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IDENTIFIED MUSCLE FIBRES IN A CRAB. DIFFERENCES IN FACILITATION PROPERTIES

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SUMMARY

1. The superficial muscle fibres in the proximal head of the closer muscle in the crab *Eriphia* belong to four distinct fibre types (I–IV). By stimulating either the 'slow' or the 'fast' axon, facilitation properties of the EJPs were investigated in eight identifiable muscle fibres which represented the four fibre types.

2. Amount, time course and frequency dependence of facilitation (f) of both 'slow' and 'fast' EJPs differed characteristically among the fibres studied, but were similar within fibres belonging to the same type.

3. In fibres receiving double excitatory innervation through the 'slow' and the 'fast' axon (type I and II fibres), the amount of facilitation of 'fast' EJPs was smaller than for 'slow' EJPs.

4. With the exception of type I fibres, there was an inverse relationship between facilitation and the size of both 'slow' and 'fast' EJPs. Facilitation increased with frequency and time of stimulation. In type I fibres, facilitation of 'slow' EJPs increased with time at low frequencies of stimulation (up to 32 Hz), but decreased at higher frequencies after an initial rise. 'Fast' EJPs in type I fibres showed little, if any, facilitation of frequencies below 8 Hz. At frequencies above 8 Hz, they were always depressed (negative values of f).

5. The functional significance of different facilitation properties is discussed with regard to the participation of different muscle fibre types in the mechanical performance of the closer muscle.

INTRODUCTION

Facilitation is a property of most nerve terminals by which the quantal content of transmitter release can be increased with maintained stimulation in a frequency-dependent way. A matching of presynaptic facilitation properties with postsynaptic electrical and mechanical properties has been demonstrated for several crustacean muscles (Atwood & Bittner, 1971; Sherman & Atwood, 1972). For other muscles, like the opener of the crayfish *Procambarus*, this matching is less evident. In general, it

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appears that synapses generating excitatory junction potentials (EJPs) with large quantal content exhibit little facilitation (low F_e-synapses), whereas synapses producing EJPs with small quantal content show high facilitation rates (high F_e-synapses; Atwood & Bittner, 1971; Sherman & Atwood, 1972; Parnas, Parnas & Dudel, 1982*c*).

Facilitation at crustacean muscles has mainly been studied in opener and stretcher muscles (Dudel & Kuffler, 1961; Atwood & Bittner, 1971; Sherman & Atwood, 1972; Zucker, 1974; Bittner & Sewell, 1976; Parnas, Dudel & Parnas, 1982*a*; Parnas *et al.* 1982*b*,*c*; Zucker & Lara-Estrella, 1983), which are innervated by a single excitatory motor axon of the 'slow' type. The closer muscle, however, receives a double excitatory innervation through a 'fast' and a 'slow' motor neurone. The two proximal heads of the closer muscle of the crab *Eriphia spinifrons*, used in the present study, contain a population of 10–12 superficial fibres, which differ significantly in their innervation pattern, electrical responses and histochemical properties (Rathmayer & Erxleben, 1983; Maier, Rathmayer & Pette, 1984). Individual fibres can be located in each preparation at identical positions, permitting a correlation of presynaptic and postsynaptic properties at the level of identifiable muscle fibres. The 10–12 superficial fibres, which have been classified into four types (Maier *et al.* 1984), are representative of the remaining fibres building up the closer muscle (Rathmayer & Erxleben, 1983). These identifiable muscle fibres are ideally suited to permit a comparison of facilitation properties between 'fast' and 'slow' axon terminals and a correlation of these features with known postsynaptic properties.

MATERIALS AND METHODS

Recordings were made from the closer muscle of the first three pairs of walking legs of the crab *Eriphia spinifrons*. The thin nerve bundle containing the 'slow' and the 'fast' axon (SCE and FCE) was isolated in the meropodite and stimulated with a suction electrode. By adjusting duration and/or intensity of stimulation and by reversing polarity, SCE and FCE could be stimulated selectively. Details of the preparation and of intracellular recording of EJPs are described in Rathmayer & Erxleben (1983).

The preparation was kept in a temperature-controlled bath (18 °C) of artificial sea water (composition in mmol l^{-1}: Na^+ 490; K^+ 8; Ca^{2+} 10; Mg^{2+} 12; Cl^- 542; buffered at pH 7·2 with 10 mmol l^{-1} TES buffer). Calcium-free solutions were obtained by substituting Na^+ for Ca^{2+}.

