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Computers in Biology and Medicine 37 (2007) 1572–1581

Computers in Biology and Medicine

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Variability of the twitch parameters of the rat medial gastrocnemius motor units—experimental and modeling study

Rositsa Raikova[a,*], Piotr Krutki[b], Hristo Aladjov[a], Jan Celichowski[b]

[a]*Bulgarian Academy of Sciences, Centre of Biomedical Engineering, Acad. G. Bonchev Str., Bl.105, 1113 Sofia, Bulgaria*
[b]*Department of Neurobiology, University School of Physical Education, 55 Grunwaldzka Str., 60-352 Poznań, Poland*

Received 7 December 2005; received in revised form 20 February 2007; accepted 22 February 2007

Abstract

In the present study a previously proposed model of a twitch based on an analytical function with four-parameters (lead, contraction and half-relaxation times and maximum force of the twitch) was validated on 115 motor units (MUs), divided into slow (S), fast-fatigue resistant (FR) and fast fatigable (FF) types. The original records were collected from electrophysiological experiments performed on MUs from the medial gastrocnemius muscle of five rats. Besides the easy calculation of the twitch parameters and their variability, the usefulness of the model was confirmed by eliminating artifacts and noise in the original twitch records, as well as by calculations of the velocity of force increase and decrease, the area under force records, and by normalization of all twitches with respect to the maximal force and contraction time. It was concluded that: (1) the four-parameter twitch model describes precisely the individual contractions of various MUs; (2) all physiological twitch parameters are distributed continuously and located within overlapping intervals for different MU types; this distribution is not linear, but exponential; (3) S MUs can be distinguished from fast ones on the basis of some twitch parameters (contraction and half-relaxation times, velocity of contraction), but the same cannot be applied for FF and FR MUs; (4) the analysis of the normalized twitches reveals the differences in shapes for different types of MUs, which shows that twitches of different MUs cannot be obtained from one standard pattern scaled in time and force. These results may have functional implications for studying effectiveness of twitch summation during tetanic contractions and the work performed by various types of MUs.

Keywords: Motor units; Twitch; Modeling; Rat; Statistics

1. Introduction

The form of a twitch of a motor unit (MU), i.e., the force developed as response to one impulse (stimulus), is important in several aspects. This form and the coordinates of its typical points can be used for classification of different MU's type. A twitch analytical model is necessary for modeling the way the individual mechanical responses are summated into tetanus when simulate different motor tasks [1,2]. The time course of the generated force and its maximal value are related to the effectiveness of the muscle contraction. The force-time area, i.e., the area under the force record, reflects the work performed by a MU [3]. Finally, precise analysis of the form of single contractions of individual MUs of all types is necessary for developing complex models of motoneuronal pool activity [4] or models of complex limb movements' control [1,2,5,6].

The twitch has well known bell-shaped form that can be approximated with an exponential analytical function [7–10]. Usually only two parameters (maximal twitch force and contraction time) are taken into consideration in this function. As shown in the study of Piotrkiewicz [7], these parameters are insufficient for describing the wide variability of MUs' twitches. The analytical form recently proposed by Raikova and Aladjov [5] takes into account also the half-relaxation time and the lead time. It has been shown that this four-parameter analytical function allows more precise description of the twitch form for various MUs, both for fast and slow ones. Values of these four parameters are widely and continuously distributed and fall into different ranges for different types of MUs. Doherty and Brown [11] have written: "A continuum of twitch tensions,

* Corresponding author. Tel.: +35 92 8700527; fax: +35 92 723787.
E-mail address: rosi.raikova@clbme.bas.bg (R. Raikova).

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doi:10.1016/j.compbiomed.2007.02.006

contractile speeds, . . . , was observed in our study with no clear division of MUs into subgroups based on their physiological characteristics".

Independently of the common usage of MUs division into different subtypes—three or four, namely: slow (S), fast-fatigue resistant (FR), fast-intermediate (FI), and fast-fatigable (FF) [12–15], many studies emphasize that limb muscles contain heterogeneous populations of MUs [16,17]. Gordon et al. [13] conclude that muscle fibers and MUs in cats form a continuum with respect to any of the studied twitch properties. They divide MUs into four types, but also mention that some MUs remain unclassifiable. Performing statistical analysis of 28 MUs of human finger muscles (both fast and slow), McNulty et al. [18] state that MUs can be labeled as fast or slow using arbitrary criteria, although human thenar MUs form a continuum rather than discrete groups. Karageorgos [19] have also indicated that a gradual transition between different MUs subtypes exists. Therefore, a question arises whether there exists a relationship between various twitch parameters and if yes, can this be used for classification of MUs? For example, Kernell and Eerbeck [20] have reported that the contraction time increases from FF, to FI, FR and finally S MUs, and that the same order relates to the decreasing maximal forces of MUs.

It is impossible to study all MUs composing one muscle in one *in vivo* experiment. Sometimes, due to a low number of investigated MUs, reasonable conclusions concerning the distribution of the twitch parameters and their relation cannot be made. Some authors state that the twitch force is not related to the contraction time, but significantly correlates to the total twitch duration [18]. Kwa et al. [17] have investigated 249 rabbit MUs and found negative correlation between the maximal force and the contraction time. On the other hand, Fuglevand et al. [21] have assumed in their model that the dependence between the contraction time and the twitch force is not a linear, but an exponential function. They demonstrated that the twitch forces of MUs vary over a wide range and that large number of MUs produces small forces, whereas few MUs generate relative large forces. These authors have also found that the twitch amplitude correlates positively with the twitch area, whereas the contraction time correlates with the half-relaxation time and the total twitch duration, but do not correlate with the duration of relaxation. The study of the twitch parameters' variability is important in two aspects. First, models of muscle individual contractions can help in studying the summation of individual twitches into tetanus and in explanation of previously described observations that parameters of successive contractions summing into tetanus change significantly [22]. Second, modeling of a population of various types of MUs within one muscle is necessary for creating complex muscle models and algorithms for motor control investigation [2].

