The Journal of Experimental Biology 199, 2169–2183 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0381

CORRELATION BETWEEN MUSCLE STRUCTURE AND FILTER CHARACTERISTICS OF THE MUSCLE–JOINT SYSTEM IN THREE ORTHOPTERAN INSECT SPECIES

DAGMAR BÄSSLER, ANSGAR BÜSCHGES*, SABINE MEDITZ AND ULRICH BÄSSLER
Fachbereich Biologie der Universität, D-67653 Kaiserslautern, Germany

Accepted 26 June 1996

Summary

In orthopteran insects, neural networks for joint control exhibit different characteristics due to behavioural specializations. We investigated whether these differences are generated purely by the neuronal networks, or whether characteristics of the muscles or joint architecture (muscle–joint system) are also involved in these behavioural specializations. We compared the properties of the muscle system moving the femur–tibia joint of the middle and hindleg of three species, *Carausius morosus*, *Cuniculina impigra* and *Locusta migratoria*. Four aspects were analysed for the tibial extensor muscle: (i) the frequency-dependence of motoneuronal activity in response to sinusoidal stimulation of the femoral chordotonal organ (fCO), (ii) the muscle structure, (iii) the innervation pattern of the muscle and (iv) the histochemical properties of the muscle fibres. These aspects were compared with the filter characteristics of the open-loop femur–tibia control system and of the muscle–joint system involved.

Whereas in both phasmid species (*Carausius morosus* and *Cuniculina impigra*) the motoneuronal activity steadily increases with sinusoidal stimulation of the fCO in the frequency range 0.01–5 Hz, in *Locusta migratoria* there is a decrease in motoneuronal activity between 0.01 and 0.3 Hz.

The muscle structure is basically similar in all three species, as the number of singly innervated muscle fibres (supplied by the fast extensor tibiae motor neurone, FETi) decreases from proximal to distal. The number of triply innervated fibres supplied by the FETi, the slow extensor tibiae (SETi) and the common inhibitor 1 (CI_1) is maximal in the middle of the muscle, and the number of dually innervated fibres (supplied by SETi, CI_1) increases from proximal to distal. Differences between the locust and the two phasmid species exist in the distal portion of the muscle. The phasmid extensor tibiae muscle contains a morphologically distinct bundle of muscle fibres, not present in the locust, which is mostly dually innervated and which is larger in *Cuniculina impigra*.

Similar results were obtained for the histochemical characterisation of the muscle fibres as revealed from their staining for myofibrillar ATPase activity. The number of histochemically identified fast fibres decreased from proximal to distal, while the number of slow fibres increased. In *Carausius morosus* and *Locusta migratoria*, the percentage of slow fibres increased by up to 60–70 % at the distal end, while this increase was to almost 100 % in *Cuniculina impigra*. Apparently, the larger this distal region and the higher the percentage of slow, dually innervated fibres in it, the lower is the upper corner frequency (the stimulus frequency at which the joint control system produces a movement with 70 % of its maximal response amplitude) of the muscle–joint system.

In summary, it appears that the upper corner frequency of the open-loop system in *Locusta migratoria* (<0.05 Hz) results at least in part from properties of the neuronal joint control network, but in *Carausius morosus* (0.5–1.0 Hz) and *Cuniculina impigra* (0.1–0.2 Hz) it results from the upper corner frequency of the muscle–joint system.

Key words: joint control, muscle fibre, orthopteran insects, stick insect, locust, *Carausius morosus*, *Cuniculina impigra*, *Locusta migratoria*.

Introduction

Investigations in the field of neuroethology have led to a detailed insight into the neuronal mechanisms underlying behaviour and behavioural specializations (e.g. Burrows, 1984; Breidbach and Kutsch, 1995; Schildberger and Elsner, 1994). Besides analysing the neuronal basis of specific behaviours in individual species, numerous investigations have also tried to compare neuronal networks in related species exhibiting (i) the same behaviours (e.g. Hennig, 1994; Stumpner *et al.* 1995; von Helversen and von Helversen, 1994; Schildberger, 1994; Elsner, 1994; Michelsen, 1994) or (ii) different behaviours due

*Author for correspondence (e-mail: bueschge@rhrk.uni-kl.de).

to different ethological requirements (Ebner and Bässler, 1978; Büschges and Wolf, 1995). Most of these investigations have dealt with the generation of the motor output pattern (e.g. Robertson and Olberg, 1988; Katz and Tazaki, 1992). However, the motor output is not the same as the movement itself. Therefore, we here attempt to correlate features of the neuromuscular system with details of movements for which the ethological significance is known.

The femur–tibia (FT) control system in orthopterans is well suited for such a comparative investigation for three reasons: (i) it is one of the best-known joint control loops (see Bässler, 1993, for summaries and comparisons with other joint control loops). (ii) The FT control loop in phasmids and locusts apparently consists of the same neuronal elements and connections (e.g. Burrows, 1989, 1992; Büschges and Wolf, 1995). (iii) There are strong quantitative differences in the performance of the reflex loop between different species that enable behavioural specializations (Bässler, 1983*a*, and below). These can, at least in part, be attributed to different ecological niches.

Phasmids exhibit catalepsy as one of the behavioural components of twig mimesis (see Bässler, 1983*a*), while locusts show no catalepsy. To be able to generate catalepsy, the joint control loops of stick insects must have a very low position sensitivity combined with a high non-linear velocity-dependency and a high gain (see Bässler, 1993). In contrast, locust joint control loops are more linear, have a considerably smaller velocity-sensitivity and a rather low gain (Ebner and Bässler, 1978; Büschges and Wolf, 1995; Field and Burrows, 1982). These differences are most obvious in the ‘open-loop configuration’ in ‘inactive’ animals (for a definition of inactive, see Bässler, 1983*a*). Open-loop configuration in the case of the FT joint means that the apodeme of the femoral chordotonal organ (fCO), the sensor of the FT control system, is cut. The fCO is then stimulated mechanically and the movement of the tibia generated by the evoked resistance reflex is monitored. From the movement responses to sinusoidal stimulation of the fCO, amplitude–frequency plots can be calculated which describe the dependency of the amplitude of the induced joint movement on stimulus frequency (see Fig. 1B). In such a plot, two characteristics are especially important, the maximum response amplitude and the stimulus frequency above which the response amplitude declines.

The response amplitude can be described by the dimensionless term ‘gain’ (output amplitude divided by input amplitude) since the mechanical stimulus to the fCO can be converted into the joint angle variation it signals (for details, see Bässler, 1983*a*). Because gain is independent of body size, it can be used in comparisons between different species.

To characterise the stimulus frequency above which there is a decline in response amplitude, the ‘upper corner frequency’ is normally used. This value is a measure of the fastest stimulus frequency that results in a movement by the joint control network. In linear systems, it is defined as the specific frequency at which a regression line through the declining part of the amplitude–frequency plot meets a horizontal line drawn through the maximal response amplitude. Since biological systems are not completely linear, only approximations of their upper corner frequencies can be obtained and they are usually taken as the frequency at which the joint control system produces a movement with 70 % of its maximal response amplitude.

The open-loop FT control system of phasmids has a high maximal gain value (up to nearly 10; Bässler, 1983*a*; Weiland *et al.* 1986). The upper corner frequency differs among species. For *Carausius morosus*, it is approximately 0.5–1.0 Hz (Bässler, 1972; Bässler *et al.* 1974; Kittmann, 1984, 1991) but for *Cuniculina impigra* it is in the range 0.1–0.2 Hz (Bässler and Foth, 1982). In contrast, the FT control system of the locust middle leg has a maximal gain of less than 1 and an upper corner frequency of less than 0.05 Hz (Ebner and Bässler, 1978). The gain and upper corner frequency of a control system affect each other; for example, control systems with high gain tend to become unstable under closed-loop conditions if the upper corner frequency is too high (for a detailed discussion, see Bässler, 1983*a*). Therefore, the relatively low upper corner frequency of the phasmid FT control loops protects them from instability and can be considered to be a consequence of the high gain that is necessary for the generation of catalepsy.

The question arises as to whether these differences between species mainly correlate with properties of the neuronal networks controlling the activity of the motoneurones in the FT joint or whether they are also correlated with species-specific properties of the muscles and joints involved. For both phasmid species, the dependence of the firing frequency of the extensor tibiae motoneurones upon fCO stimulus frequency is known (see summary in Bässler, 1983*a*). Amplitude–frequency characteristics of the responses of the slow extensor tibiae motor neurone (SETi) show that SETi firing frequency increases steadily up to a stimulus frequency of 5 Hz. Because (i) SETi is the only neurone determining extensor force under these conditions and (ii) the force of the flexor tibiae has similar characteristics (Storrer and Cruse, 1977), the decline in movement amplitude with higher fCO stimulus frequencies in phasmids must originate from properties of the tibial muscles and/or the leg joint itself. In the following, these properties are summarised by the term ‘muscle–joint system’. The muscle–joint systems of both phasmid species used in this study apparently form low-pass filters that transform only slow modulations of SETi firing into appropriate movements.

To obtain an understanding of the factors affecting the upper corner frequencies of phasmid muscle–joint systems, we used the following arguments. (i) If the structure or the arrangement of the muscle fibres is involved in the generation of the upper corner frequency of the muscle–joint system, there should be a correlation between appropriate structural variables and the upper corner frequencies of *Carausius morosus* and *Cuniculina impigra*. (ii) The locust middle leg, as an unspecialised walking leg, should not possess specializations that are directly or indirectly necessary for catalepsy. Thus, the amplitude–frequency characteristics of

the locust muscle–joint system should be compared with those of the phasmid species.

We focused on the extensor tibiae muscle for studying putative correlations between muscle structure and joint control properties because the extensor tibiae muscle in all three species has a much simpler innervation than the flexor muscle (Burns and Usherwood, 1978, 1979; Debrodt and Bässler, 1989; Theophilidis and Burns, 1983). We investigated and compared (i) the arrangement of the muscle fibres, (ii) the distribution of the innervation patterns of the muscle fibres and (iii) the distribution of muscle fibre types by histochemical staining for myofibrillar ATPase (mATPase) according to established procedures (Müller *et al.* 1992). In the companion paper (Bässler and Stein, 1996), we then analyse possible causes of the reported correlation and their consequences for other properties of this system.

Materials and methods

Animals

Adult females of two stick insect species, *Carausius morosus* Brunner and *Cuniculina impigra* Redtenbacher (syn. *Baculum impigrum* Brunner), and adults of both sexes of the locust *Locusta migratoria* L. were obtained from colonies at Kaiserslautern University, Germany.

