Model-based deconvolution of a force signal to estimate motor unit twitch parameters under low-force isometric contractions

Robin Rohlén[1*], Jan Celichowski[2]

[1] Department of Diagnostics and Intervention, Umeå University, Umeå, Sweden

[2] Department of Neurobiology, Poznan University of Physical Education, Poznań, Poland

* Correspondence to Robin Rohlén (robin.rohlen@umu.se).

Abstract

Muscle force generation is key in everyday life, yet the mechanisms regulating force generation are not fully resolved. Over the last half-century, attempts have been made to elucidate the mechanisms regulating force generation in vivo, primarily focusing on the motor unit (MU) activity. Although different methods exist to extract MU twitch parameters from the force signal, no method can accurately identify a *single* MU twitch given a *single* MU spike train. We addressed this problem by developing a model-based deconvolution method. We evaluated the method using extensive simulations mimicking low-force isometric contractions, which resulted in four main findings. First, given an accurate spike train, the method can accurately estimate twitch parameter estimates in a very weak force scenario. Second, the more missed or false spikes were used to estimate twitch parameters, the larger the underestimation is. Third, the higher the ground truth twitch amplitude, the less variance between the true and estimated twitch parameters. Fourth, the method accurately estimates a representative twitch when the force signal comprises unequal successive MU twitch profiles within each MU. To conclude,

we anticipate this method enables studying mechanisms regulating force generation during electrically stimulated or voluntarily generated forces.

Keywords: motor units, twitch, force, deconvolution

Introduction

Muscle force generation is key in everyday life, yet the mechanisms regulating force generation are not fully resolved. Over the last half-century, attempts have been made to elucidate these mechanisms in *animals* and *humans* in vivo, primarily focusing on the motor unit (MU) activity because of its role as the smallest voluntarily contractible unit. The study of MU twitches mostly relies on trains of *electrical* or *voluntary*-generated stimulus and directly recording the generated force pulling on the tendon with a force transducer. Given the recorded force signal of the partially fused tetanus, a computational method extracts the MU twitch parameters (Raikova et al., 2023). Although there are different computational methods for this purpose in the literature, they are all subject to various limitations.

Spike-triggered averaging is the most well-known computational method for extracting twitch parameter estimates for an average twitch. This method uses the time instants of the stimulus as triggers in the muscle force signal within a pre-defined window length and averages all the triggered windowed sub-signals. This method has been used in human studies, e.g., using intraneural motor-axon electrostimulation to obtain the stimulus used for averaging (McNulty and Macefield, 2005; Thomas, 1995) and voluntary contractions using discharge times from high-density surface EMG (Cescon et al., 2004) or intramuscular EMG (Milner-Brown et al., 1973; Nordstrom et al., 1989; Roatta et al., 2008; Stein et al., 1972).

Although spike-triggered averaging has been widely used in the literature, it is well-known that it may provide biased twitch parameter estimates (Calancie and Bawa, 1986; Gossen et al., 2003; Negro et al., 2014). The main reason for the resulting biasedness is that the time between two stimuli (inter-spike interval) is commonly 65-125 ms (8-15 Hz firing rate), which may be shorter than the contraction times that can be as long as 140 ms or more (Burke and Tsairis, 1974; Stephens and Usherwood, 1977) and certainly shorter than the duration of the twitch (McNulty et al., 2000). The partial fusion will lead to biased amplitude estimates as the triggers will capture the subtle oscillations of the partially fused tetanus (Lim et al., 1995).

A method overcoming such biases of the spike-triggered averaging method is a model-based peel-off method (Raikova et al., 2008, 2007). The method uses a parametrised twitch model fitted to the initial twitch in the force signal before subtraction, and the next twitch is being fitted and subtracted, etc. In this way, the method can correctly identify the twitch amplitudes, contraction times, and other twitch parameters, not only in tetanic force signals (Raikova et al., 2008, 2007) but also in tetanic displacement signals from ultrasound (Rohlén et al., 2022). This method can identify unequal successive twitch shapes, an important feature in force generation (Celichowski et al., 2014). On the other hand, if the signal-to-noise ratio is low, there may be a risk of error propagation if the preceding twitches are poorly estimated, and the error remains when estimating the consecutive twitch(es). Moreover, this method works only when a single MU is activated, likely only in cases stimulating a single axon in a specific rat model (Drzymała-Celichowska and Celichowski, 2020) or humans using microelectrodes (McNulty and Macefield, 2005). Although it is possible to electrically stimulate multiple axons simultaneously, even with irregular time intervals, to mimic the characteristics of natural contractions (Calvin and Stevens, 1967), this method is not feasible for signals recorded under voluntary contractions when a pool of MUs is co-active.

Due to the various limitations of the previous methods, a model-based deconvolution of the muscle force signal in the time domain has been proposed (Negro and Orizio, 2017). This model consists of a parametrised twitch model and an optimisation algorithm to estimate the MU twitch parameters for an average twitch by numerically solving a least mean square problem. In contrast to spike-triggered averaging and the model-based peel-off method, this method requires *multiple stimuli as input*, such as spike trains from multiple MUs, resulting in the method's output being parameters for *multiple average twitches*. Although this method fits well with the developments of decomposition of high-density surface EMG, which can decode the EMG signals into multiple MU spike trains (Negro et al., 2016), the method breaks down for a single MU spike train input. In some situations, only a single stimulus train (e.g., MU spike train from intramuscular EMG or electrical stimulation) is accessible, and it should suffice to estimate the parameters of an average twitch. If we could develop a model-based deconvolution method that can take one stimulus input and provide accurate MU twitch parameters for a single average twitch, this method may replace the widely known spike-triggered averaging method and not be dependent on having multiple MU spike trains for MU twitch parameter estimation.

In this study, we aimed to develop a model-based deconvolution method of a force-based signal (muscle voluntary activity – the unfused tetanus) that can accurately estimate the MU twitch parameters for a single average twitch based on a single MU spike train. We performed extensive simulations to evaluate the performance of the method by varying the four twitch parameters of the method (spike-to-force onset time, contraction time, half-relaxation time, and twitch amplitude), numbers of co-active MUs (6-18), firing rates (8-15 Hz), signal-to-noise levels (0-40 dB), force signal length (10-120 s), missed spikes (0-5%), and false spikes (0-5%).

