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CONTRIBUTIONS OF STRUCTURE AND INNERVATION PATTERN OF THE STICK INSECT EXTENSOR TIBIAE MUSCLE TO THE FILTER CHARACTERISTICS OF THE MUSCLE–JOINT SYSTEM

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Summary

It is shown that the low-pass filter characteristics of the muscle–joint system of the femur–tibia joint of the stick insect *Cuniculina impigra* result from co-contraction of the extensor and flexor tibiae muscles. The most distal region of the extensor muscle, which contains a high percentage of slow muscle fibres, is involved in this co-contraction. This conclusion results from the following evidence. (1) Inertial and friction forces do not affect the characteristics of the low-pass filter of the muscle–joint system. (2) There is some co-contraction of the extensor and flexor muscles during sinusoidal stimulation of the femoral chordotonal organ at high stimulus frequencies. Both muscles generate tonic forces that increase with increasing stimulus frequency and also increase with time from the beginning of stimulation until a plateau is reached. (3) For the extensor muscle, this tonic force is produced by its most distal portion only. (4) Electrical stimulation of the common inhibitory motoneurone (CI_1) reduces the tonic force generated in this most distal portion of the extensor muscle. Therefore, CI_1 stimulation reduces the amplitude of tibial movement in response to sinusoidal stimulation of the femoral chordotonal organ at stimulus frequencies below 0.5 Hz (over this frequency range, the tibial movement amplitude is a function of the force amplitude produced by the whole extensor muscle and there is no co-contraction), but at chordotonal organ stimulus frequencies of 1 Hz and above, CI_1 stimulation increases the tibial movement amplitude (in this case, movement amplitude is limited by the degree of co-contraction of the extensor and flexor muscles).

With repeated chordotonal organ stimulation at higher stimulus frequencies, the tibial movement amplitude steadily decreases. This must be a consequence of increasing levels of co-contraction of the extensor and flexor muscles, since at low stimulus frequencies (no co-contraction) there is no reduction in movement amplitude during repeated stimulations.

It is concluded that co-contraction of the extensor and flexor tibiae muscles prevents instability in the reflex loop in spite of the high gain necessary for the generation of catalepsy. Therefore, the mechanism described can be considered to be an adaptation to the ecological niche occupied by this animal. The contribution of the distal part of the extensor muscle to this system can be switched off by the CI_1 during active movements.

Key words: stick insect, *Cuniculina impigra*, femur–tibia control system, filter characteristics, co-contraction, habituation, muscle-partitioning, extensor muscle, CI_1, SETi.

Introduction

Behavioural adaptations to the requirements of certain ecological niches are usually thought to be generated within the nervous system. In this study, we report a behavioural adaptation that is based upon a muscular phenomenon. This adaptation is the extremely low upper corner frequency of the muscle–joint system of the femur–tibia (FT) feedback loop in the inactive stick insect *Cuniculina impigra* (Bässler, 1983*a*,*b*; Bässler *et al.* 1996). The term ‘muscle–joint system’ is used here to describe all of the mechanically relevant attributes of the muscles, lever arms and joint architecture. The ecological relevance of this low upper corner frequency is as follows. Twig mimesis is an important factor in the biology of phasmids, and catalepsy is a behavioural component of twig mimesis (Bässler, 1983*a*). In addition to other factors, a very high gain for each joint control system is a necessary prerequisite for the generation of catalepsy. However, control systems with very high gain tend to become unstable. A very low upper corner frequency is one possible mechanism by which instability may be prevented (Bässler, 1993).

It is likely that the low upper corner frequency of the stick insect FT feedback loop is a consequence of a functional partitioning of the muscles involved, especially of the extensor

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tibiae muscle (Bässler *et al.* 1996). Many muscles are known to be functionally partitioned in vertebrates (Windhorst *et al.* 1989 and references therein) as well as in insects (e.g. Hoyle, 1978; Burns and Usherwood, 1979; Theophilidis and Burns, 1983; Müller *et al.* 1992). The function of such partitioning in the stick insect extensor tibiae muscle (Bässler and Storrer, 1980) is indicated from the following results. In *Carausius morosus* and *Cuniculina impigra*, the relative sizes of different regions of this muscle are correlated with the filter characteristics of the FT muscle–joint system during resistance reflexes. *Carausius morosus*, with an upper corner frequency of approximately 1 Hz, has a smaller distal region containing approximately 50 % slow muscle fibres, whereas *Cuniculina impigra* has an upper corner frequency of approximately 0.1 Hz and a relatively large distal region, in which the percentage of slow fibres is almost 100 % (Bässler *et al.* 1996). The present study demonstrates that the distal region of the extensor tibiae muscle is responsible for the development of tonic forces which increase the degree of co-contraction. The degree of co-contraction, in turn, is mainly responsible for determining the upper corner frequency of the FT muscle–joint system.

The distal part of the extensor tibiae muscle extends over only 3–4 mm of the total muscle length of 25–30 mm. In contrast to the rest of the muscle, it contains two rows of muscle fibres, one originating at the dorsal roof of the femur and the other originating at its posterior side wall. Both rows mainly have fibres containing the slow isoform of myosin ATPase. In the posterior row, all except a few triply innervated fibres at its posterior end are innervated only by the slow extensor tibiae motoneurone (SETi) and the common inhibitor 1 (CI_1), the latter generating large inhibitory junctional potentials (IJPs). In the dorsal row, half of the fibres are of this innervation type, while the others are triply innervated (Bässler *et al.* 1996). The proximal main part of the extensor muscle (comprising 80–90 % of the muscle length) contains fast extensor tibiae motoneurone (FETi)-innervated fibres, triply innervated fibres and dually innervated fibres. In contrast to the distal region, the dually innervated fibres in the main body of the muscle receive only small CI_1 IJPs. Approximately 10 % of the fibres of the main part of the extensor muscle contain slow myosin ATPase, the rest are fast fibres (Bässler *et al.* 1996).

An investigation of the contribution of muscle structure to the degree of co-contraction and thus to the filter characteristics of the muscle–joint system will also have general implications.

First, during resistance reflexes in the FT control system of resting stick insects, only one of the three motoneurones innervating the extensor tibiae muscle is normally active, namely SETi. Its firing rate is highly reproducible (see Bässler, 1993). This provides an opportunity to study the dependence of the muscle force upon the defined activity of a single slow motoneurone under nearly intact conditions (e.g. under 'natural' neuromodulatory conditions; see Evans and Siegler, 1982). Comparable studies on the locust extensor tibiae muscle have been performed using extracellular electrical stimulation of single motoneurones (e.g. Hoyle, 1955, 1978; Burns and Usherwood, 1979; Wilson, 1979; Evans and Siegler, 1982), a technique that is not practicable for the stick insect SETi, since the axons of FETi and SETi are always present in the same nerve. Intracellular stimulation of motoneurones of the stick insect has not yet been performed in this context.

Second, because of their high gain, the FT feedback systems of some stick insect species operate close to the border of instability (see Bässler, 1993), as do some other joint control loops (e.g. human elbow control; Prochazka and Trend, 1988; Jacks *et al.* 1988). Without a relatively low upper corner frequency of the muscle–joint system, these stick insect control loops would be unstable. The filter characteristics of the muscle–joint system stabilize the control system (Bässler, 1993) and are therefore one possible mechanism for preventing feedback oscillations in joint control loops with high gain.

Third, the dependence of gain upon stimulus frequency in an open-loop system (i.e. the filter characteristics of an open-loop system) is a very important factor in an investigation of the stability of a closed-loop system (Stein and Oguztöreli, 1976). In all vertebrate and many invertebrate systems, this relationship can only be detected indirectly for anatomical reasons (see discussion in Stein, 1982). The FT control system of orthopteran insects allows direct measurement of gain and changes in gain (Kittmann, 1991) because its sensor (the femoral chordotonal organ, fCO) can be specifically stimulated without interfering with the muscles. Additionally, the filter characteristics of the sensory cells, of the interneurones and motoneurones and of the muscle–joint system can often be determined because most of these elements can be individually identified (Bässler, 1983*b*, 1993; Bässler *et al.* 1996). Therefore, this system may be used as a model for other joint control loops.

Details of the anatomy of the femur–tibia feedback system and of the stick insect are given in Bässler (1983*a*, 1993). The structure of the extensor muscle and its innervation pattern are described in Bässler *et al.* (1996).

Materials and methods

The experiments were carried out on hindlegs of female *Cuniculina impigra* Redtenbacher (syn. *Baculum impigrum* Brunner) obtained from a colony at Kaiserslautern University, Germany.