Stimulation of the fCO

The experimental animal was fixed dorsal side up on a foam platform and its right middle leg was fixed, using dental cement (Protemp, ESPE), perpendicular to the thorax on a foam platform. Preparation of the femur and cutting and mounting of the receptor apodeme of the fCO in a stimulation clamp were performed according to previously published procedures (e.g. stick insect: Bässler, 1983*a*; locust: Büschges and Wolf, 1995). The fCO was stimulated (Fig. 1) conventionally using a pen-motor (Hellige) and a sine-wave generator (Tektronix), as described in detail in Bässler (1986). In *Cuniculina impigra*, a movement of the fCO apodeme by 400 μm corresponds to a change in joint angle of 40 °, while in *Carausius morosus* 200 μm corresponds to 40 ° (Weiland *et al.* 1986). In *Locusta migratoria*, the fCO apodeme had to be moved by approximately 600 μm for the same joint movement of 40 ° (Field and Pflüger, 1989).

The fCO was stimulated using sinusoidal stimuli (Fig. 1B) of 40 ° amplitude around a centre position that corresponded to the 90 ° position of the FT joint. Stimulation frequency

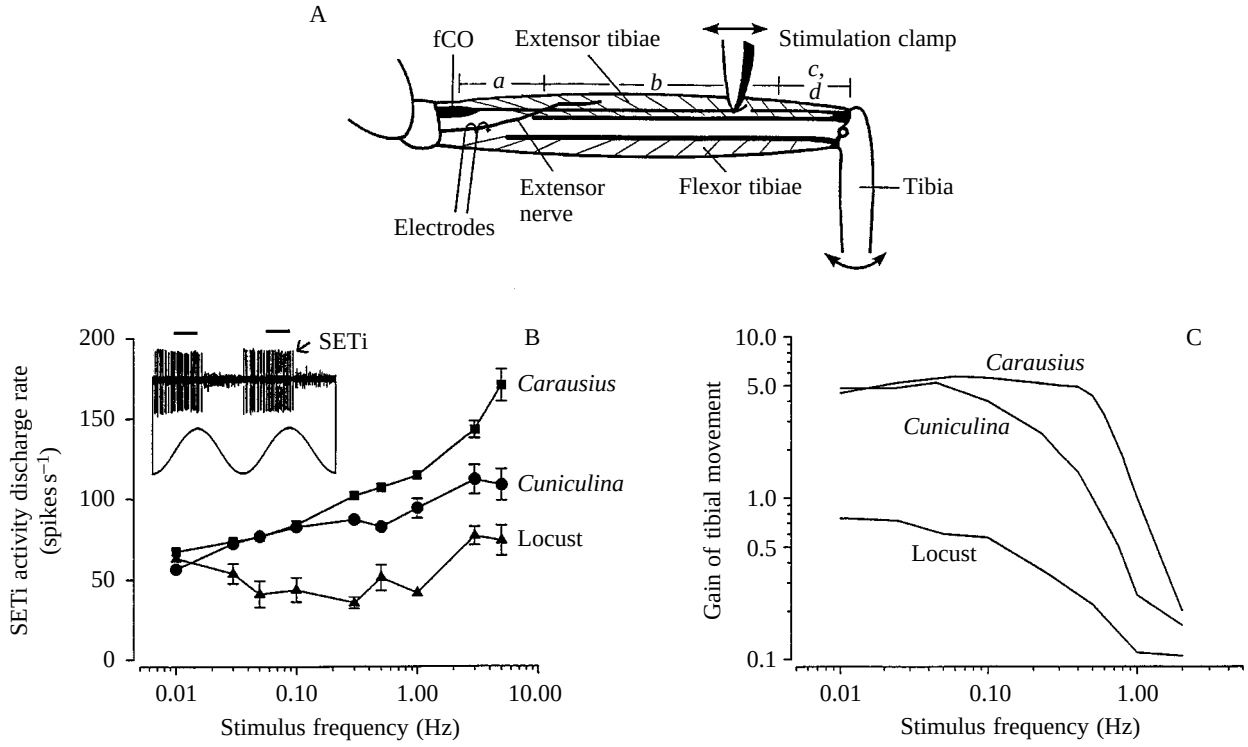

Fig. 1. Comparison of the action of the femur–tibia control system in three orthopteran insect species. (A) The experimental arrangement for measuring the frequency-dependence of motoneuronal discharge rate upon sinusoidal stimulation of the femoral chordotonal organ (fCO). The regions *a*, *b*, *c* and *d* shown along the length of the extensor tibiae muscle are explained in the text. Arrows indicate the direction of movement of the stimulation clamp. (B) Plot of the mean slow extensor tibiae (SETi) discharge rate *versus* frequency of sinusoidal stimulation for one experiment in each species. The inset shows, for two full cycles, reflex activation in extensor motoneurones (upper trace) during sinusoidal stimulation of the fCO (lower trace). The bars above the inset show the portion of the cycle evaluated (see also Materials and methods). Values are means ± S.D. for N=5 cycles at each point. Results were confirmed in four experiments for each species. (C) Plot of the gain of tibial movement (output amplitude/input amplitude) *versus* the frequency of fCO stimulation: summarised from previous investigations (*Cuniculina impigra*: Bässler, 1983*a*,*b*; locust: *Schistocerca gregaria*, Ebner and Bässler, 1978; *Carausius morosus*: Bässler, 1983*a*; Kittmann, 1984).

ranged from 0.01 to 5 Hz. Elongation of the fCO mimics imposed flexion of the FT joint and relaxation of the fCO mimics imposed extension. SETi activity was recorded (see below). As a measure of SETi response amplitude, the number of spikes that occurred during the second half of stretching was counted because the spike frequency is maximal at this time (see inset in Fig. 1B). The spike frequency could be used as a measure of response amplitude because the minimum spike frequency during fCO relaxation reached zero in all cases. As the FT control system is known to show marked habituation (Kittmann, 1991), the animal was touched with a brush between each stimulation sequence at a given frequency in order to dishabituate the experimental animal. Only the first five full cycles were considered for each stimulus frequency. The values obtained (Fig. 1B) are expressed as spikes s^{-1} and they give the average activity of SETi for the first five cycles.

Extracellular recordings

Extracellular recordings were made conventionally either by pressing the nerve against a trachea using myogram electrodes with the insulation removed from the tips (for details, see Pflüger, 1977) or by using a paraffin-oil hook electrode (Schmitz *et al.* 1988) to record *en passant* the activity of the extensor nerve.

Intracellular recordings

Intracellular recordings from muscle fibres were conventionally obtained using electrodes filled with 3 mol l^{-1} KCl for the phasmids (in order to be able to compare the data with previous recordings; Bässler and Storrer, 1980) or 2 mol l^{-1} potassium acetate for the locust, and having a resistance of 20–50 MΩ. We could not find differences in the parameters measured between electrolytes (e.g. resting membrane potential). For identification of muscle fibres, the electrodes were filled with 4% Lucifer Yellow (tip) and 1 mol l^{-1} LiCl (shaft). The experimental animals were fixed either dorsal-side-up (*Carausius morosus*, *Cuniculina impigra*) or ventral-side-up (all three species) on a foam platform. The femur of the middle or hindleg was fixed horizontally with dental cement (Protemp, ESPE) on a foam platform at an angle of approximately 90 ° to the body's longitudinal axis. The parts of the femur that were dissected were surrounded by a small basin filled with saline. In the dorsal-side-up preparations of the phasmids, a small window was cut into the dorsal cuticle of the femur to expose the muscle fibres. Care was taken to ensure that only a small number of muscle fibre origins had to be removed. In the ventral-side-up preparations, the flexor tibiae muscle, the crural nerve and the retractor unguis apodeme were removed, together with most of the ventral 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 could 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.

Histochemistry

Autotomised or cut middle and hindlegs were opened on the ventral side over the whole length in locust or *Carausius* saline (Bässler, 1997). The tibiae were cut off distal to the FT joint, the tendon of the flexor tibiae muscle was cut at mid-femur; hence, the diaphragm separating the extensor tibiae and flexor tibiae muscles was destroyed at this point. The leg was dried on cellulose tissue, embedded in Tissue Tec medium and shock-frozen in an aluminium mould in liquid nitrogen. Preparations were mounted with Tissue Tec on a cold chuck of a microtome (Reichert and Jung) at −15 to −20 °C. Serial cross sections (30 μm) of the whole leg (including flexor tibiae and extensor tibiae muscles) were made. Individual cross sections were then placed alternately onto two different cold, glycerine–albumin-coated microscope slides (both middle and hindlegs). They were quickly thawed and air-dried on a heating plate at 40 °C.

Staining for mATPase activity was performed according to Padykula and Hermann (1955) with modifications adopted from Müller *et al.* (1992). The reaction medium was adjusted to pH 8.4 (incubation time 10–15 min). Possible isoforms of mATPase were detected on the basis of their different pH stability. Therefore, the sections on one microscope slide were preincubated for 5 min at pH 4.7 and on the other slide for 10 min at pH 10.2 before they were transferred to another reaction medium at pH 9.4 (incubation time 30–45 min; Brooke and Kaiser, 1970; Guth and Samaha, 1970).

Statistics

Mean values of samples with $N<30$ were compared using a modified *t*-test according to Dixon and Massey (1969). Binomial distributions were compared using Fisher's test. Means and samples were regarded throughout the investigation as significantly different when $P<0.01$.

Results

Amplitude–frequency characteristics of SETi

In order to estimate the filter characteristics of the muscle–joint system in the FT joint, the amplitude–frequency characteristics of the activity of the extensor motoneurone during sinusoidal stimulation of the fCO must be known. For the two phasmid species, *Carausius morosus* and *Cuniculina impigra*, the amplitude–frequency characteristics of the slow extensor tibiae motoneurone (SETi) have been reported previously. For both species, the SETi response increases up to stimulus frequencies of approximately 5 Hz and decreases at higher stimulus frequencies (*Carausius morosus*: Bässler, 1983*a*; *Cuniculina impigra*: Bässler *et al.* 1982; Bässler, 1983*b*; summarized in Bässler, 1983*a*). However, these data were collected in different ways; for example, by measuring the maximum spike frequency, the number of spikes per cycle or the modulation in soma membrane potential. In addition, for locusts, only the amplitude–frequency characteristics of tibial movements have been published (Ebner and Bässler, 1978) while the frequency-dependence of the motoneuronal response

is not known. Thus, to obtain the best comparison possible, the amplitude–frequency characteristics were re-investigated in all three species and the data were evaluated in the same way (see Materials and methods section).