Methods

Theory

The force generated by a muscle can be described as a sum of forces generated by a pool of MUs, where each MU comprises a train of firings or spikes (i.e., a spike train) and a twitch occurring after each spike (Heckman and Enoka, 2004). These spike-induced twitch profiles may vary across and within a MU (Celichowski et al., 2014). The generated force $Y(t), t = [0, T]$ during an isometric contraction can be described by the following model:

$$Y(t) = \sum_{i=1}^{k} \sum_{j=1}^{n_i} h_{i,j}\left(t - t_{i,j}\right) + \varepsilon(t), \tag{1}$$

where k is the number of active MUs, n_i is the number of firings for the ith MU, $i = 1, \dots, k$, $h_{i,j}(t - t_{i,j})$ is the MU twitch response for the jth firing of the ith MU occurring at time instant $t_{i,j}$, and $\varepsilon(t)$ is additive noise. The spike train for the ith MU, containing zeros and ones, can be described as:

$$s_i(t) = \sum_{j=1}^{n_i} \delta(t - t_{i,j}), \tag{2}$$

where δ is the Dirac Delta function.

Given a force signal, and $s_i(t)$, obtained from electrostimulation or estimated from EMG, the goal is to estimate the MU twitches to obtain the contractile characteristics of a MU. However, it is not feasible to estimate all the MU twitch profiles for a given MU i (i.e., $h_{i,j}, \forall j$ for a fixed i) unless only one active MU exists, such as in studies stimulating single axons in a rat model (Drzymała-Celichowska and Celichowski, 2020). Therefore, to obtain the contractile characteristics of a MU within a pool of active MUs, one must estimate a representative MU

twitch, i.e., simplifying the complex model in Equation (1) by assuming the MU twitch responses to be identical within each MU, i.e., $h_{i,j}(t) = h_i(t)$.

One alternative to a representative MU twitch is the average twitch. Replacing the varying twitch profiles with an average twitch (Fig. 1A) still captures the dynamics of the original force signal, although they are not identical (Fig. 1B). However, as stressed in numerous studies, spike-triggered averaging leads to biased estimates of the average twitch (Calancie and Bawa, 1986; Gossen et al., 2003; Negro et al., 2014). Instead, we implemented a model-based deconvolution method to provide unbiased estimates of a representative MU twitch.

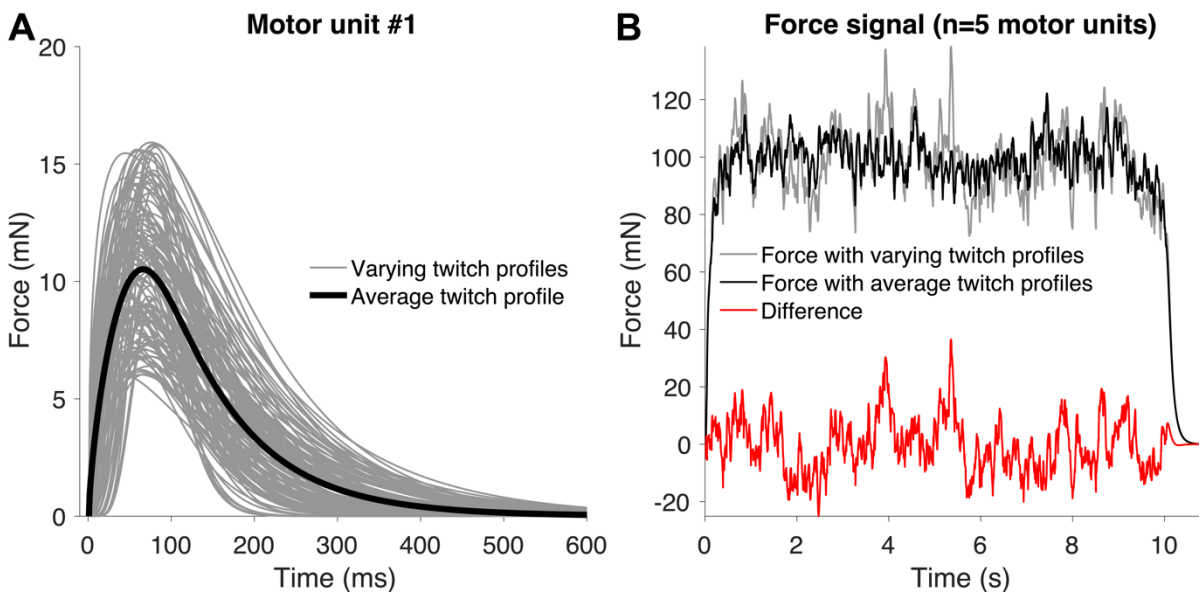

Figure 1. *The difference in tetanic force using varying successive MU twitch profiles and an average twitch profile using simulations. (**A**) Illustration of the varying MU twitch profiles (grey) and the average MU twitch profile (black). (**B**) Replacing the varying twitch profiles with an average twitch (from A), the tetanic force (black) still captures the dynamics of the original force signal (grey), although they are not identical.*

Model-based deconvolution method

Inspired by the previous work of Negro and Orizio, who proposed a model-based deconvolution method with a *multi*-spike-train-input with a *multi*-twitch-parameter-output (Negro and Orizio, 2017), we implemented a model-based deconvolution method with a *single*-spike-train-input with a *single*-twitch-parameter-output. The method is based on using 1) an existing twitch model (Raikova et al., 2007; Raikova and Aladjov, 2002), 2) a high-pass filter, and 3) an optimisation algorithm.

The twitch model has five parameters ($T_{imp}, T_{lead}, T_c, T_{hr}, F_{max}$) corresponding to the time instant of the impulse (firing or spike), the time between spike and force onset, contraction time, half-relaxation time, and maximal twitch amplitude. Given its boundary conditions (Fig. 2A); the twitch for the ith MU can be described by the following equation (Raikova et al., 2007):

$$h_i\left(t; T_{imp}, T_{lead}, T_c, T_{hr}, F_{max}\right) = p\tau^m e^{-k\tau}, \tag{3}$$

where $\tau = t - T_{imp} - T_{lead}$, $p = F_{max}e^{-kT_c(\ln T_c - 1)}$, $m = kT_c$, and $k = \frac{\ln 2}{T_{hr} - T_c - T_c \ln\left(\frac{T_{hr}}{T_c}\right)}$. The time instants of the impulses (spike train) are usually assumed known through electrostimulation or estimated through EMG.

