLoS One 9, e101614. doi:10.1371/journal.pone.0092390

Nordstrom, M.A., Fuglevand, A.J., Enoka, R.M., 1992. Estimating the strength of common input to human motoneurons from the cross-correlogram. J. Physiol. 453, 547–574. doi:10.1113/jphysiol.1992.sp019244

Nordstrom, M.A., Miles, T.S., Veale, J.L., 1989. Effect of motor unit firing pattern on twitches obtained by spike???triggered averaging. Muscle Nerve 12, 556–567. doi:10.1002/mus.880120706

Nordstrom, M. a, Miles, T.S., 1990. Fatigue of single motor units in human masseter. J. Appl. Physiol. 68, 26–34.

Orizio, C., Cogliati, M., Bissolotti, L., Diemont, B., Gobbo, M., Celichowski, J., 2016. The age related slow and fast contributions to the overall changes in tibialis anterior contractile features disclosed by maximal single twitch scan. Arch. Gerontol. Geriatr. 66, 1–6. doi:10.1016/j.archger.2016.05.003

Roatta, S., Arendt-Nielsen, L., Farina, D., 2008. Sympathetic-induced changes in discharge rate and spike-triggered average twitch torque of low-threshold motor units in humans. J. Physiol. 586, 5561–5574. doi:10.1113/jphysiol.2008.160770

Roatta, S., Farina, D., 2011. Sympathetic activation by the cold pressor test does not increase the muscle force generation capacity. J. Appl. Physiol. 110, 1526–1533. doi:10.1152/japplphysiol.00039.2011

Santo Neto, H., de Carvalho, V., Penteado, C., 1985. Motor units of the human abductor digiti minimi. Arch Ital Anat Embriol.

Stein, R.B., French, A.S., Mannard, A., Yemm, R., 1972. New methods for analysing motor function in man and animals. Brain Res. 40, 187–192. doi:10.1016/0006-8993(72)90126-6

Taylor, A.M., Steege, J.W., Enoka, R.M., 2002. Motor-unit synchronization alters spike-triggered average force in simulated contractions. J. Neurophysiol. 88, 265–276.

Thomas, C.K., 1995. Human motor units studied by spike-triggered averaging and intraneural motor axon stimulation. Adv. Exp. Med. Biol. 384, 147–160.

Thomas, C.K., Bigland-Ritchie, B., Westling, G., Johansson, R.S., 1990. A comparison of human thenar motor-unit properties studied by intraneural motor-axon stimulation and spike-triggered averaging. J. Neurophysiol. 64, 1347–51.

Thomas, C.K., Johansson, R.S., Westling, G., Bigland-Ritchie, B., 1990. Twitch properties of human thenar motor units measured in response to intraneural motor-axon stimulation. J. Neurophysiol. 64, 1339–1346.

Yavuz, U.Ş., Negro, F., Sebik, O., Holobar, A., Frömmel, C., Türker, K.S., Farina, D., 2015. Estimating reflex responses in large populations of motor units by decomposition of the high-density surface electromyogram. J. Physiol. 593, 4305–4318. doi:10.1113/JP270635

Zarkou, A., Stackhouse, S., Binder-Macleod, S.A., Lee, S.C.K., 2017. Comparison of techniques to determine human skeletal muscle voluntary activation. J. Electromyogr. Kinesiol. 36, 8–15. doi:10.1016/j.jelekin.2017.05.011

