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THE INNERVATION OF THE MESOTHORACIC FLEXOR TIBIAE MUSCLE OF THE LOCUST

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SUMMARY

The anatomy and innervation of the mesothoracic flexor tibiae muscle indicated a subdivision into proximal, middle and distal flexors.

The muscle is innervated by 12 excitors, two inhibitors and two dorsal unpaired median (DUM) neurones. The motor axons were identified by (a) the height of the action potentials recorded extracellularly from the flexor nerve branches of an intact locust, (b) the EPSPs or IPSPs and the tension which they produced in the muscle when they were stimulated and (c) the distribution on the muscle. There was some independent innervation of proximal, middle and distal flexors.

INTRODUCTION

The organization of arthropod muscles is apparently much simpler than that of vertebrate muscles since they usually receive a small number of motor axons. The extensor tibiae muscle of the locust, for example, is innervated by just two excitatory and one inhibitory neurone (Hoyle, 1955, 1978; Burns & Usherwood, 1979). The same pattern occurs in the closer muscles of *Carcinus* walking legs, while the stretcher muscle of *Graspus* is innervated by only one excitor and two inhibitors (Atwood, 1973). The smallest number of axons is found in the accessory flexor muscle of *Cancer*, which is innervated by a single excitor and one inhibitor motor axon (Dorai-Raj, 1964).

Although no arthropod muscles receive as many axons as do vertebrate muscles, there are muscles in arthropods which receive many more than three axons. Five different motor axons innervate the dorsal longitudinal flight muscle in the flesh fly (*Sarcophaga bullata*), although this muscle has only six muscle fibres (Ikeda, 1977). In the cockroach, two inhibitory and four excitatory axons have been found to innervate the posterior coxal levator muscle (Pearson & Bergman, 1969) while five motor axons have been found to innervate the cockroach coxal depressor muscle (Pearson & Iles, 1971).

Insect flexor tibiae muscles are also complex and receive inputs from as many as eight motor neurones in the cockroach (Dresden & Nijenhuis, 1958) and more than six in the locust metathoracic leg (Burrows & Hoyle, 1973; Burrows & Horridge,

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1974; Phillips, 1980). In the mesothoracic flexor tibiae muscle of the locust twelve excitatory and two inhibitory axons were found in the present studies. These numbers are higher than those reported for the metathoracic flexor and this may be for two reasons. First, the mesothoracic flexor is larger than its antagonist while the opposite is true in the metathoracic femur. Second, the mesothoracic legs seem to participate more in walking and posture, while the metathoracic legs have been adapted for the jump and the defensive kick (Heitler & Burrows, 1977*a*,*b*; Heitler, 1977).

The purpose of the present work is to attempt to answer the following questions: what is the exact number of axons innervating the mesothoracic flexor tibiae muscle? How are their endings distributed on the muscle and what are their effects? How do they control the muscle in various behaviour patterns? Why does the control of the flexor muscle require such a large number of motor axons while its antagonist muscle operates equally well with only two excitors and one inhibitor?

MATERIALS AND METHODS

Most of the animals used were adult locusts, *Schistocerca gregaria americana* (Dirsch, 1974), kept in colonies at 32 °C. Females were chosen because they have a thinner exoskeleton and they are larger in size; both of which are helpful when dissecting the small mesothoracic leg. The mesothoracic femur was mounted with its ventral side up on plasticine or Takiwax. The dorsal cuticle, the extensor tibiae and the retractor unguis muscles were removed to expose the flexor muscle and $N5B_2$ (Campbell, 1961), which were then immersed in saline (Usherwood & Grundfest, 1965).

Anatomy

To establish the anatomy of the muscle the flexor nerve branches were back-filled with $CoCl_2$ contained in a glass suction electrode. Occasionally the flexor nerve branches were stained using a low concentration of reduced methylene blue injected into the femur of an intact locust. For transmission electron microscopy both the flexor muscle and $N5B_2$ were fixed (Karnovsky, 1965) and then $N5B_2$ with its flexor nerve branches was removed and subjected to standard electron microscope preparatory techniques.

For scanning electron-microscopy the flexor muscle and $N5B_2$ were fixed with 2% glutaraldehyde, washed with distilled water for 30 min and dehydrated in a series of acetones. Specimens were dried in a Polaron Critical Point Drier and coated in a Polaron Sputter Coater.

Physiology

Records were simultaneously obtained from intracellular glass microelectrodes inserted in different parts of the flexor muscle and from gold-plated glass suction electrodes on the flexor nerve branches (Theophilidis & Burns, 1982). The flexor nerve was stimulated directly or the flexor motor axons were reflexly activated by extending the tibia at constant velocity.

To measure the mechanical properties of the flexor muscle the animal was mount

ventral side up (Fig. 5C), a small window was cut in the cuticle of the mesothorax to expose the ganglion and a fine hook electrode (modified from Wilkens & Wolfe, 1974) was attached to nerve 5 for stimulation. The tendon of the extensor tibiae muscle was cut and the mesothoracic ganglion was destroyed. Passive and active forces were measured from the distal end of the tibia under nearly isometric conditions using a silicon strain gauge (compliance, 0·05 mm g^{-1}). The forces measured were converted to equivalent muscle tension by multiplying by a factor of 12 derived from measurements of the articulation and muscle insertion points on mesothoracic tibiae. To avoid inducing artificial variation in muscle tension no saline was used in these experiments.

RESULTS

The flexor tibiae muscle

The two antagonistic muscles in the mesothoracic femur of the locust show very similar morphological features; they are both pinnate in form with almost the same number of muscle bundles. However, although the extensor tibiae muscle is considered to be a single muscle (Burns & Usherwood, 1978), in the present study it was found necessary to examine the flexor in three parts. Initially, when the anatomy and innervation of the flexor was studied, no subdivision was made, but the evidence soon suggested a division of the muscle into three parts named the proximal, middle and