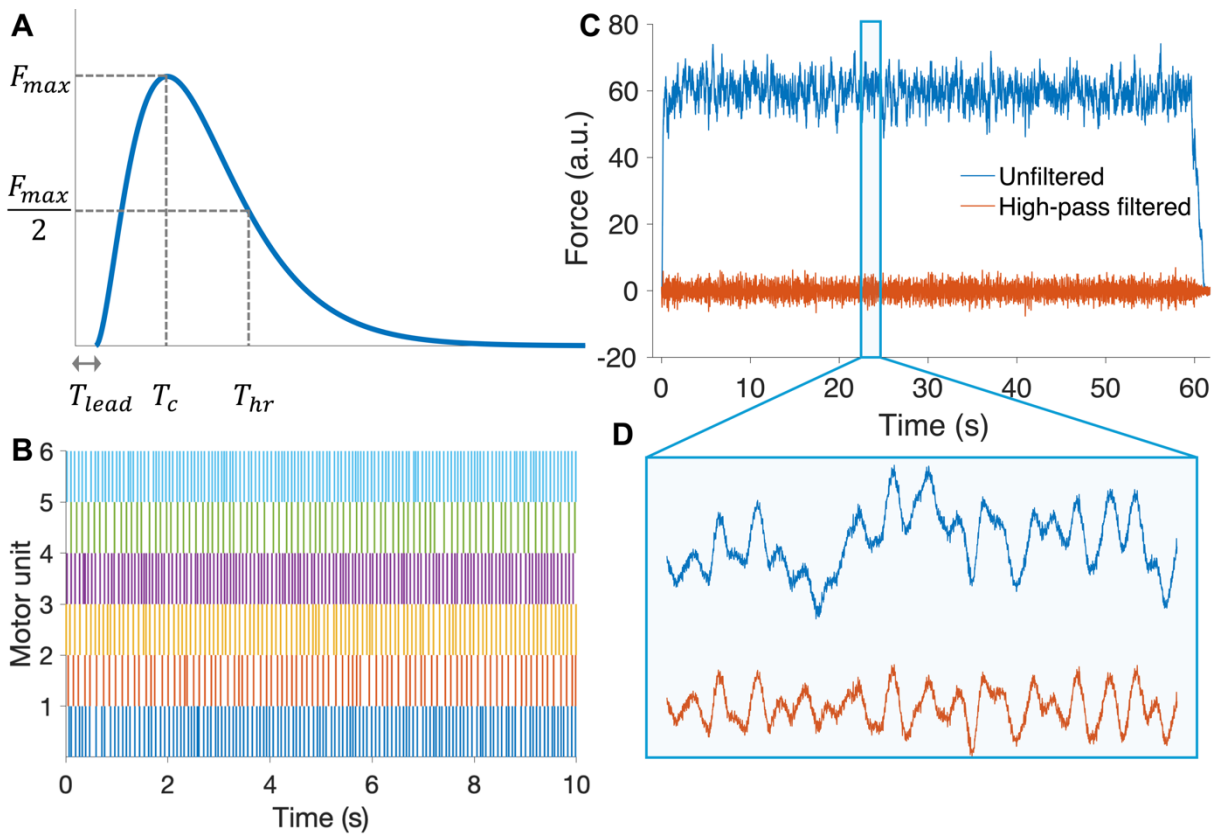

Figure 2. *(**A**) The twitch model has five parameters ($T_{imp}, T_{lead}, T_c, T_{hr}, F_{max}$) corresponding to the time instant of the impulse (firing or spike), the time between spike and force onset, contraction time, half-relaxation time, and maximal twitch amplitude. The time instant is zero in the figure. (**B**) The simulated motor unit (MU) spike trains and the simulated twitch profiles in (A) were used to obtain a tetanic force signal. (**C**) Integrating the twitch profiles over each MU spike train results in the simulated tetanic force signal (blue), a sum of forces generated by a pool of MUs. Given this tetanic force signal and a single MU spike train (B-C), it is difficult to estimate a MU twitch accurately using the existing model-based deconvolution method (Negro and Orizio, 2017). The reason is that the tetanic signal of a single MU is much smaller than the (total) tetanic force signal requiring an additional bias parameter intended to explain the remaining MU activities, and there are substantial amplitude modulations, making the task for the optimisation algorithm very complicated and easily converging in local minima. (**D**) We used a high pass filter to make the tetanic signal oscillate around zero and have less amplitude modulation, which makes the task easier for the optimisation algorithm to estimate the twitch parameters for a single MU.*

The integration of twitch profiles over each MU spike train (Fig. 2B) results in the tetanic force signal (Fig. 2C, blue line), which is a sum of forces generated by a pool of MUs. Given this

tetanic force signal and a *single* MU spike train (Fig. 2B-C), it is difficult to estimate a MU twitch accurately using the existing model-based deconvolution method (Negro and Orizio, 2017). The reason is that the tetanic signal of a single MU is much smaller than the (total) tetanic force signal requiring an additional bias parameter intended to explain the remaining MU activities, and there are substantial amplitude modulations, making the task for the optimisation algorithm very complicated and easily converging in local minima. To overcome this problem, we used a high pass filter to make the tetanic signal oscillate around zero and have less amplitude modulation (Fig. 2D), which makes the task easier for the optimisation algorithm to estimate the twitch parameters for a single MU. Empirically, we found that a 4 Hz high-pass Butterworth zero-phase filter with filter order 2x3, provided a stable performance for various simulation parameters.

The next step was to estimate the twitch parameters for a single MU (average) twitch based on the twitch model and the high-pass filter. We considered the following problem formulation to estimate the twitch parameters for the ith MU:

$$\hat{\theta}_i = \arg\min_{\theta_i} \left\| Y^{filt}(t) - \hat{Y}_i^{filt}(t) \right\|_2^2, \tag{4}$$

where $\theta_i = (T_{lead}, T_c, T_{hr}, F_{max})$, $Y^{filt}(t)$ denote the high-pass filtered tetanic signal described in Equation (1), and $\hat{Y}_i^{filt}(t) = h_i(t; \hat{\theta}_i) * s_i(t)$ denote the high-pass filtered tetanic signal for the ith MU.

