Dynamic changes of twitchlike responses to successive stimuli studied by decomposition of motor unit tetanic contractions in rat medial gastrocnemius

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The force of a skeletal muscle is a result of summation of forces generated by numerous coactive motor units (MUs) and mechanical activity of each MU evoked by trains of action potentials generated by a motoneuron at variable time intervals (Adam et al. 1998; Boe et al. 2005; Masuda and De Luca 1991; Moritz et al. 2005; Westergaard and De Luca 2011). In general, successive MU action potentials recorded in a muscle are quite stable in amplitude and shape (Mambrino and De Luca 1983; Stålberg 2003), although small changes in duration and amplitude of MU action potentials, related to a decrease in muscle fiber conduction velocity, were also reported during 30 s of voluntary activity (Furina and Falla 2007). Contrary to this, the twitchlike responses to successive motoneuronal action potentials within a tetanic contraction are highly variable, as reported in several studies on cat (Stein and Parmiggiani 1979, 1981) and rat (MacIntosh et al. 2007) muscles. These studies have shown that the amplitude of a single muscle twitch may be significantly smaller than a twitchlike response to one of the stimuli within a train. When the same method was used for tetanic contractions of one fast and one slow MU in a rat muscle (Raikova et al. 2008), apparent differences were noted between these two MUs, with considerably more variability in time and force parameters of decomposed twitches for the slow MU. Moreover, a progressive increase in force and time parameters of the decomposed twitches was observed in parallel to a tetanus force increase. It should be stressed that the above-mentioned studies involved muscle or MU forces evoked by constant stimulation frequencies, which are not observed during voluntary activity.

In our previous papers we have studied MU contractions evoked by trains of pulses at variable interpulse intervals (IPIs), more closely resembling physiological conditions (Celichowski et al. 2008; Krutki et al. 2008; Raikova et al. 2010). A new mathematical algorithm for decomposition of a MU tetanic force into a series of twitch responses has been proposed. These studies have revealed for a small sample of slow and fast MUs significant correlations between the parameters of the successive decomposed twitches and the initial force level at which the next contraction starts. However, these studies have been limited to fast units only (Celichowski et al. 2008) or restricted to a few MUs, selected only to illustrate methods proposed in the paper (Raikova et al. 2010). Because of the inconsistent behavior of this small group of units, no common rules with respect to particular MU types could be derived.

Experiments based on constant stimulation frequencies (Celichowski et al. 2000) have revealed that for each MU type the maximum force-time area corresponding to one stimulus is reached in tetanic contractions with a force amounting to ~75% of the maximum tetanus force. However, for slow MUs absolute values of this maximum force-time area per pulse may be up to 25% higher than the respective area for fast resistant (FR) MUs, and only ~43% lower than for fast fatigable (FF) MUs, although twitch forces of slow (S) MUs are on average one-third and one-ninth of the respective forces of FR and FF MUs. This clearly indicates that some major processes of force generation are different between the three MU types. In addition, slow MUs have the lowest twitch-to-tetanus ratio (Burke 1981), and this ratio positively correlates to the twitch force within all studied populations of MUs as well as within MUs of the same type (Celichowski and Grottel 1993). These observations suggest that several biomechanical phenomena are involved in the processes of force development.
From the above-mentioned reports and the initial decomposition studies it can be hypothesized that much larger variability of responses to individual stimuli would exist for tetanic contractions of slow MUs compared with fast MUs, despite the fact that fast MUs develop higher tetanic forces. This hypothesis needs verification on a representative group of each of the three physiological types of MUs, especially because forces of slow MUs vary in a relatively narrow range whereas forces of fast units show considerably high variability (Burke 1981). It should also be emphasized that the decomposition effects have never been compared between FF and FR MUs. Therefore, in this study we analyzed unfused tetani of 30 MUs (10 MUs of S, 10 of FR, and 10 of FF type) recorded from experiments on functionally isolated MUs of the rat medial gastrocnemius (MG) muscle. The second aim of the study was to gain insight into mechanisms of generation of tetanic forces by an analysis of variability of force output in response to individual activations. The tetanic curves were decomposed into successive (twitchlike) responses to individual stimuli, and these contractions were modeled with a six-parameter analytical function. The variability of these parameters (twitch force, contraction and half-relaxation times, twitch area) was presented with their direct values and the values normalized to the first decomposed twitch both for contractions obtained for one MU and for various MU types. To enable a comparison of differences between the successive contractions within one decomposed tetanus as well as a comparison of variability of decomposed responses between MUs of the three types, a normalization procedure taking the values of parameters of individual twitch responses of each MU as 1.0 was used. Since there are still gaps in knowledge concerning force generation processes in tetani evoked at frequencies that are not constant, trains of stimuli at variable, random intervals were used.