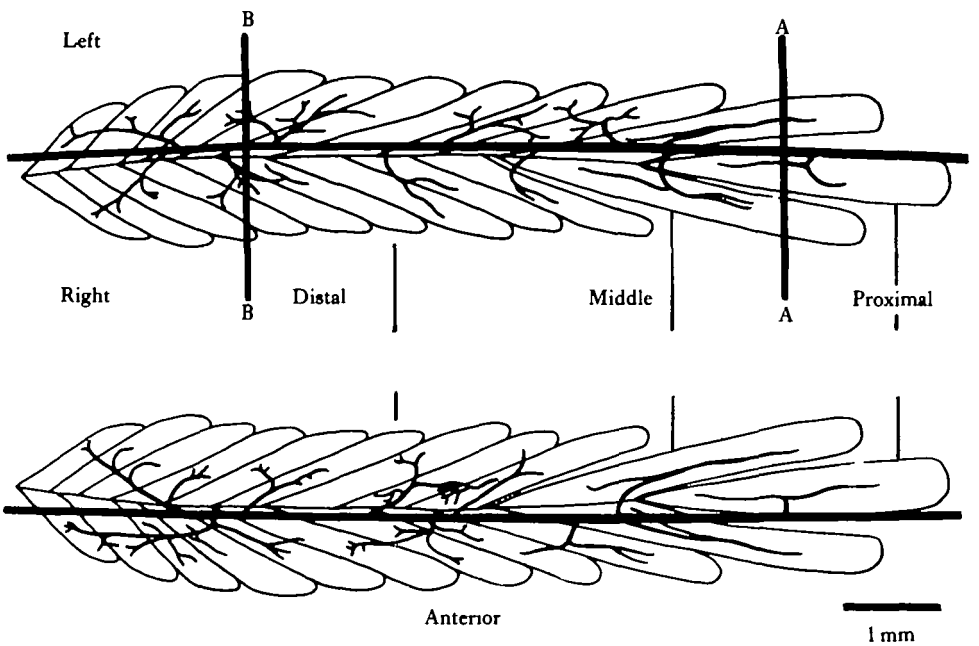

Fig. 1. Diagram of the mesothoracic flexor tibiae muscle and its motor nerve. The muscle bundles and the three different parts of the muscle are shown. There is no systematic difference between right and left legs. AA and BB show the approximate positions of the transverse sections in Fig. 2. The nerve branches shown are the commonest patterns found in cobalt fills of 10 right and 10 left nerves. Although there was some variation between animals in the number of distal nerve branches and the ways in which they approached the distal flexor, the branches to the proximal and middle flexors were constant.

distal flexors (Fig. 1). The distal flexor is the largest part and might possibly be divided further, but no physiological evidence was found to support such division. Snodgrass (1929) also divided the mesothoracic flexor of *Locusta* into three parts, 107a, b and c, on purely morphological grounds.

The proximal flexor is the only fusiform part of the pinnate flexor and consists of a single large muscle bundle, approximately 3·45 mm long, arising from a single proximal insertion on the ventral cuticle of the femur close to the trochanter (Fig. 1). A diagram of a transverse section through the middle of this muscle bundle can be seen in Fig. 2A and the dimensions of the muscle fibres are listed in Table 1. The mesothoracic extensor tibiae muscle is similarly constructed but the difference between the mean effective diameter of fibres in the proximal and distal parts is much smaller.

The middle flexor consists of the largest, most proximal pair of muscle bundles in the pinnate part of the flexor muscle (Fig. 1). These bundles are attached to the anterior and posterior sides of the femur with a pinnation angle of about 9°. Each muscle bundle contains approximately the same number of muscle fibres as the proximal flexor but the effective diameter of the middle muscle fibres is about 30% smaller. A major difference between the flexor and extensor muscles is that there is no part of the extensor muscle obviously homologous to the middle flexor.

The distal flexor forms the rest of the pinnate part of the flexor muscle. There are 8 to 10 anterior muscle bundles (Fig. 1) arising from a row of more or less circular, discrete insertions on the anterior cuticle of the femur and a similar number of bundles arising from a posterior row of elongated insertions very close to those of the extensor

Table 1. *Dimensions and structural characteristics of the mesothoracic femoral muscles of the locust*

Muscle	Length of m.b. (mm)	Number of m.f.	Mean diameter of m.f. (μm)	Cross sectional area (μm²)
Flexor muscle				
Proximal	3·45 (±0·3)	31 (±5)	66·0 (±7·0)	102·456 (±8·200)
Middle anterior	3·14 (±0·4)	33 (±3)	44·1 (±6·1)	51·525 (±5·258)
Posterior	3·80 (±0·7)	30 (±4)	46·2 (±5·3)	54·411 (±4·000)
Distal Anterior				
+	2·60 (±0·3)	28 (±5)	42·7 (±3·5)	27·737 (±5·600)
*	1·90 (±0·2)	20 (±3)		
Posterior				
+	2·40 (±0·3)	19 (±3)	46·1 (±6·7)	32·947 (±3·100)
*	1·70 (±0·1)	10 (±2)		
Extensor Muscle				
Proximal		32 (±5)	41·15 (±5·2)	42·777 (±4·600)
Distal				
Anterior +		28 (±3)	37·30 (±3·8)	30·737 (±3·610)
Posterior +		19 (±5)	36·1 (±4)	20·806 (±4·100)
Retractor unguis muscle		25 (±4)	32·3 (±5·5)	21·974 (±2·800)

m.b. = muscle bundles, m.f. = muscle fibres, + = first muscle bundle of this part of the muscle, * = last muscle bundle. Figures in brackets are standard deviations.

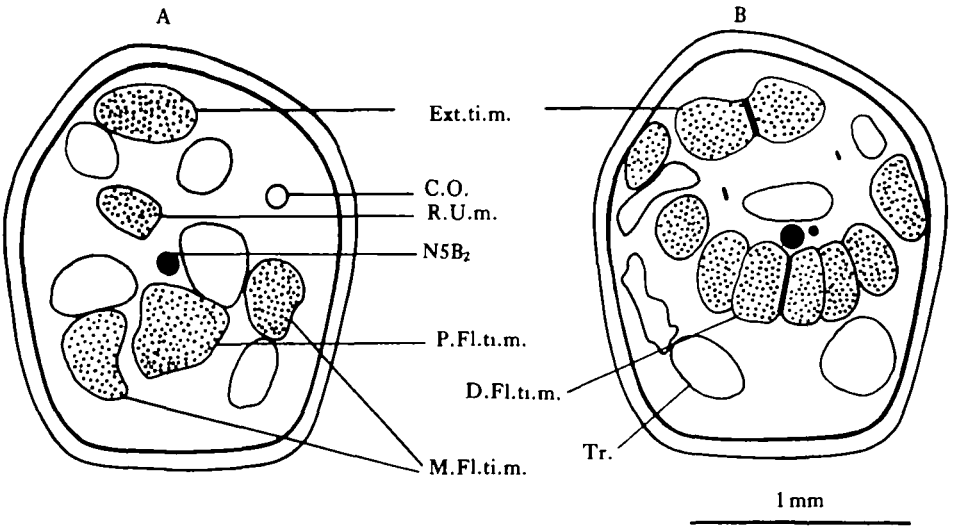

Fig. 2. Diagrams of cross-sections through (A) the proximal part of the mesothoracic femur of the locust and (B) the distal part of the femur. The levels of the sections are shown in Fig. 1 (AA, BB). Ext. ti. m., extensor tibiae muscle; C.O., chordotonal organ; R.U.m., retractor unguis muscle; P.Fl.ti.m., proximal flexor tibiae muscle; M.Fl.ti.m., middle flexor tibiae muscle; D.Fl.ti.m., distal flexor tibiae muscle; Tr., trachea; $N5B_2$, main femoral nerve.