The aims of the present paper are: (1) to verify the previously proposed analytical model of the MU single twitch on a large, statistically significant group of MUs of the rat medial gastrocnemius (MG) muscle; (2) to perform detailed statistics of models of experimentally recorded twitches in order to show the distribution of the twitch parameters (the lead, contraction and half-relaxation times, duration of the twitch, maximal twitch force, the twitch area and the contraction velocity) and their interrelations; (3) to compare the above parameters between slow and fast as well as between FR and FF MUs, both using the models of the original twitches and different normalization procedures.

2. Methods

2.1. Experimental procedure

Properties of the MG MUs were electrophysiologically investigated in five female Wistar rats (weighing 250–340 g), under general anesthesia with pentobarbital sodium (60 mg, i.p., the initial dose, supplemented as required). The depth of the anesthesia was verified throughout the experiment by controlling the shape of pupils and pinna reflexes. All procedures were approved by Local Ethics Committee and followed the European Union guidelines for animal care.

The detailed description of surgical procedure was given elsewhere [23,24]. Briefly, the spinal cord was exposed to laminectomy at the level of L2–S1 segments, and the ventral and the dorsal roots were cut proximally to the spinal cord. The MG muscle was isolated from the lateral head of the muscle and dissected free from surrounding tissues. The blood vessels and the respective nerve branch to this muscle were left intact, while other branches of the sciatic nerve innervating other muscles of the leg were cut. The Achilles tendon was connected to a force transducer and the hind limb was immobilized by steel clamps. The operated limb and the exposed areas of the spinal cord were covered with paraffin oil. Its temperature was automatically kept at $37 \pm 1\,^{\circ}\mathrm{C}$.

The MG muscle was stretched up to a passive tension of 100 mN, to generate the maximal twitch forces of MUs [25]. The L5 ventral root was split into very thin filaments to reach functional isolation of single MUs. MUs were stimulated by a silver bipolar electrode with rectangular electrical pulses of 0.1 ms duration and amplitude up to 0.5 V. The evoked twitches were accepted as single MU activity when both the force and the action potential were of the 'all or none' type at near-threshold stimulation. The muscle fibers action potentials were recorded with bipolar silver electrode inserted into the muscle. The force (1 kHz sampling rate) and the EMG (10 kHz sampling rate) signal were stored on a computer disc using analog-to-digital converter (RTI-800 Utilities).

Five stimuli at 1 Hz were initially applied and the evoked individual twitches were recorded. Then, a series of stimuli at 10, 20, 30, 40, 50, 60, 75, 100 and 150 Hz were delivered every 10 s to evoke tetanic contractions. Finally, all MUs were tested with a standard fatigue test (series of 14 pulses at 40 Hz repeated each second within 3 min) [16]. MUs were classified as fast or slow based on sag visible in fast units during tetanic stimulations at 40 Hz stimulation [26] and then fast MUs were divided into FF and FR based on calculated fatigue index. For FF MUs this fatigue index was below 0.5 and for FR MUs over 0.5 [27,28].

2.2. Model of single twitch

The analytical function used for describing the twitch form was proposed in the study of Raikova and Aladjov [5], see Appendix. Four parameters were used as inputs in the model: T_{lead}—lead time, the time between the stimulus and the start of the force development; F_{max}—maximum force of the twitch; T_c—contraction time, the time from the start of the MU mechanical activity to the time where MU force reaches its maximal value; T_{hr}—half-relaxation time, the time from the start of the MU mechanical activity to the time where MU force decreases to $F_{max}/2$. One should note that the half-relaxation time is given here with respect to the start of the force developing (and not measured from the force peak), aiming to simplify the analytical function. Software application developed by the authors was used to calculate all twitch parameters. It is based on the minimization of the root-mean square estimation of the deviation of the model from the experimental data. For some low-force MUs, the noise could make the automatic calculation imprecise, and therefore the parameters were manually adjusted in addition. This adjustment was made by a visual inspection of the experimental and modeled curves (see [22] for details). For the modeled twitches two additional parameters were calculated: T_{tw}—the total duration of the twitch, defined between the moment when the force starts and the moment when the contraction force decreases to 0.5% of F_{max}, and *Area*—the area under the record of the force up to the T_{tw}. Moreover, the rate of change of the force, contractile velocity, was calculated for all modeled twitches by numerical differentiation. The maximal (V_{max}) and minimal (V_{min}) values of this velocity were also computed.

Several normalization procedures of all 15 modeled twitches were performed. Firstly, the force of each MU twitch was divided to its maximal force accepting T_{lead} as zero. Secondly, the contraction time was assumed to have value of 100 for each MU, and the half-relaxation time for all MUs was calculated as $100T_{hr}/T_c$.

3. Results

One hundred and fifteen MUs in total were recorded and processed. Seventeen of them were classified as S (14.78% of the total population), 58 as FR (50.44%) and 40 as FF (34.78%) types. The proportions of MUs of each type were similar for all animals. Nineteen MUs were recorded from the MG muscle of the first animal (2 S, 10 FR and 7 FF), and for the next animals they were as follows: 25 (3 S, 8 FR and 14 FF), 24 (5 S, 16 FR and 3 FF), 21 (1 S, 13 FR and 7 FF) and 26 (6 S, 11 FR and 9 FF).

The most noiseless single twitch from the five recorded at the beginning of the stimulation procedure was used for modeling. It was proved during the processing of the experimental data that the four-parameter analytical function described precisely all twitches, regardless of the twitch shape and MUs' type (see Fig. 1).