The degree of facilitation (f) was defined according to Mallart & Martin (1967) as the ratio between the amplitude of the second (or the nth) and the first EJP minus one:

$$f = h/h_o - 1,$$

where h_o is the amplitude of the first EJP (unfacilitated) and h is the amplitude of a subsequent EJP. The summated synaptic depolarization was corrected for non-linear summation according to Martin (1955).

RESULTS

The fibres forming the dorsal surface of the closer muscle in its proximal part were numbered starting from the innermost fibre, number 1, to the outer fibres, numbers 10 to 12. The fibres have been described previously. They belong to four distinct types (Rathmayer & Erxleben, 1983; Maier *et al.* 1984). Type I is represented by fibres 2, 3 and 4, type II by fibre 5, type III by fibre 6 and type IV by fibres 1 and 7 to 10 or 12. In some preparations one or two additional fibres can reach the surface, emerging for short distances from the second layer of muscle fibres. All fibres are innervated by the 'fast' excitatory neurone (FCE); fibres type I and type II receive additional innervation through the 'slow' excitatory neurone (SCE) and a branch of the common inhibitor (CI).

Facilitation differs greatly among fibres 1 to 10. Fig. 1 shows recordings from the four types, taken from nine adjacent fibres of one preparation by averaging five sweeps for each fibre (with the exception of fibre 6). Fibres 10 and 11 in this preparation showed similar EJP responses to those of fibre 9. In the following, facilitation is described for EJPs in fibres 1 to 8. Since occasional recordings from other fibres belonging to the types indicated above gave very similar data, the results can be generalized for the four muscle fibre types defined previously (Maier *et al.* 1984).

Facilitation of 'slow' EJPs

Differences in facilitation properties between type I and II fibres became very prominent when repetitive stimulation with trains of varying frequencies was employed (Fig. 2). The results were very consistent among many preparations. The time course of facilitation was similar in fibres 2, 3 and 4, all belonging to type I. There was always an increase in facilitation with time of stimulation up to frequencies of 16 Hz, sometimes even 32 Hz. After an initial growth facilitation often reached a steady value, i.e. EJPs did not further increase in amplitude. The amount of facilitation was frequency dependent, ranging from 2–4 at 4 Hz up to 9–20 at 16 or 32 Hz stimulation. At higher frequencies, beginning in some fibres with 32 Hz, but in particular at 64 and 100 Hz, facilitation rose steeply at the beginning of a train to values between 9 and 16, but then declined to lower values. At the end of a 100-Hz train the EJPs were still facilitated. Their amplitudes, however, were smaller than those of the EJPs immediately following the first EJP.

The decline in amplitude during high-frequency, repetitive activity could be due to rectification in the muscle fibre membrane (Sherman & Atwood, 1972). Type I fibres have a higher input resistance than fibres of the other types and show a non-linear current-voltage relationship at depolarizations above 25 mV from resting potential (V_M). As judged from the decrease of the time constant of EJP decay at different membrane potentials, not more than 10 % of the decline in facilitation is attributable to membrane rectification. Applying calcium-free Ringer reduced the size of the EJPs three- to four-fold and thus also the total depolarization obtained during repetitive stimulation. Amount and time course of facilitation, however, remained unaffected (Fig. 3). This is an argument that the observed depression of facilitation at high

frequencies must be due mainly to intrinsic properties of the 'slow' axon terminals of this fibre type.