muscle and tending to merge into one another. A diagram of a transverse section through the distal flexor is shown in Fig. 2B, but the pinnate form means that only four pairs of muscle bundles can be seen. In the distal flexor there is an asymmetry between the anterior and posterior halves, since the anterior muscle bundles are slightly longer and contain more muscle fibres (larger cross-sectional area, Table 1) than their posterior counterparts. This suggests that there could be a significant asymmetry in the distribution of the forces developed by the contraction of the muscle. This asymmetry is also emphasized by the difference in the pinnation angle of the distal muscle bundles. The posterior muscle bundles have smaller pinnation angles, producing more force parallel to the tendon than the anterior bundles, but the force perpendicular to the tendon would be smaller than the equivalent and opposite force developed by the anterior part. The effect of this difference in the forces can be seen during tetanic contraction of the muscle, since the apodeme not only moves in the direction of the contraction, but also towards the anterior part of the muscle. A possible purpose for this anterior movement of the whole flexor inside the femur is that it might help the flow of the haemolymph (Alsop, 1978). A similar function was suggested by Usherwood (1974) for the intrinsic rhythm of the metathoracic extensor muscle.

The flexor nerve branches

The flexor tibiae muscle is innervated by a series of short branches from the main femoral nerve $N5B_2$ (Fig. 1). Using transmission electron-microscopy it was possible to count the number of axons in the main flexor nerve branches. In the nerve branch innervating the most distal part of the flexor (Fig. 3C) there are 17 to 19 axons, in the

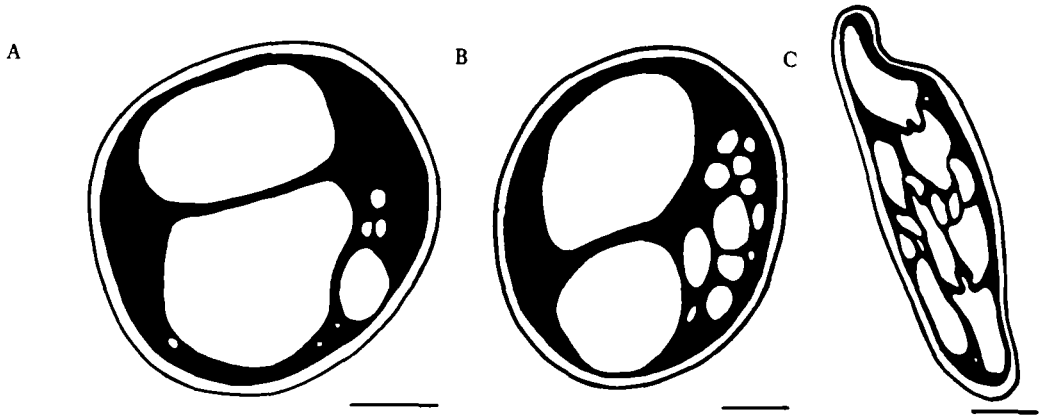

Fig. 3. Diagrams of electron micrographs of transverse sections through the nerve branches to the mesothoracic flexor tibiae muscle. (A) Proximal flexor branch; (B) middle flexor branch; (C) distal-most branch to the distal flexor. The locations of the nerve branches can be seen in Fig. 1. Scale bars = 10 μm.

middle flexor branch (Fig. 3B) 14 to 16 axons, and in the proximal flexor branch 8 to 10 axons (Fig. 3A). These results were obtained from three different animals. The effective diameters of the largest axons in the proximal nerve branch were 25·5 μm and 23·5 μm. In the middle nerve branch the two largest axons had diameters of 19 and 20 μm but the effective diameters of the largest axons in the distal nerve branch were about half this size. In some cases two axons in the same nerve branch were found to have very similar effective diameters and it seems likely that some of the profiles represent the same axon branching before the nerve does.

To study the fine details of the flexor nerve branches the scanning electron microscope was used. It was found that most of the nerve terminals were characteristic of the orthopteran diffuse type (described by Hamory, 1961). In some cases, however, the terminals approached the surface of the muscle fibres with a connecting tissue link between them (Fig. 4A, B). This tissue link seems to be the perineurium merging with the basal lamina-reticular fibre complex covering the muscle cells. This structure is very unusual since in typical neuromuscular junctions the sheath (lemnoblast) cells continue the terminal filament where they lie over the axon (Hoyle, 1974). From Fig. 4 it is obvious that this tissue link (l) is shorter than the motor axons attached to the same muscle fibre. This suggests that its function may be to protect the neuromuscular junctions by absorbing any mechanical tension developed between the short flexor nerve branch (Fig. 1) and the muscle. A similar structure was described in the retractor unguis muscle of the locust (Rees & Usherwood, 1972).

A brief examination of the prothoracic flexor tibiae muscle revealed that, apart from a reduction in the number of nerve branches innervating both sides at the distal flexor, there were no major differences in muscle or nerve anatomy from that of the mesothoracic femur.

Flexor muscle tension

To investigate changes in the resting tension of the muscle at different lengths the isometric length-tension curve was plotted for passive tension during stretching. T[redacted]

