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Transitory force decrease following a sudden reduction in stimulation frequency in motor units of rat medial gastrocnemius

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ABSTRACT

Effects of a sudden decrease in the stimulation frequency for motor unit force were studied in rat medial gastrocnemius. For 161 functionally isolated single motor units of three types (S, FR, FF), unfused tetanic contractions were evoked by three-phase trains of stimuli (low–high–low frequency). The course of the tetanus at the onset of the third phase of the force recording was analyzed in tetani with variable fusion degree. For 78 units within the third phase of tetanus, a transitory force decrease to a level lower than in the first phase (identical frequency), was observed. This phenomenon was more frequent for fast fatigue resistant (65.9%) than for fast fatigable and slow motor units (27.1% and 35.5%, respectively). Moreover, the force decrease was strongest for fast resistant motor units (up to 36.5%) and when contractions evoked at variable frequencies of stimulation were compared, the highest amplitudes of the studied force decrease were noted for middle-fused tetani (0.50–0.90). A new phenomenon of transitory force decrease in tetanic contractions of motor units with a decrease in stimulation frequency was found. Most probably, the phenomenon is dependent on disturbances in the force transmission by collagen surrounding active muscles fibers.

1. Introduction

In classical experiments, the force of motor units evoked by trains of stimuli at several constant frequencies of stimulation was measured and the sigmoid-shape force–frequency relationship was presented (Kernell et al., 1983; Reinking et al., 1975). However, the force regulation is a complex process, dependent also on changes in instantaneous or mean stimulation frequency (MacIntosh et al., 2007). The history of preceding motor unit activity considerably influences the force production. Several phenomena related to the effects of specific patterns of stimuli were described. First, when motoneurons begin the activity with a doublet, i.e., two action potentials at a short (up to 10 ms) time interval (Garland and Griffin, 1999; Person and Kudina, 1972) the doublet causes a rapid increase in developed force, known as the catch effect (Binder-Macleod and Clamann, 1989; Burke et al., 1976; Sandercock and Heckmann, 1997). This phenomenon was observed in fast (F) and slow (S) motor units. In other experiments, the force development at linearly increasing and decreasing frequencies of stimulation was studied and the hysteresis in the force–frequency relationships (at increasing and decreasing stimulation frequency) was observed. In addition, the force was also compared with values measured at a constant-rate stimulation, at given frequencies. When motor units were activated with trains of pulses at linearly decreasing frequency, the force decrease was slower than expected when compared with the constant stimulation frequency (Clamann and Schelhorn, 1988). On the other hand, it is worth emphasizing that at linearly increasing stimulation rate, the force increase was slower than expected (Binder-Macleod and Clamann, 1989; Frigon et al., 2011). This effect is related to a later-described phenomenon: reduction in the force at a constant frequency of stimulation conditioned by initial activation at lower frequency (Celichowski et al., 2004). The force reduction, reported as tetanic depression, was noted for fast motor units and was observed even when only one interpulse interval was prolonged (Celichowski, 2000; Grottel and Celichowski, 1999).

The catch effect and tetanic depression are effects of changes in the stimulation frequency at the onset of activity. However, the effects of a decrease in the activation frequency during evoked activity of motor units have not been studied before. During preliminary experiments, testing effects of a decrease in the stimulation frequency (a switch from high to low frequency), we unexpectedly found for a part of the motor units a sudden decrease in the force. Namely, the motor unit force temporarily decreased to a level lower than expected for the reduced stimulation frequency. This observed phenomenon potentially influences motor unit force when motoneurons are reducing their activity. Therefore, the aim of the present study was to recognize the occurrence of this force decrease in the three types of motor units, define the

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amplitude of the force decrease, and its dependence on the fusion degree of tetanic contractions.

2. Materials and methods

2.1. Animals

The experiments were performed on 11 female Wistar rats (4–5 months old), of mean body mass 254.6 ± 17.0 g. Rats were kept in standard laboratory cages (two per cage) in an animal house with a 12:12 light/dark cycle, controlled temperature (22 ± 2 °C) and humidity (55 ± 10%). All rats had unrestricted access to standard laboratory food and tap water throughout the study period, ensuring a balanced nutrient diet.

All procedures were approved by the Local Ethics Committee for Experiments on Animals (Permission Number: 2/2015) and were conducted in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, the Polish Law on the Protection of Animals as well as EU regulations. All experiments were performed under anesthesia and all efforts were made to minimize the suffering of examined animals.