To estimate $\theta_i = (T_{lead}, T_c, T_{hr}, F_{max})$ based on the problem formulation in Equation (4), we found that running a simplex algorithm to estimate all parameters at once did not provide robust estimates likely because of the dependence between the time parameters (T_{lead}, T_c, T_{hr}). Our solution to this was to use the Nelder-Mead simplex algorithm (Lagarias et al., 1998) to estimate (T_c, F_{max}) while fixing the remaining parameters (T_{lead}, T_{hr}) to particular values, where T_{hr}

was a numerical ratio applied to the T_c value. This numerical ratio approach has been used in previous studies, e.g., fixing the ratio to 5/3, resulting in $T_{hr} = \frac{5}{3}T_c$ (Negro and Orizio, 2017). Then, we re-ran the simplex algorithm with updated fixed values for the two parameters (T_{lead}, T_{hr}). This procedure was repeated until we had gone through all combinations of parameters for T_{lead} and T_{hr}, and at each fixed parameter combination performing the Nelder-Mead simplex algorithm (Lagarias et al., 1998) to estimate (T_c, F_{max}). Based on the criteria of a minimised norm in Equation (4), we selected the parameter set that resulted in the least mean square.

The bounds of the parameters with respect to the simplex algorithm were set to be $50 < T_c < 160$ ms and $0.05 < F_{max} < 12$ a.u., where the algorithm achieved the bounds using the sine function, and the start values were set to $(T_c, F_{max}) = (100.0; 5.0)$. Since this study considered simulations to evaluate the method, we set the twitch amplitudes to be arbitrary units, which must be adapted to the scale of the experimental signals in future studies. The fixed parameter ranges for T_{lead} and T_{hr}/T_c ratios were set to be $\{0,1, \dots ,20\}$ ms and $\{4.0; 4.1; \dots ; 8.0\}/3$.

Simulations

To evaluate the proposed model-based deconvolution method's performance in estimating a MU twitch based on an unfused tetanic signal, we performed extensive simulations mimicking the characteristics expected at low-force levels with the *baseline* parameters being 6 co-active MUs with a randomly generated firing rate between 8 and 15 Hz (using a Gaussian renewal process), with 20% inter-spike interval coefficient of variation, and the number of firings adjusted with respect to the firing rate such that the total length of the force signal is 60 s. Finally, Gaussian white noise was added so that the signal-to-noise ratio was 20 dB. The sample rate was 1 kHz. The number of MUs and firing rates are approximately the output one would

get from the standard onion skin model at 2.3% of maximum excitation from the MU recruitment model by Fuglevand and colleagues (Fuglevand et al., 1993). However, a 10-fold twitch amplitude difference in the Fuglevand model corresponds to 150 co-active MUs, whereas we consider 6 to 18 MUs to evaluate the performance with respect to varying MU twitch amplitudes (see the Discussion section).

To evaluate the performance of the method with respect to changes in the *given force signal*, we varied the numbers of co-active MUs (6, 12 or 18), signal-to-noise levels (0, 20 or 40 dB), and force signal length (10, 60, or 120 s). We also evaluated the performance with respect to changes in the *given spike trains,* including missed or false spikes (0, 2.5 or 5%) by removing spikes or adding spikes from another MU spike train. Each scenario was repeated 50 times to increase the number of MU twitch parameter comparisons (true vs estimated) for statistical means.

Throughout all these scenarios, we randomly generated the MU twitch parameters: $T_{lead}\sim\text{Uniform}(0,20)$ ms, $T_c\sim\text{Uniform}(75,110)$ ms, $T_{hr}/T_c\sim\text{Uniform}(4.5;7.5)/3$, and $F_{max}\sim\text{Uniform}(1,10)$ a.u. Note that we generated only one twitch profile per MU throughout these scenarios. To evaluate the method performance for changing MU profiles within each MU, we considered the baseline parameters (6 MUs, 20 dB, 60 s) repeated 50 times with slightly different MU twitch parameters since we do not expect the differences to be as big as in the equal successive twitch case above. Thus, for the unequal successive twitch case, we randomly generated the following twitch parameters within each MU twitch: $T_c\sim\text{Uniform}(90,110)$ ms, $T_{hr}/T_c\sim\text{Uniform}(5.5;6.5)/3$, and $F_{max}\sim\text{Uniform}(MU_{ind},MU_{ind}+2)$ a.u, where MU_{ind} denote the MU index from 1 to 6. The

reason for varying the average twitch amplitude for each MU is to enable the study of the performance with respect to varying amplitudes.

Statistical analysis

We tested whether the difference between the true and estimated twitch parameters for each scenario described in the previous section significantly differed from zero with the nonparametric Sign Test. We also tested whether there was any difference in variation between the true and estimated twitch parameters by dividing them into two groups based on 1) the ground truth amplitude or 2) the ground truth firing rate, using the nonparametric Ansari-Bradley Test (after removing the median from each sample). For both tests, Bonferroni correction was performed given a 0.05 significance level.

Results

A representative 60 s tetanic force signal with 6 MUs and 20 dB signal-to-noise ratio is shown in Fig. 3A, comprised of six co-active MUs with spike trains varying between 8 and 15 Hz (Fig. 3B). Given the force signal and the spike trains (Fig. 3A-B), the proposed deconvolution method can accurately estimate twitch parameter estimates, whereas the STA does not (Fig. 3C). However, occasionally STA may provide accurate contraction times, although the twitch amplitude is poor and becomes even worse for higher twitch amplitudes (Fig. 3D).