MATERIALS AND METHODS

Ethical approval. All procedures applied within the study were accepted by the Local Ethics Committee in Poznan and conducted in accordance with the Polish Law on the Protection of Animals as well as European Union regulations. Experimental procedure. The experiments were performed on eight adult male rats of mean body weight 451.1 ± 33.8 g under pentobarbital anesthesia (60 mg/kg ip, supplemented with additional doses of 10 mg/kg when necessary). The depth of anesthesia was controlled by pinna and hindlimb withdrawal reflexes. At the end of the experiments, animals were killed by a lethal dose of anesthetic (180 mg/kg ip).

The experimental procedures were described in detail in previous papers (Celichowski et al. 2008; Raikova et al. 2007b, 2010). Briefly, initial surgical preparation concerned left sciatic nerve and MG muscle isolation and denervation of all muscles of the hindlimb except the one studied. The distal tendon of the MG was cut and sutured to connect it to a force transducer. Then a laminctomy of L2-L5 vertebrae was performed, and L4-L5 ventral roots of spinal nerves, innervating the studied muscle, were cut close to the spinal cord. Animals were immobilized with steel clamps on L4 vertebra and the sacral bone, and the hindlimb was additionally clamped by the tibia. The skin around the laminctomy was hung up to form a pool for paraffin oil, which covered the spinal cord and the spinal nerves. The hindlimb and the MG muscle were also immersed in a pool filled with paraffin oil. The animal’s rectal temperature was automatically kept at 37°C.

The functional isolation of a single MU was achieved by electrical stimulation of very thin filaments of ventral roots containing only one axon to the MG muscle. Rectangular electrical pulses (duration of 0.1 ms, amplitude up to 0.5 V) were generated by a dual-channel square pulse stimulator (Grass S88). The MU force was recorded under isometric conditions, with an inductive force transducer (compliance of the active element of 100 μm/100 mN) in a muscle stretched with a force of 100 mN (Celichowski and Grottel 1992). MU action potentials were recorded with a bipolar electrode (2 silver wires, not insulated, inserted into the middle part of a muscle perpendicularly to its long axis). The response evoked in the muscle was accepted as a single MU activity when the twitch and the action potentials were all-or-none.

After isolation of a single MU was achieved, the following protocol was performed in each case: 1) a single twitch was evoked; 2) an unfused tetanus was evoked by a 500-ms train of stimuli at 40 Hz; 3) the maximum tetanus was evoked by a 200-ms train of stimuli at 150 Hz; 4) four unfused tetani were evoked by 500-ms trains of stimuli at 40 Hz in order to potentiate the MU force; 5) tetanic contractions were evoked by trains of 41 electrical pulses at four (slow units) or five (fast units) different frequencies, at constant and then at random, variable IPIs, but with the same mean frequency. This means that for each applied mean stimulation frequency two tetanic contractions were recorded. The stimulation patterns were prepared with the random number generator (function rand in MATLAB). For slow MUs the following mean (±SE) IPIs were applied: 60.0 ± 2.6, 70.0 ± 3.5, 80.0 ± 3.4, and 100.0 ± 5.1 ms (i.e., mean frequencies were 16.6, 14.2, 12.5, and 10 Hz, respectively). For fast MUs the applied mean IPIs were 20.0 ± 1.2, 25.0 ± 1.2, 30.0 ± 1.3, 40.0 ± 1.8, and 50.0 ± 2.2 ms (i.e., mean frequencies amounted to 50, 40, 33.3, 25, and 20 Hz, respectively). For random stimulation patterns variability in the IPIs for each train of stimuli was generated randomly, but the range of variability in each case covered ±50% of the mean IPI, i.e., the respective ranges of IPIs were 10–30 ms, 12.5–37.5 ms, 15–45 ms, 20–60 ms, 25–75 ms, 30–90 ms, 35–105 ms, 40–120 ms, and 50–150 ms. Ten seconds after each random stimulation a single twitch was recorded to ensure that no major fatigue or potentiation of a MU force took place. After the above procedure was completed a standard fatigue test was performed: stimulation with trains of 14 pulses at 40 Hz repeated each second for 3 min (Burke et al. 1973). Ten-second intervals were applied between all successive steps of the above protocol and between all tetanic contractions evoked when point 5 of the protocol was performed.

