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The influence of a doublet of stimuli at the beginning of the tetanus on its time course

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Abstract. The influence of a pair of stimuli generated in a short time sequence (doublet) at the beginning of stimulation on the time course of the following tetanus was investigated. Experiments were performed on single motor units in rat's medial gastrocnemius. The doublet evoked an increase in tetanic tension, tetanic fusion and the area under tension record. These effects were measured in tetani fused to varying degrees. It was found that for all types of motor units the strongest influence of the doublet was observed in half-fused tetani. Moreover, the doublet influenced the first part of tetanus significantly more as compared to the second. Slow motor units showed greater sensitivity of the tension and the tetanus area to the doublet than fast units. The results show that slow units are characterized by better summation of their tension at the beginning of a tetanus.

Key words: motor unit, tension, tetanic fusion, doublet

INTRODUCTION

Muscle fibers are able to reach higher tension levels when the stimulation pattern starts with two pulses over a short time span (doublet) (Burke et al. 1970). This phenomenon (catch effect) is of interest because motoneurons during natural activity can begin their firing with a doublet, followed by a lower rate of discharges (Bawa and Calancie 1983, Lomo 1985, Hennig and Lomo 1987, Spielmann et al. 1993). Moreover, the optimal pattern of stimulation (giving the biggest area under the record of tetanic tension) also starts with two pulses in a short time period (Zajac and Young 1980a). However, the influence of this doublet on the fusion of a tetanus and on tension in different parts of a tetanus and, finally, in different motor unit types has not been investigated. Similarly, we have no answer to the other physiological question - at which tetanic fusion is the influence of the doublet the strongest in the following part of the tetanus? The present paper attempted to find the answers to the above problems.

METHODS

Experiments were performed on 7 adult Wistar rats (weight 282 ± 36 g), under pentobarbital anesthesia (Vetbutal, 30 mg/kg I.P., supplemented as required).

The medial gastrocnemius muscle was partly isolated from the side of Achilles tendon. Muscle innervation and blood supply were left intact, but remaining branches of the sciatic nerve were cut. A laminectomy was performed on L2-S1 segments of the vertebral column. Ventral and dorsal roots of L3-S2 segments were cut as close to the spinal cord as possible. The animal was immobilized with special steel clamps on vertebrae (L1 and S2) and tibia. The hindlimb was immersed in a pool filled with paraffin oil. Paraffin oil also covered the isolated spinal cord. The rectal temperature of the animal and the oil bath were kept at 37 ± 1°C.

Isolation of single motor units in the investigated muscle was obtained by splitting the L5 ventral root on thin filaments, stimulated with electrical pulses (Celichowski 1992). The activity evoked with this stimulation was accepted as the contraction of single motor unit when it was of "all-or-none" type (both in the action potential and tension) and when in electroneurogram (recorded in the medial gastrocnemius nerve branch) only a single potential was visible.

The investigated muscle was connected to an isometric force transducer. Contractile tension was measured when the muscle was stretched up to a passive tension of 100 mN, optimal for contraction of its single motor units (Celichowski and Grottel 1992). Muscle fiber action potentials were recorded with two silver wires inserted into the muscle. Action potentials of motoneuron axons were recorded with a thin silver electrode placed under the medial gastrocnemius nerve branch.

Muscle tension and action potentials were stored on a computer disc using A/D converter (sampling rate of 1 kHz for tension and of 10 kHz for action potentials).

A special computer program was used to create patterns of stimuli. First, the motor unit was stimulated with a single pulse repeated 10 times and the twiches were averaged. Then, the unfused tetanus (20 pulses at 40 Hz) and fused tetanus (30 pulses at 150 Hz) were evoked. Units with a "sag" in unfused tetani were classified as fast, whereas these with no sag were classified as slow (S) (Grottel and Celichowski 1990).

The influence of the doublet on tetanic tension for fast motor units was tested at stimulation frequencies corresponding to their unfused tetani: 30, 40, 50 and 60 Hz (Grottel et al. 1993, Celichowski and Grottel 1995). For each of these frequencies first a tetanus at a train of 19 pulses in regular distances was recorded and then a tetanus at a 20-pulse train, but for the first two pulses the time period was shortened up to 5 ms (doublet). These tetani were named "doublet tetani" whereas the previous ones were called "non-doublet tetani".