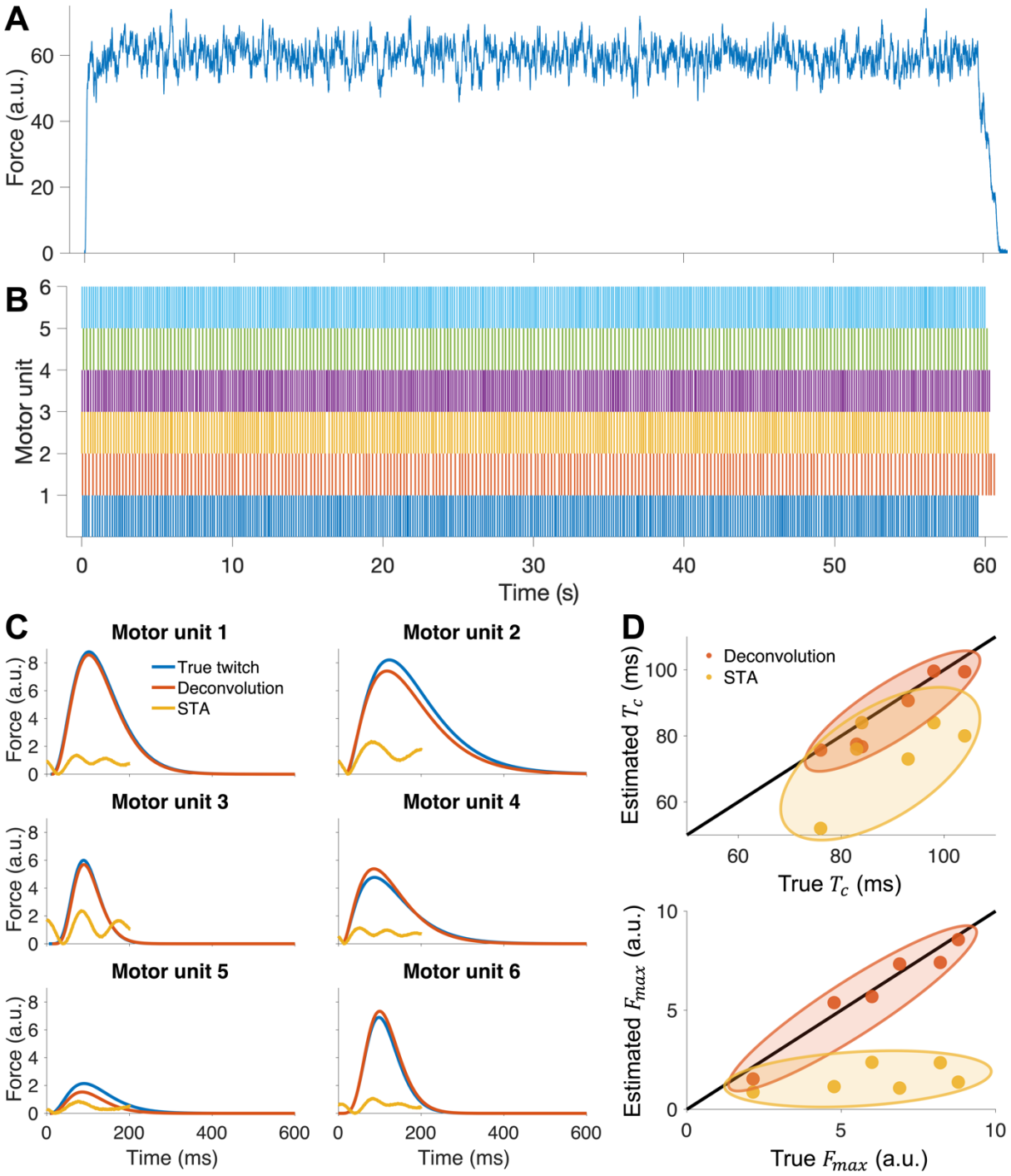

Figure 3. *A representative simulation with 6 MUs, 20 dB signal-to-noise ratio, and 60 s force signal (**A**) comprised of six co-active MUs with spike trains varying between 8 and 15 Hz (**B**). Given the force signal and the spike trains, the deconvolution method can accurately estimate twitch parameter estimates, whereas the spike-triggered averaging (STA) does not (**C**). Occasionally, STA may provide accurate contraction times, although the twitch amplitude is poor (**D**). Note that the STA twitch has been adjusted such that its minimum value is zero.*

By comparing the estimated twitch parameters against the ground truth ones, we found that there is no bias for the *baseline* case (as illustrated in Fig. 3), i.e., 6 co-active MUs, 20 dB, and 60 s (Fig. 4). However, there is a varying degree of variation with respect to the different twitch parameter estimations where the twitch amplitude had the least variance. T_{lead} had overall the highest variance and therefore, it cannot be reliably estimated. It turns out that the method is robust to the force signal's SNR, and it does not work as well for 10 s recordings (Fig. 4). Also, increasing the number of MUs from 6 to 12 or higher, there is a slight overestimation and increased variation in the T_c and F_{max} estimates.