Force measurements

The forces exerted by the extensor tibiae muscle were measured using force transducers (Swema SG4-25 for indirect measurements, and SG3-0.25 for direct measurements) connected to a balanced bridge (Hellige TF19). Both force transducers measured the force nearly isometrically. The force transducer was either directly attached to the muscle apodeme using a clamp (direct measurement), or attached to the tibia (the femur–tibia angle was 90 °) some distance away from the femur–tibia joint and the apodeme of the flexor muscle was cut (indirect measurement). The indirectly measured forces were

converted into forces at the end of the apodeme using the known lever arms. The lever arm between the axis of rotation of the joint and the insertion of the muscle apodeme on the tibia was measured in the following way. The tibia was cut exactly 2 cm distal to the femur–tibia joint and the cut end was moved for 1 cm around the 90 ° position while the movement of the muscle apodeme in the femur was measured simultaneously. From these values, the lever arm was calculated.

Preparation

The animals were restrained on a horizontal foam plastic (Veneret) plate under crossed pins with either the dorsal or ventral side uppermost; except for the experiments investigating the roles of inertia and friction and habituation of movement, in which the plate was vertical. The right hindleg was fixed, using dental cement (Protemp, Espe), perpendicular to the body with the femur horizontal. The parts of the femur that had to be opened were surrounded by a small basin filled with *Carausius* saline (Bässler, 1977). In the dorsal-side-up preparation, a small window was cut into the dorsal femoral cuticle, taking care that only a small number of muscle fibre origins were removed. In the ventral-side-up preparation, the flexor tibiae muscle, the crural nerve and the retractor unguis apodeme were removed together with most of the ventral femoral cuticle in that region of the leg. The large leg trachea (supplying the flexor tibiae muscle) was cut distally and the cut end was raised above the surface of the saline so that saline would not be sucked into it. The diaphragm between the flexor and extensor haemolymph space was carefully removed, leaving the smaller leg trachea (supplying the extensor tibiae muscle) intact.

Intracellular recordings

Intracellullar recording from the muscle fibres was performed conventionally using electrodes filled with 3 mol l^{-1} KCl and having a resistance of 20–40 MΩ.

Stimulation of the fCO

The femoral chordotonal organ (fCO) was mechanically stimulated by inserting the receptor apodeme into a clamp and cutting it distal to the clamp. The clamp was attached to a pen-motor (Hellige, upper corner frequency 100 Hz) controlled by a function generator (Tektronix). The stimulation amplitude was usually 350 μm (corresponding to 35 ° joint movement; Weiland *et al.* 1986), except for during the habituation experiments, when it was 400 μm. Sequences of sinusoidal and, in some cases, triangular wave stimuli were separated by intervals of 30–60 s, during which time the animals were briefly touched with a paint brush to ensure a high gain. The sequence in which sinusoidal stimulus trains with different frequencies were applied was random.

Extracellular recordings

Extracellular recordings were made using paraffin-oil hook electrodes (Schmitz *et al.* 1988) or by pressing the nerve against a trachea using two steel myogram wires insulated except at the tip (according to Pflüger, 1977).

Stimulation of CI_1

The common inhibitor 1 (CI_1) neurone was electrically stimulated in the following way. The body of the animal was opened dorsally (dorsal-side-up preparation), the gut was removed and the body cavity filled with *Carausius* saline. The fluid in the body cavity had no direct contact with the saline surrounding the femur except through the coxa and trochanter. A bipolar extracellular stimulation electrode was placed on the retractor nerve (nl_5). Rectangular pulses of 1 ms duration and with an amplitude of 1–3 V were sufficient to stimulate CI_1 but did not produce stimulus artefacts in intracellular recordings from muscle fibres. The success of the stimulation was monitored by extracellular recording from the protractor nerve (nl_2), which contains an axon branch of CI_1. The stimulus frequency was 20 Hz. This is close to the lower end of the CI_1 frequency range (10–100 Hz) during active movements (Büschges *et al.* 1994).

Movement of the tibia

The movement of the tibia was measured in two ways. When investigating the effects of CI_1 stimulation, a dorsal-side-up preparation was used. The hindleg was perpendicular to the body, the femur–tibia joint being approximately 1.5 cm outside the Veneret plate. The plane of movement of the tibia was vertical (with the tibia pointing downwards in the 90 ° position). Movement of the tibia was filmed in front of a protractor using a Leicina Super-8 camera at 24 frames s^{-1} and the films were analysed during projection at a reduced rate (1–4 frames s^{-1}). Only maximum and minimum femur–tibia angles were measured (accuracy 2 °). In other experiments, the animal's body was vertical and the movement plane of the tibia was horizontal. Tibial movement was measured from film recordings as described above or using an optical device as described by Weiland *et al.* (1986).

Evaluation of force records

This investigation aimed to identify the factors determining the low upper corner frequency present in the transfer function between motoneuronal activity and movement in the inactive stick insect. To this end, muscle force characteristics were related to motoneuronal as well as to movement variables. Most of the terms normally used for the description of muscle force characteristics are not appropriate in the present context and additionally they involve a certain amount of theory regarding muscle mechanics. We therefore defined the following purely descriptive terms (see Fig. 1), as Storrer and Cruse (1977) did for a similar reason. The spontaneous force is the force that is developed under the influence of spontaneously active motoneurones (in the case of the extensor muscle, SETi only). Zero force is the force that is present after approximately 10 s of no motoneuronal activity. SETi activity can be reproducibly suppressed by relaxation of the fCO even at low velocities (Bässler *et al.* 1982). Therefore, the force

produced at the end of a relaxation stimulus longer than 5 s was taken as zero force for the extensor muscle. Zero force is indicated at the beginning of each force record by a horizontal line. Force maximum and force minimum describe maximum and minimum forces recorded during repeated fCO stimuli. The difference between them is the force amplitude.

Results

The role of inertia and friction

Before the contribution of muscle partitioning to the filter characteristics of the FT muscle–joint system can be studied, other possible influences on these filter characteristics must be investigated. The upper corner frequency of the open-loop system (obtained from movement measurements, Bässler *et al.* 1996) is determined by the low-pass filter characteristics of the muscle–joint system (Bässler *et al.* 1996). The filter characteristics of this system may theoretically derive from muscle characteristics, from the degree of co-contraction or from joint friction and inertial forces.

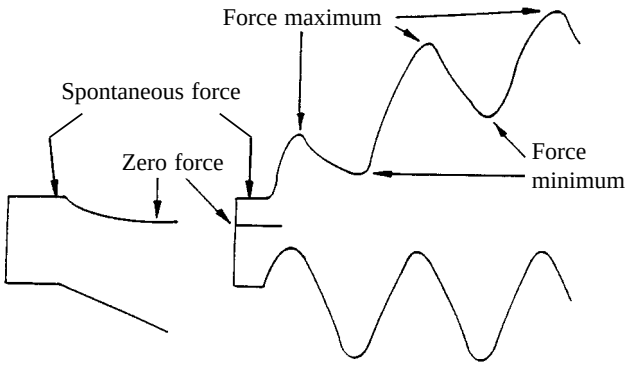

Fig. 1. Schematized example force records defining the terms used in the evaluation of force records. Upper traces: muscle force record, lower traces: fCO stimulus (relaxation is downwards). The first example shows the definition of zero force by a rampwise relaxation stimulus. The second example illustrates the terms used for evaluation of responses to sinusoidal stimuli. See text for further details.

To investigate whether inertial forces and joint friction contribute significantly to the filter characteristics, the amplitude–frequency characteristics of the open-loop system were measured for three hindlegs, first with the leg intact (length of tibia plus tarsus approximately 4 cm) and then with the tibia cut 0.5 cm distal to the femur–tibia joint. Movement of the tibia was similar to that in previous studies (Bässler 1983*a*, see also Fig. 10). Response amplitudes were measured, averaged and plotted from the first five complete cycles (three cycles for 0.01 Hz). For intact legs, these plots were very similar to those obtained in previous experiments (Bässler and Foth, 1982; Weiland *et al.* 1986). An example is given in Fig. 2. After cutting the tibia, the same values were obtained, indicating that inertial forces and possibly also joint friction (which would both have been altered by removal of the tibia) do not contribute to the filter characteristics, at least not for the frequency range investigated.

Co-contraction of the extensor and flexor tibiae muscles

From previous experiments (Storrer and Cruse, 1977), it is known that, during sinusoidal stimulation of the fCO of *Carausius morosus* at frequencies higher than approximately 0.3 Hz, the minimum forces recorded from the extensor tibiae and flexor tibiae muscles do not return to the zero-force level (see Fig. 1). Therefore, during higher-frequency stimulation, there is always a certain amount of co-contraction of these two muscles which may contribute to the filter characteristics of the muscle–joint system.