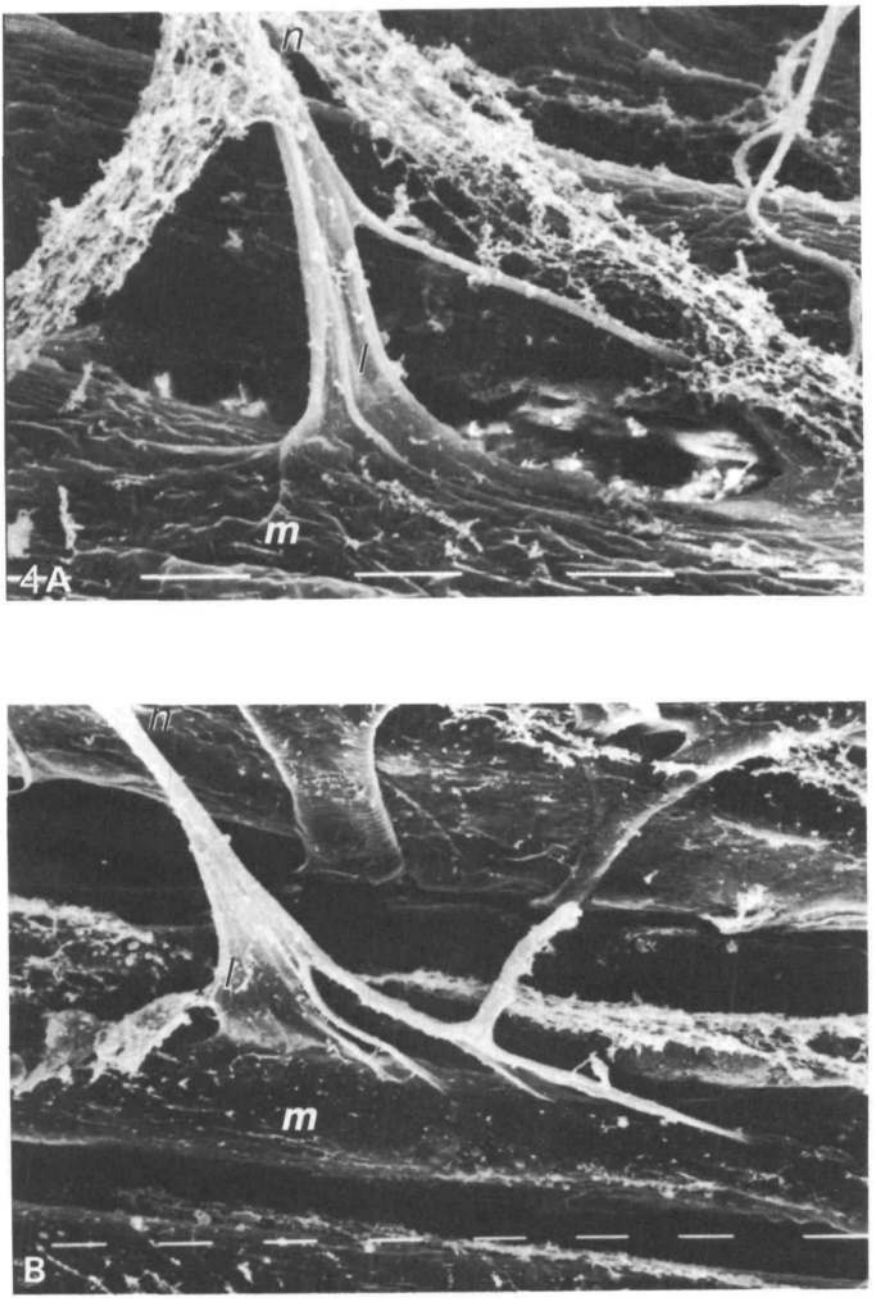

Fig. 4. (A), (B) Scanning electronmicrographs of the axon terminals on the surface of the mesothoracic flexor tibiae muscle. *l*, Tissue link between the nerve branch (*n*) and the muscle fibre (*m*). Scale bars 20 = 1 μm.

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tibia was extended from 90° to 175° in steps with a constant velocity of 150° s^{-1}, which is the speed at which tibial extension occurs when a locust walks at 2 steps s^{-1} (Burns, 1973). Typical records are shown in Fig. 5A and the average length-tension curve is plotted in Fig. 5B, curve 1. At each muscle length a large single shock was also given to nerve 5 so that all the flexor motor axons were excited. The isometric twitch that resulted was recorded, and the amplitude was measured and plotted against muscle length (Fig. 5A, B curve 2). Maximum active tension occurred at the normal rest length of the muscle for a flexor tibiae angle (FTA) of 90–100°.

The innervation of the flexor

From the transverse section of the main flexor nerve branches (Fig. 3) the number

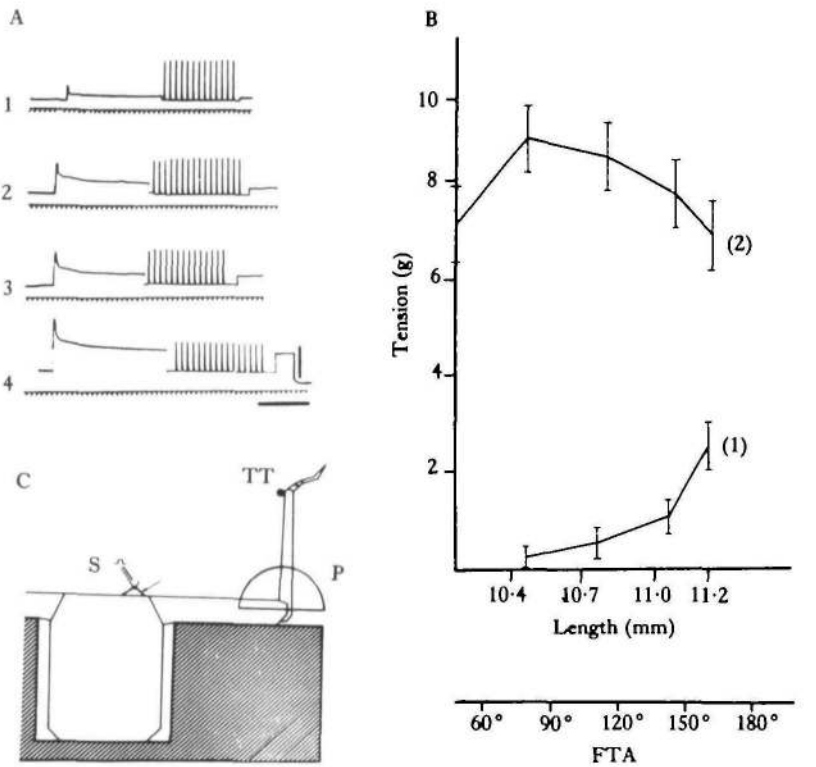

Fig. 5. (A) Tension from the mesothoracic flexor tibiae muscle recorded at the distal end of the tibia (see C). The first peak of all four records represents changes in muscle tension caused by passive tibial extension: (1) from 60° to 90°; (2) from 90° to 120°; (3) from 120° to 150° and (4) from 150° to 175°, each with an angular velocity of 150° s^{-1}. The remaining tension peaks are twitch contractions of the flexor muscle induced 20–30 s after the passive extension. Scale bars: horizontal, 10 s; vertical, 2 g (4 g during twitch contractions). (B) The tension recorded as in (A) plotted against flexor muscle length, (1) passive plateau tension, (2) amplitude of twitch. The data were obtained from experiments on three different locusts, 15 repeats on each. FTA, femur–tibia angle. (C) The apparatus used to record tension from the mesothoracic flexor tibiae muscle in its own haemolymph. The dissection to expose N5 was small. No saline was used and care was taken to leave most of the tracheal system intact. To measure tension from either extensor or flexor tibiae muscle the apodeme of the antagonistic muscle was cut near its connection with the tibia. S, Stimulation of N5 through a hook electrode; TT, tension transducer attached always at the end of the tibia; P, miniature protractor.

