Modeling of summation of individual twitches into unfused tetanus for various types of rat motor units

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Abstract

Repeated stimulation of motor units (MUs) causes an increase of the force output that cannot be explained by linear summation of equal twitches evoked by the same stimulation pattern. To explain this phenomenon, an algorithm for reconstructing the individual twitches, that summate into an unfused tetanus is described in the paper. The algorithm is based on an analytical function for the twitch course modeling. The input parameters of this twitch model are lead time, contraction and half-relaxation times and maximal force. The measured individual twitches and unfused tetani at 10, 20, 30 and 40 Hz stimulation frequency of three rat motor units (slow, fast resistant to fatigue and fast fatigable) are processed. It is concluded that: (1) the analytical function describes precisely the course of individual twitches; (2) the summation of equal twitches does not follow the results from the experimentally measured unfused tetani, the differences depend on the type of the MU and are bigger for higher values of stimulation frequency and fusion index; (3) the reconstruction of individual twitches from experimental tetanic records can be successful if the tetanus is feebly fused (fusion index up to 0.7); (4) both the maximal forces and time parameters of individual twitches subtracted from unfused tetani change and influence the course of each tetanus. A discrepancy with respect to the relaxation phase was observed between experimental results and model prediction for tetani with fusion index exceeding 0.7. This phase was predicted longer than the experimental one for better fused tetani. Therefore, a separate series of physiological experiments and then, more complex model are necessary for explanation of this distinction.
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1. Introduction

It is well known that the maximal force generated by a motor unit (MU) after repetitive (random or uniform) stimulations is higher than that predicted by linear summation of individual, equal twitches, while applying the same stimulation pattern [2,24,27]. This phenomenon can be observed for fused and unfused tetani when the next stimulus appears before the force of the previous contraction falls to zero, hence when the twitches overlap in time. For slow MUs, unfused tetani are formed at lower stimulation frequencies than for fast ones, due to longer contraction and relaxation times. Moreover, the summation of twitches into tetanus is more effective in slow MUs since they have lower twitch-to-tetanus ratio in comparison to fast MUs [8,28]. The MU force development increases from the minimal twitch force, throughout the unfused tetanus, in parallel to a rise of stimulation frequency, up to the maximum in the fused tetanus [6,18–20].

Experimental investigations of the course of unfused tetani [11] have shown changes not only in the maximal forces, but also in the time parameters (contraction time and relaxation course) of the successive twitches composing tetanus. However, conclusions concerning mainly the course of unfused tetani have been made only, since the

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courses of individual twitches are usually obscured within the tetani. These experiments have raised several questions. How do successive twitches summate into tetanus? How far from the experimental evidences is the supposition, originally formulated by Helmholz (cited in [17]), that the tetanus is composed of linearly summed individual, equal twitches (see also [29,30])? Is it possible to extract individual twitches from the unfused tetanus, and if yes, how do these twitches differ at various stimulation patterns and for various types of MUs? Modeling is an adequate tool for detailed investigation of changes of MUs' contractile properties during unfused tetani that can help to answer the above questions.

First, an analytical model of the twitch, capable of describing its form for many different types of MUs, is necessary. After that, the process of summation can be modeled. Existing analytical models of the twitch [22–24,31] are based on only two parameters – contraction time and maximal force amplitude. The analytical form recently proposed by Raikova and Aladjov [25] takes into account also the half-relaxation time and lead time, thus allowing more precise description of the twitch form for various MUs. Few papers in the literature report approaches for tetanus modeling. The simulation results presented by van Zandwijk et al. [29,30] are based on linear summation of individual twitches calculated using Hill type muscle model, with length and contraction velocity of the muscle as input parameters. Fuglevand et al. [12] have modeled the force increase during tetanus by introducing a gain factor in the function describing the twitch, i.e., the tetanus has been simulated by linear summation of twitches with only one variable parameter – different maximal forces. The simulated unfused tetani in the cited studies have quickly reached stable force levels. They have not been compared with experimental data, and moreover, they have not matched phenomena such as sag and force potentiation, observed for different types of MUs [1–5,10,11].

The aim of the present paper is to develop an algorithm for subtracting (reconstructing) the individual twitches that compose experimentally measured unfused tetanus, and to demonstrate its applicability for various types of MUs and tetani with different fusion index.

2. Methods

2.1. Experiments

The experimental procedure was described in details elsewhere [4,11]. Briefly, the activity of individual MUs was evoked by electrical stimulation of bundles of axons isolated from the L5 ventral root. The "all or none" response confirmed the activity of a single MU. The contractile force of MUs of the rat medial gastrocnemius muscle was measured under isometric conditions with the muscle stretched up to passive force of 100 mN (force transducer deformation 100 μm/100 mN). Initially, five stimuli at 1 Hz were applied and respective individual

twitches were recorded. Then, series of stimuli at 10, 20, 30, 40 Hz were delivered every 10 s to evoke tetanic contractions. MUs were classified as fast or slow based on sag appearance and then fast MUs were divided into fast fatigable and fast resistant to fatigue based on the fatigue index [1,10,19]. The experimental data for three representative MUs (one slow, S, one fast resistant to fatigue, FR, and one fast fatigable, FF) were used for the present study. The best single twitch (most noiseless) from the five recorded ones and the unfused tetani obtained for stimulation at constant frequencies of 10, 20, 30 and 40 Hz were processed and afterwards used for current modeling. The fusion index was calculated for analyzed unfused tetani as a ratio of the distance between the baseline and the lowest relaxation before the last contraction to the maximal amplitude of the last contraction of the tetanus [9].