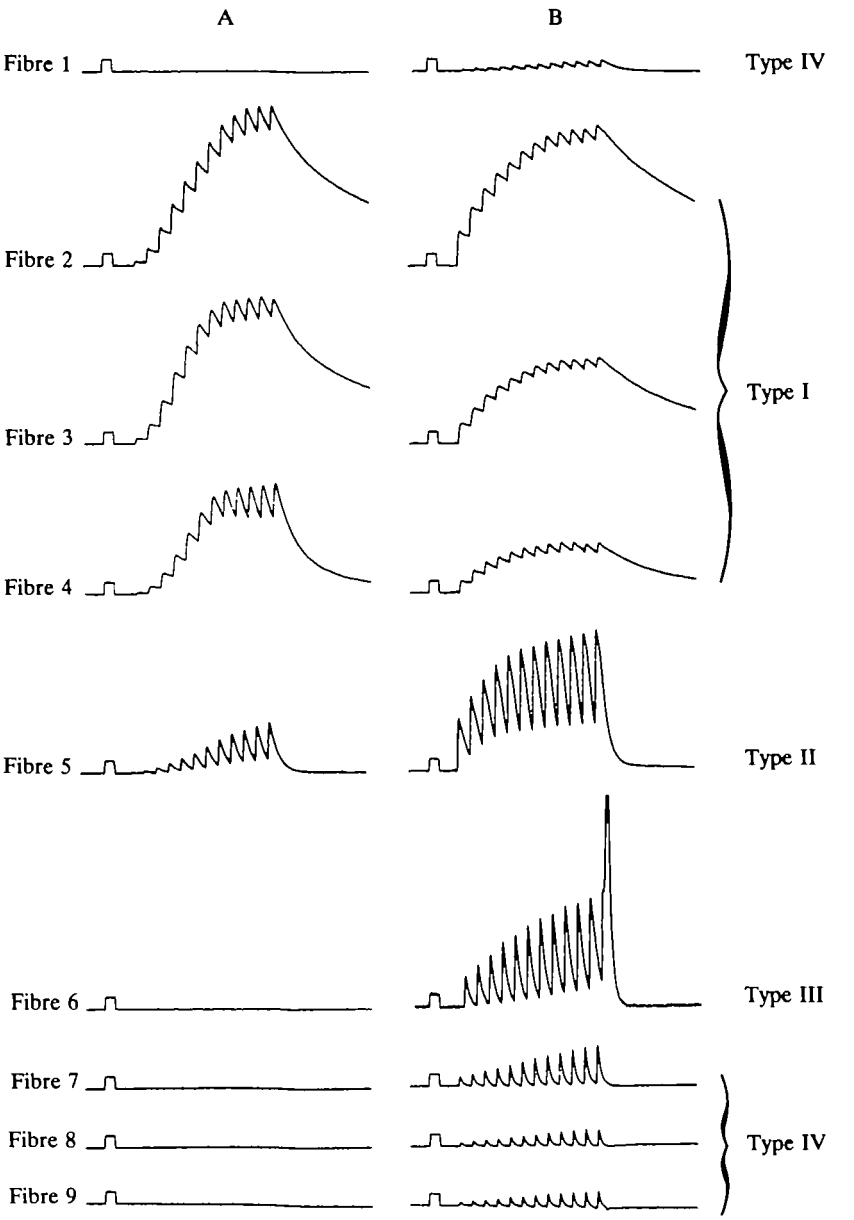

Fig. 1. Typical recording of EJPs from fibres 1 to 9 upon stimulation of SCE (A) or FCE (B) with 12 pulses at 40 Hz. Each recording represents the average of five sweeps, with the exception of fibre 6, for which, because of the generation of an action potential, only one sweep was photographed for documentation. All recordings are from one preparation. τ of fibre 4 during recording of the 'slow' EJPs was reduced because of fibre injury. The calibration pulse at the beginning of each sweep is 2 mV, 20 ms.

Facilitation in type II fibres (represented in Fig. 2 by fibre 5) at low frequencies (4 and 8 Hz) was similar to or even smaller than that in type I fibres. At higher