The statistics of the calculated parameters of all modeled twitches of MUs as well as of the separate three types is given

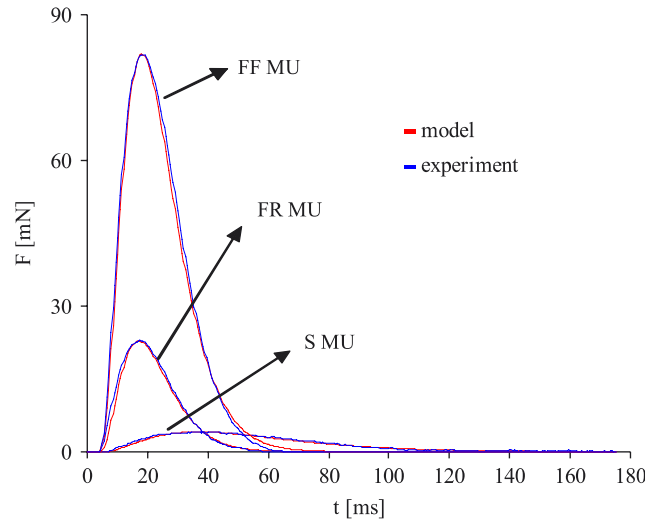
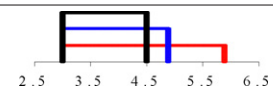
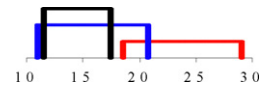
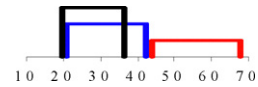
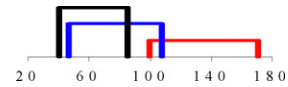
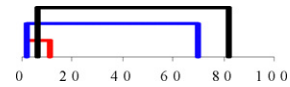
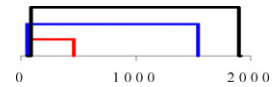
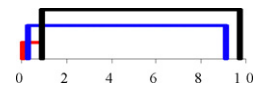
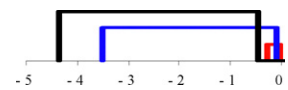


Fig. 1. Comparison between experimental individual contractions and their analytical models for three MUs representative for the three types. The modeled twitch parameters are as follows: S MU—$T_{lead} = 5.0$ ms, $T_c = 29.0$ ms, $T_{hr} = 65.0$ ms, $F_{max} = 4.1$ mN; FR MU—$T_{lead} = 4.0$ ms, $T_c = 13.1$ ms, $T_{hr} = 25.2$ ms, $F_{max} = 22.8$ mN; FF MU—$T_{lead} = 3.8$ ms, $T_c = 14.4$ ms, $T_{hr} = 27.7$ ms, $F_{max} = 81.8$ mN.

in Table 1. The maximum force of the strongest MU was 51 times higher than that of the weakest MU. However, this proportion for the twitch area was smaller, about 37 times. The lead time changed in a very narrow range and showed no strict regularity for various MUs' types. The analysis of the remaining time parameters for separate types of MUs indicated a possible division between slow and fast MUs based on the twitch contraction and half-relaxation times. The lowest value for the contraction time of the S MUs was 18.5 ms. None of the FF MUs and only four out of 58 FR units had T_c longer. With respect to T_{hr}, there was no overlapping between fast and S MUs, and the border value between them amounted to 43 ms. Such a differentiation between FR and FF units was impossible on the basis of the twitch time parameters. Values of T_{tw} overlapped between FF and FR MUs as well as between FR and S MUs. Similar observation with respect to overlapping ranges of values concerned F_{max}, V_{max} and V_{min}. MUs with the lowest areas under the force curve were these for some FR MUs, but not for the slow ones. The largest range of *Area* values had FR MUs (see Table 1) and this range included all values for S MUs. However, the remaining experimental data (recorded tetanus curves) indicated that there was strict division between FR and FF MUs with respect to the fatigue index, excepting four FR units for which this parameter was between 0.5 and 0.55 (see Fig. 2c). All other FR units had fatigue indices above 0.72, and all FF units less than 0.4.

The continuous distribution of the twitch' forces is evident from Fig. 2a. Despite the differences between the mean and the maximal values of all twitch parameters for all distinguished types of MUs (see Table 1) they were not strictly separated and were considerably overlapped (Figs. 2b–d). However, it should be pointed out that some of the strongest FR units (4 MUs, indicated by arrows in Fig. 2c) had fatigue indices near

Table 1
Statistics of the twitch parameters (mean value ± standard deviation and minimal–maximal value) of all 115 MUs and separately for S, FR and FF types

	All MUs	S MUs	FR MUs	FF MUs	Overlapping of the ranges
T_{lead}	4.02 ± 0.56	4.59 ± 0.70	4.02 ± 0.48	3.78 ± 0.42	
(ms)	(3.00–5.90)	(3.00–5.90)	(3.00–4.90)	(3.00–4.50)	
T_c	15.60 ± 4.29	24.52 ± 3.23	14.53 ± 2.01	13.35 ± 1.40	
(ms)	(11.00–29.00)	(18.50–29.00)	(11.00–20.80)	(11.50–17.50)	
T_{hr}	30.86 ± 11.09	53.55 ± 7.42	28.84 ± 5.64	24.17 ± 3.76	
(ms)	(19.50–68.00)	(44.00–68.00)	(21.00–42.50)	(19.50–36.30)	
T_{tw}	74.98 ± 32.54	139.85 ± 21.50	70.3759 ± 17.8901	54.08 ± 11.1505	
(ms)	(40.50–170.50)	(99.00–170.50)	(46.00–107.80)	(40.50–85.50)	
F_{max}	19.67 ± 17.50	4.49 ± 2.22	13.91 ± 12.30	34.48 ± 17.25	
(mN)	(1.60–81.80)	(1.60–11.00)	(2.10–70.00)	(5.90–81.80)	
Area	431.65 ± 345.67	218.25 ± 104.88	330.03 ± 274.88	669.69 ± 375.51	
(mN.ms)	(50.86–1901.00)	(88.10–458.56)	(50.86–1544.00)	(90.89–1901.00)	
V_{max}	2.50 ± 2.35	0.32 ± 0.17	1.70 ± 1.56	4.59 ± 2.26	
(mN/ms)	(0.10–9.70)	(0.10–0.88)	(0.25–9.08)	(0.87–9.70)	
V_{min}	−1.10 ± 1.11	−0.10 ± 0.06	−0.69 ± 0.69	−2.11 ± 1.09	
(mN/ms)	(−4.36– − 0.03)	(−0.28– − 0.03)	(−3.51– − 0.08)	(−4.36– − 0.45)	

0.5. One may also notice that some of the weakest fast MUs had similarly low F_{max} of their twitches as S MUs (Fig. 2c versus b).