2.2. Model of the individual twitch and the summation of twitches

The analytical function used for describing the twitch form has been given in [25] and is explained in details below by the Eqs. (1)–(5). Five parameters are necessary for summation of many modeled twitches (see Fig. 1): $T_{imp}^{(i)}$ – moment of the ith stimulus; $T_{lead}^{(i)}$ – time between the stimulus and the start of force development; $F_{max}^{(i)}$ – maximal force of the ith twitch; $T_c^{(i)}$ – contraction time, the time from the start of the MU mechanical activity to the time where MU force reaches its maximal value; $T_{hr}^{(i)}$ – half-relaxation time, the time from the start of the MU mechanical activity to the time where MU force decreases to $F_{max}^{(i)}/2$. Note that the half-relaxation time is given here with respect to the start of the twitch, to simplify the analytical function. The following algorithm for summation of modeled twitches with different parameters (see Fig. 1) is applied. The force developed as a result of the ith stimulus
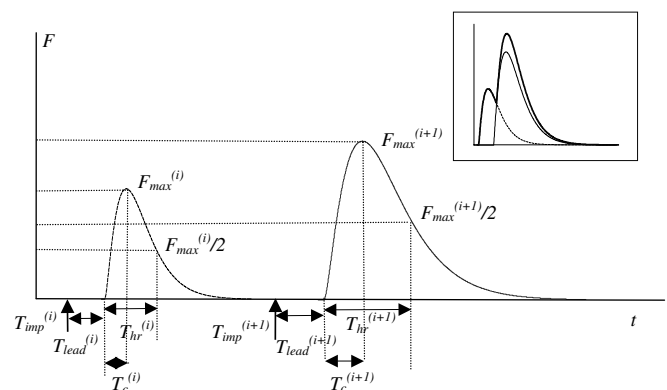

Fig. 1. Parameters of the individual twitches (for explanation see in the text) and illustration of twitch summation. Two twitches of one and the same MU, the ith and the $(i + 1)$th, that do not overlap, are shown. The case with overlapping twitches is shown in a box upright (black broken line – the ith twitch, black thin line – the $(i + 1)$th twitch, black bold line – the sum of the two twitches). The algorithm for summation is identical for both cases (non-overlapping and overlapping).

is calculated for $t \in [0,T]$ (where T is the duration of the experiment) as follows:

if $t < T_{imp}^{(i)} + T_{lead}^{(i)}$ then $F_i(t, T_{imp}^{(i)}, T_{lead}^{(i)}, F_{max}^{(i)}, T_c^{(i)}, T_{hr}^{(i)}) = 0$,

else :

$$F_i(t, T_{imp}^{(i)}, T_{lead}^{(i)}, F_{max}^{(i)}, T_c^{(i)}, T_{hr}^{(i)}) = p\tau^m \exp(-k\tau),$$

(1)

where

$$\tau = t - T_{imp}^{(i)} - T_{lead}^{(i)}, \tag{2}$$

$$k = \ln 2 / \{T_{hr}^{(i)} - T_c^{(i)} - T_c^{(i)} \ln(T_{hr}^{(i)}/T_c^{(i)})\}, \tag{3}$$

$$m = kT_c^{(i)}, \tag{4}$$

$$p = F_{max}^{(i)} \exp\{-kT_c^{(i)}(\ln T_c^{(i)} - 1)\}. \tag{5}$$

The force $F(t)$ developed by a MU as response to many impulses is the sum:

$$F(t) = \sum_{j=1}^{N} F_j(t, T_{imp}^{(j)}, T_{lead}^{(j)}, F_{max}^{(j)}, T_c^{(j)}, T_{hr}^{(j)}), \tag{6}$$

where N is the number of impulses and $t \in [0,T]$. This algorithm is applied independently whether the twitches overlap or not (see Fig. 1). Hence, the amplitudes of different twitches are summed for each discrete t.

2.3. Algorithm for establishing the individual twitch parameters and for subtracting individual twitches from experimentally measured unfused tetani

The twitch parameters can be calculated easily from the experimental data stored in digital text format in cases when the individual twitches do not overlap (see Fig. 1). Knowing $T_{imp}^{(i)}$, the value of the maximal force $F_{max}^{(i)}$, the time moments when the mechanical activity begins and $F_{max}^{(i)}$ and after that $F_{max}^{(i)}/2$ are reached, i.e., $T_{lead}^{(i)}, T_c^{(i)}$ and $T_{hr}^{(i)}$, are automatically calculated. The calculation is based on minimization of root mean square (RMS) error between experimental and the modeled curves of the non-overlapping twitches. Visual inspection of the experimental and modeled curves and possibilities for manual adjustment of the twitch parameters is also available on-line in the software implementation of the algorithm (the software is free for download on the web page http://www.clbme.bas.bg/projects/motco/).