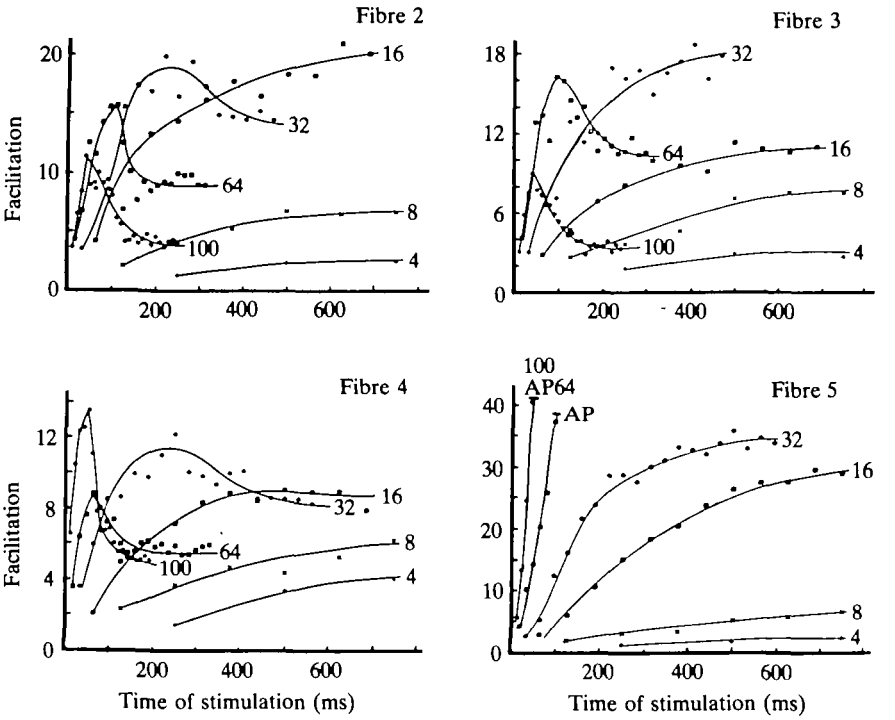

Fig. 2. Time course of facilitation of 'slow' EJPs in identified muscle fibres of type I (fibres 2, 3 and 4) and type II (fibre 5) upon repetitive stimulation of SCE with different frequencies (in Hz, indicated at the end of each line). Each diagram represents values from one experiment. Lines were fitted by eye. AP, generation of action potentials.

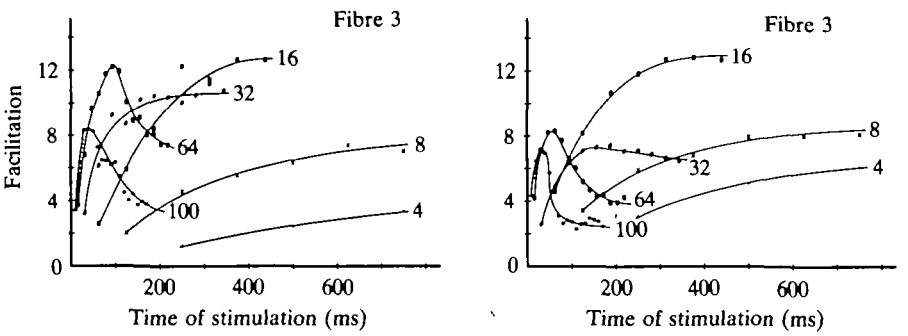

Fig. 3. Time course of facilitation in a fibre 3 in a solution containing normal Ca^{2+} (left) and no Ca^{2+} (right) upon stimulation of SCE with different frequencies. Explanation as in legend of Fig. 2.

frequencies, however, differences became apparent. Both rate and amount of facilitation increased rapidly with frequency. In no case was a decline of facilitation observed during continued stimulation. Values for facilitation were twice as high as those for type I fibres, reaching 40 within a few EJPs at 64 or 100 Hz stimulation. The highly facilitated EJPs depolarized the muscle membrane to the threshold for the generation of action potentials.

Although facilitation at low frequencies in type II fibres was often similar to that in type I fibres, the total depolarization achieved in type II fibres was much smaller than that in type I fibres because of the small initial size of the EJPs. In addition, the short time constant of type II fibres ($\tau = 20$ ms) did not permit summation of EJPs at low frequencies. In type I fibres substantial summation of EJPs was present because of their large time constant ($\tau = 100$–200 ms). This, and the large facilitating EJPs, achieved a large depolarization at low frequency discharges of the 'slow' neurone in these fibres. Only at higher frequencies (>16 Hz) did the strong increase in facilitation in type II fibres guarantee rapid and considerable depolarizations.

Facilitation of 'fast' EJPs

Repetitive stimulation also revealed significant differences in facilitation properties for EJPs generated by the 'fast' axon among the muscle fibres studied (Fig. 4). At low stimulation frequencies, 'fast' EJPs in type I fibres (number 2 to 4) were barely, if at all, facilitated. Beginning with 8 Hz stimulation the 'fast' EJPs declined in amplitude and became even smaller than the first EJP in the train. The rate and amount of this depression depended on stimulation frequency. The decline in EJP amplitude was not due to membrane rectification. The overall depolarization of type I fibres produced by the 'fast' EJPs was usually much smaller than that produced by 'slow' EJPs (see also Fig. 1).

The 'fast' EJPs for all other fibre types (represented by numbers 1, 5 to 8 in Fig. 4) showed facilitation in a frequency-dependent manner, without signs of depression. Facilitation was highest at junctions where output of transmitter was low (type IV fibres), values for facilitation of 25 to 30 often being obtained upon high-frequency stimulation. In type II and III fibres, represented in Fig. 4 by numbers 5 and 6, where 'fast' EJPs were large, facilitation was lower by about 40–70 % when compared with the results obtained from type IV fibres.