FIGURE CAPTIONS

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

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

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

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

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

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

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

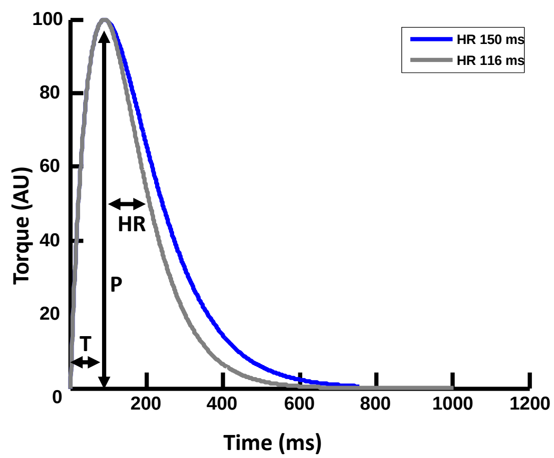
Figure 8. Comparison of the STA and the proposed technique during sustained contractions at 5 % MVC. A. Twitch estimations calculated using the proposed method (blue), average

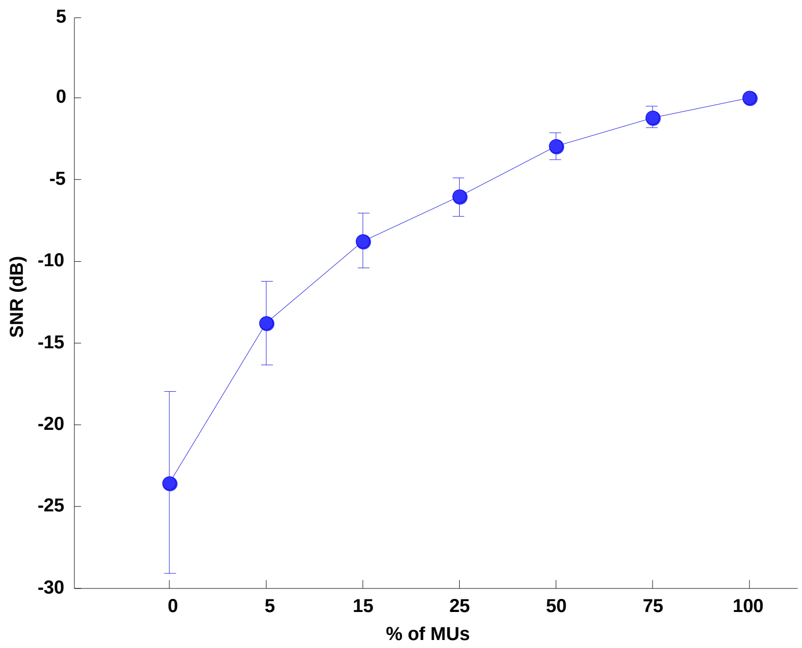
value (red) and the STA twitch estimation (black) for one representative subject. B. Averaged values of P, T, and HR for all subjects.

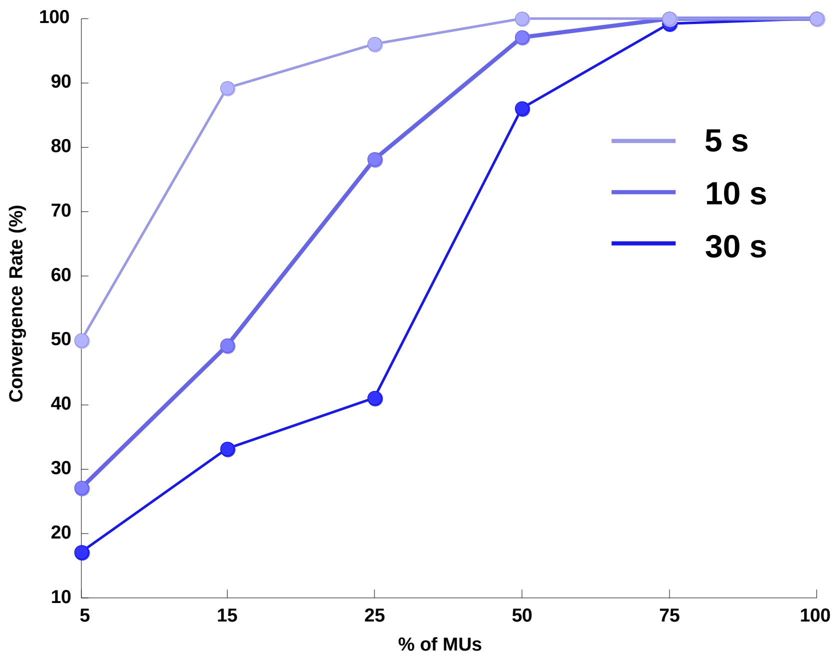
TABLE 1. **Parameters estimated for the five subjects during voluntary contractions.**