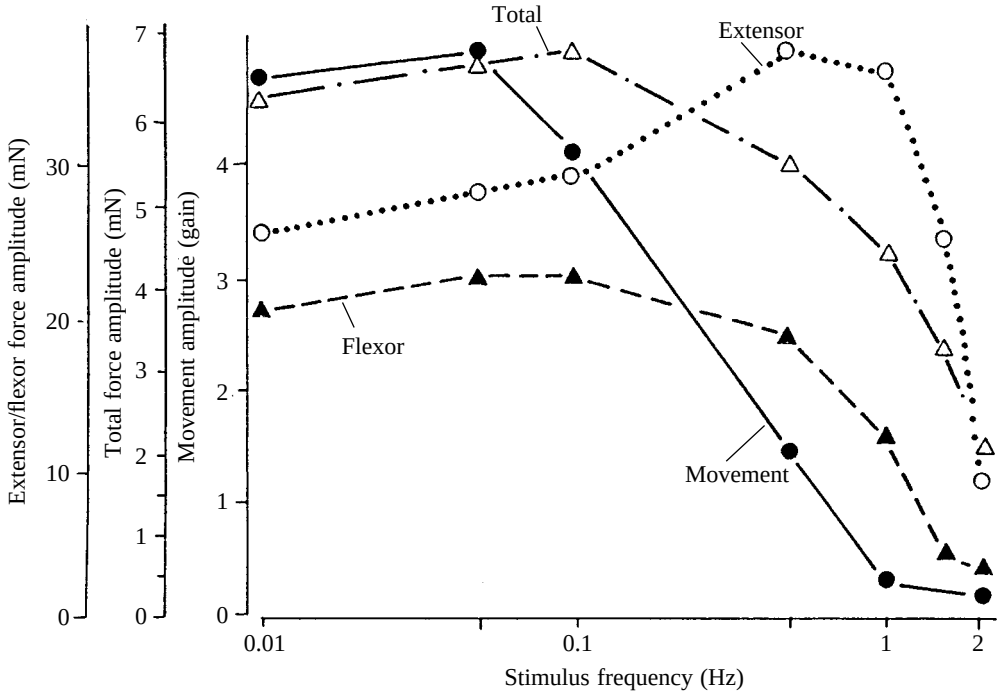

Fig. 2. Movement amplitude of the tibia, force amplitude of the flexor and extensor tibiae muscles and total force measured at the tibia 25 mm from the FT joint *versus* fCO stimulation frequency (amplitude 350 μm). Force values for the flexor and total force are means of 10 measurements on a single intact leg; values for the extensor are from this study (see Fig. 8) and Bässler *et al.* (1982). The ordinates are scaled such that the maximum values are at the same height, except for the flexor force amplitudes. The latter values were usually smaller than the extensor force values owing to the longer lever arm of the flexor. These amplitude–frequency plots were derived from force records similar to those shown in Fig. 3.

Is this co-contraction also present in *Cuniculina impigra* and is it evident from the beginning of a sinusoidal stimulation or does it slowly increase? To answer these questions, the forces produced by the extensor and flexor muscles were recorded during sinusoidal fCO stimulation. Four legs were used. Fig. 3 shows typical results from one animal for different stimulus frequencies between 0.5 and 3 Hz. Fig. 3 confirms that the degree of co-contraction increases with increasing stimulus frequency above 0.5 Hz in *Cuniculina impigra*. In addition, Fig. 3 demonstrates that this co-contraction increases with the number of stimulus cycles after the beginning of stimulation (note the increase in minimum force with time). The extensor muscle minimum force reached a plateau earlier than did the flexor muscle minimum force in all cases. However, the time course of the force increase as well as its magnitude differed from animal to animal.

The spontaneous force produced by the extensor muscle was always larger than its zero force (for definitions, see Materials and methods and Fig. 1), but was the same as the zero force for the flexor. Apparently, only the extensor muscle develops force in response to spontaneous motoneurone activity.

To estimate the contribution of flexor force characteristics to the filter characteristics of the muscle–joint system, the force amplitudes of the flexor muscle after they had reached a constant level were measured in records similar to those shown in Fig. 3. Fig. 2 (flexor) gives the mean force amplitude values for one animal. The absolute values of mean force amplitude differed between the four legs, but in all cases the flexor force amplitudes decreased above 0.1 Hz stimulus frequency. This frequency is considerably lower than that at which the extensor muscle values decrease (Fig. 2; Bässler *et al.* 1982; see also Fig. 8C) but is larger than the upper corner frequency of movement amplitude (0.05 Hz; Fig. 2).

To understand the contribution of co-contraction to the movement of the leg, the sum of the extensor and flexor forces (or the sum of the torques exerted by them) must be known. The amplitude of this resulting (total) force is not simply the sum of the amplitudes of the extensor and flexor forces for two reasons: (1) the lever arm of the extensor muscle is considerably smaller than that of the flexor muscle; (2) the force increase during sinusoidal fCO stimulation is faster than its decline in both muscles. Therefore, the maximum force in one muscle occurs earlier than the minimum force in the other (see dotted lines in Fig. 3).

The resulting force (total force) exerted on a force transducer 25 mm distal to the femur–tibia joint in response to a sinusoidal fCO stimulus was recorded from four legs. Fig. 2 shows the mean values for one of these animals. The absolute values differed to some extent, but in all four cases the force amplitudes were maximal at 0.1 Hz (in one case, the amplitude at 0.05 Hz was the same as at 0.1 Hz). The decrease in the amplitude of total force between 0.1 and 0.5 Hz cannot be attributed to a decrease in the amplitude of both of the two muscles, since only the flexor amplitude decreased whereas the extensor amplitude increased over this frequency range in all cases (Fig. 2; Bässler *et al.* 1982). Hence, the total force amplitude decrease must be the result of the phase shift between maximum force in one muscle and minimum force in the other.

It is clear that any movement is the result of the total force produced as well as the amount of co-contraction occurring. For example, when the total force is directed towards extension and the flexor is not fully relaxed, the flexor is stiffer than in the totally relaxed state and therefore exerts a higher resistance against the movement. The higher the isometric force of the antagonist, the higher will be this resistance. The force of the

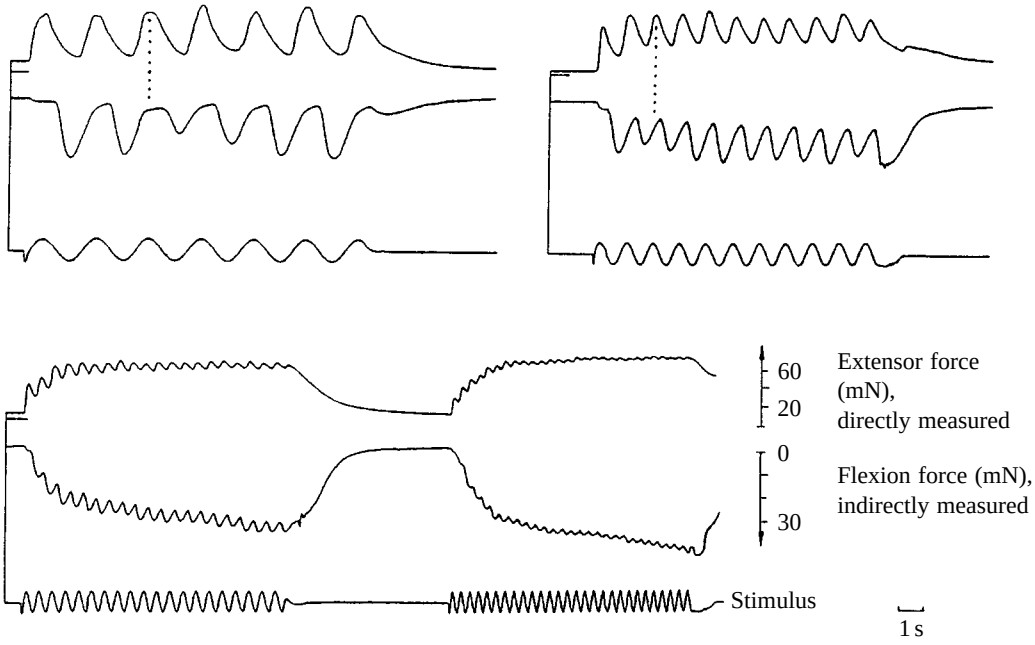

Fig. 3. Simultaneously recorded forces produced by the extensor tibiae muscle (directly measured, top traces) and the flexor tibiae muscle (indirectly measured; see Materials and methods; middle traces, force increase downwards) in response to sinusoidal stimulation of the fCO at four different frequencies (0.5, 1.0, 2.0 and 3.0 Hz; amplitude 350 µm, bottom traces; relaxation is downwards). The inverse axis and expanded scale were used for the flexor force because the direction and amount of the corresponding torques are then approximately equal for both muscles. For the extensor, zero force is indicated by the horizontal line at the beginning of each record; for the flexor, the force was zero at the beginning in all cases. At maximum extensor force, the flexor force had not yet reached its minimum (dotted lines). Scales are the same for all examples.