In case of unfused tetanus, i.e., when the twitches overlap, the following algorithm is developed for automatic decomposition (see Fig. 2). Since at first the individual, initial twitch of current MU is processed, its parameters are known. Supposing that it is not very different from the first contraction into the tetanus the parameters of this initial twitch are slightly adjusted (varying them into preliminary given limits). The aim is to find such a twitch form that describes best the experimental curve between $T_{imp}^{(1)}$ and $T_{imp}^{(2)}$ (see Fig. 2a). The estimation is made using RMS error between experimental and modeled curve within the mentioned time interval. The twitch that has minimal RMS
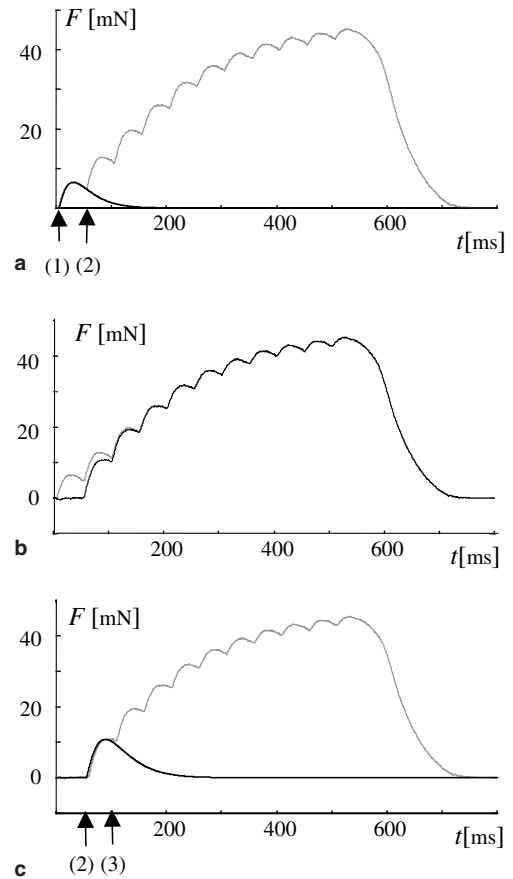

Fig. 2. Illustration of the algorithm for decomposition of an unfused tetanus into individual twitches. (a) The experimental unfused tetanus (grey line) and the first modeled twitch (black line); (b) subtraction of the first modeled twitch from the experimental curve (grey line), the curve obtained after this subtraction is shown in black; (c) the second modeled twitch (black line) versus the experimental curve (grey line) from which the first modeled twitch is subtracted. Arrows show the time moments of the successive impulses.

error is fixed. During the next step, the first modeled twitch is subtracted numerically from the experimental curve (see Fig. 2b). Aiming to recognize the second twitch, the obtained parameters of the first modeled twitch are varied as this was described up, so that the remaining part of the experimental force record (after subtracting the first modeled twitch) between $T_{imp}^{(2)}$ and $T_{imp}^{(3)}$ is described well by the second modeled twitch (see Fig. 2c). The so obtained parameters of the second twitch become initial values for the recognition of the third twitch. This process continues up to the last impulse, subtracting numerically all preceding modeled twitches from the experimental curve in order to obtain parameters of the current twitch. After the automatic reconstruction a visual inspection and manual adjusting of all twitch parameters is available. An interactive table with these parameters is appeared. Changing here some of the parameters, the respective in number experimental contraction and model are shown magnified, and the manual fitting of the parameters allows to follow more precise the form of the contraction. Two possibilities are

realized in the software: manual fitting of all parameters or their automatic re-optimization after this twitch which is processed manually.

If the tetanus is fused in such a degree that only short fragment of relaxation is visible because of the next overlapping contraction (as seen in Fig. 2b), the twitch parameters: $T_{\mathrm{lead}}^{(i)}, F_{\max}^{(i)}$ and $T_{\mathrm{c}}^{(i)}$, can be calculated with a good precision. $T_{\mathrm{hr}}^{(i)}$ cannot be calculated, however, since this moment is invisible. In case of the tetanus shown in Fig. 2, for example, the third impulse comes before the force of the second twitch falls to one half of its maximal force (see Fig. 2b). Hence, $T_{\mathrm{hr}}^{(i)}$ is estimated approximately. This presumes many solutions for this parameter, but our supposition is that it can be varied in restricted limits which are preliminary manually set in the software. Something else that can reduce this indeterminateness is the visual inspection which helps much to follow just the form of the curve. For nearly fused tetanus, $F_{\max}^{(i)}$ and $T_{\mathrm{c}}^{(i)}$ also become invisible (see Section 4) after subtracting preceding twitches. Hence the accuracy of the algorithm depends on the fusion index (see Section 4).

3. Results

The best (most noiseless among the five initially measured twitches) twitch recordings for the three types of MUs and their models are presented in Fig. 3. In general, the model describes enough well the twitch shapes of the three MUs, independently that the parameters of the twitches are within a wide range. It has to be noted here that some filtering of the experimental data is not performed independently that such an option is realized in the software. The reason is that the filtering can destroy the specific form of the twitch, especially in the time interval between the impulse and the start of the mechanical response, which will reflect to the correct estimation of the lead time. Since the S MU is the weakest, its experimental data contain more noise (see Fig. 3a) and the discrepancy between the model and the experimental curve is the biggest. The RMS errors between the experimental and modeled curves are given in the text under the figures. Independently that this quantitative estimation is the smallest

for S MU (since its maximal force is the lowest) the fitting of the twitch shape is the worst. This fact enforced the adding of possibilities for visual inspection of the form of the experimental and modeled twitches and manual adjusting of the parameters.