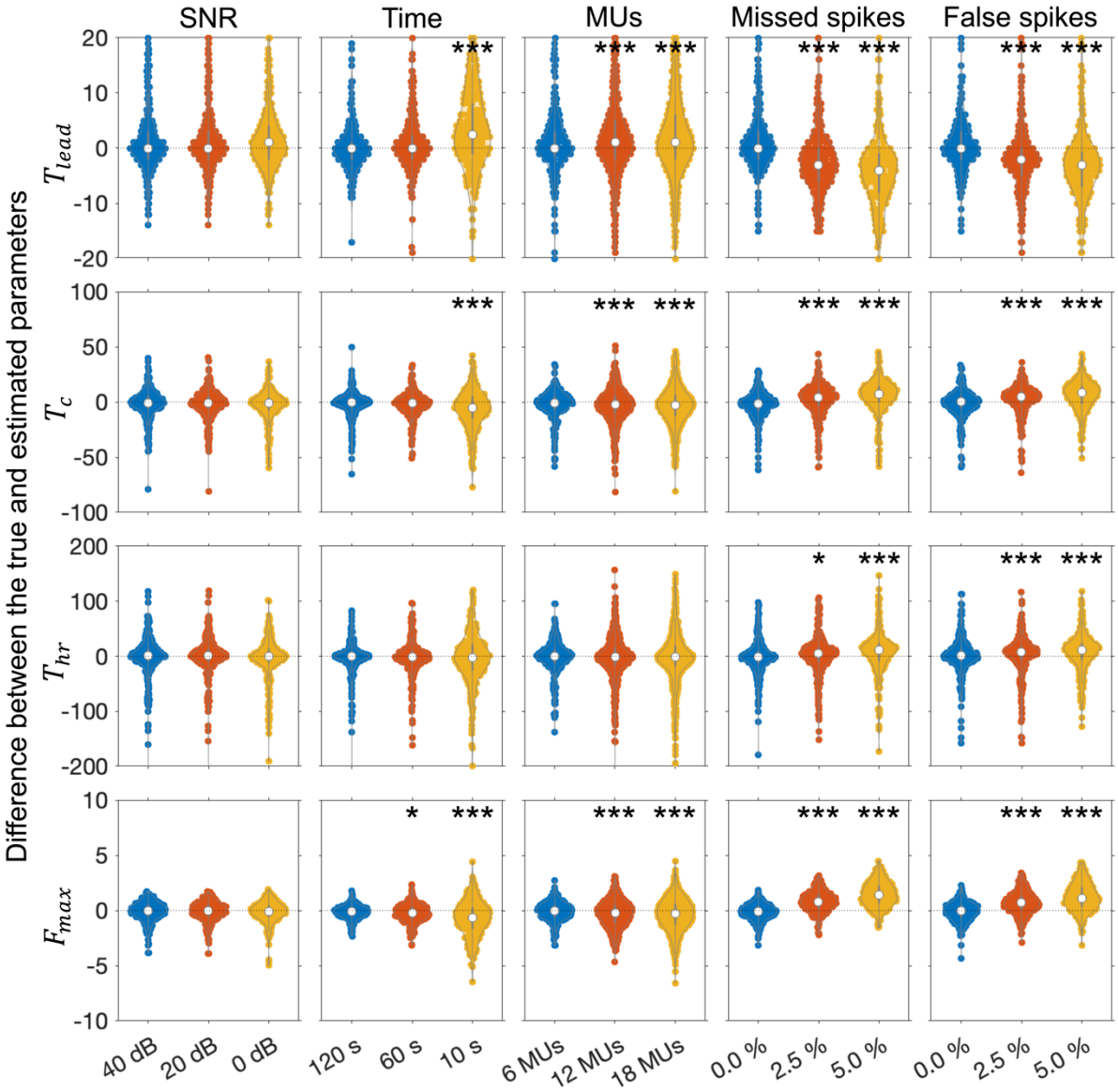

Figure 4. *Difference between the true and estimated twitch parameters from a tetanic force signal with varying parameters using violin plots. There is none or negligible bias for the baseline case, i.e., 6 motor units (MUs), 20 dB signal-to-noise ratio (SNR), and 60 s. There is a varying degree of variation with respect to the different twitch parameter estimations, where the twitch amplitude (F_{max}) had the least variance. T_{lead} had overall the highest variance. The method is robust to the force signal's SNR, and it does not work as well for 10 s recordings. Also, increasing the number of MUs from 6 to 12 or higher, there is a slight overestimation and increased variation in the T_c and F_{max} estimates. The more missed or false spikes were used to estimate twitch parameters, the larger the underestimation is for the contraction time, half-relaxation time, and twitch amplitude parameters. Even at 2.5% missed or false spikes, there is an underestimation in the twitch parameter estimates, except for the T_{lead} parameter. Although the variation slightly increased for increased misses or false spikes, the bias was more prominent. *p<0.05; **p<0.01; ***p<0.001.*

We also found that the more missed or false spikes were used to estimate twitch parameters, the larger the underestimation is for the contraction time, half-relaxation time, and twitch amplitude parameters (Fig. 4). And even at 2.5% missed or false spikes, there is an underestimation in the twitch parameter estimates, except for the T_{lead} parameter (Fig. 4). Although the variation slightly increased for increased misses or false spikes, the bias was more prominent.

Next, we studied how the variance in twitch parameter estimates depends on ground truth twitch amplitude and firing rate. We found that the higher the ground truth twitch amplitude, the less variance (Fig. 5). However, this does not concern the twitch amplitude, which has the same variance across all ground truth twitch amplitudes. The variance is slightly higher for the missed or false spikes than the baseline case. Interestingly, the variance in twitch parameter estimates does not depend on the firing rate (mean inter-spike interval).

