RESEARCH ARTICLE

The tetanic depression in fast motor units of mammalian skeletal muscle can be evoked by lengthening of one initial interpulse interval

J. Celichowski · Z. Dobrzyńska · D. Łochyński · P. Krutki

Abstract A lower than expected tetanic force (the tetanic depression) is regularly observed in fast motor units (MUs) when a higher stimulation frequency immediately follows a lower one. The aim of the present study was to determine whether prolongation of only the first interpulse interval (IPI) resulted in tetanic depression. The experiments were carried out on fast MUs of the medial gastrocnemius muscle in cats and rats. The tetanic depression was measured in each case as the force decrease of a tetanus with one IPI prolonged in relation to the tetanic force at the respective constant stimulation frequency. Force depression was observed in all cases studied and was considerably greater in cats. For cats, the mean values of force depression amounted to 28.64% for FR and 10.86% for FF MUs whereas for rats 9.30 and 7.21% for FR and FF motor units, respectively. Since the phenomenon of tetanic depression in mammalian muscle is commonly observed even after a change in only the initial interpulse interval within a stimulation pattern, it can effectively influence processes of force regulation during voluntary activity of a muscle, when motoneurones progressively increase the firing rate.

Keywords Tetanic depression · Motor units · Medial gastrocnemius · Rat · Cat · Interpulse interval

Introduction

The force of motor units (MUs) can be regulated by changes in frequency and the pattern of motoneuronal firing. In the majority of experimental studies concerned with muscle force, constant stimulation frequencies have been applied (Celichowski and Grottel 1995b; Gardiner and Olha 1987; Gordon et al. 1990a, b) although the influence of irregular stimulation pattern on the MU force development has also been analyzed in several papers (Burke et al. 1970, 1976; Celichowski et al. 2008; Grottel and Celichowski 1999; Krutki et al. 2008). It is well known that shortening of the first interpulse interval (IPI) in a train of pulses considerably influences the MU force development: the catch-like effect (a force increase) occurs (Burke et al. 1970, 1976; Celichowski and Grottel 1998) when motoneurons begin their activity with two firings in a short interval (a doublet) (Bigland-Ritchie et al. 2000; Hennig and Lømo 1985). However, the effects of lengthening of the first IPI have not been studied, although it is known that a train of a low-frequency stimuli at the beginning of MU activity evokes a reduction of the force in the following part of a tetanic contraction in relation to respective constant-frequency stimulation. Such a depressive effect, called the tetanic depression, concerns fast MUs when a higher stimulation frequency immediately follows a lower one. This phenomenon has been documented in the medial gastrocnemius muscle for either rat (Celichowski 2001) or cat (Celichowski et al. 2004; Celichowski 2005). A similar effect of force depression has been demonstrated for MU tetanic contractions in which stimulation frequency linearly increases and then is kept at a constant level; the tetanic force at this constant phase of stimulation is always lower than the force generated at the respective fixed frequency (Łochyński and Celichowski 2009).

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It has been well documented that during voluntary movements, motoneurones generate successive action potentials at variable IPIs (e.g. Conwit et al. 1999; Masakado et al. 1995; Masuda and De Luca 1991). A parallel increase in muscle force and a MU firing rate has been observed in several human muscles: first dorsal interosseous (Adam et al. 1998; Boe et al. 2005; Moritz et al. 2005; Westergaard and De Luca 2011), anconeus and triceps brachii (Le Bozec and Maton 1987) or rectus femoris (Person and Kudina 1972). De Luca et al. (1982) have observed in human deltoid muscle sharp rising in MUs' firing rates just after their recruitment and a subsequent little increase in firing frequency with the increasing force. Although the shortening of the second and next IPIs in relation to the first one has not been directly analyzed, it has been documented that the first IPI can be twice as long as that the following ones (Merletti et al. 2008, Fig. 6). Similar examples of this kind of MU behavior (the first IPI 2–3 times longer than the following ones) have also been provided by De Luca et al. (1993) for several MUs in the first dorsal interosseous muscle during a phase of force increase. Person and Kudina (1972) have revealed considerable (even by about 50%) increase in firing frequency of active motoneurones parallel to the force increase, showing that the mean frequency of the first three IPIs is longer than those 0.5 s later. However, the functional consequences of these changes in the IPIs for the force development still need to be analyzed.