For each studied MU the following parameters were calculated from their single-twitch recordings: the contraction time (from the beginning of force recording to the force peak), the half-relaxation time (from the force peak to a moment when the force decreased to half of the peak value), and the twitch force. From the 150-Hz tetanus recordings the maximum MU force was determined. The studied MUs were divided into fast and slow according to the presence of sag in fast MUs in 40-Hz tetanus and a lack of this phenomenon in slow units (Burke et al. 1973; Grottel and Celichowski 1990). The fatigue index was calculated for each MU as a ratio of the force generated 2 min after the highest force generated at the beginning of the test to the highest initial force (Burke et al. 1973; Kermell et al. 1983). The value of this index varied between 0.0 (most fatigable) and 1.0 (most resistant). The division of fast MUs into FF and FR was based on the fatigue index; for FF it was lower than 0.5, while for FR MUs it was higher than 0.5 (Kermell et al. 1983). For each unfused tetanus recorded at constant IPIs within step 5 of the experimental procedure, the fusion index was calculated as a ratio of the minimum force when the response to the last stimulus began to the peak force of the last response (Bakels and Kernell 1995; Celichowski and Grottel 1995). The relative force level was determined as a percentage of the maximum tetanus force (developed at 150-Hz stimulation).

Among 152 MUs recorded in the experiments, 10 units of each type were chosen for the present study (10 of 16 S MUs, 10 of 59 FR MUs, 10 of 77 FF MUs). The selection criteria (especially important with
were different stimulation patterns for selected MUs and different aiming to better represent the whole MU population in the muscle random selection was done: one or two first-recorded FF or FR MUs were lower for much stronger FR and FF units, so in those cases selection was based on visual estimation. The signal-to-noise ratios artifacts from the nondenervated tail and hip muscles), and theings that sporadically appeared (due to respiratory movements or were high signal-to-noise ratios and lack of artifacts in force record-ings for decomposition was described in detail by Raikova et al. (2007a) and Celichowski et al. (2008). In general, it is supposed that a tetanic force development amounts to 98.3 ± 3.1% (92–106%) of the first subtracted twitch within the analyzed tetanus. Therefore, in the results all relative measures are expressed in relation to properties of a potentiated twitch (the first decomposed twitch-shape response).

**Mathematical processing.** Each of the chosen 30 tetanic curves was decomposed into 41 successive contractions (twitches). The algorithm for decomposition was described in detail by Raikova et al. (2007a) and Celichowski et al. (2008). In general, it is supposed that a tetanic force curve developed as a result from n pulses is constituted from n contractions with twitchlike forms with different parameters (maximal force amplitude, lead time, contraction and half-relaxation times). This supposition was verified by specially designed experiments (Raikova et al. 2008) in which MUs were stimulated with a succes-

respect to the smallest group of S units with relatively low forces) were high signal-to-noise ratios and lack of artifacts in force recordings that sporadically appeared (due to respiratory movements or artifacts from the nondenervated tail and hip muscles), and the selection was based on visual estimation. The signal-to-noise ratios were lower for much stronger FR and FF units, so in those cases random selection was done: one or two first-recorded FF or FR MUs from each animal were taken for the final analysis. Additional criteria aiming to better represent the whole MU population in the muscle were different stimulation patterns for selected MUs and different twitches (with respect to their force and time course) of individual units.