2.2. Surgery

Animals were deeply anesthetized throughout the experiments with sodium pentobarbital (initial dose of 60 mg/kg i.p.) and the depth of anesthesia was monitored by controlling pinna and withdrawal reflexes. The studied medial gastrocnemius muscle was carefully separated from surrounding tissues, while the supplying blood vessels and nerve branches were left intact. Remaining collaterals of the sciatic nerve were cut. Laminectomy was made over five lumbar and sacral vertebrae (L2–S1). The dura mater over the spinal cord was cut and retracted. Rats were immobilized through L1 and S1 vertebrae with steel clamps and the hind limb was stabilized with an additional clamp on the tibia in a chamber filled with oil and the temperature was kept automatically at 37 ± 1 °C. At the end of the experiment, animals were euthanized with sodium pentobarbital (180–200 mg/kg, i.p.).

To evoke an isolated activity of motor units, the ventral roots of L4–L5 spinal nerves were cut close to the spinal cord and split into the thinnest possible filaments. Filaments, containing bundles of axons, were then 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) to evoke a contractile activity in a studied muscle; all remaining muscles of the hind limb were denervated. The "all-or-none" appearance of twitch contraction and action potential evoked when each of separated filament was stimulated with stimuli of amplitude around and twice above the threshold were accepted as criteria of a single motor unit isolation.

Force and electromyogram were monitored on an oscilloscope and stored on a computer disk using an analog-to-digital converter (sampling rate of 1 kHz for force and 10 kHz for action potentials).

During recordings, the Achilles tendon of the medial gastrocnemius was connected to the inductive force transducer (custom-made model: FT–100A, deflection of recording elements of 100 μm per 100 mN). The force generated by active motor units were recorded under isometric conditions. The muscle was stretched up to 100 mN tension, optimal to attain the highest contractile force for single motor units (Celichowski and Grottel, 1992). The action potentials were recorded with a silver-wire electrode (not insulated, 150 μm in diameter) inserted into a muscle perpendicularly to its long axis, whereas the reference electrode was placed on denervated hip muscles.

2.3. Stimulation protocol

When the isolation of a single motor unit was reached, the following stimulation protocol was applied:

1. 5 pulses at 1 Hz (the force of five evoked twitches and action potentials were recorded and averaged);
2. 500 ms train of pulses at 40 Hz (the force of unfused tetanus was recorded and the presence of "sag" was verified);
3. 300 ms train of pulses at 150 Hz (the fused tetanus was recorded and the maximum force was determined);
4. the main recordings of tetanic contractions evoked with three-phase trains of pulses at changing frequencies in a general pattern: low–-high–low frequency (Fig. 1).
   (a) For F motor units, the protocol based on the following stimulation pattern was applied: 500 ms train of stimuli at low frequency, 300 ms at high frequency and 500 ms at the same low frequency as the initial. The low frequencies were 15, 20, 25, 30, 35 and 40 Hz, and high frequencies were 75, 90 and 150 Hz and all possible combinations of low and high frequencies were tested, i.e., this stimulation protocol included 18 trials;
   (b) For S motor units, the stimulation pattern was matched to longer twitch time parameters and trains were longer because the plateau phase of S motor units tetanic contractions is reached in a longer time. Therefore, 1000 ms train of stimuli at low frequency, 300 ms at high frequency and 1000 ms at the same low frequency as the initial stimuli were applied. The low frequencies were 10, 12.5, 15, 17.5, 20 and 25 Hz and high frequencies were 30, 40 and 50 Hz and all possible combinations of low and high frequencies were tested, i.e., this stimulation protocol included 18 trials.
5. the fatigue test, i.e., trains of 14 pulses at 40 Hz repeated every second during 3 min (the recordings were used to calculate the fatigue index) (Burke et al., 1973; Kernell et al., 1983).

All force recordings within the above points of the stimulation protocol were separated by 10-second intervals.