Therefore, to approach this problem, we addressed the question: Can the phenomenon of tetanic depression be observed following the smallest possible changes in MU stimulation pattern, i.e., a prolongation of only one IPI? The main purpose of experiments described in this paper was to find out whether a brief decrease in the instantaneous stimulation frequency, by the prolongation of the first IPI in a train of constant-frequency pulses, evokes a depression of the tetanic force. The method of functional isolation of MUs of medial gastrocnemius muscle was applied to cats and rats, two commonly investigated species, to determine whether the observed force modulations caused by changes in activation pattern follow any general rules, despite morphological and physiological interspecies differences.

Materials and methods

The research was performed on adult mammals of the two species, cats and rats (female cats weighing 2.2–3.0 kg, and *Wistar* female rats weighing 240–280 g).

Cats were initially anesthetized by ketamine hydrochloride (30–40 mg/kg, i.m) for the surgery, and anesthesia was sustained during the next stages of experiments by

α-chloralose (5–7 mg/kg, i.v., supplemented as required, up to a total dose of 50–60 mg/kg). The depth of anesthesia was controlled by observation of withdrawal reflexes, diameter of pupils and by monitoring of the heart rate and systolic blood pressure (kept between 90 and 130 mm Hg). Rats were anesthetized with sodium pentobarbital (initial dose 60 mg/kg, i.p., supplemented approximately every hour by additional dose of 10 mg/kg), and the depth of anesthesia was monitored by controlling pinna and withdrawal reflexes. At the end of the experiment, animals were euthanized with an overdose of sodium pentobarbital (the lethal dose of 180–200 mg/kg). The core temperature of both cats and rats was maintained by the automatic heating system at $37 \pm 1°C$ throughout the experiments. All procedures were approved by the Local Ethics Committee and conducted in accordance with the Polish Law on the Protection of Animals as well as European Union regulations.

The MUs of the medial gastrocnemius muscle were studied. In both species, the muscle was carefully separated from surrounding tissues, while the blood vessels and nerve branches to the studied muscle were left intact. Remaining collaterals of the sciatic nerve were cut. Laminectomies were made over the lumbar and sacral segments of the spinal cord (L2-S1). The dura mater was cut and retracted. Cats were fixed with steel clamps at the thoracic and lumbar vertebrae, the hip and the tibia. These clamps were immobilized with special steel instruments. The spinal cord was covered with paraffin oil in a pool formed of the skin, and the skin flaps were pulled up around the back side of the leg and filled with the paraffin oil. In rat experiments, L1 and S1 vertebrae were hung up by the steel clamps, and the hind limb was immobilized in a special chamber filled with oil whose temperature was kept automatically at $37 \pm 1°C$. The muscle tendon of medial gastrocnemius was cut distally and connected to the force transducer (custom-made models: FT-510 for cats and FT-100A for rats, deflection of measuring element of 100 μm per 100 mN in both cases). To attain the highest contractile force for single MUs, the studied muscle was stretched up to a passive tension of 400 mN in cats (Celichowski et al. 2004) or 100 mN in rats (Celichowski and Grottel 1992). The force was recorded under isometric conditions. The MU action potentials were recorded with a pair of silver-wire electrodes (not insulated, 150 μm in diameter) inserted into a muscle perpendicularly to its long axis.

The dorsal and ventral roots of L7-S1 (in cats) or L4-L5 (in rats) spinal nerves were cut close to the spinal cord, and the respective ventral roots were split into the thinnest possible filaments. Filaments were electrically stimulated by a bipolar silver electrode, with electrical rectangular pulses (amplitude up to 0.5 V, duration 0.1 ms) produced by a dual channel square pulse stimulator (model S88, Grass Instrument Company). The "all-or-none"

appearance of a twitch contraction and of a motor unit action potential were used as criteria for isolation of a single MU. Force and electromyogram were monitored on an oscilloscope screen and stored on a computer disk using an AD converter (sampling rate of 1 kHz for force and 10 kHz for action potentials).