In *Carausius morosus* and *Cuniculina impigra*, the SETi firing rate increased steadily with increasing stimulus frequency (Fig. 1B), thus confirming previous reports (Bässler, 1983*a*,*b*; Bässler *et al.* 1982; Kittmann, 1984). In contrast, SETi firing rate in *Locusta migratoria* dropped significantly between stimulus frequencies of 0.01 and 0.3 Hz. SETi activity showed a significant increase compared with the value at 0.01 Hz only at higher stimulus frequencies (>1 Hz).

Comparisons between the amplitude–frequency characteristics of SETi (Fig. 1B) and values for gain of tibial movement taken from the literature (Fig. 1C) confirm that in both phasmid species the upper corner frequency in the movement response occurs some way down the slope of the increase in SETi activity with stimulus frequency. Thus, the upper corner frequency in these phasmids must be a result of the filter characteristics of the muscle–joint system. These filter characteristics differ for the two species investigated.

In locusts, the upper corner frequency (<0.05 Hz; Ebner and Bässler, 1978) appears, at least in part, to be determined by neuronal mechanisms that are also responsible for the decrease in SETi activity with increasing stimulus frequencies between 0.01 and 0.3 Hz. This decrease in SETi activity corresponds to the much smaller velocity-dependence of the locust FT control loop found at the motoneuronal as well as the interneuronal level (for details, see Büschges and Wolf, 1995). The comparison also shows that the higher gain in the movement response (Fig. 1C) of the phasmids is mirrored by a higher SETi spike frequency (Fig. 1B), i.e. the high gain of the joint control system in phasmids is, at least in part, a neuronal attribute.

General anatomy of the extensor tibiae muscle

The extensor tibiae is a pinnate muscle which extends over almost the entire length of the femur (length of femur; *Cuniculina impigra*, middle leg 2.0–2.5 cm, hindleg 2.4–3.0 cm; *Carausius morosus*, middle leg 1.0–1.3 cm; hindleg 1.2–1.6 cm; *Locusta migratoria*, middle leg 0.8–1.0 cm). In locusts, accessory extensor and flexor muscles are known in the tibiae of the middle and hindleg (e.g. Theophilidis and Burns, 1979); however, these were not investigated in the present study.

In both phasmid species, the extensor muscle fibre bundles originate only from the dorsal roof of the femur, except in the most distal region (Fig. 2; Bässler and Storrer, 1980). To aid comparison among the three species in this study, the extensor muscle was divided into four regions, which were defined with a FT joint angle of approximately 90 ° (Fig. 1A). Region *a* covered the most proximal third of the muscle, ending in the region of the beginning of the extensor muscle apodeme. Region *b* covered the middle portion of the muscle from approximately 30 % to approximately 85 % of its length. The most distal portion, where we identified in both phasmids a previously undescribed row of muscle fibres originating on the posterior cuticle, was divided into two regions (Fig. 2). The posterior row of muscle fibres started at approximately 85 % of the muscle length, and region *c* was defined as the anterior part of the muscle and region *d* as the posterior part. This distal posterior row (region *d*) is larger in *Cuniculina impigra* than in *Carausius morosus*, but even in *Cuniculina impigra* it only occupies approximately 10–15 % of the muscle length (Fig. 2). The fibres of this row are separated from the rest of the extensor muscle by the small leg trachea, which runs dorsal to these fibres. In both middle and hindlegs of the phasmids, the extensor muscle has the same anatomy.

In *Locusta migratoria* middle legs, extensor muscle fibre bundles originate on the cuticle in two rows, an anterior–dorsal row and a posterior row (see also Fig. 6). The most proximal fibre bundle inserts on the dorsal roof of the femur (Burns and Usherwood, 1979). The most distal bundle in the posterior row

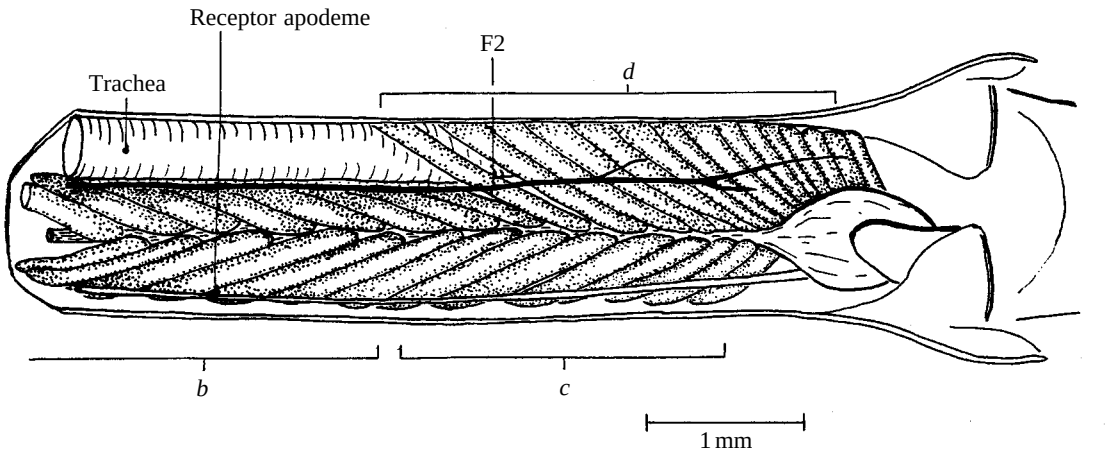

Fig. 2. Ventral view of the most distal 5 mm of a 29 mm long hindleg of *Cuniculina impigra* showing the extensor tibiae muscle and the insertion of its apodeme on the tibia (at the right-hand side). The femur–tibia joint is fully extended. The flexor tibiae muscle, the ventral part of the cuticle, the nervus cruris, the retractor unguis apodeme and the main leg trachea were removed. For definition of regions *b*, *c* and *d*, see text. F2, extensor nerve.

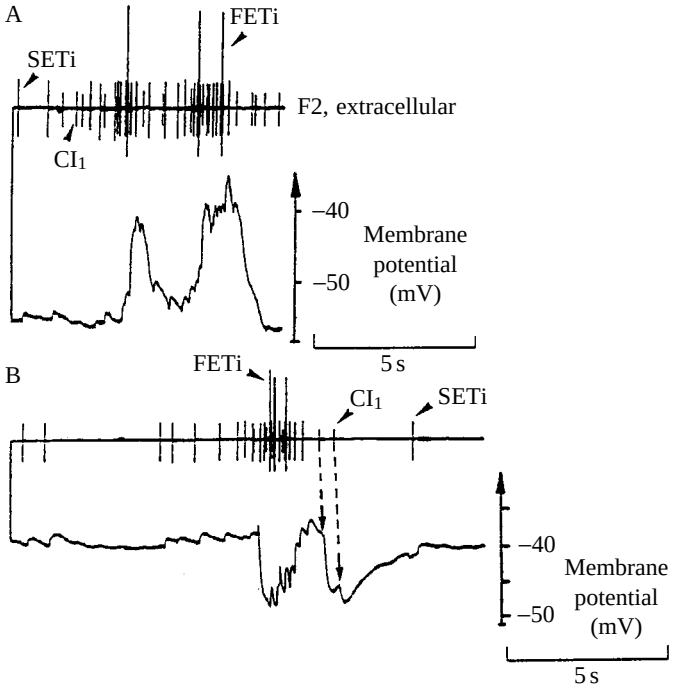

Fig. 3. Simultaneous recordings from the extensor nerve (F2, upper traces) and muscle fibres of the extensor tibiae muscle (lower traces) in *Cuniculina impigra*. (A) Recording from a triply innervated muscle fibre in region *c* (see Fig. 2). To activate the motoneurones, the animal was briefly disturbed by touching its abdomen. Action potentials in the three motoneurones (FETi, SETi, CI_1) are shown. (B) Recording from a dually innervated muscle fibre in the distal posterior region (region *d* in Fig. 2) of the muscle. Note the differences in the inhibitory junctional potential (IJP) sizes induced by CI_1 activity between recordings (individual IJPs are marked in B by vertical dashed lines and arrowheads). See also Fig. 4B,D.

has a considerably broader insertion base on the cuticle than the other bundles and its fibres appear to be shorter, thus showing some structural similarities with phasmids.

Innervation pattern of the extensor tibiae muscle

The innervation pattern of the extensor muscle was investigated using intracellular recordings from muscle fibres and simultaneous extracellular recordings from the extensor nerve (F2 in phasmids, N5B2 in the locust). As previously described for insects (cockroach, Atwood *et al.* 1969; locust, Burns and Usherwood, 1979; stick insect, Bässler and Storrer, 1980), we found three types of muscle fibre innervation: (1) only FETi (singly innervated; not shown), (2) FETi, SETi and CI_1 (triply innervated; Fig. 3A), (3) SETi and CI_1 (dually innervated; Fig. 3B). The frequency of these three fibre types changed as a function of location within the muscle (locust, Burns and Usherwood, 1979; stick insect, Bässler and Storrer, 1980).

For *Locusta migratoria*, in agreement with previous investigations (e.g. Burns and Usherwood, 1979), we found mostly singly innervated fibres in the most proximal fibre bundle (region *a*, Fig. 1A; Table 1). Towards the middle of the femur (region *b*, Fig. 1A), there was a decrease in the percentage of singly innervated fibres, an increase in the number of triply innervated fibres and a small, but increasing, number of dually innervated fibres (Table 1). In the distal region (regions *c* and *d*, Fig. 1A), only triply and dually innervated fibres were present (Table 1).

Table 1. *Distribution of fibre types in the extensor tibiae muscle of three orthopteran species*

	Singly innervated	Dually innervated	Triply innervated
Cuniculina impigra			
Region *a*	88 % (15)	6 % (1)	6 % (1)
Region *b*	34 % (25)	11 % (8)	55 % (40)
Region *c*	0 % (0)	46 % (31)	54 % (36)
Region *d*	0 % (0)	90 % (83)	10 % (9)
Carausius morosus			
Region *a*	83 % (45)	4 % (2)	13 % (7)
Region *b*	46 % (32)	3 % (2)	51 % (35)
Region *c*	0 % (0)	33 % (2)	67 % (4)
Region *d*	0 % (0)	58 % (7)	42 % (5)
Locusta migratoria			
Region *a*	82 % (116)	0 % (0)	18 % (25)
Region *b*	62 % (16)	3 % (1)	35 % (9)
Region *c*	0 % (0)	30 % (7)	70 % (17)
Region *d*	0 % (0)	43 % (17)	57 % (22)

Values are percentages of singly, dually and triply innervated fibres for the proximal (region *a*), medial (region *b*), anterior distal (region *c*) and posterior distal (region *d*) portions of the extensor tibiae muscle.