	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)

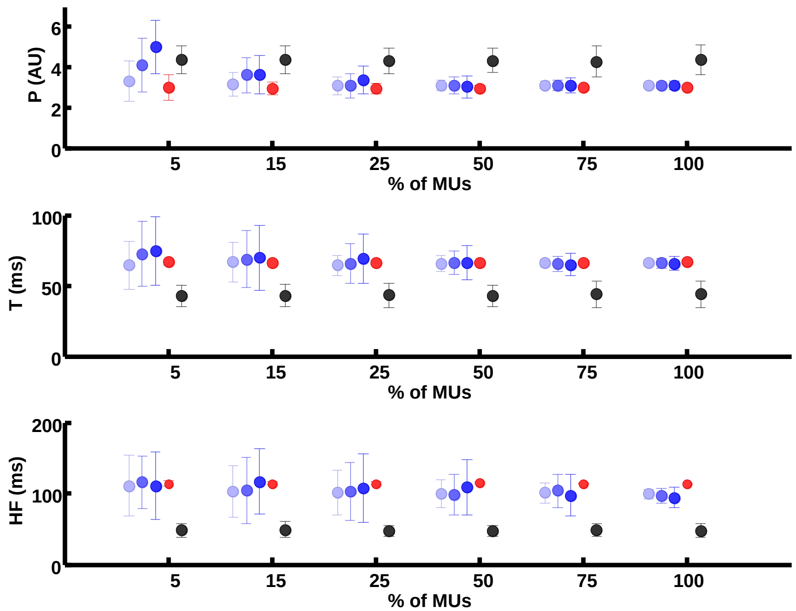
P: Peak amplitude (Nm). T: Time peak (ms); HR: Half relaxation time (ms); PM: Proposed method; STA: Spike triggered averaging technique.


HR 150 ms
HR 116 ms
Torque (AU)
100
80
60
40
20
0
200
400
600
800
1000
1200
Time (ms)
HR
P
T