2.4. Studied parameters

The basic contractile properties of 161 motor units were investigated (31 slow motor units (S), 82 fast resistant motor units (FR) and 48 fast fatigue motor units (FF). The twitch force (TwF, measured from the baseline to peak force), the contraction time (CT, measured as a time from the onset of force recording to the highest amplitude of twitch force), the half-relaxation time (HRT, measured between the peak of the twitch force and half of this value) were calculated from the average twitch force profile. Then, for the fused tetanus (third step of the stimulation protocol at 150 Hz stimulation) the maximum tetanus force (TetF, from the baseline to the peak of contraction) was measured, and the ratio of the twitch-to-tetanus forces (TwF/TetF) 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. Motor units were classified into F and S based on the presence of sag in 40 Hz contraction: in fast units, a sag phenomenon was visible in unfused tetani evoked at 40 Hz (the second step of the stimulation protocol) whereas the sag was not observed in S motor units (Burke et al., 1973). The further division of fast motor units was based on the fatigue index, which was below 0.5 for FF and above 0.5 for FR motor units (Grottel and Celichowski, 1990; Kernell et al., 1983).

The main studied parameter, in the fourth step of the stimulation

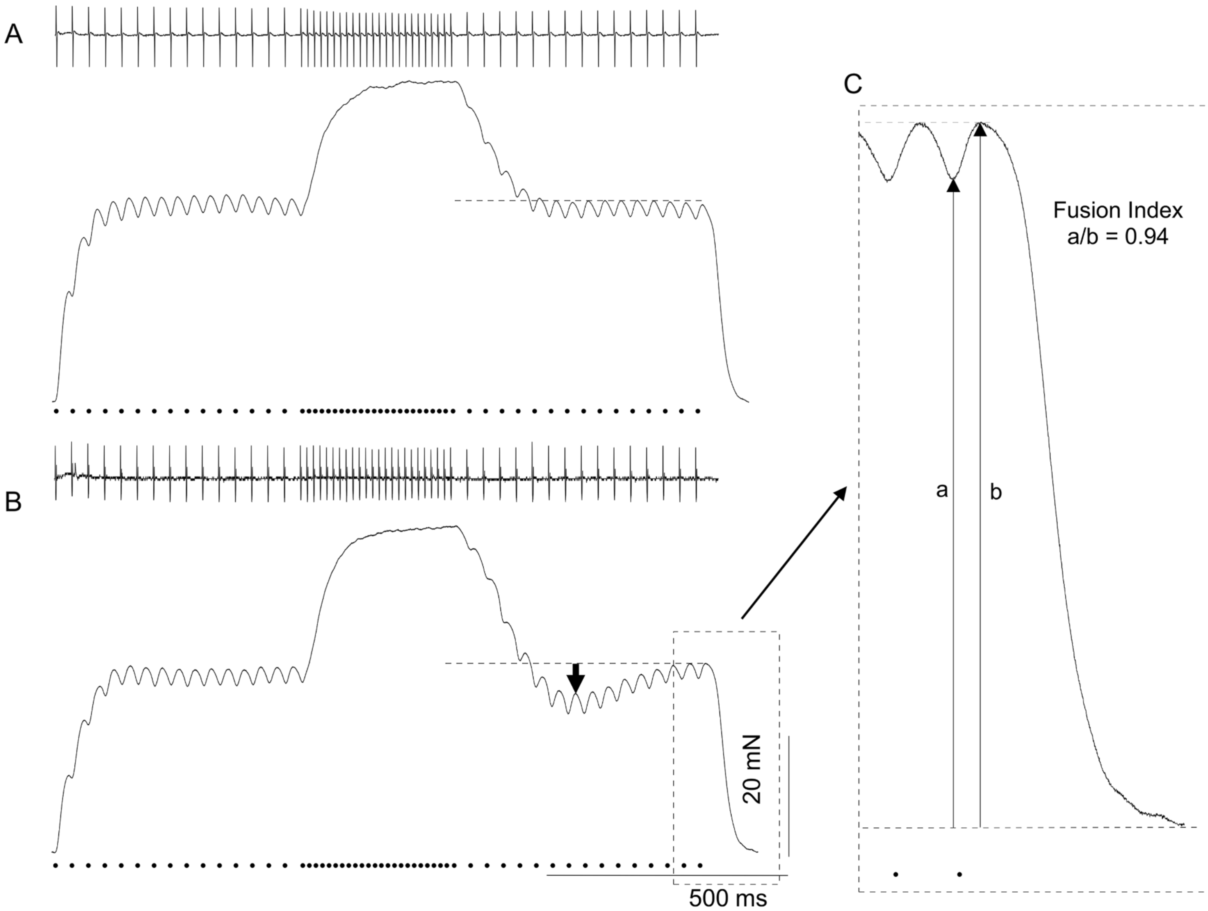

Fig. 1. Calculation of the amplitude of the transitory force decrease and fusion index. The sample recordings of the tetanic contraction for two FR motor units (A and B) stimulated with the same 30–75–30 Hz frequency pattern. The distribution of applied stimuli is indicated by dots under the force recordings. Trains of motor units action potentials are indicated above the force. Transitory force decrease (indicated by bold arrow) following the sudden reduction in stimulation frequency is visible only in (B). The horizontal interrupted line indicates the reference force value at the end of tetanus used for the amplitude of the force decrease calculation. The enlarged part of recording in (C) shows the method of the fusion index calculation (a/b).