Similar conclusions concerning regular distribution of MUs properties in the whole population can be drawn from the analysis of the contractile speed (Figs. 2e–h). However, the S MUs had much lower mean velocities of force increase and decrease than the fast ones, and only slight overlapping between them was observed (see also Table 1). It is worth mentioning that the maximum velocities of force increase for the whole population were higher than the maximum velocities observed during relaxation (Table 1), i.e., V_{max} for all MUs was higher than the absolute value of V_{min}.

To see more clearly the differences between S, FR and FF MUs, all twitches were normalized with respect to the maximum twitch force (Fig. 3a–d), and with respect to both the maximum force and the contraction time (Figs. 3e–h). Figs. 3a–d show that in general the contraction time, the half-relaxation time and the duration of the twitch are the longest for S MUs and the shortest for FF MUs. Moreover, the differences between particular MUs' types were better exhibited for T_{hr} than for T_c. Fig. 3e demonstrates that the interrelations between the contraction and half-relaxation times are also different for particular types of MUs (see also in [29]). Twitch shapes with normalized force and time are displayed on the outside part of the bell-shaped chart for the majority of S units, while curves of FF units are predominantly on the inside part of the chart. These differences in the shape of the twitches for the three types of MUs were confirmed by shorter half-contraction and longer half-relaxation times calculated for these normalized twitches (nT_{hc} and nT_{hr}, respectively, see arrows in Fig. 3h) for S MUs in comparison to fast ones. Similar relations were observed between these parameters for FR and FF MUs. The normalized half-contraction time, nT_{hc} (see Fig. 3h), is the time when the normalized twitch force reaches value of 0.5. The normalized half-relaxation time, nT_{hr}, is the time when during relaxation the twitch force decreases to the same value (as shown in Figs. 3f–h by horizontal lines). The mean values (±SD) and variability ranges of values of nT_{hc} (expresssed in percents of the normalized contraction time) amounted to: 36 ± 5.4% (29–51%) for S units, 43 ± 6.5% (31–55%) for FR units, and 49 ± 5.4% (36–56%) for FF units. The respective values of nT_{hr} were 120 ± 17.5% (74–142%) for S units, 98 ± 18.6% (66–136%) for FR units, and 81 ± 13.6% (63–115%) for FF units.

The main calculated twitch parameters, sorted for all studied MUs in an ascending order (Fig. 4), showed a continuous distribution that was not linear, but rather exponential. The same referred to T_{tw} (not shown by figure). Since T_{lead} changed in a

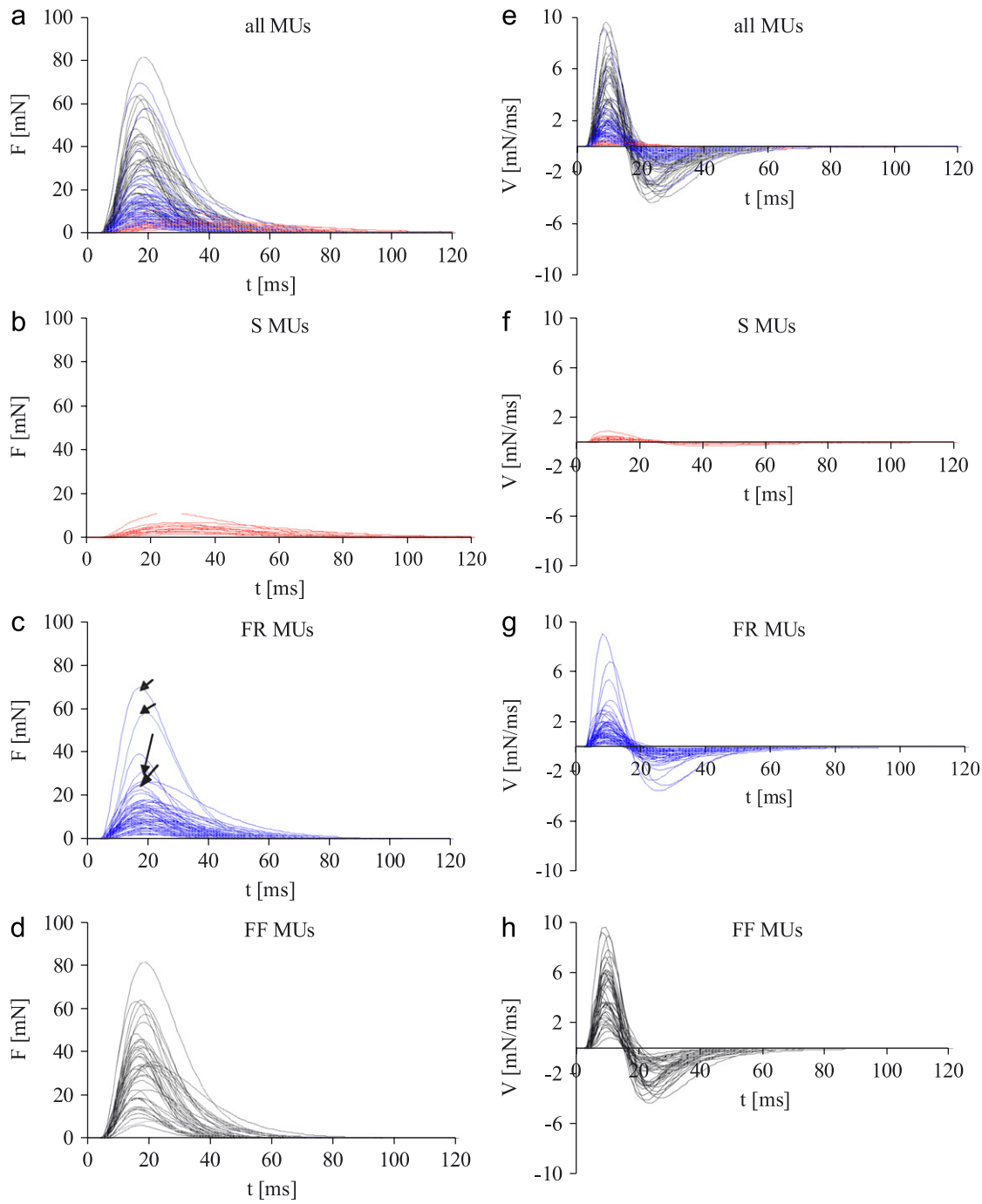


Fig. 2. Individual twitches (left panel) and corresponding velocities of force increase and decrease (right panel) for all MUs studied (a, e), and separately for S MUs (b, f), FR MUs (c, g) and FF MUS (d, h). Note that the four MUs, indicated by arrows in Fig. 2c, had intermediate contractile properties, i.e., relatively high force and fatigue index that was between 0.5 and 0.55 (see in the text).