For slow motor units the influence of the doublet on the tetanus was tested at lower frequencies of stimulation: 10, 13, 20 and 27 Hz, because these frequencies corresponded to their unfused tetani (Grottel et al. 1993, Celichowski and Grottel 1995).

Finally, all motor units were subjected to the fatigue test (trains of 14 stimuli at 40 Hz, repeated each second during 4 min) (Burke et al. 1973). The fatigue index was calculated for each unit (Kernell et al. 1983a). Fast units were classified as fast fatigable (FF) when their fatigue index was lower than 0.5 and as fast fatigue resistant (FR) when this index was higher than 0.5 (Grottel and Celichowski 1990).

Figure 1 shows examples of non-doublet and doublet tetani and all measured parameters are indicated. The area measured between the tension record and the baseline can be used as a measure of the work done by the contracting motor unit (Celichowski et al., in preparation). The area was measured for the first part of the tetanus (undotted area left to the vertical broken line) and for its second part (dotted area right to the broken line).

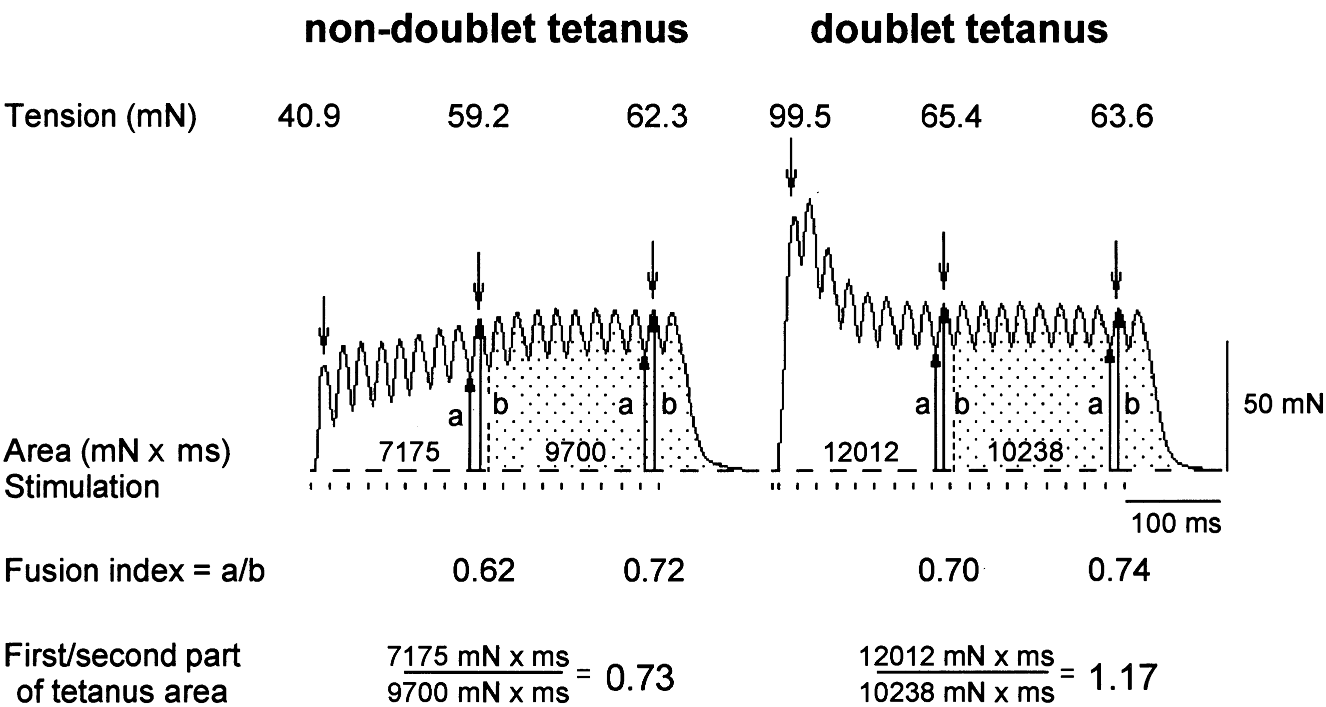

Fig. 1. Examples of "non-doublet" and "doublet" tetani of FF motor unit recorded at 50 Hz stimulation and parameters measured in the first and second parts of tetani. The area of the first part of tetanus (left to the interrupted, vertical line) is undotted, whereas the area of the second part (right to the interrupted line) is dotted. Fusion index (*a/b*) was calculated for 9th and 18th components of the tetanus. Tension was also measured for these two components (showed by arrows) and additionally for the first one (the response to the first pulse or to the doublet). Stimulation pattern is shown under tension records. Analyzed values of tension, tetanic area and fusion index are shown. In lower part the ratio of areas of the first to the second part of tetanus is calculated. For further explanations see text.