protocol, was an amplitude of the transitory force decrease at the beginning of the third phase of the force recording (Fig. 1B, bold arrow). The amplitude was determined as a difference from the reference force at the end of the last phase of the three phases of tetanus. The presence of a phenomenon in the recording was accepted when the amplitude of the force decline exceeded 3% of the force recorded at the end of the last phase of stimulation.

Finally, the fusion index (FuI) was determined as a ratio of the lowest force value before the last stimulus in the third phase (the second low stimulation phase) of stimulation (*a* in Fig. 1C) to the peak force following the last stimulus (*b* in Fig. 1C).

Table 1
Basic contractile properties (mean ± SD) of the three types of motor units (S, FR, FF). Motor units of each type were subdivided into two groups: with transitory force decrease (+) and without this phenomenon (−).

Type of MUs	CT [ms]	HRT [ms]	TwF [mN]	TetF [mN]	TwF/TetF
S (+)	24.7 ± 2.5 (21–31)	35.6 ± 4.9 (30–42)	5.4 ± 1.6 (2.2–7.8)	42.8 ± 9.8 (24.9–56.3)	0.12 ± 0.02 (0.09–0.17)
S (−)	23.5 ± 2.7 (19–29)	33.0 ± 3.4 (27–42)	4.7 ± 1.9 (1.7–9.2)	42.5 ± 13.1 (19.6–60.5)	0.10 ± 0.03 (0.08–0.16)
FR (+)	15.4 ± 3.7† (11–20)	18.0 ± 4.9 (11–30)	21.3 ± 15.6* (4.7–69.7)	91.5 ± 47.1* (27.3–217.8)	0.22 ± 0.09 (0.08–0.50)
FR (−)	14.1 ± 2.2 (10–20)	17.4 ± 4.3 (10–29)	35.8 ± 25.5 (7.5–87.1)	130.5 ± 63.9 (21.6–246.4)	0.26 ± 0.13 (0.12–0.57)
FF (+)	13.4 ± 1.3 (10–16)	17.0 ± 2.6 (14–23)	52.4 ± 32.2 † (12.9–131.2)	154.9 ± 55.1† (79.5–248.3)	0.33 ± 0.12 (0.16–0.54)
FF (−)	12.6 ± 1.6 (10–16)	16.0 ± 3.2 (10–23)	81.7 ± 41.4 (12.2–168.4)	208.5 ± 85.3 (80.7–378.1)	0.36 ± 0.11 (0.12–0.51)

MUs, motor units; *CT*, contraction time; *HRT*, half relaxation time; *TwF*, twitch force; *TetF*, tetanus force; *TwF/TetF*, the ratio between twitch and tetanus force; *S,* slow motor unit; *FR,* fast resistant motor unit; *FF,* fast fatigable motor unit; (+), phenomenon of force decrease; (−), without phenomenon of force decrease. Statistical significance between groups (+) and (−) is represented by * $p < 0.05$ (U Mann-Whitney) or † $p > 0.05$ (the Student's *t*-test).

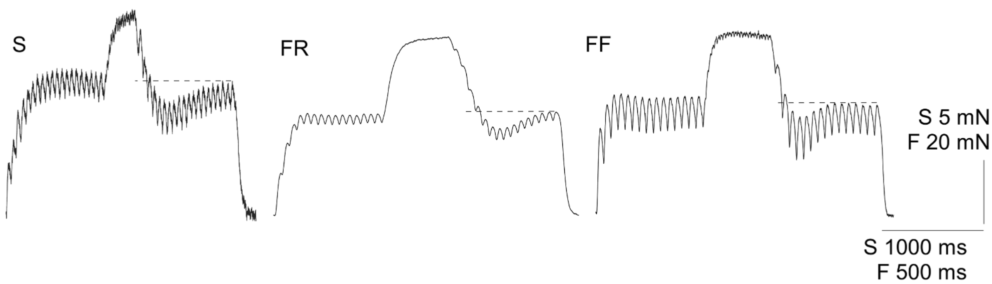

Fig. 2. Sample recordings of tetanic contractions of the three types of motor units. Stimulation patterns: 20–50–20 Hz; 30–75–30 Hz; 30–75–30 Hz for S, FR and FF motor unit, respectively. The horizontal interrupted line indicates the reference force value at the end of tetanus used for the amplitude of the force decrease calculation. Note that the transitory force decrease after high-frequency stimulation is visible in all presented recordings.