Cat MUs have twitch contraction and half-relaxation times about twice a long as rats (Burke et al. 1973; Carp et al. 1999; Kadhireresean et al. 1996; Krutki et al. 2006). Therefore, to evoke tetanic contractions of similar fusion, about twice higher stimulation frequency for rat MUs is necessary (Mrówczyński et al. 2006). The following stimulation protocol was applied: 1/five single pulses at 1 Hz (twitch forces and action potentials were averaged); 2/a 500 ms train of pulses at 20 Hz (cat) or 40 Hz (rat) to observe 'sag' appearance in studied fast MUs; 3/a 300 ms train of pulses at 150 Hz (a fused tetanus was recorded); 4/a train of 6 pulses at 20 Hz (cat) or 40 Hz (rat) at constant IPIs at 50 and 25 ms, in cat and rat, respectively; 5/a train of 6 pulses as in 4/, but with a longer first IPI (100 ms in the cat, and 50 ms in the rat); 6/a train of 6 pulses as in 4/; 7/the fatigue test (stimulation with trains of 14 pulses at 40 Hz repeated every second for 3 min (Burke et al. 1973; Grottel and Celichowski 1990). All steps were separated by 10-s intervals. During experiments on rat MUs, before the fatigue test, the effect of a prolongation of the first IPI by 50% was additionally tested, i.e., a train of 6 pulses at 40 Hz, but with the first IPI prolonged to 33 ms was applied, and after 10-s interval, it was followed again by a train of 6 pulses at 40 Hz.

For all studied MUs, their basic contractile properties were determined. For the averaged twitch recording, the twitch force (TwF), the contraction time (CT, measured as a time from the beginning of force recording to the highest amplitude of twitch force), and the half-relaxation time (HRT, measured between the highest amplitude of the twitch force and half of this value) were calculated. Then, for the fused tetanus (at 150-Hz stimulation), the maximum tetanus force (TetF) was measured, and the ratio of the twitch-to-tetanus forces (Tw/Tet) was calculated. Finally, the fatigue index (FatI) was calculated on a basis of the fatigue test, as a ratio of the tetanus force generated 2 min after the most potentiated contraction at the beginning of the fatigue test to the highest initial force (Kernell et al. 1983).

The tetanic depression (TD) was presented with two calculation methods. The first method presented a relative difference (in percent) between the force of the response to the last, sixth stimulus within a tetanus with the first IPI prolonged and the force of the respective response of a constant-frequency tetanus (Fig. 1a, arrow 1). The second method presented a relative difference (in percent) between the force of the response to the sixth stimulus within a

tetanus with the first IPI prolonged and the force of a response to the fifth stimulus in a constant-frequency tetanus (Fig. 1a, arrow 2). To describe and compare the studied tetanic contractions for all constant-frequency 6-pulses tetani (20 Hz in the cat or 40 Hz in the rat), the fusion index (FuI) was calculated as the ratio of the amplitude of the maximal relaxation before the response to the last stimulus in a tetanus and the amplitude of the last response (Celichowski and Grottel 1995b) (Fig. 1b). Moreover, a ratio of the peak force of the 6-pulses constant-frequency tetanus to the maximum tetanus force was calculated for each MU and expressed in percents (%Fmax) (Fig. 1b).