The tension was controlled at three points: in the middle and at the end of the tetanus (9th and 18th responses) as well as at the first component of the tetanus (the response to the first pulse or to the doublet). Moreover, the fusion of the tetanus was measured at two points: at its 9th and 18th components as *a/b* ratio, where *a* is the distance from the baseline to the maximal relaxation before the following contraction and *b* is the peak tension of this subsequent component of the tetanus (Bakels and Kernell 1995, Celichowski and Grottel 1995). The fusion index is a measure of tetanic fusion which can change even within a single tetanus. All the above measures were used to study and to compare the effects of the doublet in the first and second parts of a tetanus.

Forty-six motor units were used in the present study, 14 of FF type, 20 of FR type and 12 of S type.

TABLE I

Mean values (and their standard deviations) of investigated contractile properties for three types of motor units

Motor unit type	Contraction time (ms)	Half-relaxation time (ms)	Twitch tension (mN)	Tetanic tension (mN)	Fatigue index
FF (n = 14)	11.6 ± 1.2	12.0 ± 1.8	35.9 ± 25.5	158.0 ± 77.1	0.24 ± 0.12
FR (n = 20)	13.7 ± 2.4	15.4 ± 3.8	7.6 ± 6.1	44.3 ± 29.0	0.91 ± 0.09
S (n = 12)	28.1 ± 4.4	38.2 ± 9.7	4.0 ± 1.9	32.7 ± 9.4	0.96 ± 0.05

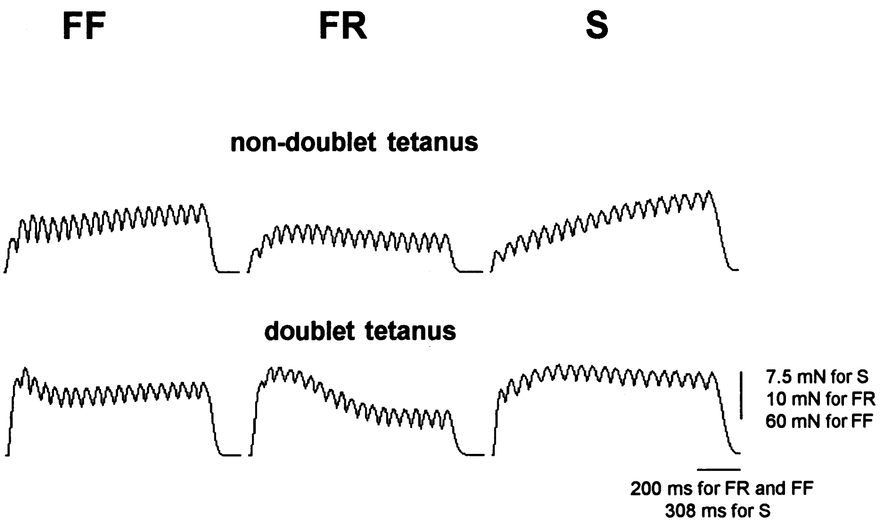

Fig. 2. Tension records of doublet and non-doublet tetani. Examples of FF, FR and S motor units. Stimulation frequency: 40 Hz for fast and 13 Hz for slow motor units.