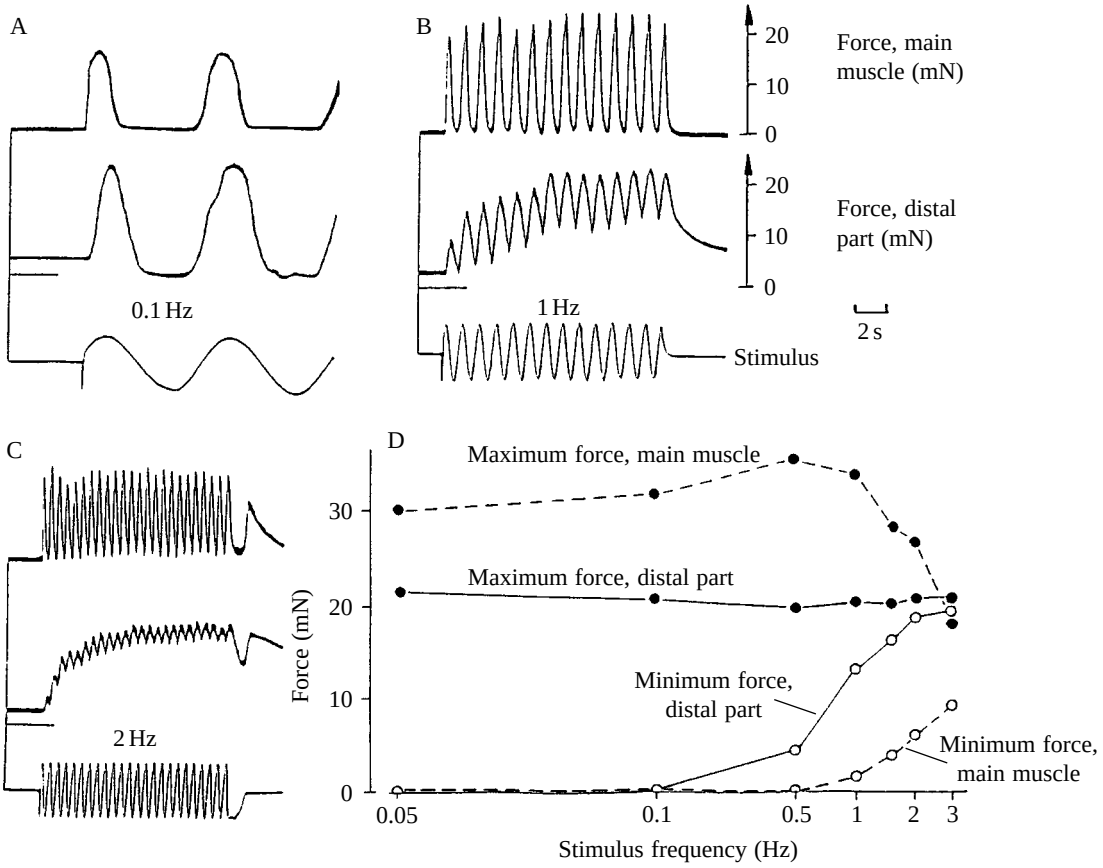

Fig. 4. Extensor tibiae forces evoked by sinusoidal stimulation of the fCO (relaxation downwards) recorded separately but simultaneously for the main muscle region (85–90 % of muscle length, upper traces) and the distal muscle region (10–15 % of muscle length, middle traces). The extensor apodeme was cut approximately at the border between the distal region and the main region. Muscle force for the main region was measured directly, distal muscle force was measured indirectly. (A–C) Three typical examples with differing stimulus frequencies (lower traces; scales apply for A–C). In the distal muscle record, zero force is indicated by the short horizontal line at the beginning of each trace. (D) Results from a single series of experiments after the plateau had been reached in the distal muscle force (means of four experiments). A–C and D are from two different animals.

antagonist can be formally divided into two components: (i) the difference between zero force and minimum force and (ii) the difference between the actual force of the antagonist and its minimum force. This second component is a consequence of the fact that the maximum force in one muscle occurs earlier than the minimum force in its antagonist. At fCO stimulus frequencies during which the minimum force reaches zero (0.1 Hz and lower, see Figs 3, 4, 8), only the second component is present. At higher fCO stimulus frequencies, the first component increasingly dominates (see Figs 3, 4, 8).

A comparison of the different plots in Fig. 2 shows that the movement amplitude decreases at frequencies higher than 0.05 Hz, while the total force amplitude only starts to decrease at frequencies higher than 0.1 Hz. Since the movement amplitude results from both the total force amplitude and the amount of co-contraction, this implies that the degree of co-contraction must be the decisive factor that limits movement amplitude, at least for stimulus frequencies close to 0.1 Hz. The much steeper decrease in movement amplitude compared with total force amplitude indicates that co-contraction also plays a role at higher stimulus frequencies.

Forces produced by different parts of the extensor muscle

As mentioned in the Introduction, a comparative study of the fibre composition and innervation pattern of the extensor tibiae muscles of *Carausius morosus* and *Cuniculina impigra* demonstrated that *Cuniculina impigra* (which has a considerably lower movement upper corner frequency) has a larger distal region of this muscle. Additionally, the distal region of the extensor tibiae muscle in *Cuniculina impigra* contains a much larger percentage of slow muscle fibres compared with that of *Carausius morosus* (Bässler *et al.* 1996). Is this distal region involved in co-contraction? To answer this question, we cut the extensor apodeme in *Cuniculina impigra* hindlegs close to the beginning of this distal region (approximately 3 mm away from the femur–tibia joint) and simultaneously measured the forces of the two separate parts (dorsal-side-up preparation). The activity of the extensor motoneurones was not measured because the motoneuronal response is very reproducible. The haemolymph and oxygen supply to the muscle remained nearly undisturbed. Four animals were investigated.

Fig. 4 shows three examples for sinusoidal stimuli of different frequencies. In all experiments with stimulus frequencies below 1 Hz, the forces of the main region of the muscle had minima that were equal to the spontaneous forces before the stimulus and these were equal to the zero forces (for definitions, see Fig. 1). The minimum forces of the distal region were smaller than the spontaneous forces before the stimulus at low stimulus frequencies and equal to the zero

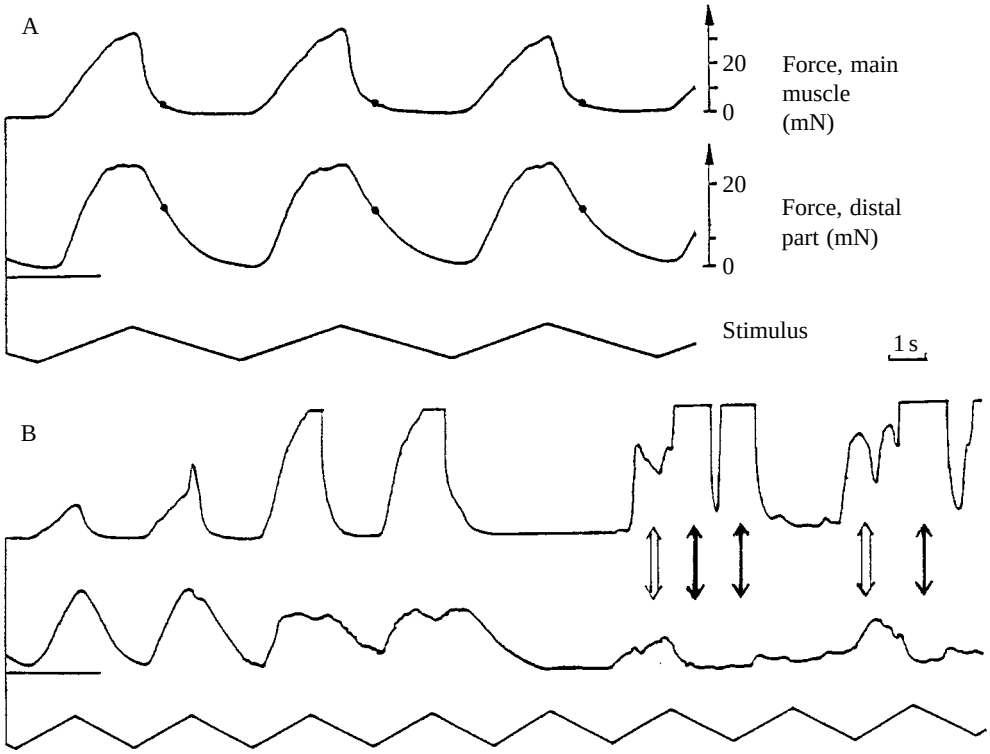

Fig. 5. Extensor tibiae forces evoked by triangular stimulation of the fCO (relaxation downwards) recorded separately but simultaneously from the main and distal regions of the muscle (other details as in Fig. 4). Sections from a longer recording. (A) Inactive animal. The forces 1 s after the stimulus peak are marked by a filled circle on both traces (compare with Fig. 6A, which shows the membrane potential of the muscle fibres). (B) The animal was inactive during the first cycle and then became increasingy active as a result of touching the abdomen. Filled arrows indicate strong forces in the main muscle region. Open arrows indicate weak forces in the main muscle region.

forces (horizontal lines at the beginning of the records; Fig. 4A, middle trace). Apparently, only the distal part of the extensor muscle develops force in response to the spontaneous SETi discharge rate (see also Fig. 6B). At stimulus frequencies of 0.5 Hz and higher, distal region minimum and maximum forces increased steadily until a plateau was reached. After reaching this plateau, the maximum force was independent of stimulus frequency but minimum forces increased strongly with increasing stimulus frequency (Fig. 4D). All records showed the same characteristics, although the absolute force values differed to some extent from animal to animal.