very narrow range (from 3.0 to 5.9 ms), reasonable conclusions about its distribution were impossible. As mentioned earlier, S MUs had longer T_c and T_{hr} in comparison to fast MUs, and these parameters could be successfully used for division of MUs into fast and slow types (Fig. 4a and b). However, despite S MUs are usually considered weaker, there are no strict division with respect to F_{max} between them and FR MUs (Fig. 4c). The same concerns values of the area under force records (Fig. 4d) as well as maximal and minimal values of contractile speed during contraction and relaxation phases (Figs. 4e and f). For all parameters, presented in Fig. 4, considerable overlapping was observed between FR and FF units. The best approximation with an exponential function proved to have the following form: $f(n) = a + b * \exp(c * n)$. Here n is the number of MUs and a, b and c are constants. The values of these constants are given in Table 2. The Levenberg–Marquardt

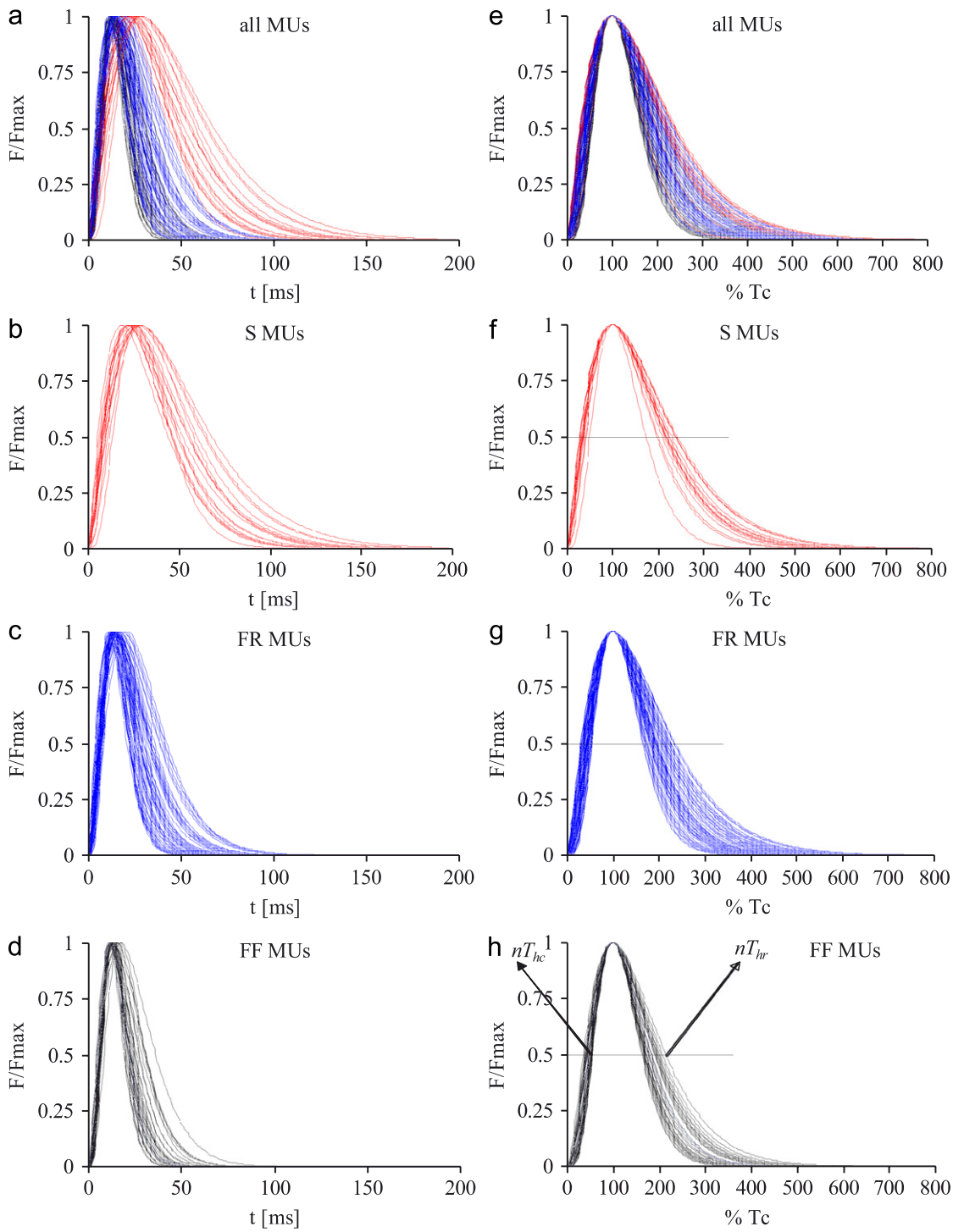


Fig. 3. Two types of normalization of twitches. Left column—the contraction force of each MUs is divided to its maximal value and the lead time is zero, right column—the force of each MU is divided to its maximal force, the lead time is zero, the contraction time is assumed to have value of 100 for all MUs and the corresponding half-relaxation time is calculated as $100T_{hr}/T_c$. (a, e)—all MUs studied, (b, f)—S MUs, (c, g)—FR MUs, (d, h)—FF MUs. The horizontal lines in Figs. 3f–h show one-half of normalized force, at which the normalized half-contraction (nT_{hc}) and the normalized half-relaxation (nT_{hr}) times (shown by arrows in Fig. 3h) were determined.

[30,31] algorithm was used for calculation of the best approximation.