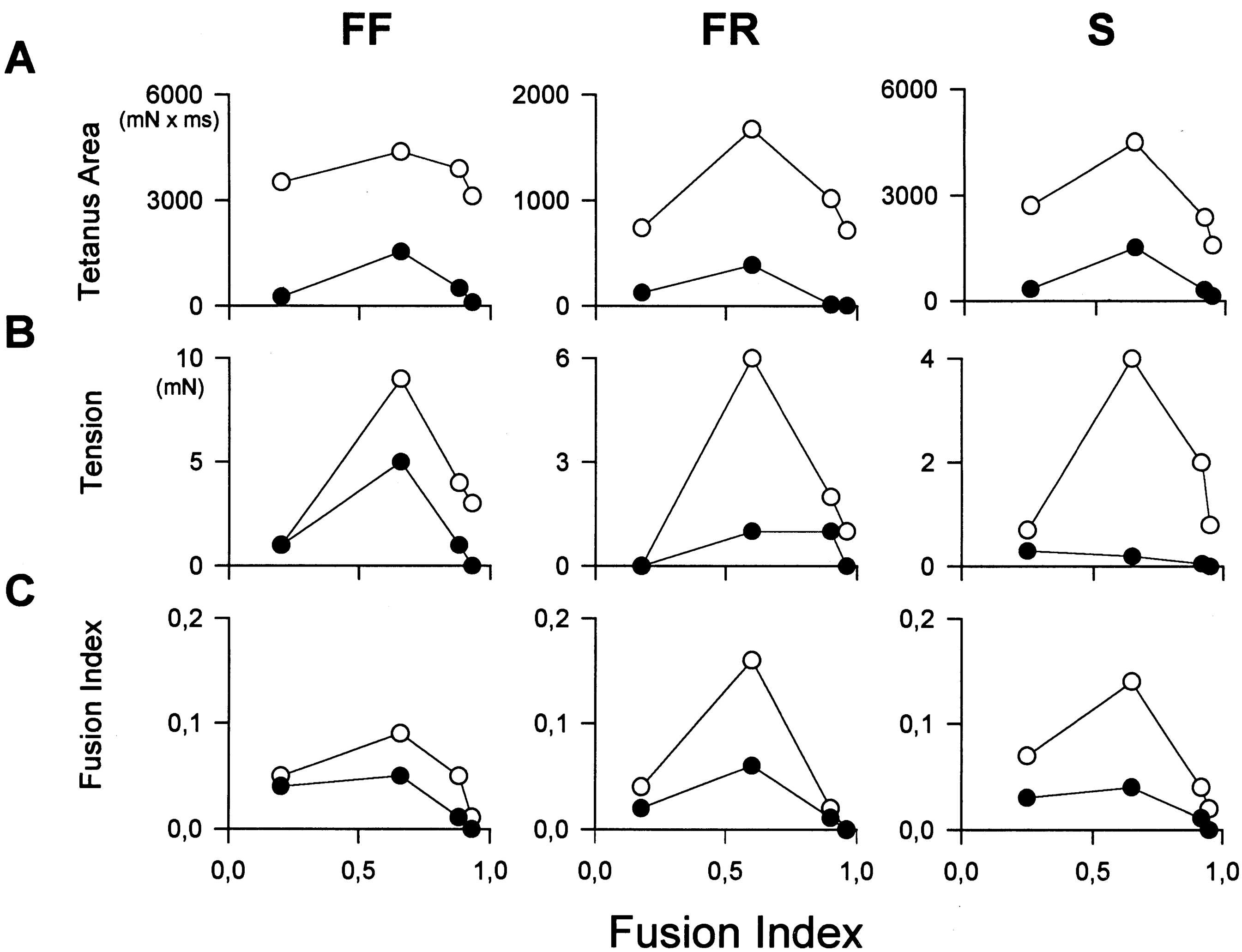

Fig. 3. Effects of the doublet: increase in area (A), tension (B) and fusion index (C) calculated as a difference between doublet and non-doublet tetani. These effects are presented as a function of tetanic fusion index at four tested frequencies of stimulation: 30, 40, 50 and 60 Hz for fast and 10, 13, 20 and 27 Hz for slow units. The FF, FR and S motor units shown are the same as in Fig. 2. Effects were stronger in the first than in the second part of the tetanus (open and filled circles, respectively).