For regular low frequency stimulation at 10 Hz (see Fig. 4), the identification process is easy for FR and FF MUs (Fig. 4d and e) since the individual twitches do not overlap. Nevertheless, the parameters of these six twitches are not constant. In both types of fast units, the staircase effect is visible [26]. The maximal force of the twitches decreases for FR MU up to the 5th impulse (from 26.05 to 23.73 mN). For FF MU the twitch forces decrease up to the 3rd impulse (from 40.16 to 38.82 mN) and after that increase. Such a potentiation is a characteristic property of FF units [5,11,13]. On the other hand, the time parameters of the twitches of the fast MUs remain nearly unchanged. At the same stimulation frequency, twitches of the S MU overlap (see Fig. 4a) due to the longer contraction and relaxation. The fusion index for this tetanus is 0.16. All parameters (except $T_{\mathrm{lead}}^{(i)}$) of the modeled twitches (see Fig. 4c) change much more than those of the fast MUs (see for comparison Fig. 4d and e). As can be seen from Fig. 4a the difference between the experimental tetanus and the summation of six equal twitches (identical to the one given in Fig. 3a) is large. The summation of subtracted modeled twitches shown in Fig. 4c, however, resembles well the experimental tetanus (see Fig. 4b). Parameters of these twitches undergo the following changes: $T_{\mathrm{c}}^{(i)}$ increases from 26.4 ms (for $i = 1$) to 31.8 ms (for $i = 4$) and after that decreases to 30.1 ms; the half-relaxation time increases from $T_{\mathrm{hr}}^{(1)} = 60.6$ ms to $T_{\mathrm{hr}}^{(6)} = 70.2$ ms; the lead time is nearly constant – between 5 and 5.5 ms; the maximal force increases from $F_{\max}^{(1)} = 6.7$ mN to $F_{\max}^{(6)} = 8.9$ mN. It is worth to note that the maximal value of the experimental tetanus is 9.49 mN. The comparison between the six subtracted twitches for the tetani of the three MUs (see Fig. 4) and the respective initial MU twitches given in Fig. 3 reveals that all they have different parameters, but bigger changes concern the modeled twitches of the S MU, in which tetanic activity begins for this stimulation frequency.
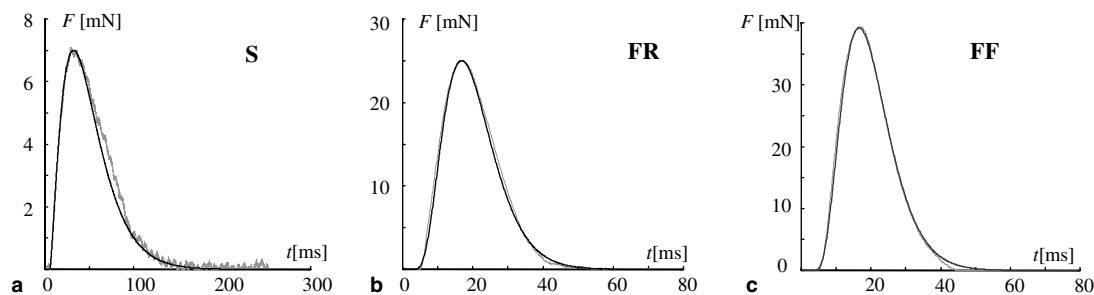

Fig. 3. The individual twitches and their models (grey line – experimental data, black line – the model). (a) S MU, twitch parameters: $T_{\mathrm{lead}} = 5.0$ ms, $T_{\mathrm{c}} = 27.0$ ms, $T_{\mathrm{hr}}^{(i)} = 61.4$ ms, $F_{\max}^{(i)} = 7.0$ mN, RMS error $= 0.2334$; (b) FR MU, twitch parameters: $T_{\mathrm{lead}} = 3.0$ ms, $T_{\mathrm{c}} = 13.8$ ms, $T_{\mathrm{hr}}^{(i)} = 23.8$ ms, $F_{\max}^{(i)} = 25.01$ mN, RMS error $= 0.5614$; (c) FF MU, twitch parameters: $T_{\mathrm{lead}} = 3.5$ ms, $T_{\mathrm{c}} = 13.1$ ms, $T_{\mathrm{hr}}^{(i)} = 22.7$ ms, $F_{\max}^{(i)} = 39.18$ mN, RMS error $= 0.6324$. Here $T_{\mathrm{imp}} = 0$ ms for all twitches. Note that the scales are different for different MUs.
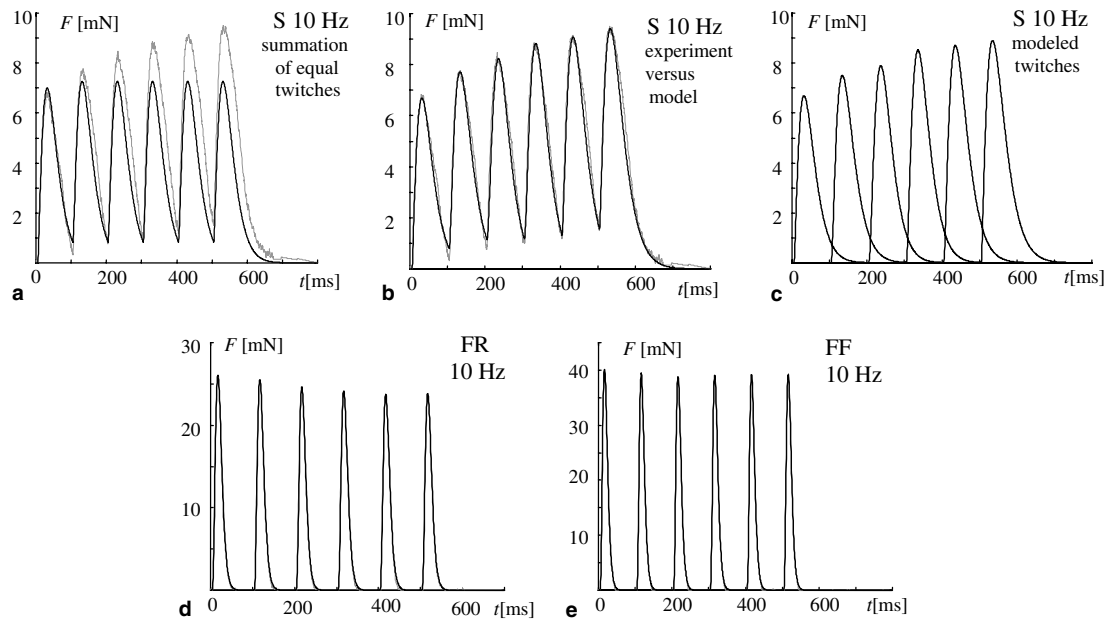
Fig. 4. Experimental data and model results for regular stimulation at 10 Hz (6 impulses with interpulse intervals of 100 ms). Upper row – S MU, the fusion index is 0.16. Lower row – two types of fast motor units, FR and FF, both with fusion index 0.00. (a) The experimental curve (grey line) versus summation of equal twitches (black line) with the same parameters as those in Fig. 3a, RMS error = 1.3380; (b) the experimental curve (grey line) versus modeled tetanus (black line) by using the algorithm for reconstruction of individual twitches, RMS error = 0.4162; (c) reconstructed individual twitches which summation gives the model in (b); (d) the experimental curve (grey line) versus modeled tetanus (black line) for FR MU (the two curves nearly coincide), RMS error = 0.4408; (e) the experimental curve (grey line) versus modeled tetanus (black line) for FF MU (the two curves nearly coincide), RMS error = 0.3967. Since the twitches in (d) and (e) do not overlap and the model matches well the little changes during this stimulation, the summation of equal twitches and subtracted twitches are not shown for FR and FF MUs. Note that the force scale is different for S, FR and FF MUs.