N is given in parentheses.

There were no significant differences in the fibre distributions between the three species for regions *a* and *b*. However, the fibre distribution in regions *c* and *d* in *Cuniculina impigra* did differ significantly from those of the other two species (see text).

In contrast, in both phasmid species, we found a different innervation pattern from that previously reported (Bässler and Storrer, 1980). In the proximal region (region *a*), as in the locust, mostly singly innervated fibres were found (Table 1). Triply innervated fibres were present throughout the muscle, with a lower percentage at both ends (Table 1). However, small numbers of dually innervated fibres were detected over the whole length of the muscle, especially in the dorsal-side-up preparation. In the distal region, their number showed a steep and marked increase (see below). The percentage of dually innervated fibres found in the distal region in *Cuniculina impigra* was significantly higher than that in *Carausius morosus*. These results thus differ markedly from previous descriptions of fibre type distribution in the extensor tibiae of phasmids (Bässler and Storrer, 1980). These differences are likely to be due to the fact that in the present investigation muscle fibre recordings were made from the dorsal as well as the ventral side of the muscle, whereas in previous investigations on phasmids recordings were made only from the anterior side.

In the proximal (*a*) and middle (*b*) regions of the extensor muscle, there were no significant differences in the percentage of fibre types between the three species. It was only in the distal region of the extensor muscle that we detected significant differences in innervation pattern between the three species investigated. Between *Locusta migratoria* and *Carausius morosus*, we found no difference in fibre type distribution for regions *c* and *d* (Table 1). In contrast fibre type composition in regions *c* and *d* in *Cuniculina impigra* did differ significantly (Table 1). In region *c* (dorsally originating distal fibres), triply innervated and dually innervated fibres were detected in approximately equal numbers (Table 1). In region *d*, however, most of the fibres were dually innervated. Only a few triply innervated fibres were found and all were located at the proximal end of this region.

From the relative sizes of the excitatory junctional potentials (EJPs), there appeared to be gradual transitions between the three fibre types [e.g. for triply innervated muscle fibres (Fig. 4A–C) and dually innervated fibres (Fig. 4D) in a *Cuniculina impigra* hindleg]. Similar results were obtained for *Cuniculina impigra* middle legs and for the middle legs of *Carausius morosus* and *Locusta migratoria*.

In triply and dually innervated muscle fibres in the proximal and middle region of the extensor muscle, CI_1 usually elicited relatively small IJPs (Fig. 4; see also Fig. 8A), while they were always large in dually innervated fibres of the distal region (Fig. 3B). Thus, in many cases, IJPs could only be detected unequivocally in dually innervated fibres of the proximal and middle regions and in triply innervated fibres when they occurred at relatively depolarised membrane potentials, e.g. during spontaneous bursts of motoneurone activity (Fig. 3A). This was true for all three species investigated. In addition, in the companion paper (Bässler and Stein, 1996), it will be shown that CI_1 activity decreases the force of contraction of dually innervated muscle fibres in the distal portion of the extensor muscle much more strongly than in the other portions of the muscle, indicating that there might be different types of dually innervated fibres.

As a consequence, a distinction between dually and triply innervated fibres was clearest in the distal portion of the

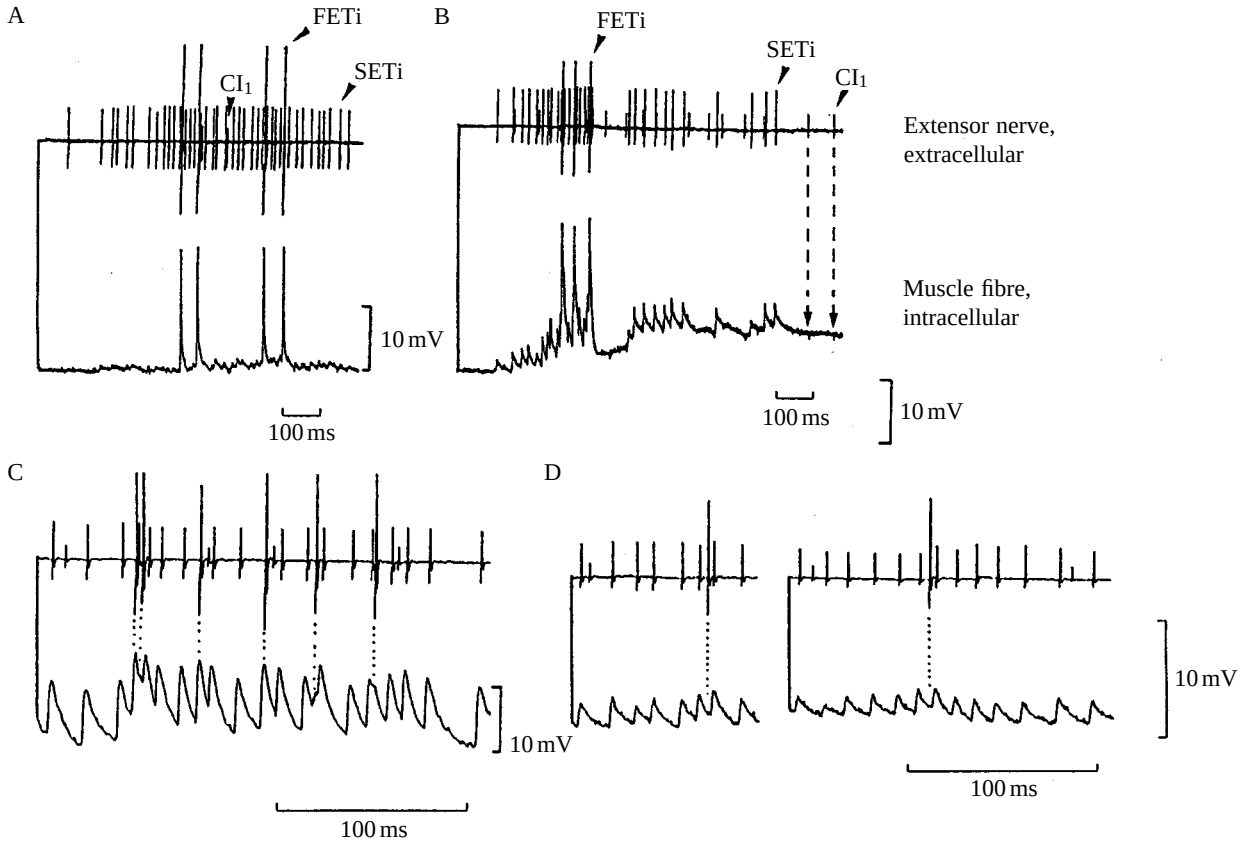

Fig. 4. Simultaneous recordings from the extensor nerve F2 (upper traces) and from muscle fibres (lower traces, dorsal-side-up preparation) in the proximal half of a *Cuniculina impigra* hindleg showing that there are no clear distinctions between dually and triply innervated muscle fibres in the extensor tibiae muscle. Inhibitory junctional potentials (IJPs) from CI_1 are very small in all fibres (IJPs are marked in B by vertical lines and arrowheads; compare with Fig. 3B). (A) Recordings from a triply innervated muscle fibre with very small SETi-induced excitatory junctional potentitals (EJPs) and barely detectable CI_1-induced IJPs (marked by an arrowhead in one case). Note that EJPs induced by SETi are only visible with increasing SETi discharge rates, indicating facilitation. (B) Recordings from a triply innervated fibre with clearly distinguishable EJPs from FETi and SETi. Two IJPs induced by CI_1 are marked by arrowheads. (C) Recordings from a triply innervated muscle fibre with similar EJP sizes for FETi or SETI. (D) Recordings from a dually innervated fibre.