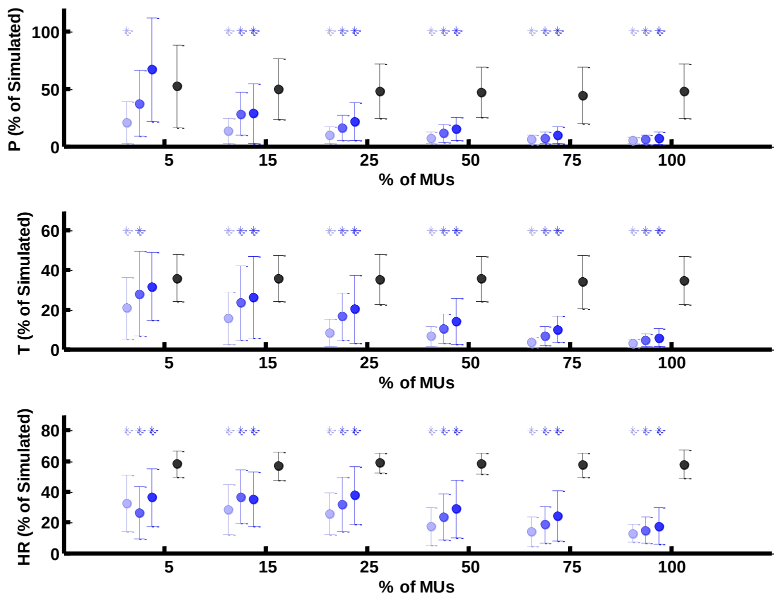

5
0
-5
-10
-15
-20
-25
-30
SNR (dB)
0
5
15
25
50
75
100
% of MUs



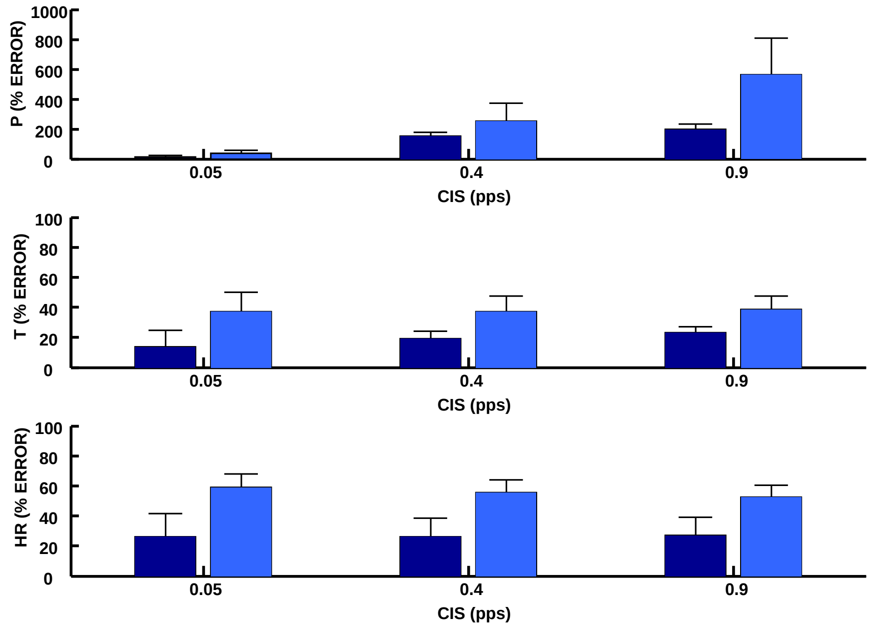
100
90
80
70
60
50
40
30
20
10
Convergence Rate (%)
5
15
25
50
75
100
% of MUs
5 s
10 s
30 s