At higher stimulation frequency, i.e., 20 Hz, and respective higher fusion index, the difference between experimentally observed tetanus for the S MU and the modeled one, obtained by summation of equal twitches, becomes evident (see Fig. 5a). The fusion index amounts to 0.94 in this case. The parameters of the successive twitches, calculated using the described algorithm, change considerably (see Fig. 5c, see also Table 1). The experimental tetanus curve is well
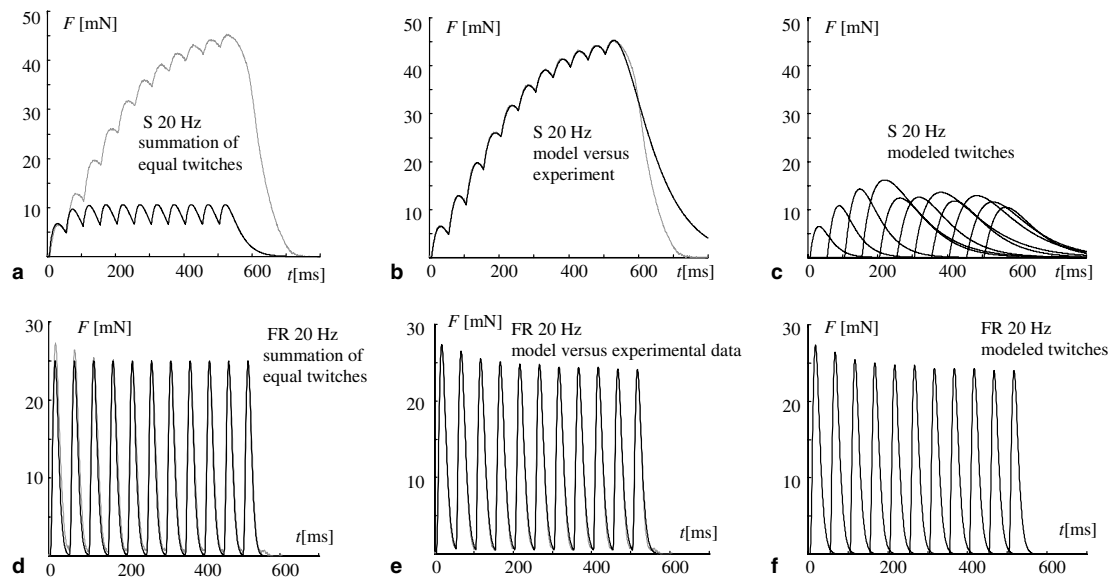

Fig. 5. Experimental data and model for regular stimulation at 20 Hz (11 impulses with interpulse intervals of 50 ms). Upper row – S MU, fusion index 0.94; lower row – FR MU, fusion index 0.02. The first column – experimental curves (grey lines) versus summation of equal twitches (black lines) with the same parameters as those in Fig. 3a and b, respectively. The second column – experimental curves (grey lines) versus modeled tetanus (black lines). The third column – individual twitches reconstructed from the experimental tetani presented in (b) and (e), respectively, their summation gives the curves in (b) and (e). The respective RMS errors are 23.6980 for (a), 3.9910 for (b), 1.7713 for (d) and 0.6463 for (e).