By adding the forces of both muscle parts in Fig. 4A, it is clear that the total force amplitude is produced by the whole muscle at low stimulus frequencies. For higher stimulus frequencies (Fig. 4B,C), the contribution of the main muscle to the overall force amplitude increases progressively with increasing stimulus frequency. The minimum total force is mainly determined by the distal muscle region.

Fig. 5A shows a section from a longer record with triangular fCO stimulation. From a large number of electrophysiological records, it is known that, under these conditions, FETi fires only during the first few stimulus cycles (i.e. it will not be active in the record shown in Fig. 5A). SETi stops firing just after the stimulus peak and CI_1 shows an action potential only rarely, if at all (see also Fig. 6A). It is clear that the forces increase more slowly and decrease faster in the main region of the muscle than in the distal part (compare the force values 1 s after the stimulus peak; dots in Fig. 5A). Therefore, the effect on that portion of co-contraction that results from the slow decrease of the antagonistic force is also mainly produced by the distal muscle region.

In Fig. 5B, the animal was touched on its abdomen after the first stimulus cycle. For three cycles, this led to an increase in the overall force (disturbance increases the gain as long as the animal does not become active, Bässler, 1983*a*; Kittmann, 1991; for definitions of active and inactive, see Bässler, 1983*a*). The disturbance only increased the force produced by the main muscle region (probably due to FETi activation) but not that of the distal region, which decreased (probably due to CI_1 activation). After a period of no extensor force (probably corresponding to flexor force), the animal became active (i.e. it tried to perform active movements). When the main muscle then developed low-level forces (open arrows), the distal muscle region usually also produced some force. However, when the main muscle developed strong forces (filled arrows, the force record became saturated), the force produced by the distal muscle region dropped, usually down to the level of the zero force. Similar results were obtained with all other animals (36 trials altogether).

These results led to the following conclusions. (1) During repetitive fCO stimuli, i.e. when only SETi activates both muscle regions, the distal part of the muscle exerts forces that fall much more slowly than those of the main muscle region. This causes slowly increasing force minima at higher stimulus frequencies, which will contribute to co-contraction in the intact leg. The main muscle region contributes only minimally to co-contraction. If, therefore, co-contraction really does decrease movement amplitude, this would explain why a larger distal muscle region in *Cuniculina impigra* is accompanied by a lower upper corner frequency of the muscle–joint system. (2) Only the distal muscle region exerts a measurable force in response to spontaneous SETi discharge

rates. Since the main muscle part also possesses a number of dually innervated muscle fibres, this implies that there are two different types of these fibres, those that produce force in response to spontaneous SETi discharge rates (located in the distal muscle region) and those that do not produce force under these conditions (located in the main muscle region). (3) The animal is apparently able to switch off the distal muscle region in cases when fast active movements are performed. Since SETi is always active together with FETi during fast active extension movements (Weiland and Koch, 1987; Nothof and Bässler, 1990), this can only be due to CI_1 activity (CI_1 normally fires in active animals; Büschges *et al.* 1994), the influence of which should be stronger on the distal muscle part (which has larger IJPs).

In two animals, the roof of the distal femur was removed to eliminate functionally the triply innervated muscle fibres of the distal dorsal row. Although this operation also destroys part of the trachea in this region and disturbs haemolymph flow, the rest of the distal muscle region (now almost exclusively containing dually innervated fibres) produced considerable forces, but not for long periods. Except for the absolute values, all the other force characteristics of this altered distal part were similar to those for an intact dorsal distal row. Since, after functional elimination of the distal dorsal row of muscle fibres, only dually innervated fibres remained to exert force, the force characteristics of the complete distal muscle region must be mainly attributable to its dually innervated muscle fibres.

Intracellular responses of the muscle fibres to stimulation of the fCO

Are the differences in force characteristics between the two parts of the muscle also evident in the electrophysiological responses of the muscle fibres? To answer this question, intracellular records from muscle fibres were made during stimulation of the fCO (ventral-side-up preparation; five inactive animals). Triangular and sinusoidal stimuli with increasing frequency were used.

Apart from differing resting potentials, no fundamental differences could be found between records from the middle of the main muscle part and those from the posterior row of the distal part, nor between records from triply or dually innervated muscle fibres when FETi and CI_1 did not fire (more than 20 fibres of each fibre type in each muscle part were investigated). Fig. 6 shows examples from three dually innervated fibres in the posterior distal row (compare with Fig. 7A from a triply innervated fibre in the main muscle region). In response to triangular stimulation, the membrane potential dropped to the resting level immediately after the last EJP in each cycle (Fig. 6A). During sinusoidal stimulation, the minimum membrane potential also approached the resting level. In none of nearly 100 records obtained was there any increase in minimum membrane potential observed that was comparable to the increase in minimum force recorded in the distal muscle part (Fig. 4). In other words, when only SETi is active, the difference in the characteristics of force production between the distal and main regions of the muscle is not mirrored by their intracellular membrane potential characteristics.

Influence of electrical stimulation of CI_1 upon the forces produced by the two muscle parts

The strong reduction in the forces produced by the distal muscle region in the active animal (Fig. 5B) could be explained if the effects of CI_1 stimulation differed between the main and distal muscle parts. Therefore, the effect of electrical stimulation of CI_1 on the two muscle parts was studied in 11 dorsal-side-up preparations (five for the main muscle and six for the distal muscle part). The main muscle measurements were combined with intracellular recordings from muscle fibres. In three additional preparations, the force of the distal part of the muscle was measured indirectly, together with intracellular recordings from distal muscle fibres of the dorsal row.

CI_1 stimulation only slightly decreased the membrane potential in muscle fibres of the main muscle part, irrespective of whether they were dually (SETi, CI_1) or triply innervated. During sinusoidal fCO stimulation, the forces of the main muscle were also not very strongly reduced by CI_1 stimulation (Fig. 7A). There was no influence of CI_1 stimulation on the spontaneous force produced by the main muscle. In contrast, the forces produced by the distal muscle part were strongly decreased by 20 Hz CI_1 stimulation (Fig. 7B,C) and large IJPs were seen in most dually innervated muscle fibres of that region (Fig. 7B; see also Bässler *et al.* 1996). In the inactive animal, CI_1 stimulation reduced the spontaneous force of the distal muscle region measured before the fCO was stimulated (Fig. 7B). The force during such CI_1 stimulation dropped to the zero-force value obtained with slow relaxation stimuli (see Fig. 1). CI_1 stimulation is apparently able to counterbalance the influence of spontaneous SETi discharge on the muscle fibres of the distal muscle part. It can also counterbalance most of the influence of stronger SETi activity on the distal muscle fibres, but not on those of the main muscle. It is, therefore, conceivable that strong CI_1 activity (as is often present in active animals) is able to switch off the force production of the distal part of the extensor muscle, whereas that of the main muscle is only minimally affected by CI_1 activity.

Effect of CI_1 stimulation on muscle force depends upon fCO stimulation frequency

The contribution of the distal and main muscle parts to the determination of force amplitude and minimum force differs for different fCO stimulus frequencies (Fig. 4). Therefore, the influence of CI_1 stimulation on these two parameters of muscle force should also differ for different fCO stimulus frequencies. This deduction was tested using a dorsal-side-up preparation and combined mechanical fCO and electrical CI_1 stimulation (five animals).

Without CI_1 stimulation, minimum extensor force (measured after the plateau of force had been reached; see Fig. 3) was different from zero at stimulus frequencies larger than 0.1 Hz (Fig. 8). In all cases, force amplitude was maximal