The comparison of the main parameters calculated by using the modeled twitches (Fig. 5) showed near linear relationships between T_c and T_{hr} and between F_{max} and *Area*. The division of slow from fast MUs was apparent from the first (Fig. 5d) and, less clearly, from the second relationship (Fig. 5c). However, the separation of the two types of fast MUs was impossible

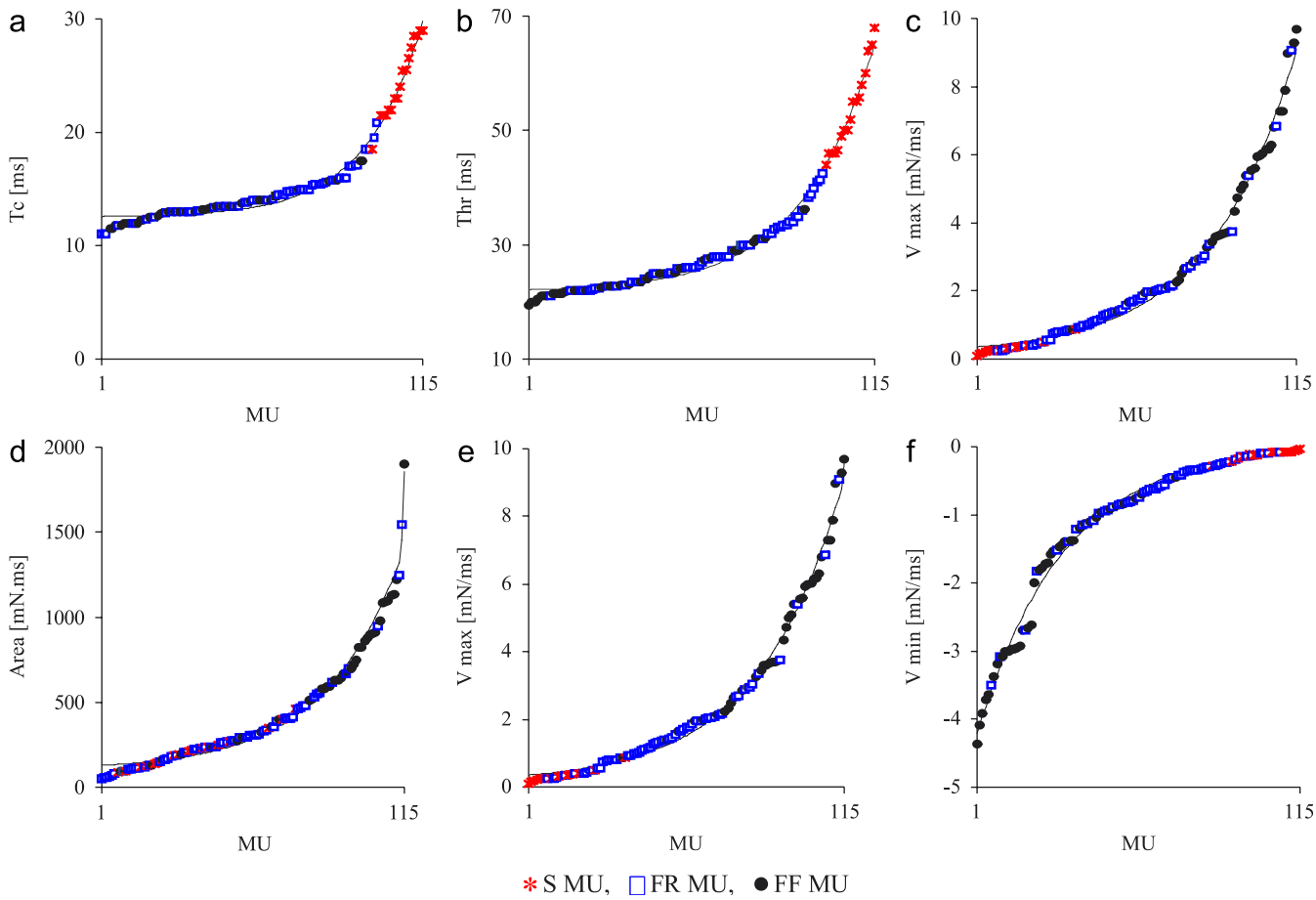


Fig. 4. Distribution of the twitch parameters arranged for all MUs in an ascending order: (a) the contraction time (T_c); (b) the half-relaxation time (T_{hr}); (c) the maximal twitch force (F_{max}); (d) the force-time area (*Area*); (e) the maximal velocity of force increase during contraction (V_{max}); and (f) the minimal velocity of force decrease during relaxation (V_{min}). The best exponential function fitting the correspondent distribution is shown by a solid black line.

Table 2
Constants of the exponential function $f(n)=a+b*\exp(c*n)$ that approximates best the distribution shown in Fig. 4, where n is the number of MUs

	a	b	c
T_c (ms)	12.5098	0.0594	0.0498
T_{hr} (ms)	21.7101	0.3705	0.0418
F_{max} (mN)	2.3061	1.5124	0.0332
Area (mN.ms)	102.6838	25.5941	0.0345
V_{max} (mN/ms)	0.1389	0.2136	0.0328
V_{min} (mN/ms)	0.0151	−4.2310	−0.0328

using such reasons. Relationships between the maximal twitch forces and the time parameters (Figs. 5a and b) or between the area under the twitch records and the time parameters (Figs. 5e and f) were of the L-like shape, showing a regular distribution of all types of MUs, with overlapping slightly noticeable between S and FR types and better exhibited between FR and FF units.

4. Discussion

In the present study, the previously proposed four-parameter model of a single twitch [5] has been validated for a large group of 115 MUs, representative for the whole population in the muscle studied. This sample exceeds the number of MUs in MG muscle in rats. Previous reports [32–34] have estimated this number as smaller than 100 (80–98). The proportions of particular types of MUs in the population investigated are also close to the proportions actually found in the MG muscle [35–37]. The study gave evidence that the mean values and the ranges of all main parameters of MUs individual contractions (twitch contraction time, half-relaxation time, maximum twitch force, area under the twitch record) calculated from the modeled twitches, either for the whole population or for particular types of units (S, FR and FF, see Table 1), are in agreement with the experimental data obtained previously from the rat MG MUs by various research groups [3,35,37,38].