To obtain similar force variability and similar fusion degree among the contractions evoked by several stimulation frequencies at constant IPIs, we selected the tetani with force levels ranging from 30% to 70% of the maximum force and with fusion indexes in the range 0.40–0.95; then in each case the respective tetanus evoked at the same mean frequency, but with random stimulation pattern, was chosen for further processing. Such force levels correspond to the steep parts of the force-frequency curves, where an increase in the stimulation rate by 1 Hz evokes on average 4.9%, 2.6%, and 1.9% force increase in relation to the maximum force for S, FR, and FF MUs, respectively (Celichowski et al. 2000). It should be stressed that the biggest changes in force as a response to temporary changes in the pattern of pulses for the three MU types have been observed for subfused contractions within this range of force level and similar fusion degree (i.e., for different MUs the mean value of force level has varied between 39% and 50% of the maximum force and the fusion index has varied in the range 0.42–0.87) (Celichowski and Grottel 2001).

The early potentiation (point 4 of the stimulation protocol) induced 9–91% increase of the twitch force for FF-type MUs, which have the highest changes in force. Under these conditions, no significant further changes in the force output were observed for the analyzed recording periods: the twitch force recorded 10 s after the chosen tetanus amounted to 98.3 ± 3.1% (92–106%) of the first subtracted twitch within the analyzed tetanus. Therefore, in the results all relative measures are expressed in relation to properties of a potentiated twitch (the first decomposed twitch-shape response).

![Graph](http://jn.physiology.org/)

**Fig. 1.** Parameters of the decomposed twitches. $F_{\text{max}}$, maximal twitch force; $T_I$, time between the stimulus time position (indicated by impulse arrow) and the beginning of force development; $T_{\text{hc}}$, time from the beginning of the contraction to the time where the force reaches one-half of its maximal value; $T_r$, time from the beginning of the contraction to the time when the force reaches its maximal value; $T_{\text{hr}}$, time from the beginning of the contraction to the moment where during the relaxation phase the motor unit (MU) force decreases to half of its maximal value; $T_{\text{tw}}$, duration of the twitch; $\text{Area}$, area under the curve of contractile force.

![Graphs](http://jn.physiology.org/)

**Fig. 2.** The decomposition method and a procedure for estimation of the parameter $T_{\text{hr}}$. The 21st contraction within the tetanic force development of a fast MU is shown. Solid line, model of the decomposed 21st twitch; dotted line, force remaining after subtraction of all preceding 20 twitches; dashed line, force remaining after subtraction of the 21st twitch. Note that 1-ms increment in $T_{\text{hr}21}$ evokes change in the model-generated force resulting in an evident difference with respect to the force recording. The accepted value for $T_{\text{hr}21}$ was 54 ms, whereas the remaining parameters of the 21st contraction were $T_{\text{I}21} = 3.9$ ms, $T_{\text{c}21} = 23.5$ ms, $T_{\text{r}21} = 6.7$ ms, $F_{\text{max}21} = 225.0$ mN, and $T_{\text{tw}21} = 120$ ms.
sively increasing number of pulses. When the force curve developed by \( (i - 1) \) pulses is mathematically subtracted from that developed by \( i \) pulses, the \( i \)th supplement of force (the \( i \)th contraction) is calculated. It was shown that these contractions had twitchlike forms (MacIntosh et al. 2007; Raikova et al. 2008; Stein and Parmiggiani 1981). For an analytical description of these twitch-form curves, a six-parameter analytical function was proposed and tested in several series of experiments to prove its ability to describe precisely all twitch forms (Raikova et al. 2008). The six parameters are (Fig. 1) the lead time \( (T_l) \); four time parameters describing times calculated from the beginning of the contraction to the force increase up to half of the peak value \( (T_hc) \), to the force peak \( (T_c) \), to half-relaxation \( (T_hr) \), and to the end of relaxation \( (T_w) \); as well as the maximal twitch force \( (F_{max}) \) (Raikova et al. 2007a, 2008; Raikova and Aladjov 2002).