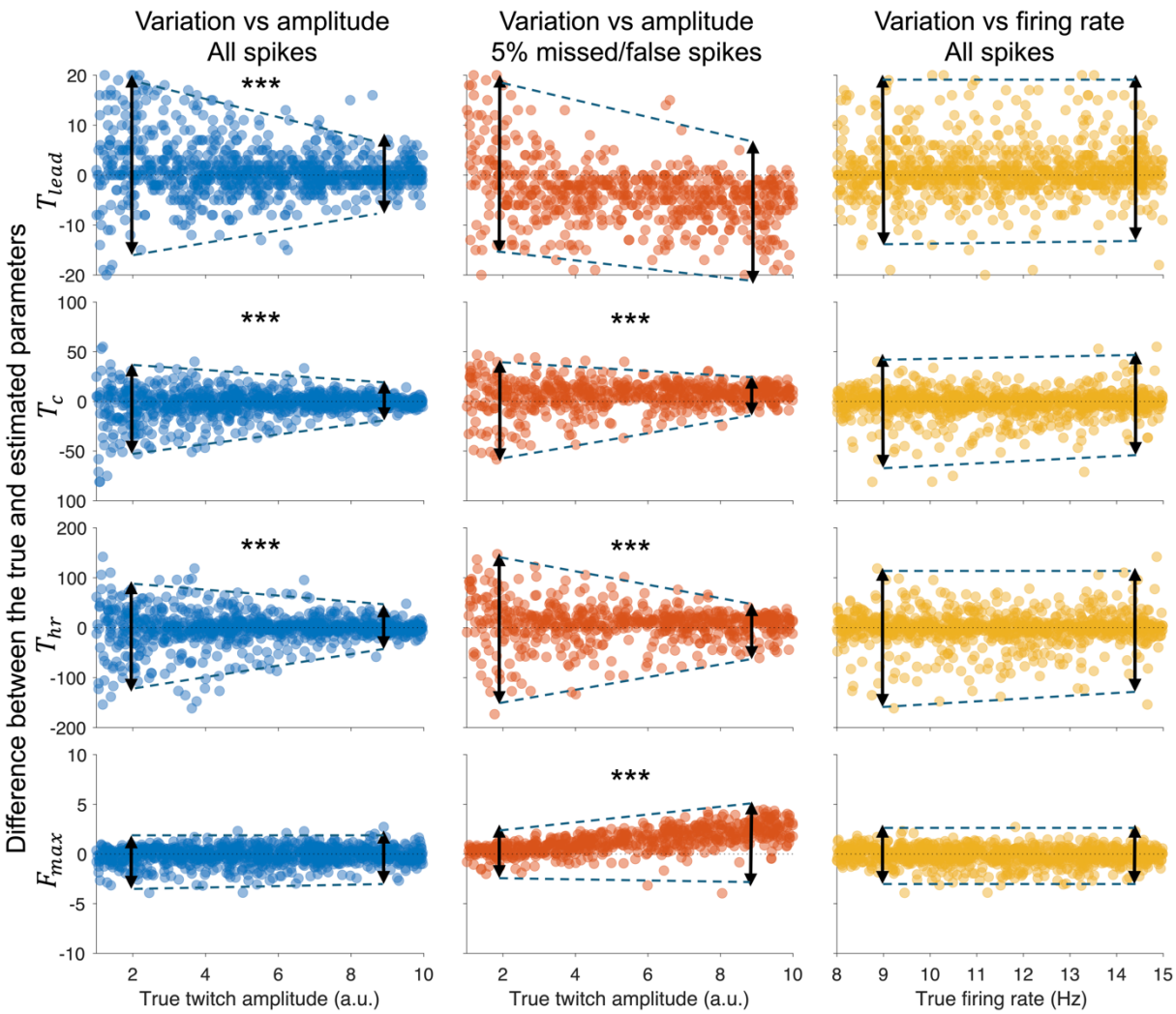

Figure 5. *Variation in difference between the true and estimated twitch parameters estimates with respect to true twitch amplitude and firing rate. We found that the higher the ground truth twitch amplitude, the less variance. However, this does not concern the twitch amplitude, which has the same variance across all ground truth twitch amplitudes. The variance is slightly higher for the missed or false spikes than the baseline case. Interestingly, the variance in twitch parameter estimates does not depend on the firing rate. Note that the arrows indicate the range of the values, and the dotted lines to highlight the (non)variation-dependent estimates on true twitch amplitude or firing rate *p<0.05; **p<0.01; ***p<0.001.*

Next, we wanted to see how the method performed when the force input comprises unequal successive twitch profiles within each MU of the tetanic contraction. We found that the method accurately estimates a representative twitch (Fig. 6). The findings are the same as in the equal successive MU cases where the estimated twitch parameters had lower variation than for higher

amplitudes and vice versa. In addition, there is no such variation-dependent relationship with firing rates.

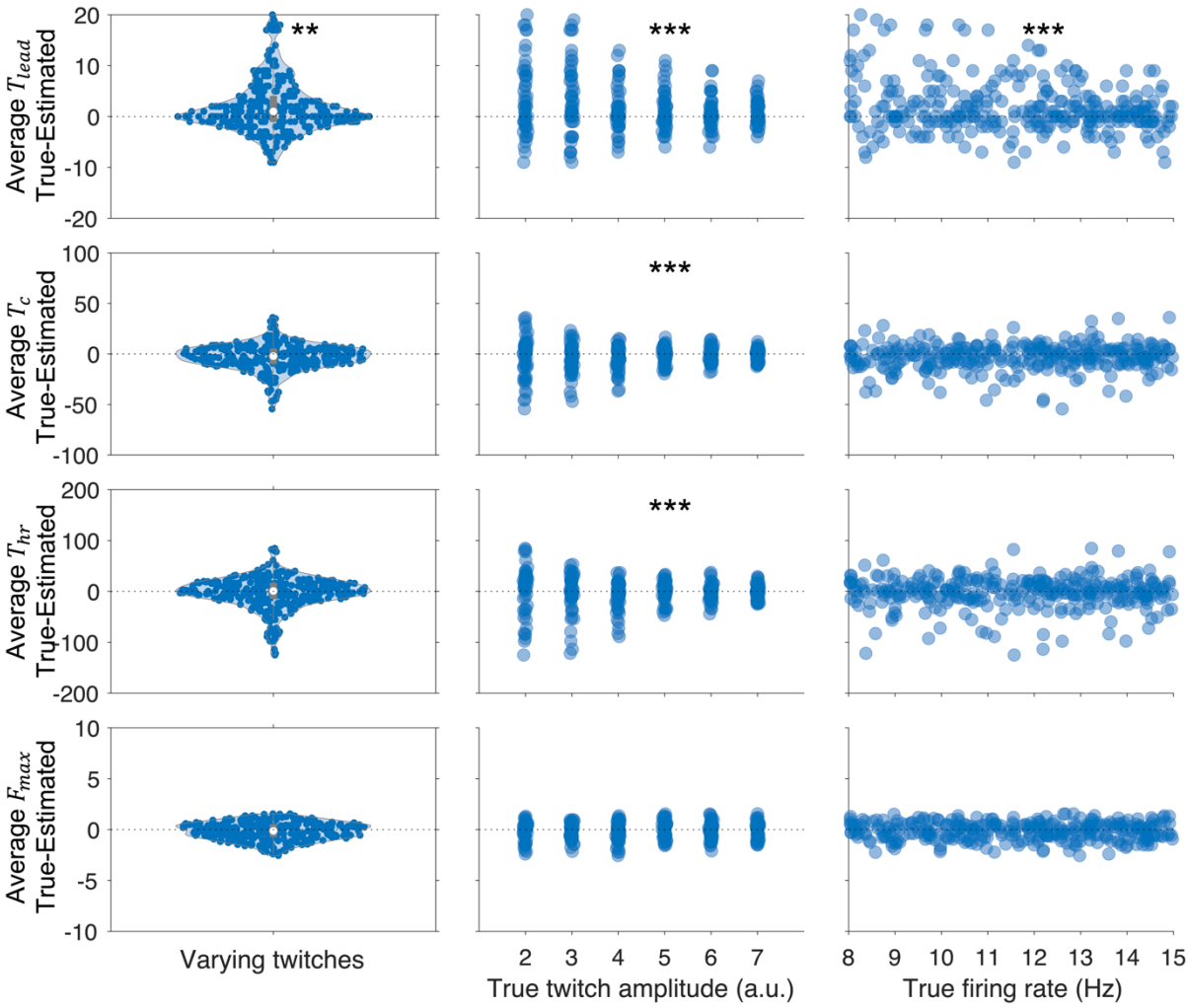

Figure 6. *Performance evaluation of the deconvolution method when the tetanic signal comprises unequal successive twitch profiles within each MU. The method accurately estimates a representative twitch. The findings are the same as in the equal successive MU cases where the estimated twitch parameters had lower variation than for higher amplitudes and vice versa. In addition, there is no such variation-dependent relationship with firing rates.*

Discussion

This study aimed to develop a model-based deconvolution method of a force-based signal of unfused tetanus that can accurately estimate the MU twitch parameters for a single average twitch based on a single MU spike train. The proposed method used a twitch model together with an optimisation algorithm to estimate the twitch parameters from a high pass filtered tetanic force signal. We performed extensive simulations to evaluate the method's performance by varying the simulation parameters to mimic low-force isometric contractions, which resulted into four main findings.