RESULTS

Table I presents some contractile parameters of the investigated units. Figure 2 shows examples of non-doublet and doublet tetani of FF, FR and S motor units.

The doublet changed significantly the shape of unfused tetani. However, these effects were different for different motor units and for their different tetani. The doublet influenced less significantly both slightly or strongly fused tetani and more significantly middle-fused tetani. Therefore the influence of the doublet on a tetanus was analyzed in relation to its fusion (Fig. 3). It was found that the biggest increase in the area under the tension record was reached for tetani of a fusion index of 0.47, 0.63 and 0.61 for FF, FR and S units, respectively (Table II) (values non-different, *t*-Student test, $P>0.01$). For the same tetani the biggest increase in tension and in fusion index were observed, with a few exceptions.

The initial doublet increased the area under the record of tetanic tension (Fig. 3A). For all three types of motor units this increase was significantly stronger in the first than in the second part of the tetanus (Table II).

Moreover, the doublet increased the tetanic tension (Fig. 3B). This effect was better visible in the first part of a tetanus than in the second part (Table II). For FR and S units the described tension increase in the first part of a tetanus was significantly stronger than in the second part ($P<0.01$), whereas for FF units the difference was smaller and non-significant ($P>0.05$).

The doublet also increased the fusion of the tetanus (Fig. 3C). This increase was also stronger in the first part of tetanus (Table II). However, the difference was significant for slow units only ($P<0.01$).

When the effects of the doublet on tetanic area and tension were compared for all three types of motor units, slow units were found to be significantly more sensitive to this doublet than FF or FR units ($P<0.01$).

Measures of tension of the first tetanic components (responses to first pulses or doublets) showed that the doublet increased the tension of this first component by 117.8 ± 46.5%, 177.7 ± 17.6% and 203.1 ± 30.3% for FF, FR and S units, respectively (differences between FF and FR or S units significant, $P<0.01$).

Finally, effects of the doublet were observed in the ratio of areas of the first to the second part of the tetanus (Fig. 1). For all types of motor units the doublet increased this ratio (Fig. 4). However, the more fused the tetanus, the lower the value of the ratio observed, and the lower its increase evoked by the doublet.

DISCUSSION

Numerous studies have shown that during their natural or evoked activity motoneurons frequently start their firings with two potentials generated in a short time period (doublet) (Zajac and Young 1980b, Bawa and Calancie 1983, Hennig and Lomo 1987, Spielmann et al. 1993). It has been previously found that this doublet can in-

TABLE II

Mean values (and their standard deviations) for the strongest effects of the doublet observed for each motor unit type. Area, increase in the area under the tension record; Tension, increase in the tetanic tension; FuI, increase in the value of fusion index. 1, effect observed in the first part of tetanus; 2, effect observed in the second part of tetanus. FuI optimum, the value of fusion index at the 9th response of tetanus when the strongest effect of the doublet was observed. In rows corresponding to the first part of tetanus asterisks show significant differences (*t*-Student test, $P<0.01$) of these values as compared to the second part of tetanus

Motor unit type		FF	FR	S
Area	1	58.30 ± 32.1% **	59.50 ± 30.1% **	106.80 ± 31.3% **
	2	22.30 ± 20.5%	9.10 ± 11.2%	24.10 ± 12.3%
Tension	1	16.30 ± 16.7%	16.70 ± 16.8% **	42.30 ± 22.9% **
	2	11.40 ± 17.7%	5.20 ± 6.4%	7.60 ± 8.1%
FuI	1	26.00 ± 26.6%	11.00 ± 12.0%	23.90 ± 18.4% **
	2	10.80 ± 13.9%	7.20 ± 16.4%	4.20 ± 05.3%
FuI optimum		0.47 ± 0.13	0.63 ± 0.20	0.61 ± 0.14