The decomposition procedure consisted of the following steps: the recorded single twitch was modeled, and its six parameters were obtained; the model was used to match the first contraction in a tetanic curve, and all twitch parameters were adjusted to fit the visible part of the contraction; this model was subtracted from the tetanic force, so a part of the second contraction was visible and used for modeling of the second twitch; and these steps were repeated until the last contraction was modeled. The present analysis was made for unfused tetanic contractions. Therefore, for all decomposed twitches the contraction phase was visible, and the parameters \( T_l, T_hc, T_c, \) and \( F_{max} \) were estimated precisely. For a majority of the decomposed twitches a substantial part of the relaxation period was also visible, so \( T_hr \) was estimated with a small error, up to 1 ms, as illustrated in Fig. 2.

The exactness of the decomposition approach was checked by reconstructing the recorded tetanic force curve by mathematical summation of all models of the subtracted twitches. To estimate numerically the degree of coincidence between the modeled and experimental curves, a fit coefficient (in \% \) was calculated as follows. First, the two curves under comparison were normalized to their total maximal force level. For each discrete time the difference between the two curves under comparison were normalized to their total maximal force level. For each discrete time the difference between the two curves under comparison were normalized to their total maximal force level. For each discrete time

\[ F(t) = \frac{F(t) - F_{min}}{F_{max} - F_{min}} \]

Fig. 3. Results of the decomposition of tetanic curves for 1 slow (A–C), 1 fast resistant (FR; D–F), and 1 fast fatigable (FF; G–I) MU. A, D, and G: experimental recordings of tetanic forces obtained for constant (blue trace) and random (red trace) stimulation patterns at the same mean frequencies. The slow MU (A) was stimulated at a frequency of 16.6 Hz [mean interpulse interval (IPI) of 60 ms], the FR MU (D) was stimulated at a frequency of 40 Hz (mean IPI of 25 ms), and the FF MU (G) was stimulated at a frequency of 20 Hz (mean IPI of 50 ms). The forces shown in green in A, D, and G were calculated by summation of equal twitches (identical with the first decomposed twitch) for the same random stimulation pattern (see text for detailed explanation). B, E, and H: consecutive decomposed contractions obtained for the respective random stimulation patterns, presented according to their time position; the force levels for the first twitch and twitches with the maximum and minimum forces are indicated by horizontal dotted lines. The same contractions are presented in C, F, and I, where they are superimposed, accepting that all pulses are delivered at time 0. For B, E, and H and C, F, and I the first decomposed twitch as well as the twitches with maximal and minimal \( F_{max} \) are marked by thick lines: the first twitch is blue, the maximum force twitch is black, and the minimum force twitch is red.
curves $\Delta_i$ was calculated. The fit coefficient was expressed (in %) as

$$\text{FitCoef} = \left(100 - \sqrt{\frac{\sum \Delta_i^2}{N}}\right),$$

where $N$ is the number of samples.

When the two curves match perfectly, this coefficient amounts to 100%. The lower the fit coefficient, the bigger are differences between the curves under comparison. The same coefficient was calculated for estimation of differences between the experimental curve (red curves, Fig. 3, $A$, $D$, and $G$) and the force curve obtained by summation of equal contractions (identical with the first decomposed twitch) with the same stimulation pattern (green curves, Fig. 3, $A$, $D$, and $G$).

The above-mentioned six parameters of the decomposed twitches of all studied tetanic contractions of FF, FR, and S MUs, as well as the force-time areas under the twitch forces (parameter $A$; Fig. 1) were analyzed in order to reveal the differences between the three types of MUs and to estimate the mechanical output evoked by individual stimuli at variable intervals. Since the parameter $T_l$ has a stable value and $T_t$ cannot be estimated with a high degree of precision, because exponential functions approach zero value slowly, only the statistics of the remaining four parameters of the twitch, i.e., $T_h$, $T_c$, $T_r$, and $F_{max}$, and $A$ area are presented in this report.