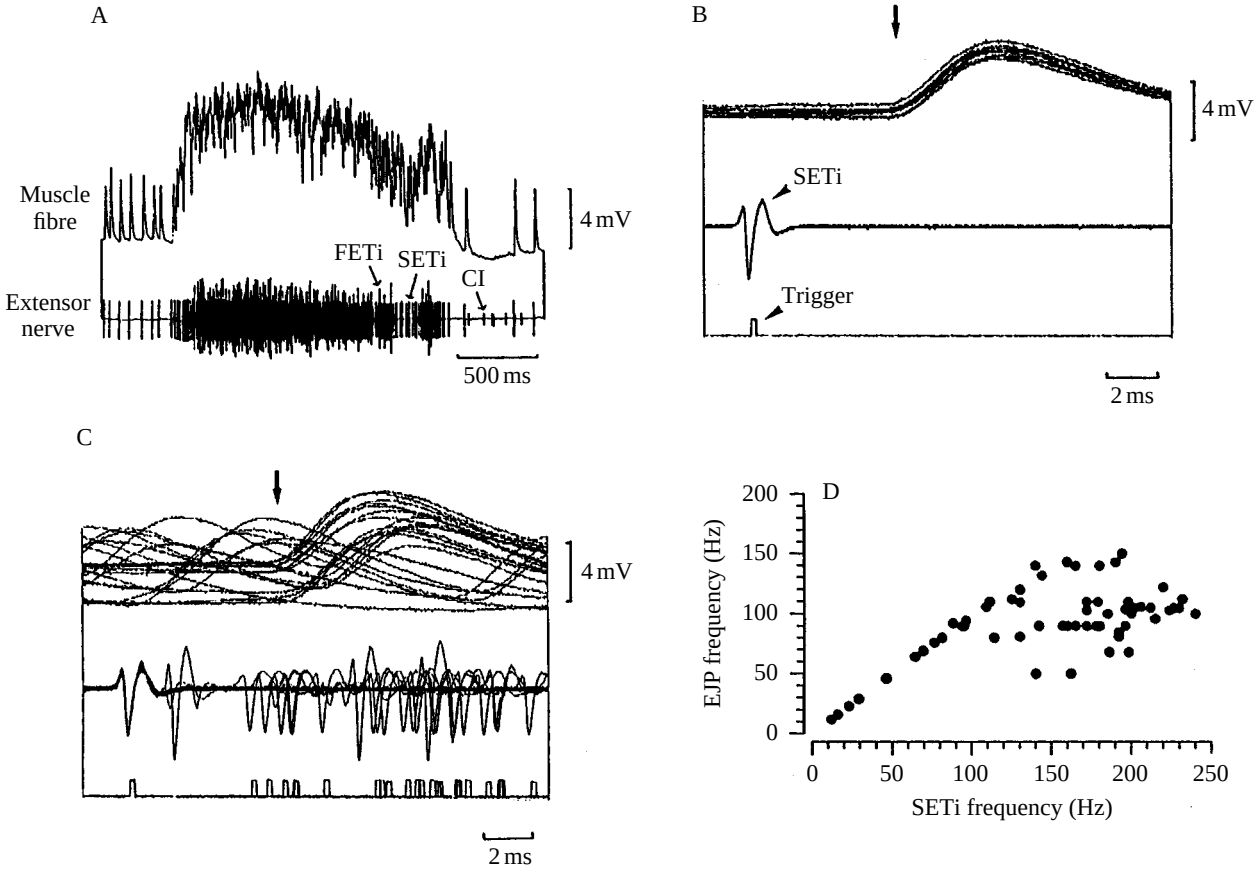

Fig. 5. EJP transmission failures in locust extensor tibiae muscle fibres. (A) Time course of membrane potential changes (upper trace) of a triply innervated muscle fibre during a spontaneous burst of activity in extensor motoneurones (lower trace). (B,C) Correlation between SETi activity (middle traces) and the occurrence of EJPs (upper traces) in the muscle fibre recorded in A. (B) With low SETi discharge rates, every SETi action potential was followed by an EJP in the muscle fibre. For better clarity, 10 successive sweeps triggered by SETi discharge (Trigger, bottom trace) were superimposed. (C) With high SETi discharge rates during bursts of motoneurone activity, as shown in A, not every SETi action potential induces an EJP in the muscle fibre. Twenty-two successive sweeps triggered by SETi action potentials were superimposed. Seven out of these 22 triggered SETi action potentials did not induce an EJP in the muscle fibre. In B and C, the muscle fibre recording was a.c.-filtered, and arrows indicate start of EJP induced in muscle fibre. (D) Quantitative description of the relationship between SETi discharge frequency and muscle fibre activity. Note that the muscle fibre only generates EJPs reliably up to SETi discharge rates of approximately 100 Hz.

extensor muscle, i.e. in regions *c* and *d* (Fig. 2). However, for comparison with previous studies, we also give fibre type percentage values for the two more proximal regions in Table 1.

In contrast to the basic similarities in the innervation pattern of the extensor muscle between the three species, we observed a phenomenon in *Locusta migratoria* muscle fibres that was not found in *Carausius morosus* or *Cuniculina impigra* muscle fibres. In 10 out of 25 dually innervated fibres and 16 out of 46 triply innervated fibres of *Locusta migratoria*, the frequency of EJPs induced by SETi activity was lower than the frequency of SETi action potentials recorded from the extensor nerve. This phenomenon was only present at high SETi discharge rates, exceeding 100 spikes s^{-1} (Fig. 5). EJPs in the muscle fibre followed SETi discharge rates reliably with a 1:1 correlation up to approximately 100 spikes s^{-1}. Between 100 and 140 spikes s^{-1}, SETi spikes sometimes did not induce EJPs in the muscle fibre, leading to an EJP frequency lower than the motoneuronal discharge rate. Above 140 spikes s^{-1}, the EJP frequency in the muscle fibre was always lower than the neuronal discharge rate. Any influence of the location of the extracellular extensor nerve recording electrode (N5B2) on this phenomenon could be excluded since the recording electrode was sometimes located proximal, and sometimes far distal, to the recorded muscle fibre. Only one out of 28 singly innervated fibres showed this phenomenon for FETi. EJP failure was also observed with orthodromic stimulation of motoneurones *via* nerve N5 for SETi and for FETi *via* nerve N3B (results not shown). In three triply innervated muscle fibres, motoneurone discharge rates above 110 spikes s^{-1} were found to be correlated with a failure of EJP occurrence. This phenomenon could, in part, be due to propagation failures of motoneurone action potentials at branching points of the extensor nerve (Theophilidis, 1988; see Discussion).

Histochemical characterisation of muscle fibres

It is known that the enzymatic status of muscle fibres, i.e.

their contractile protein composition, is correlated with their contraction properties (e.g. Stokes, 1987). For the locust coxa rotator muscle (M92, Snodgrass, 1929), it was shown that singly innervated fast-contracting muscle fibres have a high content of the enzyme myofibrillar actomyosin ATPase (mATPase), an mATPase isoform with a high reaction velocity and that inactivates at alkaline pH. In contrast, slow-contracting, dually innervated muscle fibres, contain an mATPase isoform with a low reaction velocity and insensitive to preincubation at alkaline pH (Müller *et al.* 1992). In order to characterise the muscle fibres with respect to their contraction properties, we measured mATPase activity histochemically in serial cross sections of extensor muscles. Using this technique, differences in the strength of staining correlate with differences in the reaction velocity of non-inactivated mATPase. Therefore, one can distinguish between fast- and slow-contracting fibres. The different isoforms of mATPase can be distinguished not only on the basis of staining intensity, but also on the basis of differences in their pH-stability (Morgan *et al.* 1980; Padykula and Hermann, 1955). We optimised the procedure for the extensor muscle of the species investigated. In all three species, the fast isoform of mATPase shows a high reaction velocity without preincubation (pH 8.4) and inactivates after alkaline preincubation (pH 10.2). The slow isoform of mATPase has a lower reaction velocity at pH 8.4 and remains active after alkaline preincubation. Therefore, so-called fast fibres stained darkly without preincubation but were unstained after alkaline preincubation and so-called slow fibres stained darkly after alkaline preincubation. As previously shown for the locust anterior coxa rotator muscle (Müller *et al.* 1992), some fibres (termed intermediate fibres) were lightly to strongly stained after both procedures.

At pH 8.4, the differences between the reaction velocity of the fast and the slow mATPase isoforms were smaller in the phasmid species than in the locust. Interestingly, preincubation at pH 4.7, which was reported to stain the fast isoform of mATPase and to inactivate the slow isoform in cockroach mesocoxal muscles (Stokes *et al.* 1979; Morgan *et al.* 1980), gave reasonable results in stick insects, but not in locusts (see also Müller *et al.* 1992). At pH 4.7 in locusts, both isoforms inactivated at the same time, precluding any distinction between them. This suggests that differences in the mATPase isoforms exist between locusts and stick insects.

Because of differences in staining quality without alkaline preincubation and after acidic preincubation, we quantitatively evaluated mATPase activity only after alkaline preincubation at pH 10.2. Therefore, any intermediate fibres would be counted as slow fibres (alkali-stable fibres).

Fig. 6 shows the distribution of slow fibres in middle leg cross sections typical for the three species. Cross sections were taken from the proximal and middle regions *a* and *b* (see Fig. 2) and from the distal region (i.e. regions *c* and *d* in Fig. 2) after alkaline preincubation. Slow fibres are clearly detectable by their dark staining, while the fast fibres remained unstained. There was an increase in the number of slow fibres from proximal to distal in all three species. The reverse was the case for the fast fibres. These results are in accordance with the gross fibre innervation pattern of the extensor muscle (Burns and Usherwood, 1979; Bässler and Storrer, 1980; this study).

We evaluated the number of fibres present of each type. In each cross section, the total number of fibres and the numbers of slow and fast fibres were counted. Three or four middle legs from each species were evaluated and the results are shown in Fig. 7. In the proximal and distal region of the extensor muscle of *Cuniculina impigra*, there were often tiny muscle fibres that looked as if a single fibre had been split into several pieces (see Fig. 6). Therefore, the total number of fibres in a cross section increased towards both ends in this species (Fig. 7A).

In both *Cuniculina impigra* and *Carausius morosus*, there was a small and nearly constant percentage of slow fibres (0–10 %) over more than 75 % of the muscle length (Fig. 7B,D). These fibres were found in the most dorsal region of the muscle (Fig. 6) and appeared to be shorter than the other fibres, because they had a larger insertion angle at the cuticle when consecutive cross sections were compared. Only in the distal quarter of the muscle was there a marked increase in the percentage of slow fibres. In *Cuniculina impigra*, the percentage of slow fibres approached 100 % at the most distal position, while in *Carausius morosus* this percentage reached a value of approximately 60–70 %. In *Cuniculina impigra*, the fibres of the posterior distal row of muscle fibres (region *c* in Fig. 2) were all slow fibres except for a few rare fast fibres at its proximal end. The percentage of fast fibres in these species changed correspondingly (Fig. 7B,D). The same pattern was found for the hindleg extensor muscles of *Carausius morosus* and *Cuniculina impigra*. In *Locusta migratoria* the situation was different. There was a gradual increase in the percentage of slow fibres from the most proximal region (0 % slow fibres) to the most distal region (approximately 40 % slow fibres) and a corresponding decrease in the percentage of fast fibres (Fig. 7F).

In summary, it appears that there are similarities in fibre composition between all three species. Similarities between *Carausius morosus* and *Cuniculina impigra* are clear in the dependence of percentage fibre type on muscle location (Fig. 7B,D). However, the absolute values for percentage fibre type were similar for *Carausius morosus* and *Locusta impigra* (Fig. 7D,F).

Correlation between innervation pattern and mATPase activity

Comparison of the histochemical fibre-type characterisation (Fig. 7) with the innervation pattern (Table 1) suggests that the singly (FETi) innervated fibres are fast fibres and the dually (SETi, CI_1) innervated fibres are slow fibres. The triply (FETi, SETi, CI_1) innervated fibres could not be clearly attributed to either type and they were not likely all to be intermediate fibres as they occurred more frequently than the latter. The same conclusion was drawn for the locust M92 muscle (Müller *et al.* 1992). To investigate further, we recorded from triply innervated (FETi, SETi, CI_1) fibres (Fig. 8A) and, after physiological