of axons innervating each part of the flexor muscle was roughly estimated. To find out how many of these axons were excitors, N5 of isolated muscles was stimulated and flexor tension was monitored from each of the three parts of the muscle in turn. From the records it was found that five or six excitatory axons innervate the proximal flexor, seven or eight the middle flexor and nine to ten the distal flexor. However, counting the excitatory axons does not produce any information about the distribution of the axons on the muscle surface. To investigate the innervation of the muscle, intracellular records were simultaneously obtained from the branches and muscle bundles of an intact locust (Theophilidis, 1982). For classification purposes, axons producing EPSPs larger than 20 mV were called fast axons (F1, 2, ...), axons with EPSPs between 10–20 mV intermediate (IM1, 2, ...) and axons with EPSPs between 3–10 mV slow (S1, 2, ...). The inhibitory axons were called I1, 2 and the dorsal unpaired median (DUM) motoneurones D1, D2.

Axons F1, F2, F3, IM1

In an isolated muscle, intracellular records from the proximal and middle parts were correlated with muscle tension increments simultaneously monitored only from the distal flexor (Fig. 6). This showed that there is a relatively small axon, called IM1 (EPSP < 20 mV, Fig. 6A) innervating all parts of the flexor and two large axons called F1, F2 (EPSPs > 25 mV) innervating the proximal part but not the distal part. To investigate the innervation of the middle flexor, a second microelectrode was inserted into one of its muscle fibres (Fig. 6B, middle trace). It can be seen that axons F2 and IM1 also innervate the middle flexor (Fig. 6B, see common spikes). In the middle flexor a large EPSP (F3) was also recorded (Fig. 6B, middle trace) and its activity was independent of the activities of axons F2 and F1 and of any tension increments monitored from the distal flexor. This suggests that F3 is a large axon innervating only the middle flexor. However, this leaves some doubts concerning the branching patterns of axons F1 and F3. The innervation patterns of all these axons were finally established by recording from the various parts of the flexor muscle of an intact locust (Fig. 6C). The spikes and EPSPs of axon F2 can be identified as above. Axon F1 can be seen in the top trace of Fig. 6C since it produces a large EPSP in the proximal flexor but does not innervate any other parts of the muscle. Fig. 6D shows that axon F3 innervates only the middle flexor. The innervation patterns of F1, F2, F3 and IM1 are summarized diagramatically in Fig. 9.

The shapes of the EPSPs produced by axons F1 and F2 in two different proximal muscle fibres and axons F2 and F3 in two middle muscle fibres can be seen in Fig. 7A–D. All three EPSPs (F1, F2, F3) have fast rise times and heights larger than 30 mV. However, when axons F1 and F2 innervate the same proximal muscle fibre, the height of the EPSP produced by F2 is dramatically reduced. Thus, although axon F2 produces a rapid twitch in the proximal flexor (Fig. 7F, lower trace) characteristic of a fast axon, it produces only a small EPSP (Fig. 7F, top trace, approximately 10 mV) in muscle fibres innervated by axon F1.

Axons F5, F6, IM3

These axons were found only in the nerve branches innervating the distal flexor and it seems that they do not enter the middle or proximal nerve branches. Two large a[illegible]

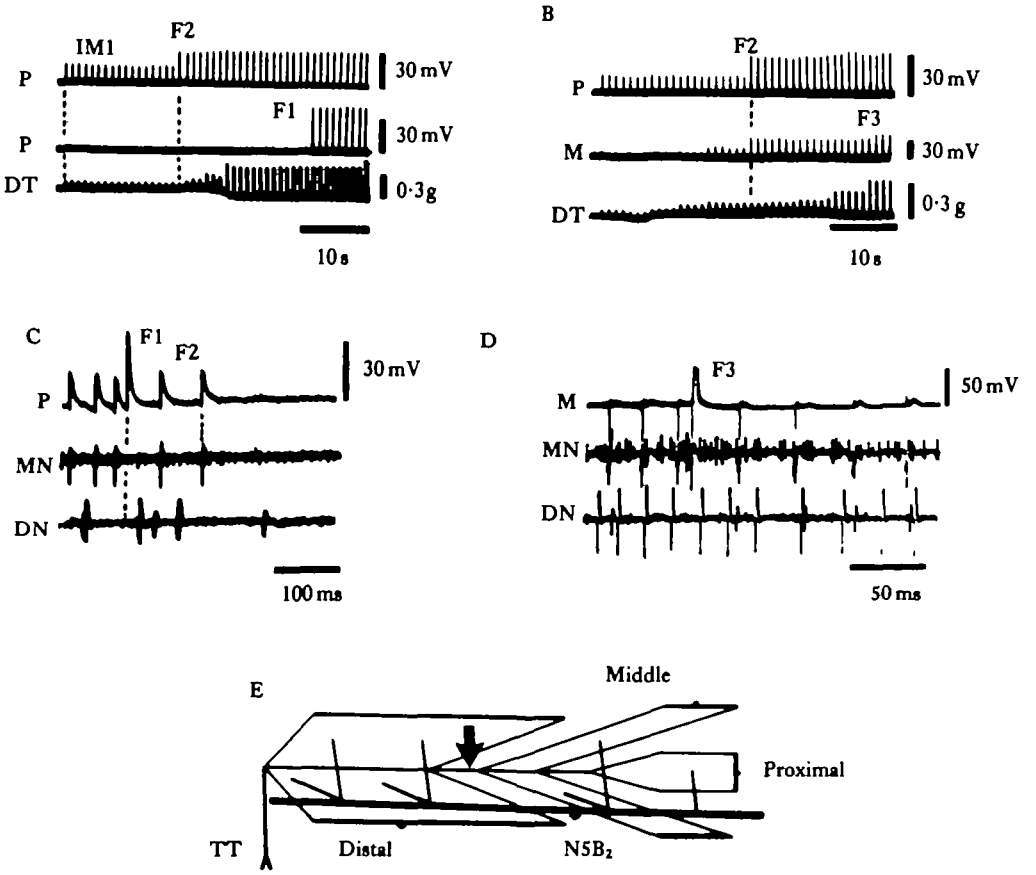

Fig. 6. (A), (B) Records from different parts of an isolated mesothoracic flexor tibiae muscle in saline. The muscle was stimulated through $N5B_2$. (See E for locations.) (A) P, intracellular records from two different proximal muscle fibres; DT, muscle tension in the distal flexor. The tension transducer was placed at the end of the muscle and the apodeme between the middle and distal flexors was cut at arrow in (E). (B) P and DT as in (A); M, intracellular record from a middle muscle fibre. (C), (D) Records from the mesothoracic flexor tibiae muscle in the dissected femur of an immobilized locust. The femur was filled with haemolymph and a small amount of saline added. The flexor motoneurones were reflexly excited by tibial extension. (C) P, intracellular record from a proximal muscle fibre; MN and DN, extracellular records from the middle and distal nerve branches respectively. (D) M, intracellular record from a middle flexor muscle fibre. Second and third traces as in (C). (E) Diagram of the mesothoracic flexor tibiae muscle showing the various parts of the muscle and the position of the tension transducer (TT).

one small spike were recorded in the distal nerve branch but not in the middle branch. The two large action potentials were correlated with EPSPs larger than 20 mV and were called F5 and F6. The axon producing the smaller action potential (EPSP < 15 mV) was called IM3. Using the same methods axons F4 and IM2 were found to innervate only the middle and distal parts of the flexors.