Finally, a coefficient of force variability within the tetanic curves was calculated for all chosen MUs of either type. It represents the relative force variation around a mean value and is formulated as follows (see Fig. 4):

$$\text{VarCoef}_i = \frac{\text{delta}_i(i)}{k_j}$$

where $i$ is the number of the successive contraction ($i = 1, 2, \ldots, N$, $N = 41$), $j$ is the number of the MU ($j = 1, 2, \ldots, 30$), $\text{delta}_i(i) = \text{TetMax}_i(i) - \text{TetMin}_i(i)$, $k_j = \sum_{i=1}^{N} \text{delta}_i(i)/N$, and $\text{TetMax}_i(i)$ is the peak force of the response to the $i$th stimulus whereas $\text{TetMin}_i(i)$ is the force level at the beginning of a response to the $i$th stimulus.

### RESULTS

The basic contractile properties of the studied MUs (Table 1), which are calculated from the single twitch of each MU (step 1 of the experimental procedure), are within ranges observed for large groups of respective types of MUs in the rat MG (e.g., Celichowski and Drzymala 2006). The properties of the MU tetani evoked at the same but constant stimulation frequency as the decomposed tetani, i.e., the fusion index and the relative force level, are given in Table 2. It is apparent that for the three types of MUs the ranges of the presented properties of studied tetani have similar values. Moreover, the force oscillations within the studied tetanic contractions for all 30 MUs were illustrated by the coefficient of force variability calculated according to Eq. 1 (Fig. 5). Figure 5 indicates that despite different stimulation frequencies and patterns applied for individual MUs, the range of normalized force variations in all tetanic curves investigated was very similar, so all recordings selected for the decomposition were comparable independently of MU type and stimulation pattern.

The decomposition of all 30 tetanic curves was made with sufficient accuracy since the fit coefficients between the experimental and modeled curves calculated by summation of the decomposed twitches amounted on average to 98.68% (98.38% for slow MUs, 99.02% for FR MUs, and 98.65% for FF MUs). The lower fit coefficients for slow MUs can be explained by a higher influence of noise since they are generally much weaker than fast MUs.

The fit coefficients between the experimental curves and those obtained by summation of equal twitches with the same stimulation pattern were considerably lower, amounting on average to 70.59% (52.57% for slow MUs, 79.24% for FR MUs, and 79.97% for FF MUs). It was evident that approximation with equal twitches is more problematic for slow MUs (Fig. 3, $A$, $D$, and $G$, green curves vs. red curves).

There was a visible difference between slow and fast MUs with respect to variability of the decomposed twitches in their

### Table 1. Basic contractile properties of studied MUs calculated for single twitch, maximum tetanic contraction, and fatigue test

<table>
<thead>
<tr>
<th>MU Type</th>
<th>Contraction Time, ms</th>
<th>Half-Relaxation Time, ms</th>
<th>Twitch Force, mN</th>
<th>Maximum Tetanus Force, mN</th>
<th>Fatigue Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF ($n = 10$)</td>
<td>$13.3 \pm 1.5$</td>
<td>$15.8 \pm 3.3$</td>
<td>$95.6 \pm 56.4$</td>
<td>$289.2 \pm 118.4$</td>
<td>$0.22 \pm 0.13$</td>
</tr>
<tr>
<td>FR ($n = 10$)</td>
<td>$13.4 \pm 1.3$</td>
<td>$15.7 \pm 3.4$</td>
<td>$49.7 \pm 34.2$</td>
<td>$196.7 \pm 105.8$</td>
<td>$0.75 \pm 0.15$</td>
</tr>
<tr>
<td>S ($n = 10$)</td>
<td>$27.9 \pm 5.0$</td>
<td>$43.1 \pm 13.0$</td>
<td>$5.4 \pm 1.8$</td>
<td>$56.7 \pm 16.2$</td>
<td>$0.98 \pm 0.02$</td>
</tr>
</tbody>
</table>

Data are means ± SD. FF, fast fatigable motor units (MUs); FR, fast resistant to fatigue MUs; S, slow MUs.