2.5. Statistical methods

The data are presented as mean ± SD. The basic contractile properties of examined motor units were submitted to statistical analysis using the Shapiro–Wilk test verifying the normal distribution for small groups. In addition, the results were statistically verified using Levene's test to determine the homogeneity of the examined three types of motor units (FF, FR, S) with the division into two groups; first, with the force decrease effect (+) and second, without the force decrease effect (−) (see Table 1). Results for the three types of motor units were compared: when the results were inhomogeneous ($p < 0.05$) nonparametric Mann–Whitney *U* test was used, otherwise (when the group was homogeneous) ($p > 0.05$) Student's *t* test was used. The differences in studied force decrease between the three types of motor units have been examined using nonparametric Kruskal–Wallis and post hoc Dunn's tests. In addition, the dependence between transitory force decrease and FuI (for each of the three types of motor units) was determined (Pearson correlation coefficient).

3. Results

The transitory force decrease after the sudden reduction in stimulation frequency in low–high–low stimulation pattern occurred in all three types of motor units in rat medial gastrocnemius muscle (Fig. 2 and Table 1), for approximately 48.5% (78 of 161) of motor units, however, the distribution of units with visible phenomenon was different between the three types. Transitory force decrease was noted for 54 of 82 (65.9%) examined FR units, but it occurred in only 13 of 48 (27.1%) FF motor units and 11 of 31 (35.5%) S motor units (Fig. 3A). The mean amplitude of force decrease was the highest for FR motor units (FR to FF $p < 0.01$, FR to S $p < 0.05$, FF to S $p < 0.05$) (Fig. 3B). The average force decrease for FR units was 13.0% of the reference force at the end of tetanus, with the maximum at 36.5%, whereas for S and FF units it was 8.9% (maximum 19.0%) and 6.2% (maximum 13.8%), respectively. It is worth noting that the transitory force decrease was not accompanied by changes in the motor unit action potentials (Fig. 1).

In addition, the analysis of basic contractile properties for the three types of motor units indicated that motor units that revealed the studied force decrease following the high-frequency stimulation phase (+) were characterized by longer contraction time (difference significant for FR motor units, $p < 0.05$). The twitch and tetanus forces were lower in FR and FF motor units with the transitory force decrease (differences significant, $p < 0.05$) (Table 1).

The dependence between the amplitude of the transitory force decrease and the fusion index was analyzed (Fig. 4A–C). For each of the three types of motor units, the force decrease did not correlate with the fusion index when data for all recorded tetani were taken together ($p > 0.05$). Nevertheless, the highest force decrease was recorded for contractions with the fusion index in a range of 0.50–0.90 (Fig. 4D). Whenever the studied effect was analyzed for several tetanic contractions of the same motor unit, it was absent in tetani with too low and too high values of the fusion index (Fig. 5). Differences in the middle, high frequency of stimulation did not influence the following transitory force decrease (Fig. 6). When amplitudes of the force decrease obtained for contractions of FR motor units with the middle high-frequency stimulation of 75, 90 and 150 Hz were compared, no significant differences ($p > 0.05$) were observed between these three types of high-frequency stimulations. Conversely, low stimulation frequencies in contractions with the strongest force decrease appeared to be different for the three motor unit types. For FF and FR motor units, the strongest studied effect was observed for low frequencies of the pattern in a range of 20–40 Hz, with the highest decrease recorded usually at 30 Hz (Fig. 4B). For S motor units the highest effect was noted for considerably lower stimulation frequencies than for F motor units as the strongest effect was observed in a range of 15–25 Hz.

4. Discussion

During voluntary contractions, muscle force is controlled by the central nervous system in two fundamental ways: recruitment of motor units (Henneman, 1957; Henneman et al., 1965) and changes in motoneuronal firing rate (rate coding) (De Luca and Erim, 1994; De Luca et al., 1982; Person and Kudina, 1972). The described phenomenon of a transitory force decrease is directly related to the second mechanism as it potentially influences force production during a phase with decreasing firing rate of motoneurons.