A question arises why should the model, based on few twitch parameters, be used instead of direct calculations from the experimental records. Firstly, the model is suitable because experimental artifacts and noise can be eliminated, and this refers mainly to the low-force MUs (predominantly slow ones). Secondly, some contractile properties may be more easily calculated from the modeled twitches rather than directly from the experimental records. This advantage concerns the velocities of contraction and relaxation, the area under the force

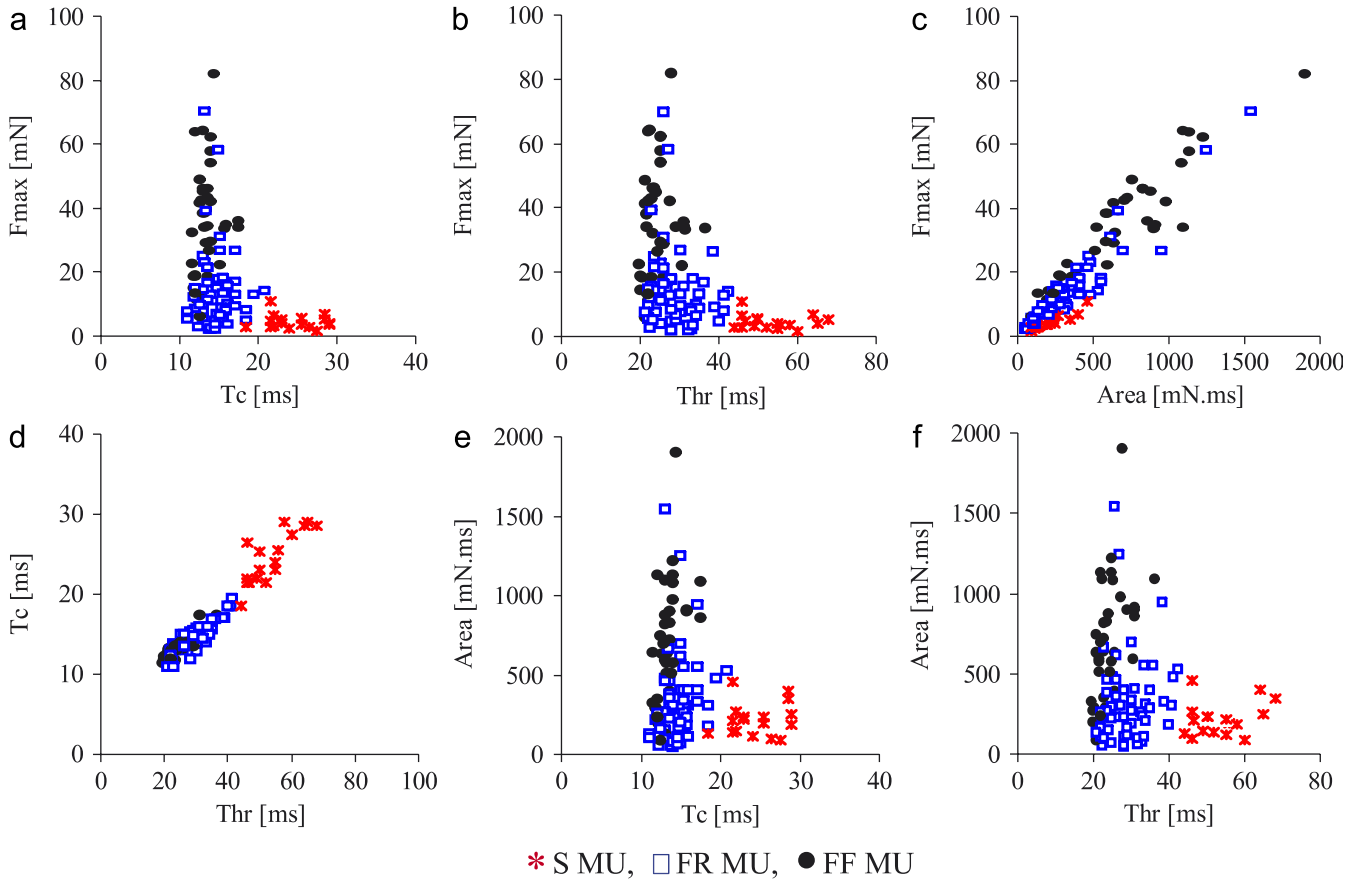


Fig. 5. Interrelations between twitch parameters for all 115 MUs. T_c—the contraction time; T_{hr}—the half-relaxation time; F_{max}—the maximal twitch force; *Area*—the force-time area.

records, the normalization of the twitches. The well known problem with filtering experimental data for the sake of precise numerical differentiation is avoided. So far, the calculations of force's rate of change during single MUs contractions (Figs. 2e–h) are missing in experimental investigations of MUs. They describe well the differences between the contraction and relaxation phases, as well as the variability of MUs with respect to the shape of the twitch. The normalization of the twitches (see Fig. 3) enables us to analyze relative differences in shapes and time courses. Finally, a precise model of a single twitch form a reliable basis for modeling MU tetanic contractions or summation of forces of numerous MUs during whole muscle activity.

In models that investigate the control of a muscle composed from many MUs [4,5,21] it is usually accepted that S MUs are weaker than FR and FF MUs. As shown in Fig. 4a, this is true to a certain degree only (see also Fig. 2b versus c). This disagreement is not essential if fatigue resistance is not important for the model, since the division of the MUs into different types is somehow arbitrary (see [12]). In the muscle model proposed in Raikova and Aladjov [5] it has been assumed that all twitch parameters are linearly distributed from a minimal to a maximal value. As evident from Fig. 4, this distribution is exponential (see also [4]). The exponential analytical functions that best approximate this distribution (Fig. 4) can help further to refine muscle models.