### Table 2. Properties of decomposed tetanic contractions

<table>
<thead>
<tr>
<th>MU Type</th>
<th>% of maximum tetanus force</th>
<th>Fusion Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF ($n = 10$)</td>
<td>$56.2 \pm 9.0$</td>
<td>$0.82 \pm 0.05$</td>
</tr>
<tr>
<td>FR ($n = 10$)</td>
<td>$45.2 \pm 10.2$</td>
<td>$0.66 \pm 0.14$</td>
</tr>
<tr>
<td>S ($n = 10$)</td>
<td>$54.4 \pm 8.6$</td>
<td>$0.83 \pm 0.07$</td>
</tr>
</tbody>
</table>

Data are means ± SD, with variability ranges in parentheses. Relative force level and fusion index were calculated for the unfused tetani of MUs stimulated at constant stimulation frequencies, which were identical to the mean frequencies of decomposed tetani evoked at random stimulation patterns.
The contraction time of the decomposed twitches was evidently longer for slow MUs than for fast MUs, but for all MUs it was prolonged in relation to the first decomposed twitch, with only a few exceptions (Fig. 6C). This prolongation was more pronounced for slow MUs, in which the longest decomposed twitches had 3.52 times longer Tc than the first twitch. There were no differences between FR and FF MUs with respect to this parameter—the ratios of the Tc of the longest to the first decomposed twitches were 1.43–2.07 for FR and 1.54–2.46 for FF MUs (Fig. 6D). Similar observations were noted for the remaining twitch time parameters, i.e., Thc and Thr, in all MU types. Note that in Fig. 6, E and F, the parameter (Thr − Tc) is shown since this time period better characterizes the course of the relaxation phase (see Fig. 1) and corresponds to the half-relaxation time classically determined for single-twitch recordings. In general, the time parameters of the decomposed twitches were the shortest for the first twitch for almost all slow MUs and for a majority of fast MUs; however, a decrease below the values of the first twitch was observed for some of the decomposed twitches of fast MUs, especially of the FR type. The time for half-relaxation as shown in Fig. 6E was obviously larger for slow MUs but comparable for FF and FR MUs. Surprisingly, the distributions of relative changes of this parameter were similar for all MU types (Fig. 6F).

**DISCUSSION**

This study presents a comparison of decomposed twitchlike responses within an unfused tetanus to successive stimuli for representative groups of the three MU types. The most striking and evident result was the difference obtained between slow and fast MUs, but the decomposition also revealed some substantial, and not previously reported, differences between FR and FF types of MUs.

A very large range of variation of the decomposed twitchlike responses to successive stimuli was found in all S MUs (we observed even 14 times difference in the force-time area and ∼7 times difference in the force amplitude in relation to the first decomposed twitch). These results are not in agreement with the effects observed in slow cat soleus muscle by Stein and Parmigiani (1981), who observed much smaller variability of the decomposed twitches. For this muscle, stimulated with four stimuli at 100-Hz frequency, an approximate doubling in force of the second twitch has been noted, whereas for further twitches (3rd, 4th) even smaller amplitudes but longer durations have been observed. This result is probably an effect of high applied stimulation frequency, which evokes a maximum, fused tetanus. Studies of force generation at different stimulation frequencies revealed that the frequency had a very high influence on mechanical output. In rat MG muscle it was observed for the three MU types that when the force-time area corresponding to one pulse was measured at different stimulation frequencies, this parameter first increased with the increase of the stimulation frequency, achieved the maximum value when the force reached ∼75% of the maximum tetanus force, and then decreased (Celichowski et al. 2000).

In male rat MG muscle fast MUs dominate and slow units generate only 5% of the total muscle force (Celichowski and Drzymała-Celichowska 2007). The decomposition of fast MU tetanic contractions reveals considerably smaller variability of area and force.
the properties of the consecutive contractions in relation to slow units. The range of forces of the decomposed twitches (up to ~3 times higher than a single twitch) found in the present study corresponds to the variability previously observed by MacIntosh et al. (2007) for the whole rat MG muscle in a study in which the sciatic nerve was stimulated, i.e., fast and slow MUs were activated in parallel.