The distribution patterns of F5, F6, IM1, F4 and IM2 are summarized in Fig. 9.

Slow axons (S1, S2, S3)

Two slow axons (S1, S2) were found to innervate the whole flexor muscle while a

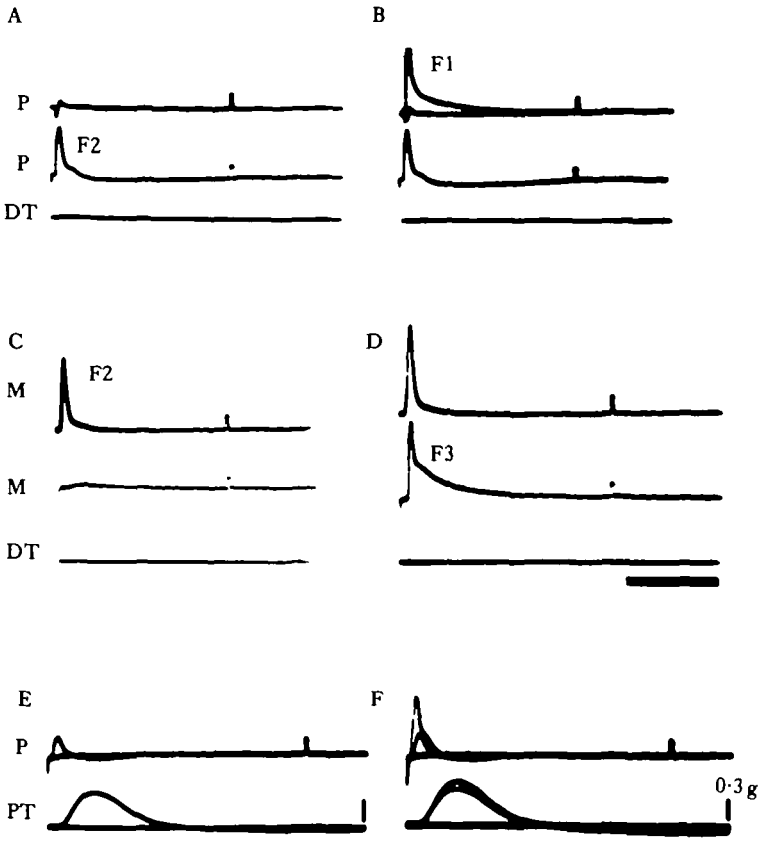

Fig. 7. Records from different parts of an isolated flexor tibiae muscle within the first 40 min immersion in saline. During this period the large axons have the lowest threshold (Theophilidis, 1982). P, intracellular record from a proximal muscle fibre; M, intracellular record from a middle muscle fibre; DT, tension in the distal flexor (as in Fig. 6A). (A), (B) Two different proximal muscle fibres; (C), (D) two different middle muscle fibres; (E), (F) a proximal muscle fibre; PT, tension in the proximal flexor isolated by denervating the middle and distal flexors. Horizontal scale bar A–D = 50 ms; E, F = 25 ms. Calibration pulses = 10 mV.

third (S3) supplied only the proximal flexor. Axon S3 produces a 7–10 mV EPSP in the proximal flexor fibres while S1 and S2 produce EPSPs of 2–5 mV in the middle flexor fibres correlated with action potentials from the distal nerve branches. A histogram of the amplitudes of these small EPSPs showed a bimodal distribution confirming that two axons were responsible. Repeated penetrations of the flexor muscle fibres showed that the slow axons innervate approximately 50–60 % of distal and middle flexor muscle fibres but have fewer endings (20–30 %) on the proximal flexor. There is also a difference in the number of excitatory axons innervating each muscle fibre. In the proximal or middle part, two, or a maximum of three, axons synapse with each muscle fibre while in the distal part up to six axons were found to synapse with the same muscle fibre.

Inhibitory axons (I1, I2)

To study the distribution of the inhibitory axons, spontaneous IPSPs were record

[illegible]m the various parts of the flexor muscle of an intact locust (Fig. 8A). The axon producing the largest IPSP (3–4 mV) was labelled I2 and the axon producing the smaller IPSP (1–2 mV), I1. The two IPSPs always appeared together in the same muscle fibres and it was found that the inhibitory axons innervate approximately 50–60 % of the distal and 20–30 % of the middle and proximal flexor fibres. Since the distal flexor receives a larger number of inhibitory endings, relaxation by the inhibitors is very obvious in this part of the muscle (Fig. 8B). Isometric twitches due to the slow or intermediate axons were reduced in amplitude when axon I1 was stimulated. When the threshold of I2 was reached, the twitch contraction of the flexor muscle decreased by about 30 % although some fast axons were now being stimulated (see arrows, Fig. 8B).

Dorsal unpaired median neurones (D1, D2)

To find the number of DUM neurones innervating the mesothoracic flexor tibiae muscle, nerve impulses were initiated in the DUM axons by stimulating the contralateral nerve branches. (Crossman, Kerkut & Walker, 1972; Hoyle, Dagan, Moberly & Colquhoun, 1974). When the left nerve branches were stimulated, impulses of about the same height were recorded in the right flexor and the right extensor nerve branches. These action potentials were almost certainly recorded from branches of the same axon (D1) since they always have the same threshold. When the intensity of the stimulus was increased, another action potential smaller than the first one was