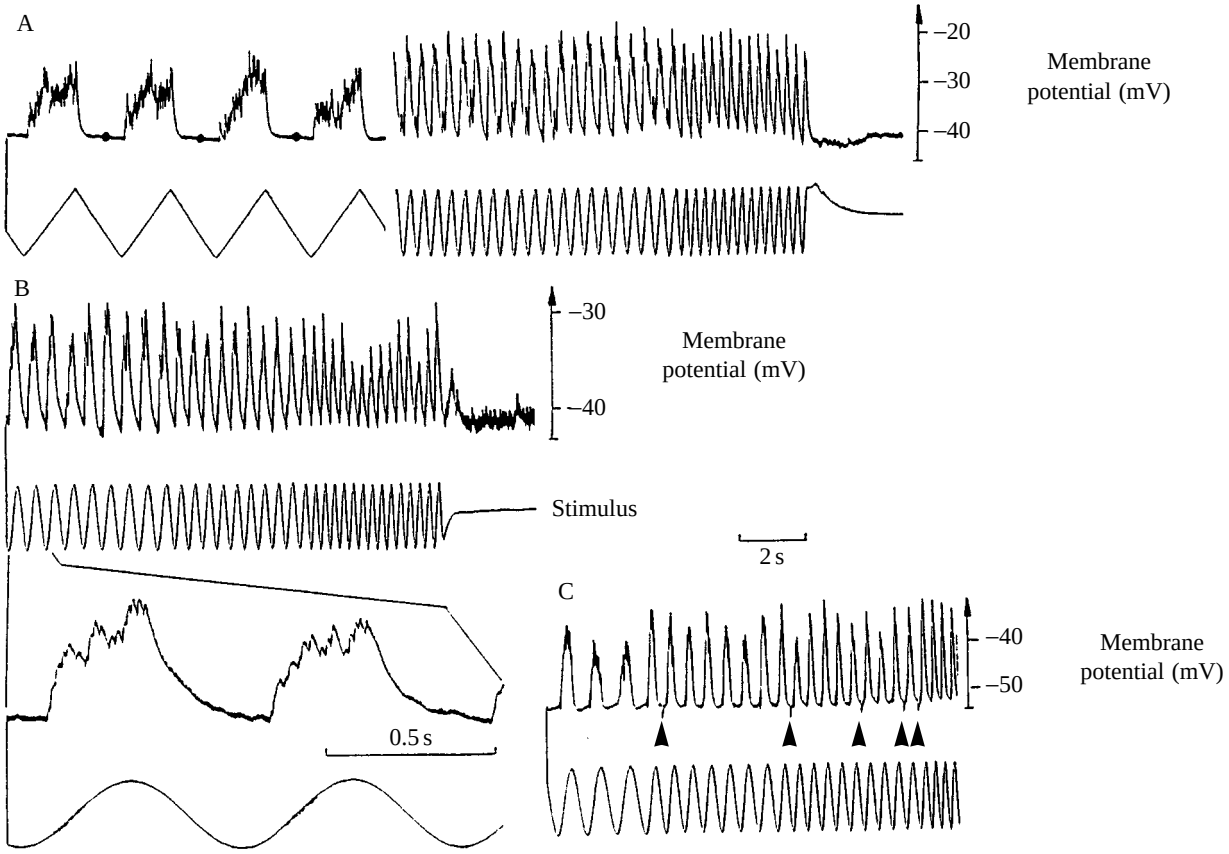

Fig. 6. Intracellular recordings of membrane potentials (upper traces) of three dually innervated muscle fibres (SETi- and CI_1-innervated) of the distal posterior row of the extensor tibiae muscle during stimulation of the fCO (lower traces). Scales apply to all recordings. (A) High SETi activity. In the left-hand panels, a dot marks the membrane potential 1 s after the peak of each triangular stimulus (compare with Fig. 5A). During sinusoidal stimulation (right-hand panels), some SETi excitatory junctional potentials (EJPs) occur even during membrane potential minima. (B) Low SETi activity (normal case). The first two cycles are also presented on an expanded time scale to demonstrate the superposition of EJPs. (C) CI_1 inhibitory junctional potentials (IJPs) at membrane potential minima (arrowheads). The resting potential is rather negative for this fibre type.

at a frequency of 0.5–1 Hz. When the minimum force had reached a plateau, the CI_1 neurone was electrically stimulated (bars in Fig. 8A,B). CI_1 stimulation decreased both force amplitude and minimum force in all cases. The decrease in force amplitude was larger at an fCO stimulus frequency of 0.1 Hz, but became relatively smaller with increasing fCO stimulus frequency. In contrast, CI_1 stimulation reduced the minimum force to a greater extent, the higher the fCO stimulus frequency. Thus, at low fCO stimulus frequencies, CI_1 stimulation mainly reduced force amplitude, whereas at high fCO stimulus frequencies it mainly reduced minimum force and with it the amount of co-contraction. This is exactly what one would expect from the experiments shown in Figs 4 and 7.

Influence of electrical CI_1 stimulation upon movement

It was suggested above that the tibial movement amplitude is determined by the amplitude of the total force and the amount of co-contraction. If this is correct, CI_1 stimulation should reduce the movement amplitude during low-frequency fCO stimulation (because it will mainly reduce force amplitude) but during high-frequency fCO stimulation it should increase the movement amplitude (by reducing mainly the degree of co-contraction).

To verify this hypothesis, the amplitudes of tibial movement in response to fCO stimulation with and without CI_1 stimulation were compared. It should be noted that, of the two antagonists, CI_1 innervates only the extensor tibiae muscle. The flexor tibiae muscle is innervated by two other common inhibitors, CI_2 and CI_3 (Hale and Burrows, 1985; Debrodt and Bässler, 1989), which normally fire together with CI_1 (Schmidt and Rathmayer, 1993; Wolf, 1990). Only those animals that had tibial resting positions larger than 120 ° were evaluated (five animals). Each fCO stimulation started without CI_1 stimulation. When maximum and minimum tibia position remained constant after a few cycles, CI_1 was stimulated until constant maxima and minima were again recorded (normally after only a few cycles). Afterwards, the movement was recorded for 3–10 additional cycles without CI_1 stimulation. Maxima and minima for the two measurements without (i.e. before and after) CI_1 stimulation were averaged.

Without fCO stimulation, CI_1 stimulation always decreased

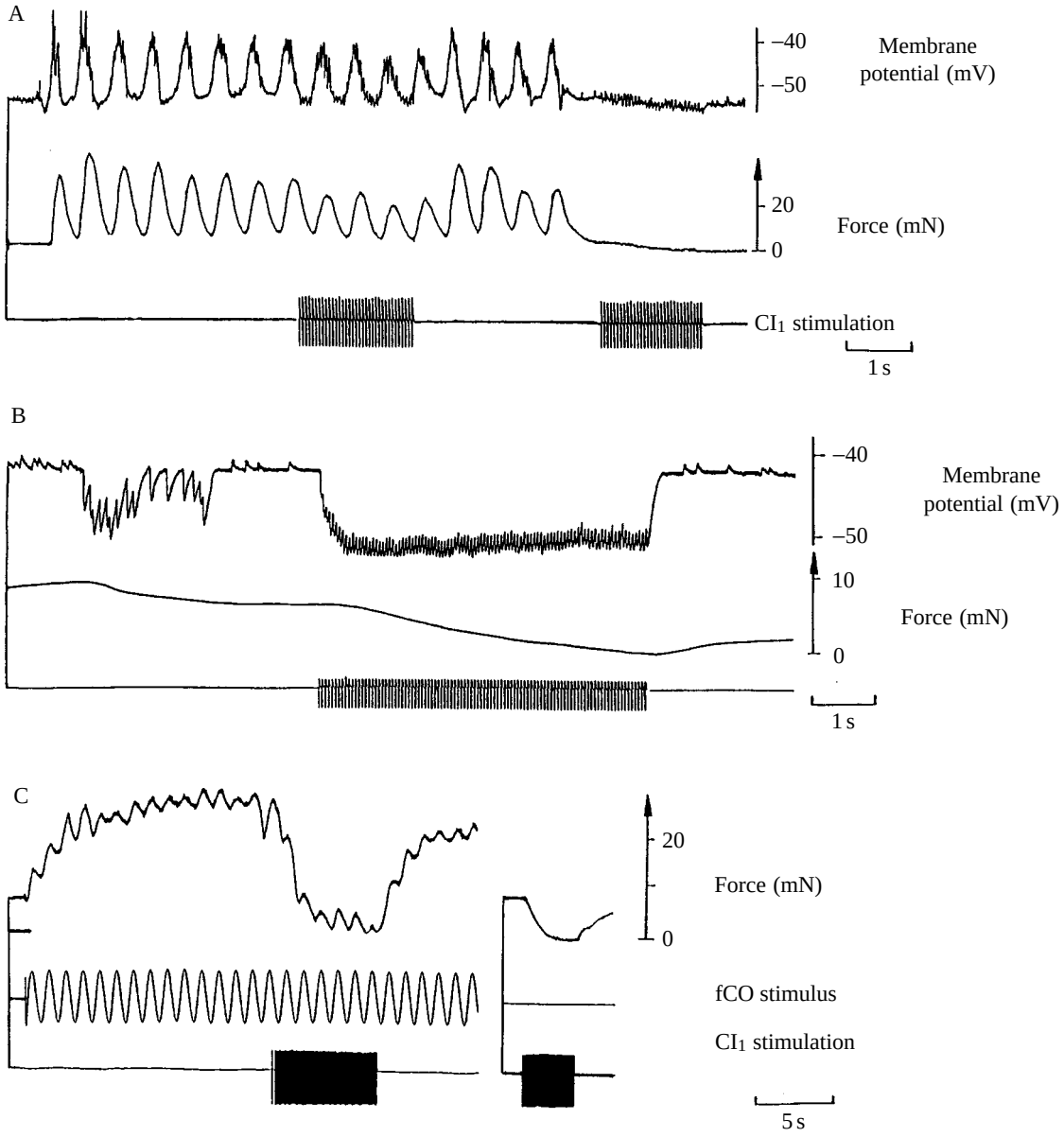

Fig. 7. (A) Intracellular membrane potential (upper trace) of a triply innervated muscle fibre in the middle region of the extensor tibiae and force (middle trace) of the main muscle (directly measured) in response to sinusoidal fCO stimulation (not shown) and electrical CI_1 stimulation at 20 Hz (lower trace). (B) Intracellular record from a dually innervated fibre of the posterior distal row of the extensor tibiae muscle and force of the distal muscle region (indirectly measured). Axis labels are as in A. The animal was first briefly disturbed (some spontaneous CI_1 inhibitory junctional potentials). Then CI_1 was electrically stimulated (20 Hz). (C, left-hand example) Force of the distal muscle part (directly measured, upper trace) in response to sinusoidal fCO stimulation (middle trace) and 20 Hz electrical CI_1 stimulation (lower trace). (C, right-hand example) The same preparation as in the left-hand example, but no fCO stimulus.

the femur–tibia angle to values of 90–100 °, irrespective of the resting position. Fig. 9 shows typical results from CI_1 stimulation at 20 Hz during fCO stimulation at different frequencies. In all cases, the movement range shifted towards flexion when CI_1 was stimulated. This must be due to the smaller maximal extensor forces since the flexor muscle is not influenced by CI_1 stimulation. The amplitude of tibial movement decreased with CI_1 stimulation at low fCO stimulus frequencies but increased at high fCO stimulus frequencies.