Comparison of the decomposition effects between FF and FR MUs also shows important differences. First, the amplitudes of the decomposed twitches for FF MUs are very similar
to the amplitude of the respective first decomposed twitch, and even for the majority of studied FF MUs numerous decomposed twiches have lower force than the first twitch (Fig. 6B). Such a situation has been observed considerably less frequently for FR MUs. This difference may partly be related to slightly lower twitch-to-tetanus ratios for FR MUs (Burke et al. 1973; Celichowski and Grottel 1993; Stephens and Stuart 1975) and/or to previously observed differences in profiles of unfused tetani of FR and FF motor units in rat MG muscle: for FR MUs the responses to five to seven stimuli within tetanic contractions summate very effectively, evoking an early force increase followed by a decrease of the force (sag), whereas in FF MUs the initial force summation is less visible (Carp et al. 1999; Celichowski et al. 2005).

There are several possible reasons for the observed high variability of the decomposed twiches of slow MUs. First, the amount of Ca$^{2+}$ ions accumulating in the cytosol can progressively increase with the development of contraction force (Pasterino and Lamb 2003), and, consequently, any Ca$^{2+}$ bound to troponin C will allow amplification of the force response to the addition of more Ca$^{2+}$. Second, the altered overlapping between actin and myosin filaments when contractions develop may influence the number of active cross bridges. This mechanism may depend on the MU twitch force. It can be expected that a weak contraction would moderately increase the overlapping, potentially contributing to an increased amplitude of the decomposed twiches. On the other hand, a strong initial contraction would immediately bring the sarcomere length to a position on the plateau of the force-length relationship, eliminating this mechanism for subsequent contractions (as was observed in the fast-twitch MUs). Third, a biomechanical mechanism should also be taken into account. Slow MUs are composed of a lower number of muscle fibers (Kanda and Hashizume 1992), which are distributed in a proximal compartment of a muscle that has shorter muscle fibers (Zuurbier and Huijing 1993). Hence slow MU forces are transmitted by longer elastic structures of connective tissue and have lower density compared with fast units (Burke 1981). This may create a situation in which during a single twitch a part of the force of these MUs is damped. When the contractile force increases and in-series elastic elements within a muscle are stretched, the twiches become progressively stronger. This effect is likely considerably dependent on muscle structure, so studies of decomposition effects for muscles with architecture (parallel, pennate) different from the MG (which is unipennate) are necessary to draw more general conclusions concerning the variability of twitch responses. On the other hand, for the strongest FF MUs the decomposed twiches were predominantly weaker than the single twitch. This effect probably has a biomechanical background, i.e., very strong single twiches are probably not damped, as was additionally reflected by the high twitch-to-tetanus ratio for the strongest FF MUs (Celichowski and Grottel 1993).

Since the present study is based on tetanic curve decomposition it has an impact on more comprehensive understanding of MU physiology. It has been revealed that the mechanical aftereffect of each motoneuronal action potential may vary over a large range, especially for slow MUs. During voluntary activity motoneurons generate series of action potentials, and their pattern is usually studied in human experiments by means of decomposition of the electromyogram into corresponding series of MU action potentials (Adam et al. 1998; Boe et al. 2005; Moritz et al. 2005; Westergaard and De Luca 2011). Each action potential has a relatively stable shape and amplitude, but as shown in this study, the contractile aftereffects are not the same, especially for low-threshold (slow) MUs, for which they are highly variable. The method of analysis of tetanic contractions used seems to be useful in further studies of functional consequences of increased and decreased MU activity or fatigue effects (Orizio et al. 2004). It is possible that a decomposition of tetani applied in the experimental models of MU plasticity might at least partly explain force generation related to increased activity (e.g., several forms of training) or decreased activity (e.g., spinal cord injuries, aging processes, deafferentation).

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