Table 1
Parameters of the modeled (subtracted) twitches for regular stimulation at 20 Hz (11 impulses with interpulse intervals of 50 ms) for the three MUs studied

No twitch (i)	S MU				FR MU				FF MU			
	$T_{lead}^{(i)}$ ms	$T_c^{(i)}$ ms	$T_{hr}^{(i)}$ ms	$F_{max}^{(i)}$ mN	$T_{lead}^{(i)}$ ms	$T_c^{(i)}$ ms	$T_{hr}^{(i)}$ ms	$F_{max}^{(i)}$ mN	$T_{lead}^{(i)}$ ms	$T_c^{(i)}$ ms	$T_{hr}^{(i)}$ ms	$F_{max}^{(i)}$ mN
1	5.5	26.3	66.0	6.5	4.0	13.9	26.2	27.4	3.5	13.4	23.7	41.5
2	5.0	34.6	85.9	10.8	4.0	13.4	25.6	26.4	3.8	13.0	22.7	39.4
3	5.0	44.2	110.0	14.3	4.0	13.2	25.2	25.4	4.0	12.9	22.0	38.5
4	5.5	65.0	180.0	16.2	4.0	13.2	25.2	25.0	4.0	12.9	22.7	38.1
5	5.5	58.7	155.0	12.4	4.0	13.2	25.2	24.7	4.0	13.1	22.0	38.2
6	5.0	64.0	165.0	12.6	4.0	13.2	25.2	24.7	4.0	13.1	22.0	38.5
7	5.5	76.1	210.0	13.6	4.0	13.0	25.0	24.3	4.0	13.1	22.0	38.5
8	5.0	66.1	170.0	11.8	4.0	13.0	25.0	24.3	4.0	13.1	22.0	38.9
9	5.0	80.0	212.0	12.9	4.0	13.0	25.0	24.3	4.0	13.1	22.0	38.9
10	5.5	75.0	196.0	11.6	4.0	13.0	25.0	24.0	4.0	13.1	22.0	39.0
11	5.0	63.0	159.0	10.5	4.0	13.0	25.0	24.0	4.0	13.1	22.0	38.8

These twitches for S and FR MUs are shown in Fig. 5c and f.

modeled until the last (11th) impulse through the summation of the modeled twitches (see Fig. 5b). As can be seen from Fig. 5b, the force during the experiment drops faster than the model prediction (see Section 4), i.e., the experimental relaxation is faster than the modeled one. For FR MU, the twitches barely overlap and the algorithm successfully performs the decomposition (see Fig. 5e and f). For FF MU, the 20 Hz stimulation evokes non-overlapping twitches (not shown in Fig. 5) and mainly the maximal forces of these twitches change. The parameters of the modeled twitches at this stimulation frequency for the three investigated MUs are given in Table 1. The comparison of the values from the first row of this table to the parameters of the recorded individual twitches given in Fig. 3 enables us to conclude that even parameters of the first twitches extracted from the tetanic contractions are different from the initially measured single twitches. One possible reason is potentiation that might develop in spite of 10 s intervals between each train of stimuli. Another reason is the inher-

ent variability of the biological objects. Even the five individual twitches, recorded initially during the experiments, differed between each other.

For 30 Hz stimulation, the experimental tetanus of S MU is more fused (see Fig. 6a, the fusion index is 0.98) and the process of subtracting the individual twitches (see Fig. 6c) from the experimental curve is much more difficult and probably imprecise. As in the previous example, the evident difference between the experimental curve and the model resulting from summation of equal twitches is observed (see Fig. 6a). Likewise, the model does not match the relaxation at the end of stimulation (see Fig. 6b). It has to be noted here that the maximal force of the experimental tetanus is 50.75 mN, while the maximal force of the modeled twitches is 17.7 mN (the 5th twitch). For less fused tetani of the two fast MUs (the fusion index is equal to 0.06 and 0.16 for FF and FR MUs, respectively), the model can successfully perform the decomposition of tetanic contractions for FR (see Fig. 6e) and FF (not shown in the figure) MUs.
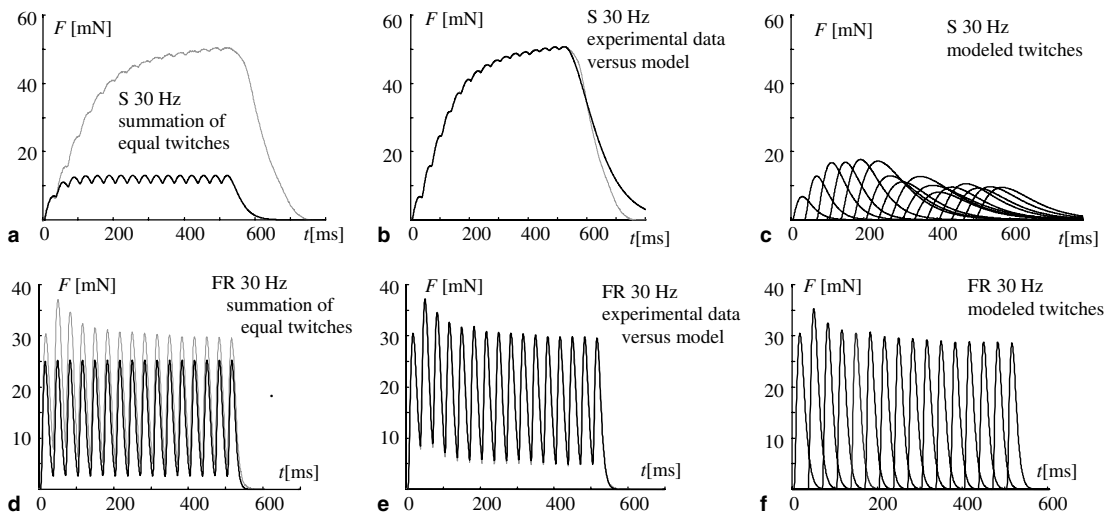

Fig. 6. Experimental data and model for regular stimulation at 30 Hz (16 impulses with interpulse intervals of 33.(3) ms). Upper row – S MU, fusion index 0.98, lower row – FR MU, fusion index 0.16. The first column – experimental curves (grey lines) versus summation of equal twitches (black lines) and all these twitches have the same parameters as those in Fig. 3a and b, respectively. The second column – experimental curves (grey lines) versus modeled tetanus (black lines). The third column – individual twitches reconstructed from the tetani presented in (a) and (d), their summation gives the curves in (b) and (e). The respective RMS errors are 27.8510 for (a), 2.6663 for (b), 6.3172 for (d) and 1.1419 for (e).