The observation highlights that the rate coding mechanism is a

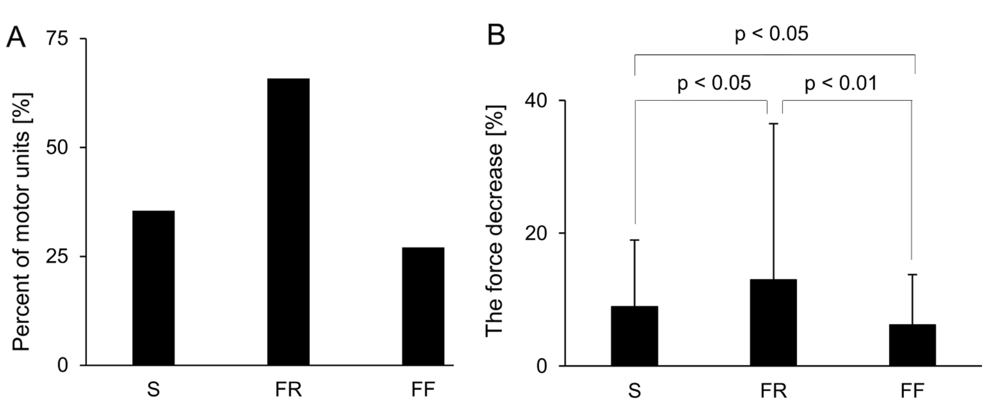

Fig. 3. The occurrence frequency and amplitudes of the transitory force decrease for the motor units. (A) Proportion of the S, FR and FF motor units with the transitory force decrease (exceeding 3% of the reference force at the end of tetanus) after a sudden reduction in stimulation frequency at low–-high–low sequence; (B) The mean values and SD of the amplitude of transitory force decrease (expressed in percent of the force at the end of the tetanus for S, FR and FF motor unit). The clamps indicate significant differences (post hoc Dunn's test).

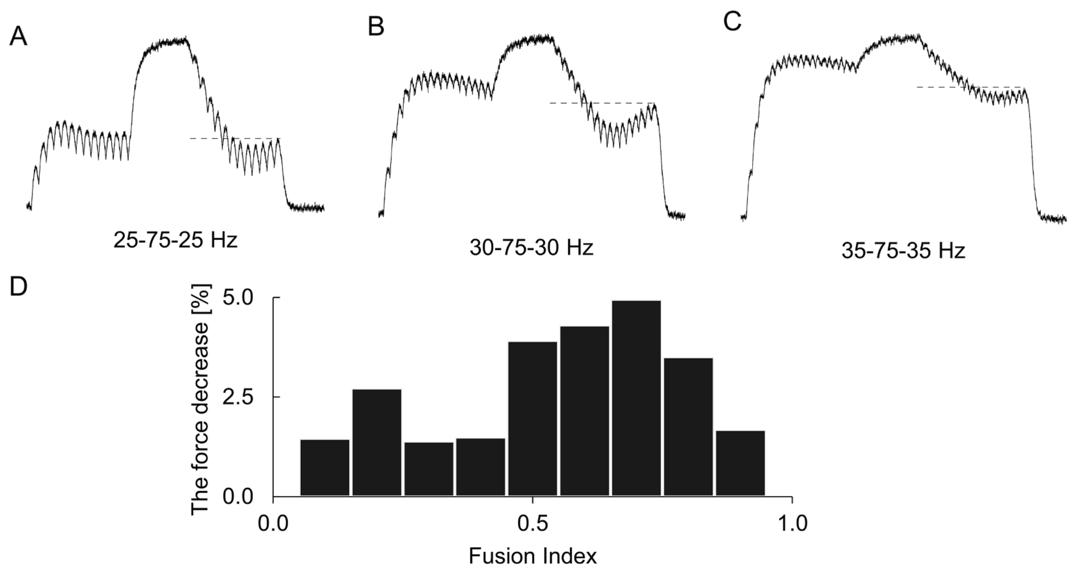

Fig. 4. The dependence of the transitory force decrease on the fusion degree of a tetanus. The examples of one FR motor unit contraction with a transitory force decrease for three stimulation patterns: (A) 25–75–25 Hz; (B) 30–75–30 Hz; (C) 35–75–35 Hz. (D) The histogram of the amplitude of transitory force decrease expressed in percent as a function of the fusion index for motor units of three types (FF, FR and S). Note that for calculations of mean values presented on the histogram all recorded tetanic contractions, i.e., also those without the transitory force decrease were included (zero value) and therefore the mean values are relatively low.