First, given an accurate spike train, the method can accurately estimate twitch parameter estimates in the baseline case (as exemplified in Fig. 3), i.e., a 60 s force signal with 6 co-active MUs and 20 dB SNR. Six co-active MUs correspond to 2.3% of maximum excitation in the onion skin model using Fuglevand's model with 300 MUs in the whole pool (Fuglevand et al., 1993), corresponding to a very weak force level. Although changes to the number of MUs (12 and 18 MUs) increased the variance and resulted in slight overestimation, it is still within an acceptable range in practice (see Fig. 4). This increased variation and slight overestimation may be reduced by longer recording than 60 s. On the other hand, we found that a 10 s force signal does not produce accurate estimates, and therefore, we recommend that this method needs at least 60 s recordings of the tetani. Moreover, it is important to stress that, T_{lead} cannot be reliably estimated, and probably explain some of the variation in the T_c and T_{hr} who both depends on the T_{lead} parameter. In previous studies with rat models, it turns out that the T_{lead} parameter values are very similar (Raikova et al., 2008, 2007). So, an alternative solution would be to use previous knowledge or estimate T_{lead} before fixing it in the optimisation algorithm. Either way, the remaining MU twitch parameters under very low-force isometric contractions can be estimated accurately.

Second, the more missed or false spikes were used to estimate twitch parameters, the larger the underestimation is. Although the variation slightly increased for increased misses or false spikes, the bias was more prominent. However, there are various solutions to this. For instance, if it is possible to estimate the number of missed firings, e.g., during an isometric and stable contraction, one may adjust the parameters. In some situations, like electro-stimulations or using intramuscular EMG, we may be much more certain to not have any missed or false spikes.

Third, the higher the ground truth twitch amplitude, the less variance between the true and estimated twitch parameters. In other words, the method is biased towards higher twitch amplitudes and, therefore, later recruited ones according to Henneman's size principle (Henneman, 1957; Henneman et al., 1965). One should stress that this is relevant for many methods interested in decoding compound signals into MU activity, including high-density surface EMG decomposition (Holobar et al., 2009) and ultrafast ultrasound (Rohlén et al., 2024). However, the twitch parameter-dependent variability does not concern the twitch amplitude, which has the same variance across all ground truth twitch amplitudes (Fig. 5). Finally, the variance in twitch parameter estimates does not depend on the firing rate (mean inter-spike interval), where the STA method clearly suffers from firing rates above 8 Hz.

Fourth, the method accurately estimates a representative twitch when the force signal comprises unequal successive MU twitch profiles within each MU. Although this representative varies around the average twitch, its variation is low enough to be a feasible representative twitch. Moreover, if the underlying unequal successive twitches are more similar, then we should expect the estimated MU twitch to be closer to the average twitch. We found that the characteristics of the unequal twitch case were the same as in the equal twitch case, with the

estimated twitch parameters having lower variation than for higher amplitudes and vice versa, and that there is no such variation-dependent relationship with firing rates.

To evaluate the performance of the proposed method, we used simulations to mimic low-force isometric contractions in natural contractions. Although we did not use a sophisticated MU recruitment model (Fuglevand et al., 1993), it may be viewed as a version of it with a stable contraction (excitatory drive of approximately 2.3% of maximum excitation with 300 MUs in the total pool) with varying average MU firing rates and twitch parameters. The upper bound 15 Hz firing rate may be exaggerated for these weak force levels since it is commonly assumed they start firing at 8 Hz once recruited (Fuglevand et al., 1993), but we intended to evaluate the model for various firing rates where we know the STA method breaks down. Moreover, the 10-fold difference in amplitude used in this study may also be exaggerated since in the Fuglevand model (with 300 MUs in the pool), such amplitude difference corresponds to 150 co-active MUs instead of the 6 to 18 MUs used here. Moreover, 5% of maximum excitation in the Fuglevand model used in (Negro and Orizio, 2017) corresponds to a 3-fold difference in twitch amplitude of the recruited MUs. Since we have made extensive simulations and evaluated the method's performance for varying simulation parameters, we are confident that the extracted twitches will be accurate if used on experimental force signals from weak force levels with low MU recruitment and an accurate spike train as input.

The present study has three main limitations. *First*, the method was tested only for low-level force modelling, and we expect that the higher the force level, the higher variation of the estimates and the highest amplitude twitches will have the least variance. *Second*, low-threshold MUs (recruited at low-level force) are usually weak, whereas the variation of the estimates is larger for the earlier recruited (low-force) MUs. If the pool of recruited units has similar

amplitudes, then we expect the variation to be the same for all MUs. As stressed in the previous paragraph, our simulations may have exaggerated the amplitude differences in the pool, and following the Fuglevand model, our twitch parameter estimates should result in less variation for the lower amplitudes. *Third*, the obtained twitches are representative twitches, likely close to the average twitch, whereas the twitches decomposed from the tetanic contractions of single MUs reveal some variability in amplitude and twitch time parameters (Celichowski et al., 2014). For most MUs, the single twitch force is lower than for twitches decomposed from the unfused tetanus, and therefore, the proposed method may overestimate the twitch amplitude.

In conclusion, we have proposed a model-based deconvolution method to accurately decode a tetanic force signal into a representative MU twitch, given a MU spike train. Although this method is limited to low-force isometric contractions, it will enable studying mechanisms regulating force generation during electrically stimulated or voluntarily generated forces.

Competing interests

The authors declare that they have no competing financial and non-financial interests.

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