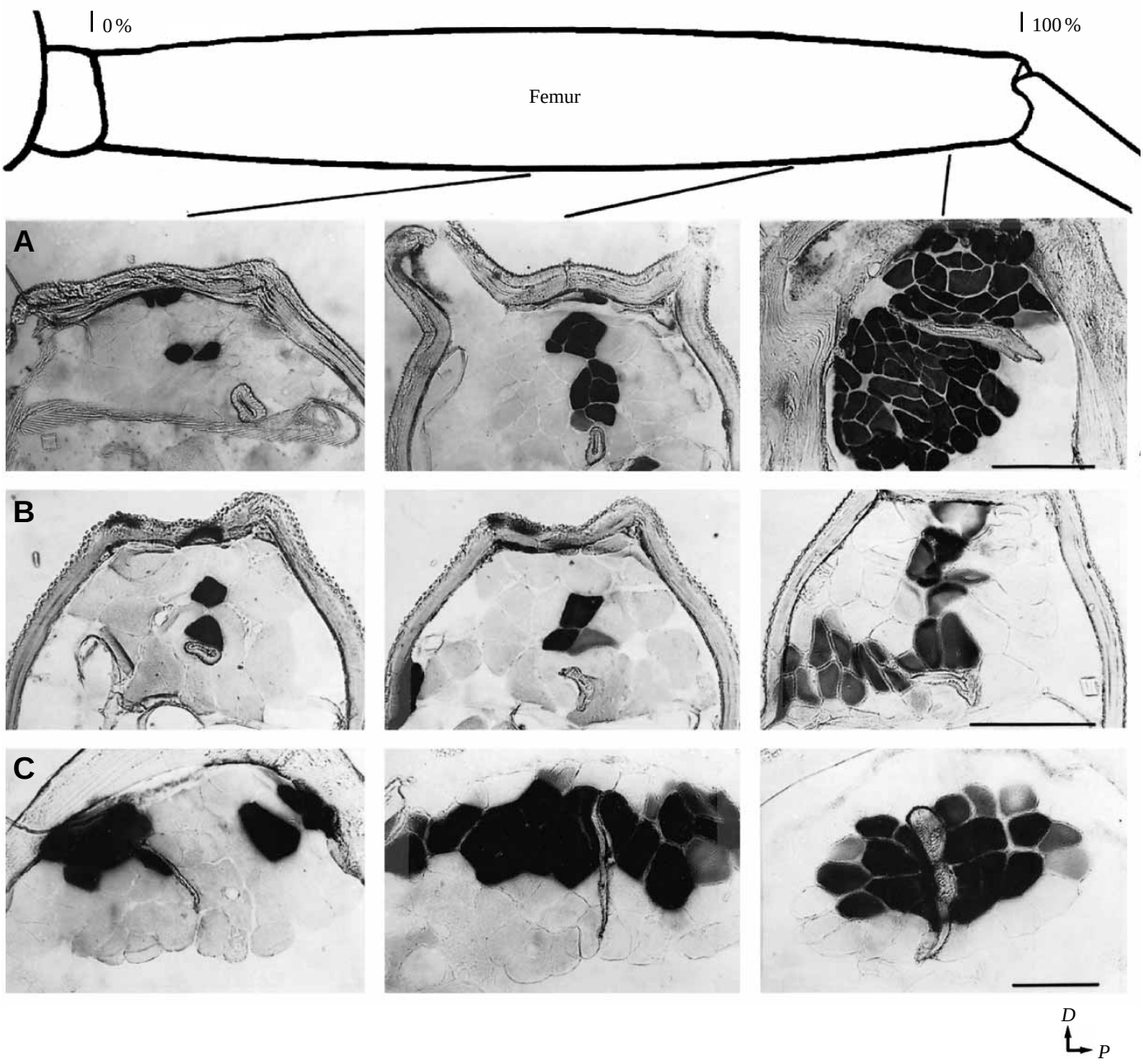

Fig. 6. Distribution of slow muscle fibres in the extensor tibiae muscle of the middle leg of *Cuniculina impigra* (A), *Carausius morosus* (B) and *Locusta migratoria* (C). Slow fibres are darkly stained. The approximate location of the cross sections chosen is indicated on the outline of the femur shown above. Note that, in both phasmid species, the number of stained fibres is small in the two sections from the proximal and median regions of the muscle and much larger in the section taken from the distal portion of the muscle (right). In contrast, the number of fibres stained in the locust gradually increases from proximal to distal (from left to right) in the sections shown. In *Cuniculina impigra*, almost all fibres in the most distal cross section (right) are stained (see text and Fig. 7). Please note that in the distal region of *Cuniculina impigra* there are tiny muscle fibres that look as if a single fibre has been split into several pieces. Scale bars, 200 μm. *D*, dorsal; *P*, posterior.

characterisation, filled them with a fluorescent dye (Lucifer Yellow). Negative current pulses of −10 nA amplitude were then injected at intervals of 1 s for 60–90 min. The dye was then given approximately 60 min for diffusion inside the muscle fibre. In unstained serial sections of the leg, the labelled fibres could be identified by their fluorescence in at least some consecutive sections close to the location of the recording electrode (Fig. 8B). In serial sections, mATPase activity was afterwards investigated with and without alkaline preincubation in order to characterise the fibre types (Fig. 8C,D). The example shown in Fig. 8 is a fibre identified as a fast fibre.

We treated a total of 10 triply innervated muscle fibres from the middle and distal regions of the locust extensor muscle in this way. Six were identified as fast fibres. Four fibres exhibited

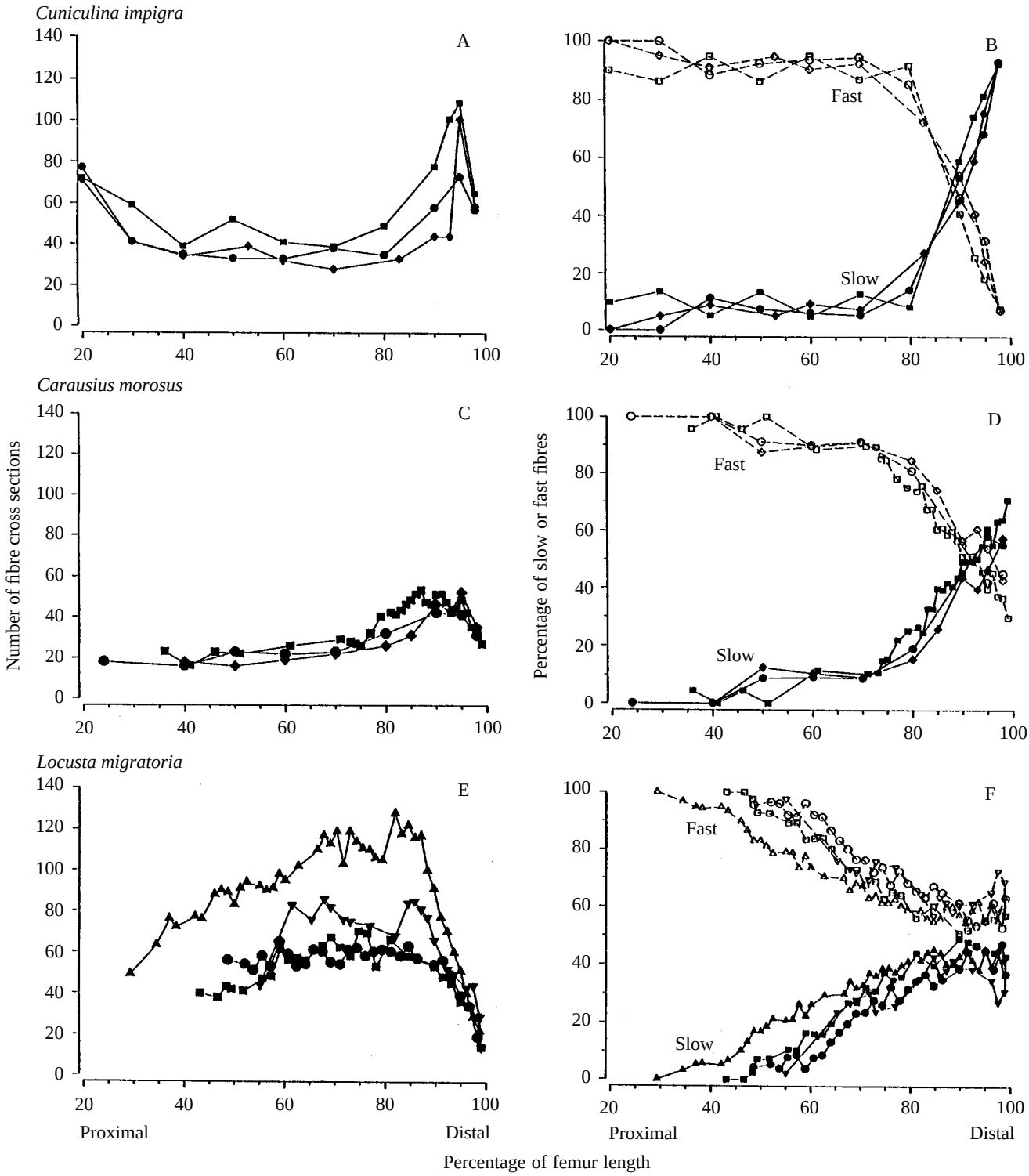

Fig. 7. Quantitative distribution of fast and slow muscle fibres in the extensor tibiae muscle of *Cuniculina impigra*, *Carausius morosus* and *Locusta migratoria*. In each cross section (see Fig. 6), the total number of fibre cross sections (A,C,E) and the percentage of slow and fast fibres (B,D,F) were evaluated and plotted *versus* the position of the cross section (as percentage femur length, where 100 % corresponds to the femur–tibia joint). The most proximal cross section evaluated was just proximal to the end of the extensor apodeme. Data are derived from three animals each, except for *Locusta migratoria* where the data from four animals are plotted.

the staining properties of intermediate fibres. From these results, it is clear that triply innervated fibres are not a uniform group as they express not only fast but also intermediate properties.