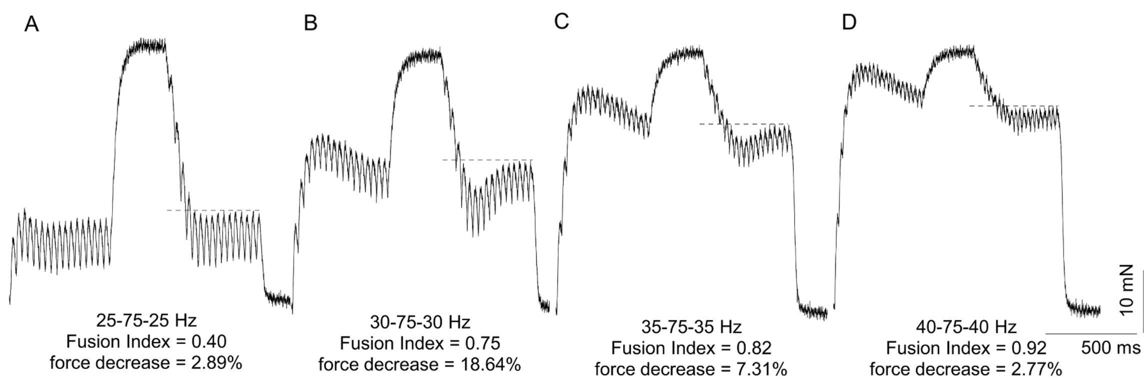

Fig. 5. The transitory force decrease is only visible in middle-fused tetanic contractions. Examples of four tetanic contractions at different stimulation patterns for one FR motor unit: (A) 25–75–25 Hz; (B) 30–75–30 Hz; (C) 35–75–35 Hz; (D) 40–75–40 Hz. Note that only in B and C was the transitory force decrease visible. The fusion index is indicated under the recordings.

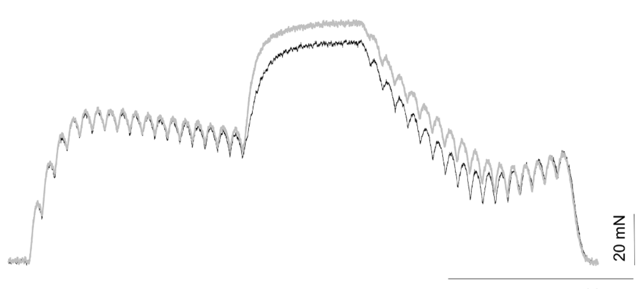

Fig. 6. Weak influence of middle high-frequency stimulation on the amplitude of the transitory force decrease. Two superimposed contractions of the same FR motor unit at two low–high–low stimulation patterns evoked with the same low frequency of stimulation but different high frequencies (30–75–30 Hz—gray line; 30–150–30 Hz—black line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nonlinear process and the motoneuronal firing (stimulation) pattern may considerably modify the force production. Effects of sudden changes of stimulation frequency (including decrease) were studied on cat and human muscles. The cat muscles (medial gastrocnemius, lateral gastrocnemius, soleus and quadriceps) were stimulated with the three-phase trials: 2 s at 20 Hz, 2 s at 40 Hz and finally 2 s at 20 Hz. Human muscles (triceps surae, tibialis anterior) were stimulated via the nerve for 2 s at 25 Hz, followed by 2 s at 100 Hz and then 2 s at 25 Hz. Under these conditions, all motor units were most likely co-activated, but no transitory decrease in force was observed (Frigon et al., 2011). In the present study performed on isolated motor units, the transitory force decrease was noted for approximately one-half of the motor units. These observations suggest that this phenomenon disappears as an effect of mechanical interaction between several co-active motor units. Further experiments testing the influence of co-contraction of other motor units in the same muscle on a studied transitory force decrease should confirm this suggestion. Moreover, it should be stressed that for different motor units, the stimulation frequency that was optimal to observe the strongest force decrease effect was different. Most likely because of the above observations, this phenomenon is not visible while the muscle is activated via nerve as the same stimulation pattern is applied to all motor units.