DISCUSSION

The approximately 320 fibres that form the closer muscle of *Eriphia* can be grouped into four types according to innervation pattern, passive electrical properties and histochemical parameters (Rathmayer & Erxleben, 1983; Maier *et al.* 1984). In the present investigation it could be shown that facilitation properties also differ significantly between the four types, as represented by eight identified fibres.

The matching concept proposed by Sherman & Atwood (1972) is only partially realized in the closer of *Eriphia*. As in the opener and stretcher muscles of other crabs

(Atwood & Bittner, 1971; Sherman & Atwood, 1972) muscle fibres of high input resistance and long time constant have large EJP amplitudes. With regard to the values of facilitation, the data are difficult to compare, because Atwood & Bittner (1971) and Sherman & Atwood (1972) studied facilitation properties only at 10 Hz stimulation. Their data at this frequency are in accordance with those obtained from

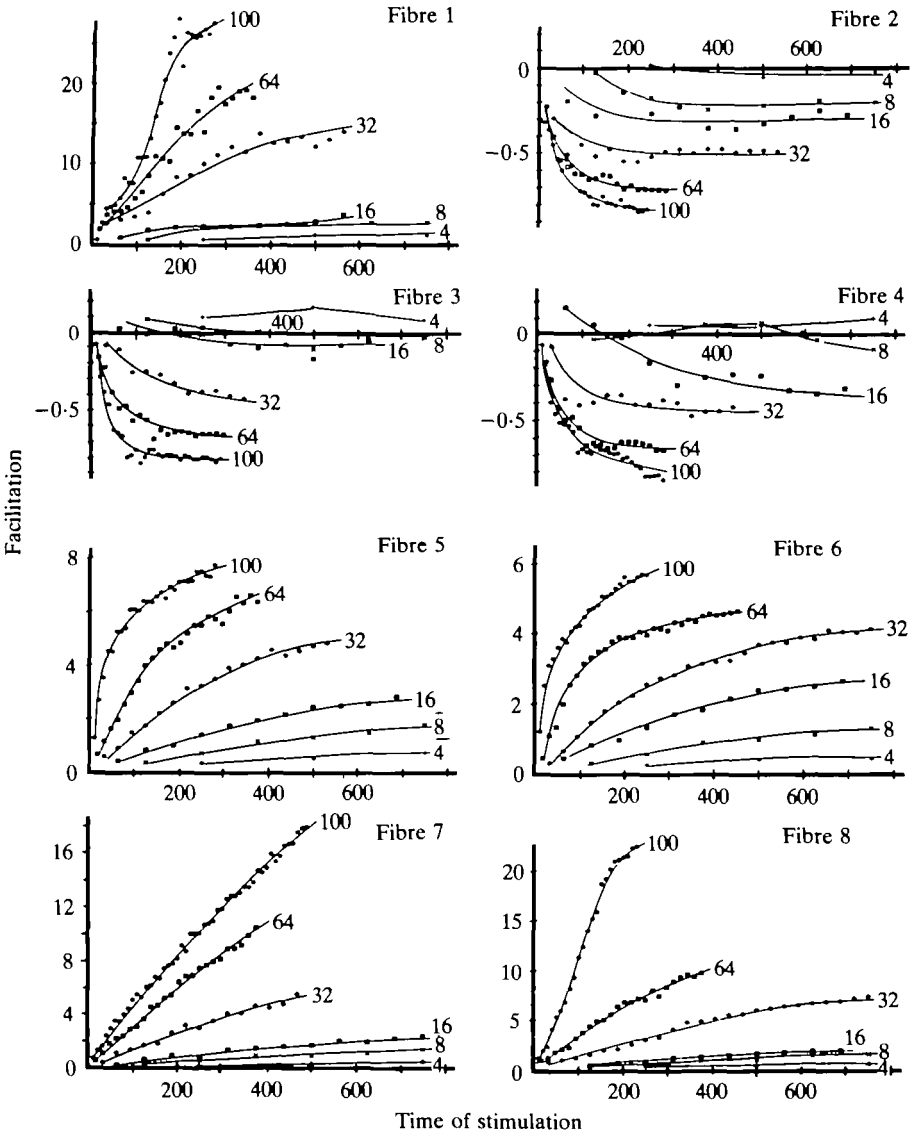

Fig. 4. Time course of facilitation of 'fast' EJPs in fibres of type I (fibres 2, 3 and 4), type II (represented by fibre 5), type III (represented by fibre 6) and type IV (fibres 1, 7 and 8) upon repetitive stimulation of FCE with different frequencies (in Hz, indicated at the end of each line). Each diagram represents measurements from one experiment. Lines were drawn by eye.