The analysis of the big sample of MUs in this modeling study confirmed the variability of the twitches, stressing on the differences between MUs types with respect to calculated parameters, and on the other hand, the continuous distribution without clear separation between MUs' types. The rat MG muscle was considered a fast one with a relatively low proportion (15% of all population) of S MUs. It was shown, however, that all units form a heterogeneous and continuous population, as this was previously reported by other authors [13,16,17] (see Fig. 2a). Fig. 4 shows an exponential distribution of the maximum force, area and contractile speed, and a clear division between slow and fast units based on T_c and T_{hr}, but not using other parameters. The relationships between MUs parameters (Fig. 5) also have shown that slow units can be differentiated from fast ones using twitch time parameters. However, it has been impossible to distinguish FF from FR MUs on the basis of contractile properties only. This can be done using ability to fatigue, hence fatigue index.

On the other hand, our study has also confirmed that the mean values of the maximum twitch forces well describe differences between the types of the MUs, despite some overlapping (see Table 1 and Fig. 2). S MUs have in general the lowest twitch forces, while FF MUs—the highest. Ker-

nell and Eerbeck [20] have stressed that the force of MUs changes gradually from slow, through fast resistant, fast intermediate, to fast fatigable. In fact, four of MUs classified in our study as FR, with relatively high twitch forces and fatigue indices near the border value for the division between fast units (Fig. 2c), have rather intermediate values of several contractile properties.

One should ask the question whether it is reasonable to divide MUs into three or four categories, especially if all main contractile parameters are continuously distributed and only few of them can be used for clear differentiation between various types? Burke in his review essay [12] has discussed this problem on several levels, from biochemical to physiological, yet without conclusions. In our study, we have also found contradictory evidences. However, the analysis of the normalized twitches has indicated variability with respect to twitch time courses between the MUs types. The most pronounced differences concerned slow and fast MUs and they had been less obvious between FR and FF units. Such differences in the course of the twitch have been previously reported for slow and fast MUs of the MG muscle in the rat when compared mean ratios between contraction and half-relaxation times (0.66 for S, 0.9 for FR and 1.07 for FF MUs, [37]). Similarly, longer relaxation phase in comparison to the contraction phase has been observed for all types of studied MUs (see Figs. 3a–d). Moreover, the analysis of the half-contraction and half-relaxation times calculated from the normalized twitches has shown that slow units have relatively shorter initial phase of contraction and relatively longer initial phase of relaxation in comparison to fast units (see Figs. 3e–h). These differences in the twitch shapes may have functional implications. They point on the relatively more effective summation of S MUs in comparison to fast MUs during tetanic contractions, as this has been previously demonstrated in muscles of various species [37]. Based on the present results, such higher relative effectiveness of S MUs seems to be caused by different time course of contraction and relaxation phases of the twitch along with its longer total duration. As previously noticed, the relative area under the contraction force is bigger for S MUs than for FR ones, so the work performed by them is more effective [3,39]. This observation supports previous hypothesis that S units play more significant role during contractions than could be estimated from their low twitch forces.

In summary, we should stress that: (1) the four-parameter twitch model (with lead, contraction and half-relaxation times and the maximal force as inputs) described well variable individual contractions of all MUs types; (2) all twitch parameters were continuously distributed and were not comprised within distinctive ranges for separate MUs types; these parameters overlapped for various types of MUs and their distribution was not linear, but exponential; (3) it was possible to distinguish slow from fast MUs on the basis of some twitch parameters (contraction and half-relaxation times, velocity of force increase and decrease), but the same could not be done for FF and FR MUs; (4) the analysis of the normalized twitches demonstrated differences in the shapes of particular types of MUs, thus showing that twitches could not be obtained from one standard pattern scaled in time and force.

Acknowledgments

This work was supported by a grant from the Polish Ministry of Science and Informatization and the bilateral agreement between Bulgarian Academy of Sciences and Polish Academy of Sciences enabled the cooperation of two research groups.

Appendix

The analytical function describing the twitch, hence the force F that a MU develops in response to a stimulus in zero moment, as function of time t, has the form:

$$F(t) = pt^m \mathrm{e}^{-kt},$$

where

$$p = F_{\max}\mathrm{e}^{-kT_c(\ln T_c - 1)}, \quad m = kT_c;$$

$$k = \frac{\ln 2}{-T_c \ln(T_{hr}/T_c) + T_{hr} - T_c}.$$

For explanation of the parameters $F_{\max}$, T_c, T_{hr} refer to the text.

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Rositsa T. Raikova was born in Shoumen, Bulgaria in 16 October 1955. She received her Ph.D. in Biomechanics in 1993. She then worked in the Institute of Mechanics and Biomechanics at the Bulgarian Academy of Sciences. Now she is an Associate Professor at the Centre of Biomedical Engineering, Bulgarian Academy of Sciences. Her research interests are in the field of biomechanics and motor control of the human limbs

Piotr Krutki was born in Poland in 1967. He graduated Karol Marcinkowski University School of Medical Sciences in Poznań (1992), he received a Ph.D. degree (1997) and the habilitation in neurophysiology from the Nencki Institute of Experimental Biology in Warsaw (2001). Since 2003, he has been an Associate Professor at the Department of Neurobiology, University School of Physical Education in Poznań. His main fields of research are: spinal neuronal networks, mechanisms of motor control, motor units and plasticity of the neuro-muscular system.

Hristo T. Aladjov was born in Sofia (Bulgaria) on 5 September 1975. He graduated in Computer Engineering in 1998, later he received his Ph.D. in Artificial Intelligence in 2002. Since 1998, he has worked in the Centre of Biomedical Engineering, Bulgarian Academy of Sciences as Research Assistant. His major research interests are related to the application of the methods of artificial intelligence for modeling in biology and particularly in motor control and biomechanics. He is also interested in new algorithm development and their implementation.

Jan Celichowski was born in Poznań, Poland in 1960. He received an M.Sc. degree from A. Cieszkowski University School of Agriculture (1983), a Ph.D. degree (1989) and the habilitation in neurophysiology from Nencki Institute of Experimental Biology in Warsaw (1996). Since 1997 he has been Professor of Neurophysiology, and since 2000 the Head of the Department of Neurobiology at the University School of Physical Education in Poznań. His main fields of research are: motor units' contractile properties and action potentials, plasticity of the neuro-muscular system, mechanomyography.