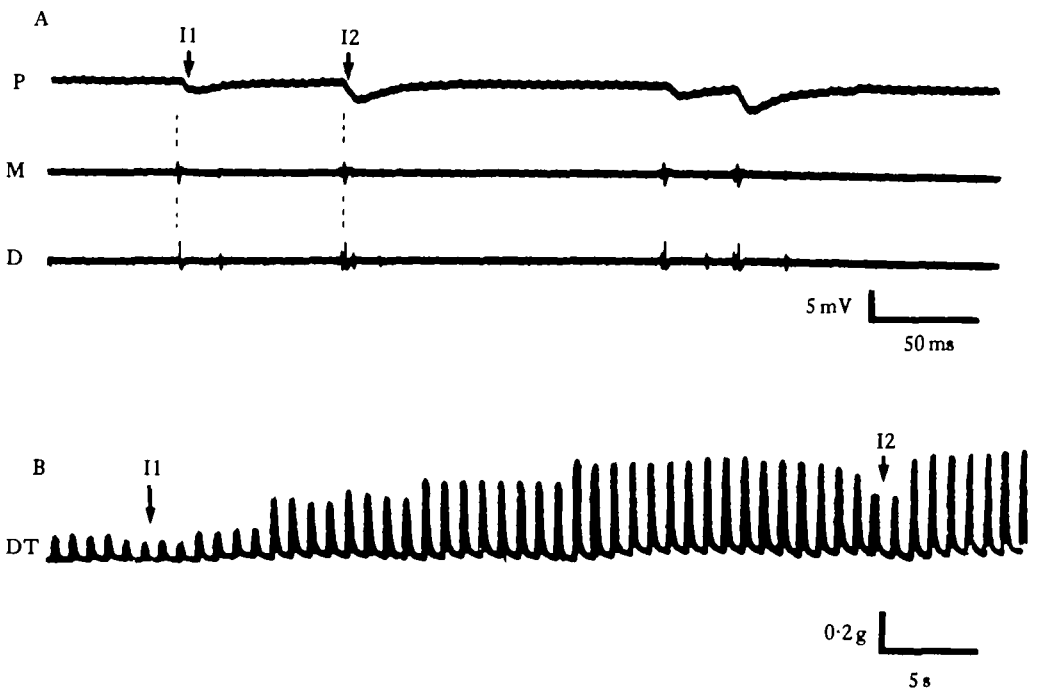

Fig. 8. Spontaneous activity recorded from the flexor muscle of an immobilized locust. (A) P, intracellular record from a proximal muscle fibre, M and D extracellular records from the middle and distal nerve branches respectively. The two inhibitors are labelled. (B) Muscle tension in the distal flexor, DT, resulting from progressively increasing amplitudes of stimulation to the nerve (as in Fig. 6A).

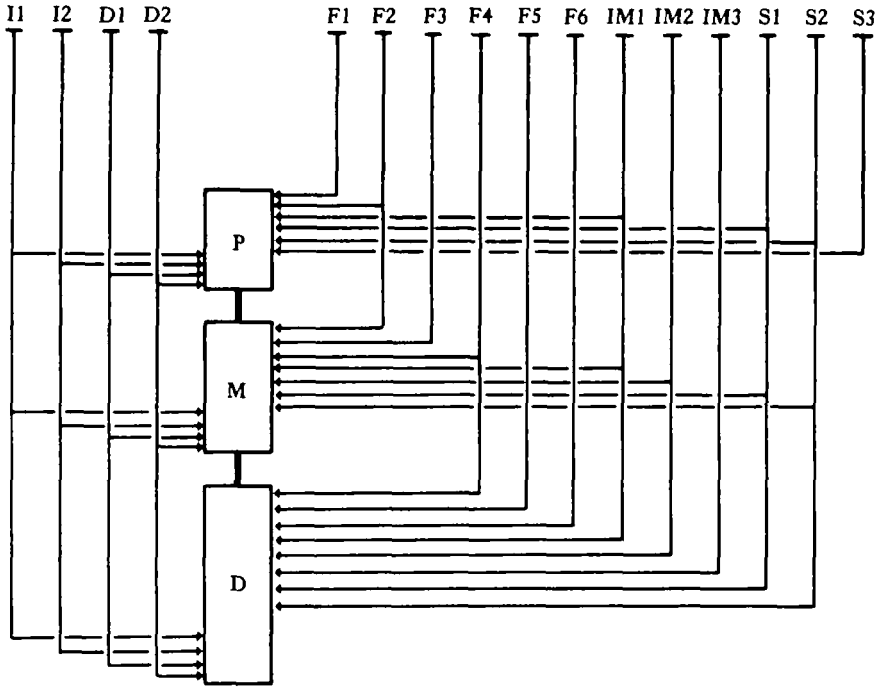

Fig. 9. Diagram of the distribution of the axons innervating the mesothoracic flexor tibiae muscle. F, fast axons; IM, intermediate axons; S, slow axons; I, inhibitory axons; D, DUM neurones; P, proximal; M, middle; D, distal regions of muscle.

recorded in the right flexor nerve branch but not in that of the extensor. This must originate in a second DUM neurone (D2).

DISCUSSION

In the pro- and mesothoracic femurs of the locust, although the two main antagonistic muscles are anatomically very similar, there are marked differences in the complexity of their innervation. The mesothoracic extensor tibiae muscle receives only four axons (Burns & Usherwood, 1979) while the mesothoracic flexor is innervated by a much larger number of axons; at least 12 excitors, two inhibitors and two DUM cells. There are similar contrasts in the number of axons in the metathoracic femur of the locust (Hoyle, 1978; Phillips, 1980) and the mesothoracic femur of the cockroach (Dresden & Nijenhuis, 1958). It is not clear why the neuronal control of the flexor muscle requires a large number of axons, while its antagonist operates adequately with only four. To study this question, physiological and anatomical studies were made in order to establish firstly the exact number of axons innervating the mesothoracic flexor tibiae muscle, secondly, the way in which the axons are distributed on the muscle and thirdly, how they are used to control the position of the tibia during various behavioural patterns (G. Theophilidis & M. D. Burns, in preparation).

The two most extreme innervation patterns which might be expected are: (a) the flexor axons might innervate all parts of the muscle, as appears to happen in the metathoracic flexor tibiae muscle (Phillips, 1980), or (b) each flexor axon innervat

[illegible]ly a specific muscle bundle (as suggested by Hoyle, 1955). The present study showed that the mesothoracic flexor tibiae receives both types of innervation.

The proximal flexor

The proximal flexor is the only part of the muscle which is directly in line with the apodeme and contains the largest muscle fibres in the whole flexor. Such a marked difference in the size of the fibres in different parts of the muscle does not occur in the extensor where the ratio of the effective diameter between the proximal and the very distal muscle fibres is 1·1/1. The ratio for the flexor muscle is 1·5/1.