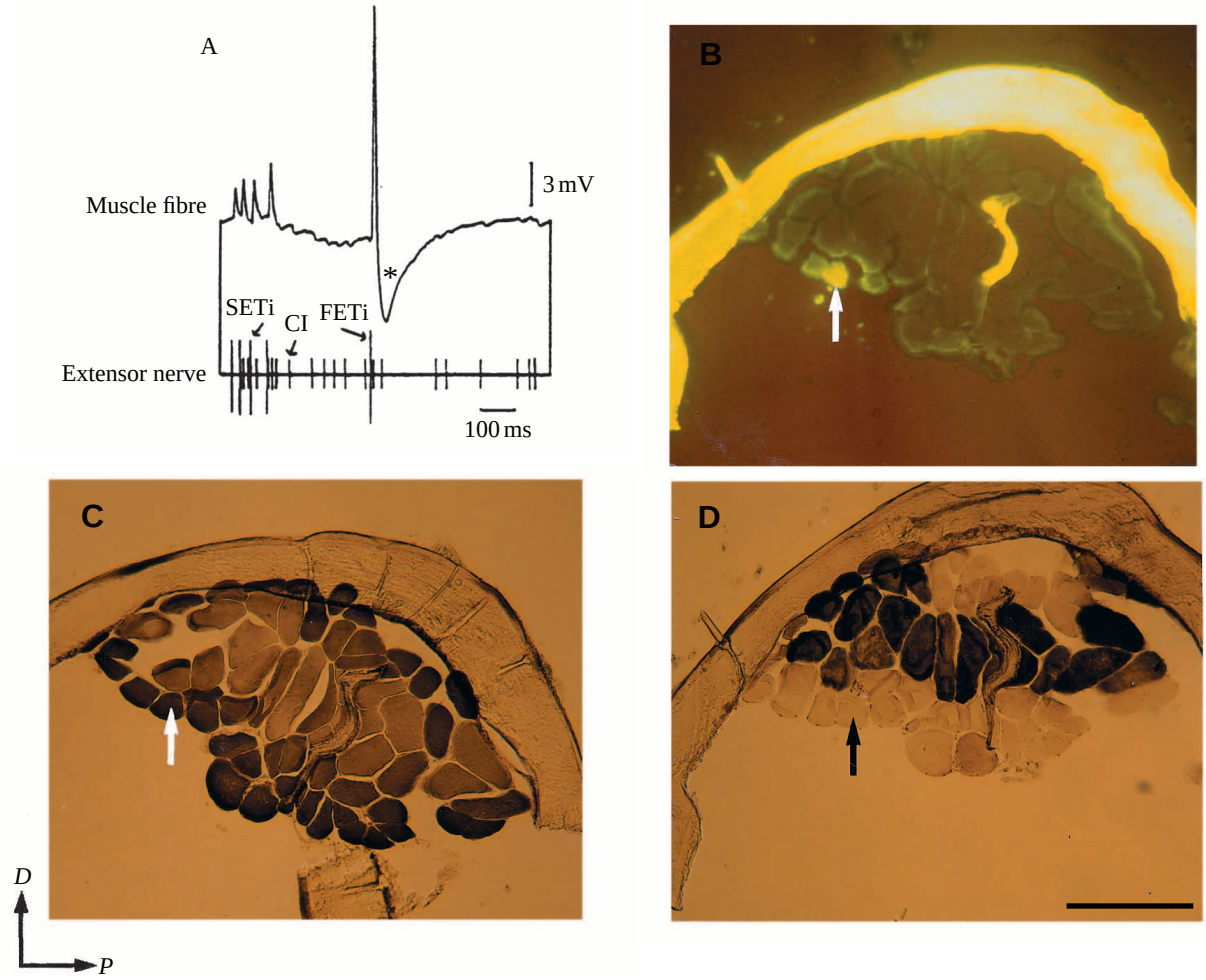

Fig. 8. Correlation between electrophysiological and histochemical properties of individual extensor tibiae muscle fibres. (A) Recording from a triply innervated muscle fibre. (B) Identification of the muscle fibre using fluorescent dye injected following physiological recordings. The cross section is through the dorsal half of the locust femur containing the extensor muscle. Note that the cuticle as well as the muscle apodeme exhibit considerable autofluorescence. The injected muscle fibre is indicated by the arrow. (C,D) Histochemical properties of the muscle fibre showing that it stains appropriately for a fast fibre: mATPase staining without preincubation (C), but no mATPase staining with preincubation at pH 10.2 (D). Scale bar, 250 µm. *D*, dorsal; *P*, posterior.

Discussion

The present investigation attempts to elucidate the correlation between neuromuscular structure and properties and the characteristics of joint control systems in three orthopteran insect species in order to detect specializations correlated with specific behavioural repertoires. We found that differences in the neuronal systems and in the muscle–joint systems are correlated with differences in the properties of the joint control systems in the three species investigated.

Neuronal properties of the femur–tibia control system and the origin of low-pass filter characteristics

Previous measurements of tibial movements and muscle forces have shown that the femur–tibia control system in orthopteran insects shows marked low-pass filter properties, although the tuning may differ as a result of different behavioural specializations (see Introduction). The response of the FT control system in phasmids and locusts, measured as movement of the tibia, can be formally described as two parallel components (for the extensor and flexor tibiae muscles). The extensor component consists of a sequence incorporating a high-pass filter (situated in the fCO), a rectifier (formed by FETi and SETi) and a low-pass filter (formed by the muscles; Cruse and Storrer, 1977; Bässler, 1983*a*,*b*). We have investigated the motoneuronal output for sinusoidal stimuli. For both phasmids investigated, *Carausius morosus* and *Cuniculina impigra*, we found, in accordance with previous investigations, that SETi activity increased over the range of fCO stimulus frequencies tested (0.01–5 Hz; Fig. 1B, Bässler, 1983*a*). In these two species, the low-pass filter is not located at the motoneuronal level; thus, the low-pass filter characteristics obviously originate from the characteristics of the muscle–joint system.

In *Locusta migratoria*, in contrast, there was a continuous decrease in SETi activity in the stimulus frequency range from 0.01 to 0.3 Hz. This decrease approximately parallels the

decrease in movement amplitude for the complete system over this frequency range and suggests that neuronal properties might contribute to the low-pass filter. SETi activity was found to increase with increasing stimulus frequency only above a stimulus frequency of 1 Hz. Thus, for faster stimuli, the decrease in movement amplitude cannot be attributed to neuronal properties and is obviously also derived from the properties of the muscle–joint system. In contrast to the situation in phasmids, an additional mechanism in locusts affects low-pass filter characteristics. We found that not all locust extensor muscle fibres are able to respond to high motoneuronal discharge rates, i.e. they do not show an EJP for every motoneuronal spike. However, this phenomenon only occurs at relatively high SETi frequencies. It is not known at present whether this phenomenon originates from the presynaptic or postsynaptic properties of neuromuscular transmission at the muscle fibres. A partial explanation for this phenomenon may be the conduction failures at branching points of the extensor nerve reported by Theophilidis (1984, 1988) for another orthopteran insect, *Decticus albifrons*.

Correlation between muscle structure and filter characteristics of the muscle–joint system

In all three species, there is a similar innervation pattern of the extensor muscle (this study; Bässler and Storrer, 1980; Burns and Usherwood, 1979). The percentage of singly innervated fibres decreases from approximately 80–90 % at the proximal end of the extensor to zero at distal locations, while the percentage of dually and triply innervated fibres increases. A similar pattern is found from the histochemical properties of the muscle fibres: the numbers of slow fibres proportionally increase while the numbers of fast fibres decrease more distally along the extensor muscle. However, marked differences between the three species were detected. In *Cuniculina impigra*, which has a low upper corner frequency of the muscle–joint system, there is a larger percentage of slow fibres in the distal portion of the extensor muscle compared with that of *Carausius morosus* and *Locusta migratoria*. Indeed, the percentage of slow fibres increases to almost 100 % in *Cuniculina impigra* in this region largely due to the characteristics of the muscle fibres in the distal posterior row. These slow muscle fibres were found to be almost exclusively dually innervated (SETi, CI_1). It is conceivable that these reported differences influence the relatively low upper corner frequency in *Cuniculina impigra* compared with that of *Carausius morosus*: the existence of a high percentage of slow innervated fibres will result in an increase in tonic muscle force with increasing stimulus frequency, leading to a relative stiffening of the leg joint at high stimulus frequencies. The companion paper (Bässler and Stein, 1996) will verify this suggestion with an analysis of the specific role of these fibres in the generation of the joint control properties of *Cuniculina impigra*.

Distinction between fibre types in insect muscles

In invertebrate muscles, muscle fibre heterogeneity has been described frequently (e.g. Govind and Atwood, 1982; Rathmayer and Maier, 1987). Nevertheless, for insect muscles innervated by only three distinct motoneurones, three classic types of muscle fibre innervation have been described (cockroach, Atwood *et al.* 1969; locust, Burns and Usherwood, 1979; Hoyle, 1978; Müller *et al.* 1992; stick insect, Bässler and Storrer, 1980). There are singly innervated muscle fibres, i.e. fibres innervated by one fast motoneurone; dually innervated muscle fibres, i.e. fibres innervated by one slow motoneurone and a common inhibitor motoneurone; and triply innervated muscle fibres, i.e. fibres innervated by all three types of motoneurone. For one insect leg muscle, the coxa rotator muscle (M92; Snodgrass, 1929) of the locust, there is evidence for a close correlation between fibre innervation type and histochemical and mechanical properties. For this muscle, three different classes of muscle fibres were distinguished and named: type I fibres are innervated by the slow excitatory motor neurone and CI_1, stain for mATPase as slow fibres and show a slow contraction velocity; type IIa fibres are triply innervated, stain for mATPase as fast or slow fibres or both and have a fast contraction velocity; type IIb fibres are innervated by the fast excitatory motoneurone only, stain for mATPase as fast fibres and exhibit a fast contraction velocity. In their study, Müller *et al.* (1992) grouped the histochemical, electrophysiological and mechanical properties of the fibres investigated into these three different classes on the basis of the fibre location within the muscle; however, they did not identify the different parameters for individual muscle fibres. Our findings on the innervation pattern of extensor muscle fibres indicate that there may be no clear distinctions in the fibre innervation pattern, i.e. they support the previous results of Hoyle (1978; see his Table 1). In addition, we have shown that triply innervated muscle fibres can exhibit differing histochemical properties. In summary, our data indicate (see also Hoyle, 1978) that the usual classification of fibres may not be applicable to triply innervated muscles of insects. These results, therefore, question the concept of clearly delimited classic fibre types.

It is highly likely that these relationships between muscle structure and filter characteristics are not the only factors affecting the properties of the joint control system. However, it is clear from our investigation that, in addition to characteristics of the neuronal network, specific properties of the muscle–joint system are strongly correlated with the properties of joint control systems. In the following paper (Bässler and Stein, 1996) it will be shown that, particularly in the case of the extensor tibiae muscle in *Cuniculina impigra*, this contribution can result in a functional partitioning of the muscle.

We thank Professor Dr W. Rathmayer, Dr H. Wolf and Dr A. E. Sauer for helpful discussions on our data. We also thank Dr A. E. Sauer and Dr J. Schmidt, as well as two anonymous referees, for their comments on the manuscript. In addition, we thank Tove Heller, Ilse Winkler and Sybille Watt for technical assistance. This work was supported by DFG grants to U.B. (Ba578) and A.B. (Bu857).

References

ATWOOD, H. L., SMYTH, T. AND JOHNSON, H. S. (1969). Neuromuscular synapses in the cockroach extensor tibiae muscle. *J. Insect Physiol.* **15**, 529–535.

BÄSSLER, U. (1972). Der "Kniesehnenreflex" bei *Carausius morosus*: Übergangsfunktion und Frequenzgang. *Kybernetik* **11**, 32–50.

BÄSSLER, U. (1977). Sense organs in the femur of the stick insect and their relevance to the control of position of the femur–tibia-joint. *J. comp. Physiol.* **121**, 99–113.

BÄSSLER, U. (1983*a*). *Neural Basis of Elementary Behavior in Stick Insects*. Berlin: Heidelberg, New York: Springer-Verlag.

BÄSSLER, U. (1983*b*). The neural basis of catalepsy in the stick insect *Cuniculina impigra*. III. Characteristics of the extensor motoneurons. *Biol. Cybernetics* **46**, 159–165.