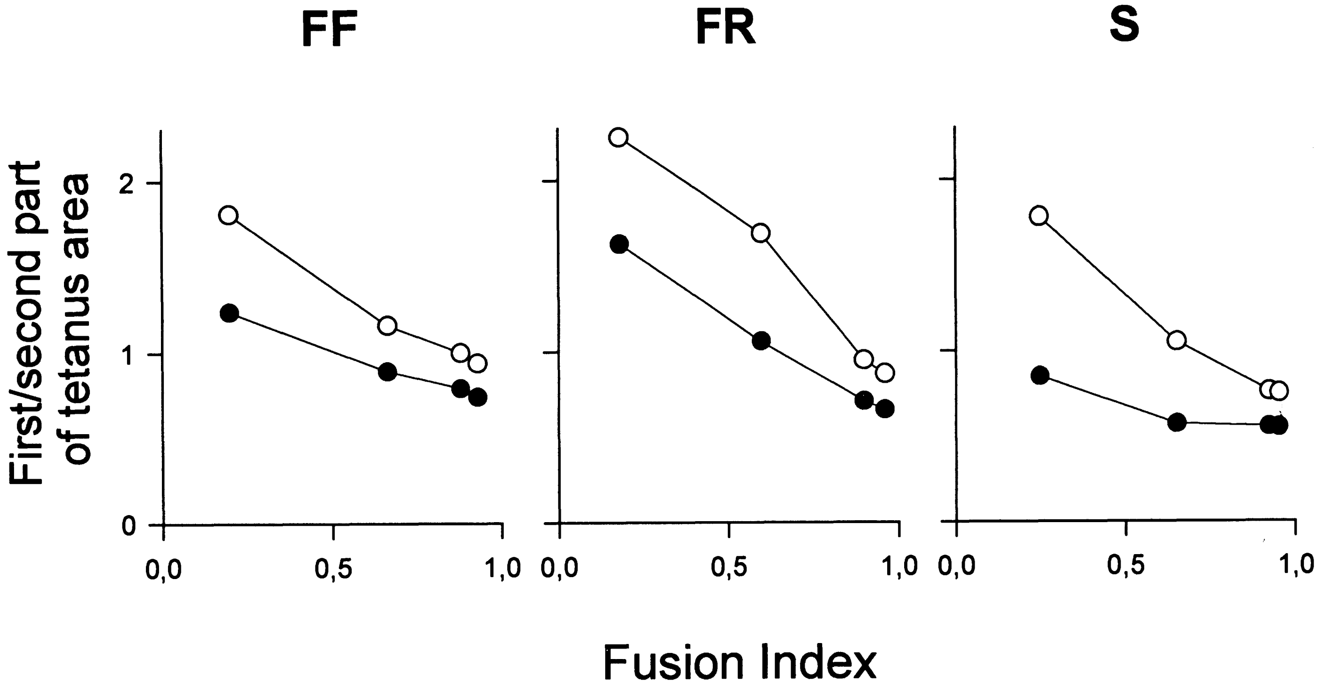

Fig. 4. The ratio of areas of the first to the second part of tetanus as a function of tetanic fusion index. Fusion index was measured at the 9th response within the tetanus. Four points correspond to four frequencies of stimulation. Open circles - doublet tetani, filled circles - non-doublet tetani. The motor units shown are the same as in Figs. 2 and 3.

crease the tetanic tension (Burke et al. 1970, Burke et al. 1976, Stein and Parmiggiani 1979). The present results show that a doublet influences the tension, the area under tension record and the tetanic fusion. Moreover, these effects were significantly better visible in the part of the tetanus directly following the doublet. We have also observed that slow units are more sensitive to stimulation with the doublet than fast units. Similarly, Burke et al. (1970) have found that "catch" property is better visible in tetani of slow motor units in cat gastrocnemius.

The standard tension-frequency relationship illustrates changes in tension of a motor unit during stimulation with trains of pulses at different frequencies. The slope in the middle part of this curve corresponds to the rate of tension summation during generation of a tetanus. The tension-frequency curve of slow motor units is more steep than for fast units (Kernell et al. 1983b, Grottel et al. 1993). Therefore, we have expected that slow units would be more sensitive to the doublet. The present results confirm this hypothesis.

The influence of the doublet on a tetanus depends on the fusion of its following part. The biggest effects have been observed in tetani with similar fusion indexes of 0.5 - 0.6 for all three types of motor units. However, these effects could be different even for motor units of the same type at each particular frequency of stimulation. Therefore, we conclude that in future studies of the tetanic course the fusion of a tetanus should also be analysed.

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