In both species, MUs were classified into fast and slow. In fast units (F), a sag phenomenon was visible in unfused tetani evoked at 20 Hz (cats) or 40 Hz (rats). The sag was not observed in slow (S) MUs (Burke et al. 1973; Grottel and Celichowski 1990). The classification of MUs appeared also fully compatible with values of the contraction time. The borderline between fast and slow MUs amounted to 45 ms for the cat medial gastrocnemius whereas 19 ms for the respective rat muscle (Mrówczyński et al. 2006). The further division of fast MUs was based on the fatigue index, which was under 0.5 for fast fatigable (FF) and over 0.5 for fast resistant (FR) MUs (Grottel and Celichowski 1990; Kernell et al. 1983). Since slow MUs do not reveal tetanic depression (Celichowski 2001;
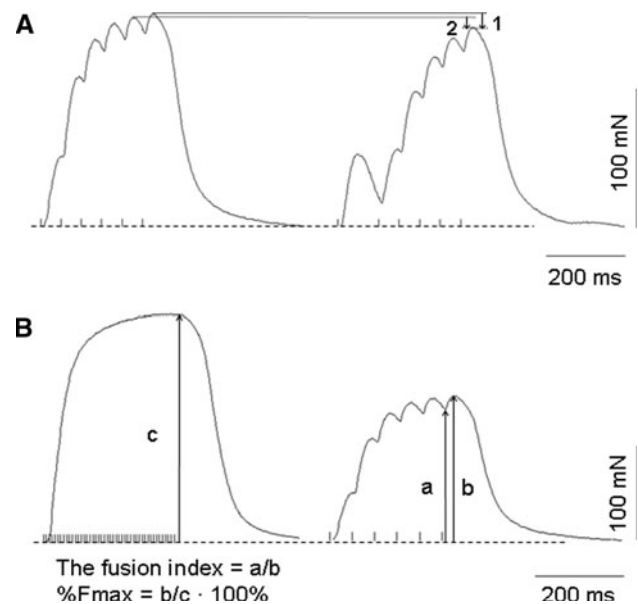
Fig. 1 **a** The two methods used to calculate the tetanic depression (indicated as *arrows 1* and *2*) presented for two compared tetani of an FF cat motor unit: the *left* recording, tetanus evoked at the constant stimulation frequency (20 Hz, 50 ms IPI), the *right* recording, tetanus evoked at a stimulation pattern with the prolonged first IPI (100 ms). **b** The method of a calculation of the two parameters: the fusion index and the relative level at the unfused tetanus force (%Fmax)
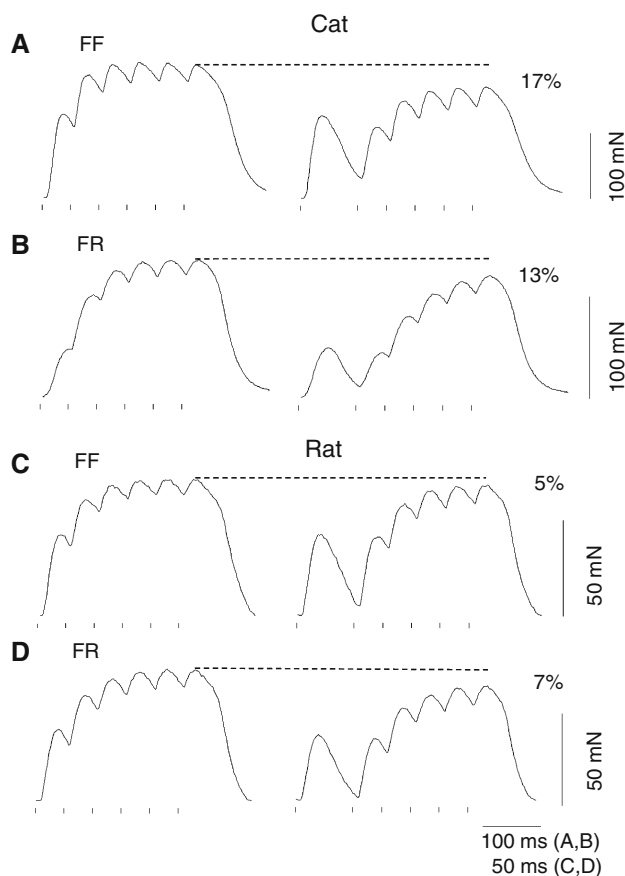
Fig. 2 The tetanic depression evoked by the prolongation of the first IPI in FF and FR motor units in the cat (**a**–**b**) and rat (**c**–**d**). On the *left*, constant-frequency force recordings (20-Hz tetani for the cat, and 40-Hz tetani for the rat) evoked by the 6-pulses trains of stimuli. On the *right*, records evoked by 6-pulses trains with the first IPI prolonged from 50 to 100 ms in the cat (**a**–**b**) or from 25 to 50 ms in the rat (**c**–**d**). The *horizontal dashed lines* indicate differences between the forces measured at the sixth component of a tetanus at constant stimulation frequency and the respective forces generated after the prolongation of the first IPI. The relative values of tetanic depression (*TD*) are given above records

Celichowski et al. 2004), only records from fast units were analyzed in the present study.

The statistical comparisons of the mean values between groups of cat and rat MUs, as well as between FR and FF types, were made with the unpaired Student's *t* test.