These results together confirm that the decrease in movement amplitude with increasing fCO stimulus frequency is mainly a result of the increased level of co-contraction since a reduction of this level by CI_1 stimulation increases the movement amplitude.

Habituation of the movement response

The amount of co-contraction builds up slowly after the beginning of a period of sinusoidal stimulation at frequencies larger than 0.1 Hz (see Figs 3, 4). If an increasing amount of co-contraction decreases the movement amplitude, the latter

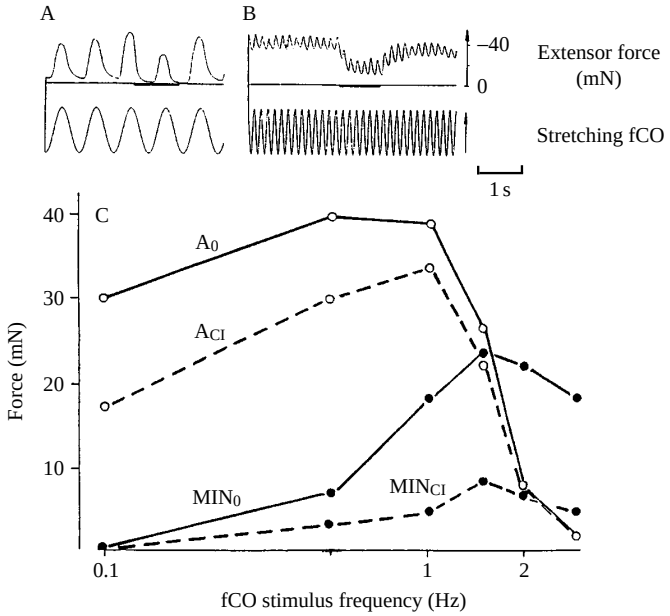

Fig. 8. Forces produced by the extensor tibiae muscle (upper traces; indirectly measured, converted into forces at the end of the apodeme; see Materials and methods) in response to sinusoidal stimulation of the fCO (lower traces; amplitude 350 µm, extension upwards). (A,B) original records. The horizontal lines denote zero force. During the time indicated by the bar, CI_1 was electrically stimulated at 20 Hz. (C) Quantitative characterization of the records after plateau values had been reached for one animal. Force amplitude (difference between maximum and minimum force) just before CI_1 stimulation (A_0) and towards the end of 20 Hz CI_1 stimulation (A_{CI}), and minimum forces just before (MIN_0) and towards the end of CI_1 stimulation (MIN_{CI}) *versus* fCO stimulus frequency. Values represent the mean of 2–4 measurements.

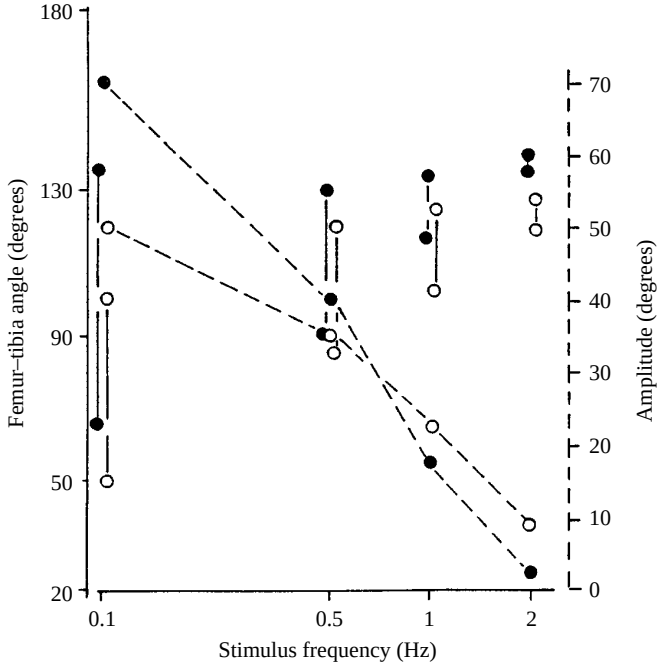

Fig. 9. Movement of the tibia in response to a 350 µm sinusoidal stimulus to the fCO at different frequencies without (filled circles) and with 20 Hz electrical stimulation of CI_1 (open circles). Means of two consecutive series of experiments on one animal. Solid vertical bars and left-hand ordinate indicate the range of tibial movement within maximal and minimal femur–tibia angles. Dashed lines and right-hand ordinate: amplitude of tibial movement (difference between maximum and minimum femur–tibia angle).

should therefore decrease more after the beginning of an ongoing sinusoidal stimulus than should the underlying SETi frequency. To test this deduction, the following experiments were performed.

The fCO was sinusoidally stimulated at 0.1 and 1 Hz and the resulting tibial movements were measured. FETi and SETi activity were also recorded using myogram wires. Four animals were investigated, with two series of experiments on each animal. Fig. 10A shows an original recording at 1 Hz stimulus frequency and Fig. 10B is an evaluation of such a record from another animal. It is obvious that SETi activity remains nearly constant; in contrast to the situation in *Carausius morosus* (Kittmann, 1984), there is only a small habituation (or none, see Fig. 10A) of the response of SETi in *Cuniculina impigra*. It is known from previous experiments that the FETi response habituates (Bässler, 1983*b*), so that there are no FETi action potentials after the first few stimulation cycles. In contrast to the SETi response, however, the movement amplitude decreases (Fig. 10) in the same way as recorded previously for *Carausius morosus* (Kittmann, 1991). Except for the first few cycles, during which FETi was sometimes active, this can only be due to an increasing amount of co-contraction.

In *Carausius morosus*, the decrease in movement amplitude with increasing stimulus number is a property of the underlying neural network and can therefore be called habituation (Kittmann, 1991). The course of habituation in that species is independent of stimulus frequency. In *Cuniculina impigra*, however, the same phenomenon (termed habituation owing to its appearance) seems to be mainly a muscular phenomenon. Since the amount of co-contraction depends on stimulus frequency, this habituation should also depend on it. This is indeed the case (Fig. 10B,C). At 0.1 Hz stimulus frequency, there was almost no decrease in either movement amplitude or SETi activity and no increase in co-contraction (Figs 3, 4). Evidently, the same phenomenon (the decrease in response amplitude with cycle number at 1 Hz) is generated in different ways in the two closely related species *Carausius morosus* and *Cuniculina impigra* (see Discussion).

Discussion

Muscle partitioning as a basis of co-contraction and low-pass filter characteristics

The results demonstrate that the characteristics of tibial movement in the open-loop configuration differ from the characteristics of the motor output. Apparently, the FT muscle–joint system forms a low-pass filter that decreases the amplitude of those movements that result from sinusoidal