BÄSSLER, U. (1986). Afferent control of walking movements in the stick insect *Cuniculina impigra*. II. Reflex reversal and the release of the swing phase in the restrained foreleg. *J. comp. Physiol.* A **158**, 351–362.

BÄSSLER, U. (1993). The femur–tibia control system of stick insects – a model system for the study of the neural basis of joint control. *Brain Res. Rev.* **18**, 207–226.

BÄSSLER, U., CRUSE, H. AND PFLÜGER, H. J. (1974). Der Regelkreis des Kniesehnenreflexes bei der Stabheuschrecke *Carausius morosus*. *Kybernetik* **15**, 117–125.

BÄSSLER, U. AND FOTH, E. (1982). The neural basis of catalepsy in the stick insect *Cuniculina impigra*. I. Catalepsy as a characteristic of the femur–tibia control system. *Biol. Cybernetics* **45**, 101–105.

BÄSSLER, U. AND STEIN, W. (1996). Contributions of structure and innervation pattern of the stick insect extensor tibiae muscle to the filter characteristics of the muscle–joint system. *J. exp. Biol*. **199**, 2185–2198.

BÄSSLER, U. AND STORRER, J. (1980). The neural basis of the femur–tibia control-system in the stick insect *Carausius morosus*. I. Motoneurons of the extensor tibiae muscle. *Biol. Cybernetics* **38**, 107–114.

BÄSSLER, U., STORRER, J. AND SAXER, K. (1982). The neural basis of catalepsy in the stick insect *Cuniculina impigra*. II. The role of the extensor motor neurons and the characteristics of the extensor tibiae muscle. *Biol. Cybernetics* **46**, 1–6.

BREIDBACH, O. AND KUTSCH, W. (1995). *The Nervous Systems of Invertebrates: an Evolutionary and Comparative Approach.* Basel, Boston, Berlin: Birkhäuser Verlag.

BROOKE, M. H. AND KAISER, K. K. (1970). Three "myosin adenosine triphosphatase" systems: The nature of their pH lability and sulfhydryl dependence. *J. Histochem. Cytochem.* **18**, 670–672.

BURNS, M. D. AND USHERWOOD, P. N. R. (1978). Mechanical properties of locust extensor tibiae muscles. *Comp. Biochem. Physiol.* **61**A, 85–95.

BURNS, M. D. AND USHERWOOD, P. N. R. (1979). The control of walking in Orthoptera. II. Motor neurone activity in normal free-walking animals. *J. exp. Biol.* **79**, 69–98.

BURROWS, M. (1984). The search for principles of neuronal organization. *J. exp. Biol.* **112**, 1–4.

BURROWS, M. (1989). Processing of mechanosensory signals in local reflex pathways of the locust. *J. exp. Biol.* **146**, 209–227.

BURROWS, M. (1992). Local circuits for the control of leg movements in an insect. *Trends Neurosci.* **15**, 226–232.

BÜSCHGES, A. AND WOLF, H. (1995). Nonspiking local interneurons in insect leg motor control. I. Common layout and species-specific response properties of femur–tibia joint control pathways in stick insect and locust. *J. Neurophysiol.* **73**, 1843–1860.

CRUSE, H. AND STORRER, J. (1977). Open loop analysis of a feedback mechanism controlling the leg position in the stick insect *Carausius morosus*: comparison between experiment and simulation. *Biol. Cybernetics* **25**, 143–153.

DEBRODT, B. AND BÄSSLER, U. (1989). Motor neurones of the flexor tibiae muscle in phasmids. *Zool. Jb. Physiol.* **93**, 481–494.

DIXON, W. J. AND MASSEY, F. J., JR (1969). *Introduction to Statistical Analysis* (3rd edn). New York: McGraw-Hill.

EBNER, I. AND BÄSSLER, U. (1978). Zur Regelung der Stellung des Femur–Tibia-Gelenkes im Mesothorax der Wanderheuschrecke *Schistocerca gregaria* (Forskal). *Biol. Cybernetics* **29**, 83–96.

ELSNER, N. (1994). The search for the neural centers of cricket and grasshopper song. In *Neural Basis of Behavioral Adaptations* (ed. K. Schilberger and N. Elsner). *Fortschr. Zool.* **39**, 167–193.

FIELD, L. H. AND BURROWS, M. (1982). Reflex effects of the femoral chordotonal organ upon leg motor neurones of the locust. *J. exp. Biol.* **101**, 265–285.

FIELD, L. H. AND PFLÜGER, H. J. (1989). The femoral chordotonal organ: a bifunctional orthopteran (*Locusta migratoria*) sense organ? *Comp. Biochem. Physiol.* **93**A, 729–743.

GOVIND, C. K. AND ATWOOD, H. L. (1982). Organization of neuromuscular systems. In *The Biology of Crustacea*, vol. 3 (ed. D. Bliss), pp. 63–103. New York: Academic Press

GUTH, L. AND SAMAHA, F. J. (1970). Procedure for the histochemical demonstration of actomyosin ATPase. *Exp. Neurol.* **28**, 365–367.

HENNIG, R. M. (1994). Neuronal organisation of the flight motor pattern in the cricket, *Teleogryllus commodus*. *J. comp. Physiol.* A **167**, 629–639.

HOYLE, G. (1978). Distribution of nerve and muscle fibre types in locust jumping muscle. *J. exp. Biol.* **78**, 205–233.

KATZ, P. S. AND TAZAKI, K. (1992). Comparative and evolutionary aspects of the crustacean stomatogastric system. In *Dynamic Biological Networks* (ed. R. M. Harris-Warrick, E. Marder, A. I. Selverston and M. Moulins), pp. 221–262. Cambridge, MA, London: MIT Press.

KITTMANN, R. (1984). Quantitative Analyse von Verstärkungsänderungen eines Gelenkstellungsregelkreises. Dissertation, Universität Kaiserslautern.

KITTMANN, R. (1991). Gain control in the femur–tibia feedback system of the stick insect. *J. exp. Biol.* **157**, 503–522.

MICHELSEN, A. (1994). Directional hearing in crickets and other small animals. In *Neural Basis of Behavioral Adaptations* (ed. K. Schildberger and N. Elsner) *Fortschr. Zool.* **39**, 195–207.

MORGAN, C. R., TARRAS, M. S. AND STOKES, D. R. (1980). Histochemical demonstration of enzymatic heterogeneity within the mesocoxal and metacoxal muscles of *Periplaneta americana*. *J. Insect Physiol.* **26**, 481–486.

MÜLLER, A. R., WOLF, H., GALLER, S. AND RATHMAYER, W. (1992). Correlation of electrophysiological, histochemical and mechanical properties in fibres of the coxa rotator muscle of the locust, *Locusta migratoria*. *J. comp. Physiol.* B **162**, 5–15.

PADYKULA, H. A. AND HERMANN, E. (1955). The specificity of the histochemical method for adenosine triphosphatase. *J. Histochem. Cytochem.* **3**, 170–195.

PFLÜGER, H.-J. (1977) The control of rocking movements of the phasmid *Carausius morosus* Br. *J. comp. Physiol*. **120**, 181–202.

RATHMAYER, W. AND MAIER, L. (1987). Muscle fibre types in crabs: Studies on single identified muscle fibres. *Am. Zool.* **27**, 1067–1077.

ROBERTSON, R. M. AND OLBERG, R. M. (1988). A comparison of the

activity of flight interneurones in locusts, crickets, dragonflies and mayflies. *Experientia* **44**, 735–738.

SCHILDBERGER, K. (1994). The auditory pathway of crickets: Adaptations for intraspecific acoustic communication. In *Neural Basis of Behavioral Adaptations* (ed. K. Schildberger and N. Elsner). *Fortschr. Zool.* **39**, 209–225.

SCHILDBERGER, K. AND ELSNER, N. (1994). *Neural Basis of Behavioral Adaptations*. *Fortschr. Zool.* **39**. Stuttgart, Jena, New York: Fischer Verlag.

SCHMITZ, J., BÜSCHGES, A. AND DELCOMYN, F. (1988). An improved electrode design for *en passant* recording from small nerves. *Comp. Biochem. Physiol.* **91**A, 769–772.

SNODGRASS, R. E. (1929). The thoracic mechanism of a grasshopper and its antecedents. *Smithson. Misc. Collns* **82**, 1–112.

STOKES, D. R. (1987). Insect muscles innervated by single motoneurones: structural and biochemical featrues. *Am. Zool.* **27**, 1001–1010.

STOKES, D. R., VITALE, A. J. AND MORGAN, C. R. (1979). Enzyme histochemistry of the mesocoxal muscles of *Periplaneta americana*. *Cell Tissue Res.* **198**, 175–189.

STORRER, J. AND CRUSE, H. (1977). Systemanalytische Untersuchungen eines aufgeschnittenen Regelkreises, der die Beinstellung der Stabheuschrecke *Carausius morosus* kontrolliert: Kraftmessungen an den Antagonisten Flexor und Extensor Tibiae. *Biol. Cybern*etics **25**, 131–142.

STUMPNER, A., ATKINS, G. AND STOUT, J. F. (1995). Processing of unilateral and bilateral auditory inputs by the ON1 and L1 interneurons of the cricket *Acheta domesticus* and comparison to other cricket species. *J. comp. Physiol.* A **177**, 379–388.

THEOPHILIDIS, G. (1984). Propagation failure of action potentials at the bifurcation point of the slow axon innervating the extensor tibiae muscle of *Decticus albifrons* (Orthoptera). *J. comp. Physiol.* **154**, 431–434.

THEOPHILIDIS, G. (1988). The study of action potential propagation failure at the branching point of an insect motor axon. *Comp. Biochem. Physiol.* **90**, 257–263.

THEOPHILIDIS, G. AND BURNS, M. D. (1979). A muscle tension receptor in the locust leg. *J. comp. Physiol*. **131**, 247–254.

THEOPHILIDIS, G. AND BURNS, M. D. (1983). The firing pattern of the locust (*Schistocerca gregaria americana*) mesothoracic femoral motor axons in resistance reflexes and during walking on a treadmill. *J. Insect Physiol.* **36**, 513–522.

VON HELVERSEN, D. AND VON HELVERSEN, O. (1994). Forces driving coevolution of song and song recognition in grasshoppers. In *Neural Basis of Behavioral Adaptations* (ed. K. Schildberger and N. Elsner). *Fortschr. Zool.* **39**, 253–284.

WEILAND, G., BÄSSLER, U. AND BRUNNER, M. (1986). A biological feedback control system with electronic input: the artificially closed femur–tibia control system of stick insects. *J. exp. Biol.* **120**, 369–385.