Results

During experiments, 81 MUs were recorded. MUs with very short contraction and relaxation times developed twitches or tetani of a very low level of fusion at 20 Hz (cat) or 40 Hz (rat). In eight such cases with FuI below 0.5, changes in the force of successive components were barely detectable (Grottel and Celichowski 1999) and the tetanic

depression which is a property visible in middle fused tetani (Celichowski et al. 2004) was not evident, and therefore, these recordings were excluded from the final analysis. For all remaining MUs, the analyzed groups were uniform with respect to the mean FuI calculated for analyzed tetani and there were no significant differences between the mean values of the FuI for fast MUs of cats and rats (Table 1). The total sample of 73 fast MUs consisted of 27 fast MUs of the cat and 46 MUs of rat medial gastrocnemius muscle. The basic contractile properties of the studied population of cat and rat MUs are summarized in Table 1. One may notice that the characteristics of the group are fully comparable with contractile properties obtained by different authors in several previously published papers concerning cat or rat MUs (Burke et al. 1973; Grottel and Celichowski 1990; Reinking et al. 1975).

The tetanic depression as a result of prolongation of the first IPI in a train of pulses was observed in all fast MUs, either in cats and in rats, but its degree varied between species. Sample recordings of the analyzed unfused tetani for FF and FR MUs are presented in Fig. 2. When the levels of force were compared between contractions with the first IPI prolonged and the constant-frequency stimulation pattern for the responses to the last, sixth stimulus in a train of pulses, the TD in cat MUs ranged from 5 to 47% (Table 1). Values of the TD in rat MUs, measured the same way, were lower and ranged from 1 to 16% (Table 1, see also recordings in Fig. 2). Moreover, there was a wider range (minimum to maximum) of values of TD than for rat fast MUs (Table 1). The interspecies differences between the respective values of TD were statistically significant for FF units. It is worth noticing here that the mean ratios of tetanic forces evoked with the analyzed 6-pulses constant-frequency stimulation (20 Hz in cats and 40 Hz in rats) to the maximum tetanus forces (150 Hz), calculated as %Fmax (Table 1), were significantly lower in cats than in rats, whereas the fusion of analyzed tetani in the two species was similar.

The response to the second stimulus following the prolonged first IPI usually began at very low force (Fig. 2). To exclude a possibility that observed depression of the tetanic force is due to lower number of pulses at the constant frequency of a tetanus with the first IPI prolonged in comparison with a respective regular constant-frequency tetanus, the second method of analysis was performed. For this purpose, the difference between the response to the sixth stimulus with the first IPI prolonged and the force of fifth response in a contraction at the constant-frequency stimulation was measured in each case: in both situations, the tetanus forces developed as responses to five pulses at a constant rate. The results were almost identical. The mean values of TD in the 20-Hz tetani in cats amounted to 29.15 ± 15.43% (10.91–46.78%) for FR MUs and 11.85 ± 6.95% (4.59–33.34%) for FF MUs. For 40-Hz tetani in rats, the mean TD values amounted to 5.70 ±

Table 1 The contractile properties of the cat and rat medial gastrocnemius motor units studied

CT (ms)	HRT (ms)	TwF (mN)	TetF (mN)	Tw/Tet	FatI	FuI	%Fmax (%)	TD (%)
Cats								
FR (*n* = 5)								
35.6 ± 7.8	35.0 ± 8.5	10.3 ± 8.3	151.7 ± 69.8	0.07 ± 0.04	0.70 ± 0.05	0.89 ± 0.02	42.68 ± 17.03	28.64 ± 13.86
22.0–42.0	27.0–48.0	5.0–25.0	84.0–246.3	0.02–0.12	0.62–0.75	0.87–0.92	20.12–61.46	11.26–41.4
FF (*n* = 22)								
32.9 ± 4.5	29.7 ± 7.9	158.0 ± 97.6	514.9 ± 193.9	0.28 ± 0.10	0.13 ± 0.10	0.82 ± 0.11	55.80 ± 18.02	10.86 ± 6.33
26.0–41.0	20.0–54.0	11.5–347.4	155.7–946.2	0.07–0.42	0.01–0.34	0.52–0.93	23.01–83.01	4.84–31.71
Rats								
FR (*n* = 18)								
16.8 ± 1.8	18.1 ± 4.1	23.5 ± 24.1	117.6 ± 78.6	0.18 ± 0.07	0.79 ± 0.16	0.88 ± 0.06	69.75 ± 11.99*	9.3 ± 3.52*
13.0–19.0	12.0–26.0	2.7–103.2	27.7–302.5	0.08–0.34	0.51–1.00	0.66–0.96	47.58–99.40	2.53–15.62
FF (*n* = 28)								
15.1 ± 1.7	14.6 ± 2.4	61.4 ± 39.2	171.5 ± 82.7	0.36 ± 0.08	0.21 ± 0.12	0.86 ± 0.07	73.82 ± 10.41**	7.21 ± 3.68**[†]
12.0–18.0	10.0–20.0	5.3–146.8	17.3–306.1	0.19–0.50	0.01–0.48	0.55–0.92	50.94–94.71	1.63–21.44