For FR MU, the difference between the experimental curve with a sag profile and the model obtained by summation of equal twitches becomes evident, too (see Fig. 6d). Thus, the summation of twitches with equal parameters does not explain polyphasic changes in the profile of fast MUs unfused tetanus, including sag phenomenon.

The S MU tetanus evoked at 40 Hz stimulation is nearly fused (fusion index 0.99) and the application of the algorithm for subtracting the individual twitches is ineffective. Therefore, Fig. 7 presents results of modeling the experimental data exclusively for fast (FR and FF) MUs. At this stimulation frequency, the unfused tetani of FF and FR
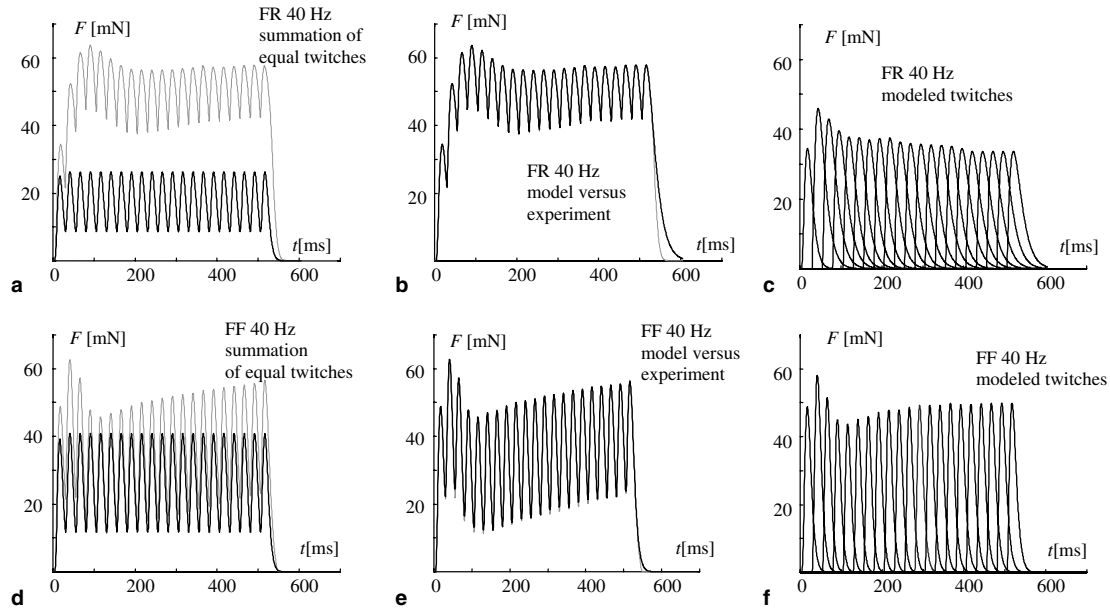

Fig. 7. Experimental data and model for regular stimulation at 40 Hz (21 impulses with interpulse intervals of 25 ms). Upper row – FR MU, fusion index 0.73, lower row – FF MU, fusion index 0.41. The first column – experimental curves (grey lines) versus summation of equal twitches (black lines) with the same parameters as those in Fig. 3b and c. The second column – experimental curves (grey lines) versus modeled tetanus (black lines). The third column – individual twitches reconstructed from the tetani presented in (a) and (d), their summation gives the curves in (b) and (e). The respective RMS errors are 30.7762 for (a), 2.8804 for (b), 9.6651 for (d) and 0.9075 for (e).

Table 2
Parameters of the modeled (subtracted) twitches for regular stimulation at 40 Hz (21 impulses with interpulse intervals of 25 ms) for the two fast MUs studied. These twitches are shown in Fig. 7c and f

No twitch (i)	FR MU				FF MU			
	$T_{lead}^{(i)}$ ms	$T_c^{(i)}$ ms	$T_{hr}^{(i)}$ ms	$F_{max}^{(i)}$ mN	$T_{lead}^{(i)}$ ms	$T_c^{(i)}$ ms	$T_{hr}^{(i)}$ ms	$F_{max}^{(i)}$ mN
1	4.0	14.6	28.8	34.5	3.5	14.0	25.1	48.8
2	4.8	15.0	34.8	46.0	3.5	13.3	24.4	58.2
3	4.8	15.9	37.1	42.9	3.5	12.5	22.5	51.6
4	5.0	15.5	37.3	39.6	3.5	12.5	22.1	45.0
5	5.3	14.8	35.1	37.8	3.5	12.5	22.1	43.7
6	5.3	14.6	34.8	37.6	3.5	12.7	22.1	45.1
7	5.3	14.4	34.1	36.9	3.5	12.9	22.4	46.0
8	5.3	14.4	34.1	37.3	3.5	12.9	22.6	47.2
9	5.3	14.6	35.2	37.5	3.5	12.9	22.9	47.9
10	5.3	14.9	35.9	36.4	3.5	13.0	23.1	47.9
11	5.3	14.9	35.9	35.9	3.5	13.2	23.3	48.6
12	5.3	15.1	36.8	35.7	3.5	13.2	23.6	49.2
13	5.3	15.1	36.8	35.5	3.5	13.2	23.8	49.2
14	5.3	15.4	38.0	35.4	3.5	13.2	24.0	49.2
15	5.3	15.4	37.5	35.1	4.0	13.0	24.2	49.9
16	5.3	15.6	39.3	34.4	4.5	12.5	24.0	49.5
17	5.3	15.6	39.3	33.6	4.5	12.5	24.0	49.4
18	5.5	15.8	39.3	33.6	4.5	12.5	24.0	49.7
19	5.5	15.8	39.3	33.6	4.5	12.5	24.3	49.9
20	5.5	15.8	39.3	33.6	4.8	12.5	24.6	49.9
21	5.5	15.8	39.3	33.6	4.8	12.5	24.6	49.9