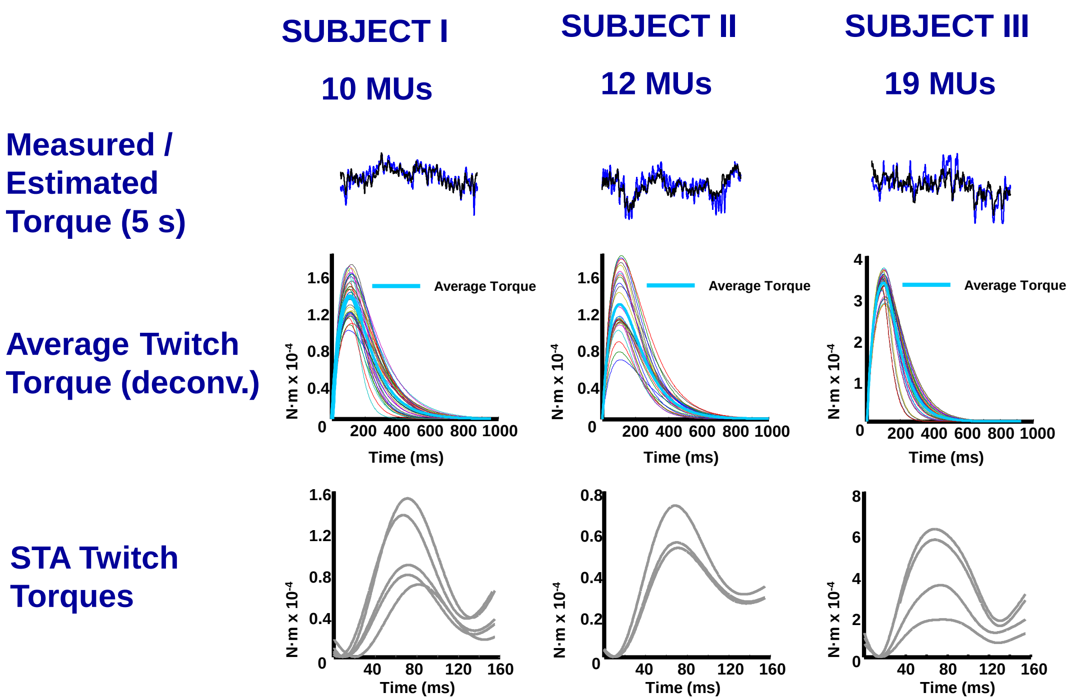


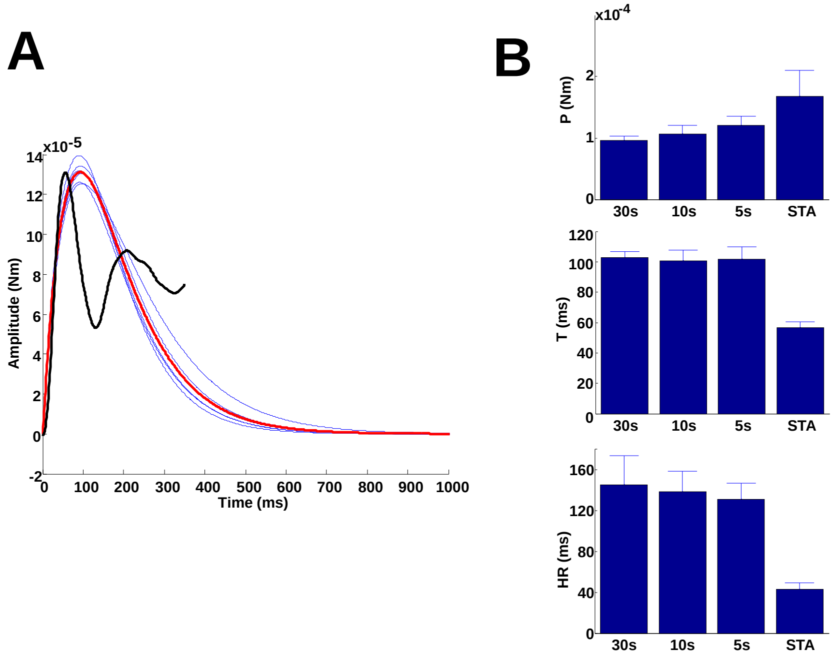
P (AU)
0
2
4
6
5
15
25
50
75
100
% of MUs
T (ms)
0
50
100
5
15
25
50
75
100
% of MUs
HF (ms)
0
100
200
5
15
25
50
75
100
% of MUs



P (% of Simulated)
100
50
0
5
15
25
50
75
100
% of MUs
T (% of Simulated)
60
40
20
0
5
15
25
50
75
100
% of MUs
HR (% of Simulated)
80
60
40
20
0
5
15
25
50
75
100
% of MUs


P (% ERROR)
1000
800
600
400
200
0
0.05
0.4
0.9
CIS (pps)
T (% ERROR)
100
80
60
40
20
0
0.05
0.4
0.9
CIS (pps)
HR (% ERROR)
100
80
60
40
20
0
0.05
0.4
0.9
CIS (pps)


SUBJECT I
10 MUs
SUBJECT II
12 MUs
SUBJECT III
19 MUs
Measured / Estimated Torque (5 s)
Average Twitch Torque (deconv.)
STA Twitch Torques
Average Torque
N·m x 10^-4
Time (ms)


A
x10-5
Amplitude (Nm)
Time (ms)
B
x10-4
P (Nm)
T (ms)
HR (ms)
30s
10s
5s
STA