The upper row values are means ± SD, and the variability ranges are given below

CT, the contraction time; *HRT*, the half-relaxation time; *TwF*, the twitch force; *TetF*, the maximal tetanic force; *Tw/Tet*, the twitch-to-tetanus ratio; *FatI*, the fatigue index; *FuI*, the fusion index of the constant-frequency tetanus (20 Hz for cats or 40 Hz for rats); *%Fmax*, the relative ratio of the force of the constant-frequency tetanus (20 Hz for cats or 40 Hz for rats) to the maximum tetanus force; *TD*, the tetanic depression for the contractions with the prolonged first interpulse interval calculated as a difference between the responses to the sixth stimulus in the two compared tetani

* $P < 0.05$; ** $P < 0.01$ statistical significance of differences between cat and rat motor units. [†] $P < 0.05$ statistical significance of differences between rat FR and FF motor units. The unpaired Student's *t*-test was used. The statistical comparison only for the last two columns is presented

3.89% (0.30–12.25%) and 6.65 ± 2.71% (2.71–15.34%) for FR and FF MUs, respectively. The differences between values of the tetanic depression obtained with the two compared methods of calculation were not statistically significant ($P > 0.05$ in all cases).

In experiments on rats, the effects of a smaller prolongation of the first IPI, by 50% of the initial IPI, were also studied. For all MUs, the TD of the comparable degree was observed (with one exception of an FR MU, in which no force depression was found). For FF MUs, the prolongation of the first IPI evoked a decrease in the force by 9.34 ± 6.01% (2.03–33.35%) and for FR MUs by 6.39 ± 2.82% (0.00–11.80%). These effects were measured as a decrease in the response to the sixth stimulus.

Finally, the relationships between the amplitude of tetanic depression and fusion index or the contraction time for the two studied populations of MUs (for rats and cats) were analyzed. However, no significant correlations between the two studied properties were found, neither for cat nor for rat MUs (Fig. 3).

Discussion

We have shown in this study that even a relatively small change in a MU stimulation pattern, i.e., a prolongation of only the first interpulse interval, leads to a considerable reduction in the developed tetanic force. This phenomenon has been found in all medium-fused MUs tested in two species, cat and rat, without any exception. However, there are differences in its magnitude, with respect to either a wide range among MUs and interspecies diversity—higher depression effects have been commonly observed for feline MUs.

We believe that the results described here make a step ahead in our understanding of the tetanic depression. In previous research studies on cat and rat MUs (Celichowski 2001; Celichowski et al. 2004), a decrease in tetanic force has been observed as a result of a train of pulses at lower frequency delivered directly before a series of stimuli at higher frequency. The striking result of the present paper is that the force reduction evoked by a prolongation of only the first IPI in the discharge pattern leads to comparable or even greater depression of force than effects of a preceding low-frequency train of several pulses. For instance, the mean tetanic depression in cat MUs with 30-Hz tetani preceded by 20-Hz tetanic contractions amounted to a mean of 15.9% for FR and 10.9% for FF MUs, whereas with 40-Hz tetani preceded by contractions evoked by 25-Hz stimulation, the mean values were 7.3 and 5.6% for FR and FF MUs, respectively (Celichowski et al. 2004). For the rat, the mean TD values observed with 60-Hz contractions preceded by 40-Hz tetani were 9.2 and 5.2%, for FR and FF units, respectively (Celichowski 2001).