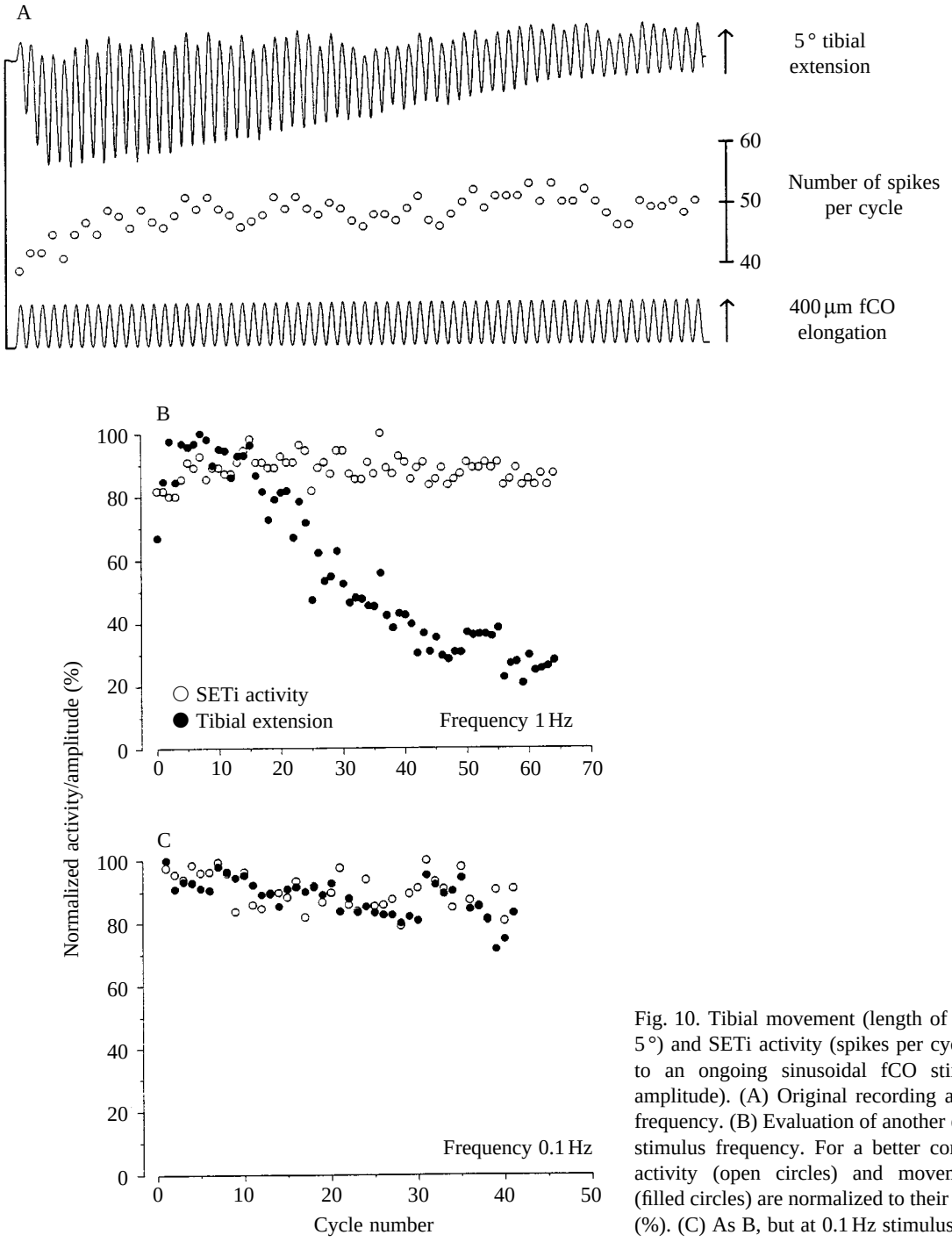

Fig. 10. Tibial movement (length of arrow indicates 5 °) and SETi activity (spikes per cycle) in response to an ongoing sinusoidal fCO stimulus (400 µm amplitude). (A) Original recording at 1 Hz stimulus frequency. (B) Evaluation of another example at 1 Hz stimulus frequency. For a better comparison, SETi activity (open circles) and movement amplitude (filled circles) are normalized to their maximal values (%). (C) As B, but at 0.1 Hz stimulus frequency.

modulation of the motor output at higher modulation frequencies. Since inertia and friction do not contribute substantially to this and given that all muscle fibres act in parallel, this filter consists of several steps in several parallel pathways. For SETi-generated forces, at least, the following parallel elements must be distinguished within the extensor muscle: (1) dually innervated muscle fibres in the most distal region; (2) dually innervated fibres in the rest of the muscle; and (3) triply innervated muscle fibres. For each of these pathways, the following information processing steps must be distinguished: (i) translation of the actual SETi spike frequency into the membrane potential of the muscle fibre (especially EJP summation and perhaps facilitation); and (ii) translation of this membrane potential into force.

The different populations of muscle fibres together produce the extensor force that interacts with the flexor force, and the result of this interaction (total force and co-contraction) is the cause of tibial movement.

These experiments have demonstrated that the amount of co-contraction is the main determinant of the low-pass filter characteristics of the muscle–joint system. The contribution of the extensor muscle to this co-contraction results nearly

exclusively from the dually innervated muscle fibres of its most distal region. In these fibres, the translation of membrane potential into force seems to be the decisive step, because the membrane potential of all these fibres followed the modulation of SETi firing rate.

Therefore, it is understandable that the larger number of these fibres in *Cuniculina impigra* compared with *Carausius morosus* and *Locusta migratoria* (Bässler *et al.* 1996) results in a lower upper corner frequency. These dually innervated muscle fibres of the distal region are the most strongly influenced by CI_1 activity. Apparently, CI_1 is able to disconnect these fibres even from strong SETi activity. CI_1 activity therefore markedly changes the filter characteristics of the muscle–joint system for modulation of SETi activity, i.e. it increases the upper corner frequency. This is important during active movements that would otherwise be impeded.

Organization of the FT control system

The reflex loop of the FT control system is organized according to the parliamentary principle, i.e. there are antagonistic influences on several consecutive levels (Bässler, 1993; Burrows, 1989). On these different levels, there are differences in the type of interaction occurring, the type of information that interacts and in the result of the interaction (for a detailed discussion, see Büschges, 1995). The following levels of antagonistic information processing are present: (1) presynaptic inhibition of fCO afferences (Sauer and Büschges, 1994; Burrows and Matheson, 1994); (2) excitation and delayed inhibition of nonspiking interneurones (Sauer *et al.* 1995, 1996); (3) supporting and opposing influences from different nonspiking interneurones on SETi (Büschges, 1990; Sauer *et al.* 1996); (4) interactions of SETi and CI_1 at the muscular level (e.g. Bässler and Storrer, 1980; present study); and (5) antagonism of co-contracting extensor and flexor tibiae muscles.

The present study contributes to points 4 and 5 above. It shows that the interaction between the co-contracting extensor and flexor muscles is responsible for two phenomena in *Cuniculina impigra*, the relatively low upper corner frequency and the decrease of movement amplitude during repeated stimulation at higher frequencies. The differences in upper corner frequencies between *Cuniculina impigra* and *Carausius morosus* can be attributed to the relative sizes of the distal muscle region and the percentage of dually innervated, slow muscle fibres within this region (Bässler *et al.* 1996). The differences between these species in the decrease of movement amplitude during repetitive stimulation – in *Carausius morosus*, it is independent of stimulus frequency (Kittmann, 1991), in *Cuniculina impigra*, the decrease only occurs at higher stimulus frequencies (see Fig. 10) – apparently originate in the different mechanisms producing this phenomenon in these species (see below). This is an example of the fact that a system with a parliamentary organization on several consecutive levels (as is present in both species) can produce a particular effect in different ways (Bässler, 1993).

Low-pass filter characteristics of the muscle–joint system as a behavioural adaptation to the ecological niche

In *Carausius morosus*, the minimum extensor force produced during sinusoidal stimulation differs from zero force at fCO stimulation frequencies above approximately 0.3 Hz (Storrer and Cruse, 1977), but it does not increase from cycle to cycle as in *Cuniculina impigra* (Kittmann, 1984). Therefore, co-contraction (at least as far as the contribution of the extensor is concerned) in *Carausius morosus* determines only the upper corner frequency, but does not contribute to habituation. Instead, maximum SETi frequency decreases from cycle to cycle independently of stimulus frequency. This neuronal phenomenon is expressed in *Cuniculina impigra* only for FETi. The amplitude of the change in soma membrane potential during sinusoidal fCO stimulation also drops from cycle to cycle, as does the minimum membrane potential (Bässler, 1983*b*).

In *Carausius morosus*, habituation is part of a gain control system that allows gain values just below the border of instability. *Cuniculina impigra* has a much lower upper corner frequency. Therefore, the FT feedback system has a much higher phase reserve, even at high gain values, and is considerably more stable than the one in *Carausius morosus* (Bässler, 1983*a*). It does not need a gain control system in order to prevent feedback oscillations at high gain values and, therefore, SETi activity does not need to decrease during ongoing sinusoidal fCO stimulation.

The high gains of the feedback loops of phasmids are a necessary prerequisite for catalepsy, an important component of twig mimesis (Bässler, 1983*a*, 1993). Twig mimesis is important in the biology of these animals. Since feedback loops with very high gain tend to become unstable, there must be mechanisms to avoid feedback oscillations. The gain control system reported for *Carausius morosus* is one such mechanism; a decrease in upper corner frequency that can be switched off during active movements, as exists in *Cuniculina impigra*, is another. The mechanism described here thus contributes to the performance of catalepsy and with it to the ecological competence of the animal.

The decrease in response amplitude with repeated stimulation in *Cuniculina impigra* is a muscular phenomenon and is therefore not habituation in the strict sense of the term. However, without knowing its causal origin, one would not hesitate to call it habituation in the same way as in *Carausius morosus*. One should thus be very careful when using this term in all cases in which its neural basis is unknown.

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