The proximal flexor has not only the largest muscle fibres but also receives the largest motor axons. The effective diameters of the two largest axons in the proximal nerve branches are 19·5 μm and 25 μm, while that of the largest axon innervating the distal flexor is only 10·5 μm. In the proximal flexor, only two large axons were identified electrophysiologically by their large EPSPs and action potentials. The larger axon, innervating only the proximal area is F1, while the other large axon branching also into the middle flexor is F2. However, when both fast axons innervate the same proximal muscle fibre the large EPSP (>35 mV) produced by F2 was dramatically reduced in height (<15 mV). This could be a simple way to avoid saturation of the contractile components of the muscle fibres receiving two large motor axons. Since axon F2 is very active in behaviour (G. Theophilidis & M. D. Burns, in preparation), it would cause a large depolarization of the muscle fibres. The small EPSP produced by axon F2 in dually-innervated fibres may ensure that these muscle fibres can produce appreciable additional tension when activated by F1.

From the construction of the proximal flexor (large muscle fibres in line with the apodeme) and its innervation (large motor axons) it seems that this area of the muscle is designed to produce rapid and powerful movements of the tibia. These are needed in walking, for example, where during protraction of the femur the tibia must flex 60° in less than 50 ms, when the locust walks at a speed of 5 steps s^{-1} (Burns, 1973). Under the same walking conditions, during retraction, the tibia takes more than 110 ms to extend again.

Middle flexor

The middle flexor is the largest, most proximal pair of muscle bundles in the pinnate part of the flexor muscle. The pinnation angle is very small so these muscle bundles are nearly in line with the apodeme and may have a similar function to the fusiform proximal flexor. The largest axons innervating this part of the muscle are F2, which also innervates the proximal flexor, and F3, which is unique to the middle flexor. Among the other smaller axons in the middle nerve branch are F4, IM1, IM2, slow axons, the inhibitors and the DUM cells.

From the innervation pattern of the mesothoracic flexor tibiae it is obvious that the middle flexor has fast axons shared with the proximal (axon F2) and the distal (F4, IM2) areas of the muscle. These common fast axons may be used to synchronize the activity of the middle flexor with the proximal or the distal parts. Thus the middle flexor can be used to reinforce the fast contraction of the fusiform phasic proximal flexor or to add phasic components to the contraction of the pinnate distal flexor. From this complicated innervation pattern of the middle flexor it seems that this area

of the flexor may be very active in behaviour and so it is not surprising that the distal scoloparium of the femoral chordotonal organ (CO) (Burns, 1974) is attached to the middle muscle fibres. Since this part of the CO is primarily a movement receptor it monitors fast contractions of the middle flexor caused by axons F2, F3 and F4. In behaviour (resistance reflexes, walking), axons F2 and IM1 have been found to be more active than the other axons (G. Theophilidis & M. D. Burns, in preparation) and one might suggest that the CO monitors mainly the muscle responses to axon F2. It would be interesting to find out if there are any central connections between the CO and the neurones innervating the middle flexor. The most surprising thing about the middle flexor is that although the flexor and extensor muscles in the mesothoracic femur have almost the same muscle bundle arrangement, there appears to be no homologue for the middle flexor in the extensor, where it may have evolved into the retractor unguis muscle and some part of the chordotonal organ.

The distal flexor

The distal flexor forms the rest of the pinnate part of the flexor muscle. The pinnate form means that most of the muscle fibres are shorter than they would be in a simple fusiform muscle and this is a way of placing more muscle fibres in parallel. A pinnate muscle can therefore exert more force per unit volume than a fusiform one, but its efficiency is lower since not all the force acts in line with the apodeme, and the maximum amplitude of apodeme movement is smaller. In this area of the flexor, EPSPs from six or more different axons were recorded in a single muscle fibre, in contrast with the proximal and middle flexor muscle fibres which were found to receive a maximum of three excitors each. Such a highly polyneuronal innervation combined with the pinnate arrangement of the distal flexor suggest that this part of the muscle can create finely controlled forces which may be very important in posture. Thus, it is not surprising that a tension receptor (Theophilidis & Burns, 1979) is attached almost in the centre of the distal flexor, probably to monitor the changes of muscle tension developed in this area of the muscle. The existence of the tension receptor and the connection of the phasic middle flexor with the distal scoloparium of the CO show that there is a strong coupling of the flexor tibiae muscle with sensory organs. Accurate control of such a large number of flexor motor axons may require extensive sensory inputs. In contrast, the extensor tibiae muscle is not directly coupled to any femoral proprioceptors.

Although most of the flexor tibiae muscle is pinnate in structure, its mechanical properties are more like those of a fusiform muscle than are those of the extensor. For instance, the maximum twitch tension of the mesothoracic extensor muscle occurs when the muscle is almost fully extended [femur-tibia angle (FTA) of 150–180°] which is typical of a pinnate muscle (Aidley, 1975), whereas the peak twitch tension in the flexor appears in the middle of the range of normal muscle length (FTA of 90–100°). This feature of the flexor muscle is typical of a fusiform muscle (Weis-Fogh, 1956) and it indicates a dominance of the fusiform parts of the flexor in rapid contractions. This is not surprising since the proximal muscle fibres are about 30–40 % larger in diameter than those in the distal part, and the middle flexor, which also receives large axons, is nearly in line with the apodeme. In contrast, in the extensor, the difference in muscle fibre diameter between proximal and distal parts

only 10% and there appears to be no homologue for the middle flexor, so the dominance of the pinnate distal part is expected. From the construction of the flexor and extensor muscles it is obvious that the different-sized muscle fibres are not randomly mixed in the different regions of the muscle. This construction differs from the vertebrate muscle where a mixture of different-sized muscle fibres occurs in each part of the muscle (Henneman & Olson, 1965).

The gradation of muscle fibre diameter along the flexor muscle is reflected in the diameter of the motor axons supplying them. In this case, as in the crayfish abdominal flexor muscle (Suzuki, 1977), nerve axons and muscle fibres of different size are segregated. However, in some other arthropod muscles, such as the locust extensor tibiae (Hoyle, 1955, 1978; Burns & Usherwood, 1978, 1979) and the crayfish superficial abdominal flexor (Velez & Wyman, 1978), the muscles have a gradient of properties accompanied by a tapering of individual axons through the muscle. Various explanations have been given for the matching between the size of the muscle and the size of the motor axons which innervate them. Some authors found that in development the motoneurones innervated muscle fibres before their type was determined and caused the fibres to differentiate into the proper type (Atwood, 1973; Barony & Close, 1971; Close, 1972). However, Frank (1973) suggested that the motor neurones selectively innervate those muscle fibres which are of the proper type.

Thus it is likely that the mesothoracic flexor tibiae muscle requires a large number of motor axons because it does not function as a single muscle. Some parts of the flexor are mainly used to achieve rapid movement and other parts to produce finely controlled postural tension. For these reasons each part of the flexor muscle is not only innervated by the appropriate axons but is also equipped with the appropriate sense organs.

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