Fig. 3 The relationships between the tetanic depression (ordinate) and the fusion index (abscissa, **a**, **b**), as well as between the contraction time (abscissa, **c**, **d**) for the whole studied sample of cat and rat medial gastrocnemius MUs
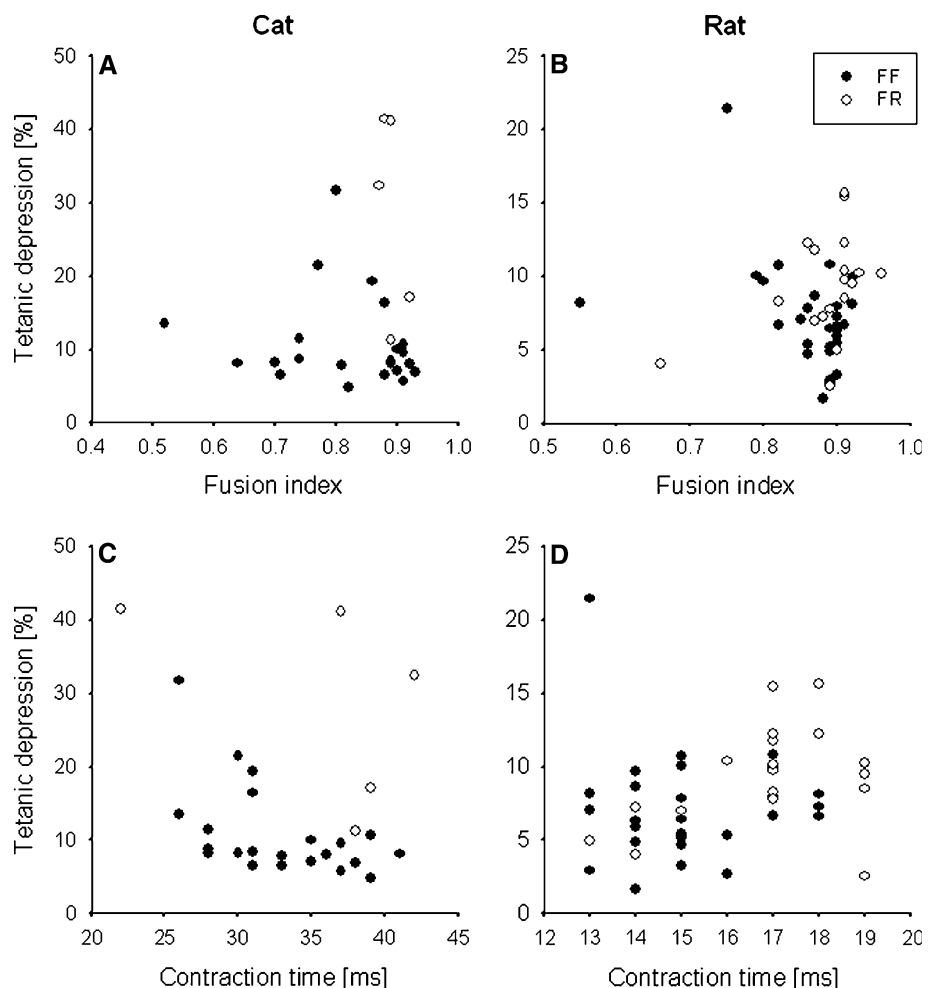
In the present study, the force reduction was significantly smaller for rat than for cat MUs. This have confirmed the previous observation that the tetanic depression in cat muscles is greater than in rat; however, one should consider methodological difficulties in such a simple comparison. Properties of MUs and mechanisms of MU contractions in both species are different even in the same muscle not only in absolute twitch force and time values, but also with respect to ranges between the weakest and the strongest MUs, and twitch-to-tetanus ratios (for details see Krutki et al. 2006). Therefore, it is impossible to set identical conditions of MU tetanic contractions for interspecies comparison, and only similar experimental arrangement could be projected, as the similar degree of fusion for cat and rat in our study. Despite the above-mentioned reservations, the interspecies differences in the magnitude and range of tetanic depression are evident (e.g., higher values and a wider range of TD in the cat), and therefore, there is a higher sensitivity of feline fast MUs to changes in motoneuronal firing rate. It is suggested that in the cat muscle, the pattern of pulses generated by motoneurones plays a more significant role in force regulation during voluntary activity. An explanation of the above might be related to differences in the force-frequency relationships, as these are considerably different for fast MU of cats and rats; greater changes in force occur for a given change in frequency for cat MUs (Mrówczyński et al. 2006).