The transitory force decrease when a sudden change in stimulation frequency occurs was most frequently visible among FR motor units, but was also noted for some FF and S units. Therefore, the studied effect appeared not to be specific to a particular muscle fiber type. It suggests that the mechanism of this phenomenon may not be related to a force production but to processes of force transmission within a muscle to a tendon. Muscle fibers of FR motor units are predominantly dispersed within the proximal compartment of medial gastrocnemius (De Ruiter et al., 1996; Gardiner et al., 1991). This compartment is relatively short; its length corresponds to 40% of the total muscle length (Taborowska et al., 2016). Therefore, the observed effect may be a response to a sudden decrease in the transmitted force whereby forces generated by motor units from the proximal compartment are transmitted to the Achilles tendon through a distal compartment (or its parts). This explanation is convergent with conclusions resulting from studies of interactions between motor units and summation of their forces. Forces recorded during co-activity of several motor units were smaller (Sandercock, 2000, 2009) or higher (Emonet-Dénand et al., 1987; Powers and Binder, 1991) than the algebraic sum of forces of these units measured during their individual activity. It was also found that with an increasing number of co-active motor units, the effects of summation of forces decreased (Drzymała-Celichowska et al., 2010). It was concluded that these differences between the recorded force and that estimated by algebraic summation of individual forces have a biomechanical background as they are dependent on topographical relations of the active motor units within the muscle and the pennated arrangement of muscle fibers (Cui et al., 2007; Sandercock, 2000; Zuurbier and Huijing, 1992). The medial gastrocnemius muscle is composed of two compartments, each containing a separate population of motor units. When the whole medial gastrocnemius muscle was stimulated and all muscle fibers of both proximal and distal compartments were co-activated, the recorded force was smaller in relation to the sum of the forces of the two compartments stimulated separately (Taborowska et al., 2016). Therefore, it is possible that the studied phenomenon of a transitory force decrease is present when contracting muscle fibers of the motor unit stretch the elastic collagen network in the muscle and a sudden decrease in the motor unit force occurs in response to a decrease in stimulation frequency that releases this passive part of the muscle. Because of a change in the pennation angle of released muscle fibers and/or elasticity of intramuscular collagen, the force from the motor unit is temporarily less efficiently transmitted to the tendon (Huijing and Jaspers, 2005; Huijing et al., 1998). This conclusion is additionally supported by an observation that within each motor unit type the studied phenomenon was more visible for weaker motor units (Table 1), which have a lower innervation ratio (Kanda and Hashizume, 1992) and their muscle fibers likely occupy a smaller territory in a muscle (Parmiggiani and Stein, 1981). On the other hand, in S motor units, which have the lowest force, the phenomenon was rare. The possible reason for this finding is that S motor units have three-times lower sensitivity to changes in instantaneous stimulation frequency (Grottel and Celichowski, 1999) and higher stiffness in relation to fast ones (Petit et al., 1990). In addition, the maximum rate of change in the force is the lowest for S motor units (Celichowski and Bichler, 1998).

Alternatively, given possible mechanisms of the observed phenomenon, one may suggest that intracellular processes should be taken into account as the muscle fiber force is related to the release of Ca^{2+} ions from the sarcoplasmic reticulum (Fitts, 2008). Cady et al. (1989) suggested that the brief tetanic muscle contractions in the fresh state cause a fast decrease in force. Changes in the Ca^{2+} level following muscle activity were reported by several authors (Cherednichenko et al., 2008; Westergaard and Allen, 1993). Chin and Allen (1996) observed that a reduction in submaximal stimulation frequency (from 70 Hz to 30 Hz) reduced intracellular Ca^{2+} concentration by approximately 30%. These authors also claimed that the force decrease could be induced by changes in the cross-bridge kinetics because a fast decrease in the rate constant could cause disorders in Ca^{2+} transmission, in the attachment of myosin and actin, and the development of the high force state. In addition, a failure of the synaptic transmission (Fuglevand et al., 1993) is not a possible explanation of the transitory force decrease as this force decrease was not accompanied by changes in the motor unit action potentials (Fig. 1).

The discovery of a new mechanism that temporarily reduces motor unit force opens the door to further questions. First, the mechanism of how the phenomenon influences force production in response to a progressive decrease in motoneuronal firing rate should be investigated. Second, it is important to observe the influence of the muscle length on the studied effect and to test the presence of this phenomenon in muscles of variable architecture. Third, several mechanisms of the studied force decrease were taken into account, however, additional experiments are necessary to explain the background of the phenomenon.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelekin.2019.02.008.

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