Eriphia: facilitation is smaller in fibres with long time constant and high input resistance (type I fibres) than in fibres with short time constant and smaller input resistance (type II fibres). The differences become even more significant in *Eriphia*, when higher stimulation frequencies are employed. At frequencies below 10 Hz this relationship, however, is not present: facilitation in type I fibres is similar to or often larger than in type II fibres.

The most obvious differences in facilitation properties of the four muscle fibre types occur in type I fibres. The 'slow' EJPs show at higher frequencies after an initial increase a decline in the amount of facilitation during repetitive stimulation. The 'fast' EJPs undergo depression (negative values for f). The mechanisms for the decline in f, as well as for the depression, have not been investigated in this study. Membrane rectification plays a negligible role. Since at high frequencies only few stimuli have been administered to the axon, we regard the observed depression as neither being caused by incomplete invasion of the nerve terminals by the action potentials nor by conduction block in axon branches. The decrease in the amount of transmitter released by each consecutive nerve impulse cannot be accounted for by a depletion of the pool of releasable transmitter, since a reduction of the quantal content in the presence of low $[Ca^{2+}]_o$ does not affect the time course of the decline of facilitation. The finding, that amount and time course of facilitation of EJPs remain unaffected by a reduction of $[Ca^{2+}]_o$ is in agreement with observations at other crustacean neuromuscular junctions (Linder, 1973; Zucker, 1974; Zucker & Bruner, 1977).

At low stimulation frequencies facilitation of 'slow' EJPs in type II fibres is similar to or even less than that in type I fibres. At higher frequencies, however, facilitation in type II fibres reaches very large values. They resemble those reported for other crustacean muscles (Atwood & Bittner, 1971) as being typical for high F_e-synapses. EJPs generated by 'fast' axons are known for their poor facilitation properties and, often, a rapid depression in amplitude with maintained stimulation (Hoyle & Wiersma, 1958; Bruner & Kennedy, 1970; Zucker & Bruner, 1977; Bryan & Atwood, 1981). In the closer muscle of *Eriphia* there is also a considerable diversity in facilitation properties for 'fast' EJPs. This is a new finding. With the exception of type I fibres, 'fast' EJPs at all other fibre types are facilitated upon repetitive stimulation. The values obtained for facilitation of 'fast' EJPs range from negative numbers to positive values as high as 25. The inverse relationship between EJP size and facilitation described for 'slow' axon terminals (Atwood & Bittner, 1971; Sherman & Atwood, 1972) applies also for certain terminals of the 'fast' axon. In all fibres exhibiting a double excitatory innervation (type I and II) the amount of facilitation for 'fast' EJPs is always smaller than that for 'slow' EJPs regardless of the amplitude of a single EJP.

The functional importance of different facilitation properties is evident. At low frequencies of SCE discharge, almost exclusively type I fibres will become depolarized as a consequence of their large EJP amplitudes, their long membrane time constant and the facilitation properties of their 'slow' axon terminals. Type I fibres are tonic fibres (Maier *et al.* 1984) and will be responsible for postural control. Type II fibres, on the other hand, will be depolarized only slightly at low SCE frequencies,

because their small EJPs tend to facilitate only weakly. During walking essentially SCE is used, FCE being activated only occasionally with two or three action potentials at high frequency at the beginning of a step (Ballantyne & Rathmayer, 1981). The frequency of SCE discharges can reach 250 Hz during a step. This will recruit type II fibres massively, since the SCE nerve terminals at this fibre type show high values of facilitation at frequencies above 16 Hz. Since, in addition, type I fibres are strongly inhibited during walking by the concomitant discharge of the CI neurone, the power during walking is almost exclusively generated by type II muscle fibres. Occasional discharges of FCE will depolarize type II fibres even more, but will also recruit type III fibres, since their EJPs are large and, in addition, exhibit facilitation. 'Fast' EJPs in type I fibres are depressed and also effectively inhibited through a presynaptic action of CI (see Rathmayer & Erxleben, 1983).

The large proportion of type IV fibres (about 50 % of the closer muscle) apparently is barely active during walking at low speed, since two or three action potentials of FCE cause only very small depolarizations in these fibres. For their activation, FCE must discharge at high frequencies. It should be interesting to determine under what conditions of locomotor activity this occurs.

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