The effects of tetanic depression were observed for cat and rat MUs at nearly the same fusion index (the mean value about 0.85 and similar range), although the relative force level of their tetani was evidently lower in cats (about 50% of the maximum force), when in rats it was higher (about 70%). This difference should also be discussed in light of results of previous experiments (Mrówczyński et al. 2006). In both species, the correlations between the stimulation frequency necessary to develop 60% of the maximum force and the contraction time were described by different regression lines, which stress the difference in a summation of twitches into the tetanic contraction. It was observed that feline fast MUs have higher sensitivity to the changes in the stimulation rate and are able to react with greater force changes even to small increase in the motoneuronal firing rate in comparison with rat MUs.

The observation that tetanic depression can be evoked even by prolongation of only one IPI turns our attention to possible mechanisms of this phenomenon. As evident on Fig. 2, especially in a case of cat FF MU (Fig. 2a), the response to the second stimulus is considerably depressed in relation to the first twitch. This resembles a negative staircase effect observed in rat fast MUs stimulated with trains of stimuli (Łochyński and Celichowski 2009 and as shown in Fig. 1). Moreover, it was shown in several papers that the twitch-shaped responses to successive stimuli within the unfused tetanic contractions show considerable variation in amplitude and time parameters, tetanic contractions evoked by trains of stimuli at constant-frequency (MacIntosh et al. 2007), task-matched IPIs (Zajac and Young 1980), or random pattern (Raikova et al. 2010). Moreover, when random pattern of stimuli was applied, very strong correlation (the correlation coefficient over 0.9) was found between the initial level of force generated by muscle fibers and the amplitude of the twitch response to the next stimulus (Celichowski et al. 2008). However, variability of amplitudes of the responses to successive stimuli appeared to be much smaller for fast than for slow MUs (Raikova et al. 2010). The study additionally revealed in fast MUs that when the stimulation frequency decreased temporarily (and as a consequence, the force considerably decreased), the twitch responses to stimuli were even smaller in amplitude than the single twitch. These observations help explain why the tetanic depression is not observed in slow motor units and suggest a mechanism for this phenomenon. Namely, a prolongation of the IPI allows greater relaxation, and therefore, the twitch response to a next stimulus becomes smaller.

The intracellular processes of tetanic depression seem to be related to a decrease intracellular calcium ions concentration, which dynamically changes during muscle activity. Rome (2006) suggests that the calcium sequestration is equal to the sum of that associated with parvalbumin present in fast muscle fibers and that associated with Ca^{2+} pumps of sarcoplasmic reticulum (SR). The most abundant Ca^{2+}-binding protein in the SR of skeletal muscle is calsequestrin, closely anchored to ryanodine receptor (RyR) Ca^{2+} release channels (Beard et al. 2004).

The present results observed in both species stress the functional significance of the tetanic depression phenomenon. It is clear that the force development is depressed even when only one, the initial IPI is longer than the following ones (so the change in the MU stimulation pattern is minimal). It was revealed in numerous studies on changes in discharge patterns of MUs during voluntary movements that during different motor tasks, MUs gradually increase the frequency of their firing rate starting from lower frequencies (Person and Kudina 1972; Søgaard et al. 1996; Tansey and Botterman 1996). In all such situations, the

longer initial IPIs can be assumed to negatively influence the force development in relation to the firing pattern beginning at higher rate of motoneuronal discharges. Studies on random stimulation patterns of MUs have also revealed evidence that the level of a tetanus force depends not only on small changes in IPIs but mostly on the initial level of force before the next contraction starts (Krutki et al. 2008). The tetanic depression, a phenomenon common in mammalian muscle, appears to be a key element in setting the initial level of force during a MU contraction. Therefore, it is a mechanism that can effectively influence processes of force regulation during voluntary activity of a muscle, when motoneurones progressively increase the firing rate.

Conflict of interest The authors declare that they have no conflict of interest.

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