MUs, with characteristic sag can be successfully modeled (see Fig. 7b and e). However, for better-fused tetanus of the FR unit (fusion index is equal to 0.73, Fig. 7b), the same discrepancy as the one detected before for the S MU is observed with respect to the relaxation between the experimental record and the curve model. The differences between the summation of equal twitches and the experimental data are also clearly visible for both FF and FR MUs (see Fig. 7a and d). The summation of equal twitches gives constant force level and cannot match the specific sag phenomenon of fast MUs. All parameters of the subtracted twitches change (see Table 2).

4. Discussion

The present study is the first attempt towards decomposition of an unfused tetanus into a series of twitches. The main supposition was that a tetanus is composed of individual summated twitches evoked by successive impulses and that these twitches have different parameters. The main effort was directed towards developing an algorithm and own software for subtracting these individual twitches from the experimentally recorded unfused tetanus. The algorithm was based on the analytical function described previously [25]. It was shown that this function described accurately the individual twitches for different MUs (see Figs. 3 and 4d and e). The algorithm matches well the experimentally observed unfused tetani with low fusion index, below 0.7. However, more investigations are necessary for explanation of the observed discrepancy between the recorded and modeled relaxation phases for more fused tetanus, i.e., with the fusion index over 0.7 (see Figs. 5b, 6b and 7b). One reason of this discrepancy can be the change in the twitch course observed for the last contraction of tetani fused to variable degree [7,16]. It was observed that the better fused is the tetanus, the shorter is the contraction, but longer the relaxation. Moreover, it has been apparent that a prolongation of relaxation is accompanied with evidently biphasic rate of relaxation, first lower and then higher. These observations concerned tetani with fusion index over 0.7. Hence, more complex analytical model probably is necessary for tetani of such relatively high fusion degree. This model should take into account a possibility of change in the relaxation speed. It should be able to perform more precise decomposition of better fused tetani.

The algorithm presented in this paper is less precise in cases of tetani with relatively high fusion degree than for slightly fused tetani, due to problems with the precise estimation of some twitch parameters that remain invisible when subtracting the previous modeled twitches from the experimental curve (see Fig. 2b). Problems became bigger when the force peak of the first twitch in the obtained curve after subtracting all previous twitches is invisible. This situation happens when the stimulus evoking the next contraction comes before the force of the current twitch begins to fall, i.e., before the start of the relaxation phase. In such a case, only the lead time can be estimated auto-

matically with good precision. Independently of this, the results obtained (especially these for tetani with fusion index less than 0.7) have enabled us to draw some general conclusions. Firstly, it has been shown that the experimentally recorded tetanus is evidently different from the curve obtained by summation of equal twitches (see Figs. 4a, 5a and d, 6a and d and 7a and d). The force of the experimental tetanus is not only bigger than the one calculated by summation of equal twitches for all studied units but also the two curves have different shapes. The summation of equal twitches gives a constant force level, while the force of experimentally recorded tetani of different MUs is not stable. For example, five-time difference between peak forces of the tetanus modeled by summation of equal twitches and the experimental tetanus has been observed for 30 Hz stimulation of the S MU (see Fig. 6a). This observation proves that the summation of successive contractions into an unfused tetanus is a non-linear process. Moreover, it has been found that variability of the contractile parameters of successive twitches within the unfused tetanus concerns not only the maximal forces of the twitches, but also time parameters (Tables 1 and 2), with the exception of $T_{lead}^{(i)}$ which undergoes only little changes.

In the present paper tetanus curves obtained at regular stimulation frequencies of 10, 20, 30 and 40 Hz, for only three representative MUs, have been processed by the proposed algorithm. Some authors recognize a fourth type MU – the so-called fast-intermediate [1,13,14]. Since our aim was to demonstrate an approach for decomposition of unfused tetani we choose three characteristic MUs with characteristic for this type of units shape of tetani [10,15]. Of course, large variability of MUs contractile properties, even within the population in one muscle has been evidenced [1,15,21]. An analysis of a greater number of MUs will help to draw more conclusions about the tendencies in the changes of the parameters of the subtracted twitches, for different MU types and for different stimulation patterns. Such analysis will help in detailed understanding of the physiological processes in MUs during repetitive stimulation.

In conclusion, one decomposition algorithm has been proposed in the study that is capable to subtract successfully the successive twitches that form an unfused tetanus. The modeling has proved that successive contractions summating into the tetanus have variable force and time parameters and that their summation is more effective than the linear summation of equal twitches for all three types of motor units.

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